EXTERNAL SCIENTIFIC REPORT

APPROVED: 30 November 2016

doi:10.2903/sp.efsa.2016.EN-1140

Occurrence of tropane alkaloids in food

Patrick P.J. Mulder[a], Monique de Nijs[a], Massimo Castellari[b], Maria Hortos[b], Susan MacDonald[c], Colin Crews[c], Jana Hajslova[d], Milena Stranska[d]

[a]RIKILT Wageningen University & Research, Wageningen, the Netherlands

[b]Institute for Research and Technology in Food and Agriculture (IRTA), Monells, Spain

[c]Fera Science Ltd., York, UK

[d]University of Chemistry and Technology (UCT), Prague, Czech Republic

Abstract

A total of 1709 samples of plant-derived food products, mainly produced in Europe, were analysed for tropane alkaloids (TAs). The samples, of which 27.4% came from organic production, were collected from retail stores, between June 2015 and August 2016, in nine European countries (Czech Republic, France, Germany, Hungary, Italy, the Netherlands, Poland, Spain, the United Kingdom). Samples analysed for the presence of 24 TAs comprised 268 single component flours (buckwheat, millet, corn), 260 cereal-based foods for young children age 6-36 months (breakfast cereals, biscuits and other cereal-based foods), 219 breakfast cereals, 164 biscuits and pastry, 114 bread, 81 pasta, 121 dry (herbal) teas, 78 legumes and stir-fry mixes. Samples analysed for six calystegines comprised 308 potato, 90 aubergine and six bell peppers. All samples were analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Two methods were developed and validated in-house and were considered fit for purpose. The limits of quantification for the various food groups were, depending on the type of TA, 0.0067-0.0333 µg/L for tea infusion, 0.5-5 µg/kg in cereal-based products, herbal tea and vegetable products, and 1-2.5 mg/kg in potatoes and aubergines. One or more TAs were detected in 21.3% of single component flours, 20.0% of cereal-based foods for young children age 6-36 months, 6.8% of breakfast cereals, 14.6% of biscuits and pastry, 15.8% of bread, 70.2% of dry (herbal) tea, 26.2% of legumes and stir-fry mixes, 100% of potatoes and 92.7% of aubergines. No TAs were detected in pasta. The highest mean TA concentration was detected in cereal-based meals for children (130.7 µg/kg), and the maximum TA concentration of was detected in a dry herbal tea sample (4357.6 µg/kg). Atropi ne and scopolamine were the most frequently detected TAs with a maximum sum concentration of 428.5 µg/kg in a dry herbal tea.

Key words: tropane alkaloids, survey, occurrence, food products, calystegines, LC-MS/MS

Requestor: European Food Safety Authority

Question number: EFSA-Q-2014-00320

Correspondence: biocontam@efsa.europa.eu

Disclaimer: The present document has been produced and adopted by the bodies identified above as author(s). In accordance with Article 36 of Regulation (EC) No 178/2002, this task has been carried out exclusively by the author(s) in the context of a grant agreement between the European Food Safety Authority and the author(s). The present document is published complying with the transparency principle to which the Authority is subject. It cannot be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Acknowledgements: The authors acknowledge the support and collaboration of:

- Netherlands Ministry of Economic Affairs, for financial support via project WOT-02-002-004 'Support function NVWA-BuRO' and project WOT-02-001-018 'Method development and surveys plant toxins'.
- Susannah de Witte, Patricia López Sánchez and Elena de Vries (RIKILT) for sample preparation and analysis.
- Carlos Terriente Palacios (IRTA) for sampling in Spain, France and Italy and for sample preparation and analysis.
- UK Food Standards Agency for financial support and the sampling of additional samples through project FS102116.
- nabim (National Association of British and Irish Flour Millers) for supply of samples.
- Joanna Stratton, Irene Leon, Julie Clough, Monika Sehlanova, Mita Parmar and Antony Lloyd (Fera) for sample preparation and analysis.
- Alena Zachariasova, Veronika Krtkova, and Petra Slavikova (UCT) for sampling in Czech Republic, Hungary and Poland, and for sample preparation and analysis. Monika Tomaniova for her support on the project related issues.

Suggested citation: Mulder PPJ, De Nijs M, Castellari M, Hortos M, MacDonald S, Crews C, Hajslova J and Stranska M, 2016. Occurrence of tropane alkaloids in food. EFSA supporting publication 2016:EN-1140, 200 pp. doi:10.2903/sp.efsa.2016.EN-1140

Summary

Tropane alkaloids (TAs) are secondary metabolites produced by a wide variety of plants from the families of Brassicaceae, Convolvulaceae, Moraceae and Solanaceae. Atropine and scopolamine, the best known representatives of this class of metabolites, are strong antimuscarinic agents. Toxic effects of other TAs and calystegines are largely unknown. Some food crops such as potatoes and aubergine, are known to produce TAs while other food crops can be contaminated when TA-containing weeds are co-harvested with the crop. Common practices for cleaning cereals are not always sufficient to remove the weed plant parts and seeds.

TAs are regarded as undesirable substances in food and feed and for that reason have been the subject of an EFSA opinion, published in 2013, and of the Commission Regulation (EU) 2016/239 on 'maximum levels of tropane alkaloids in certain cereal-based foods for infants and young children, containing millet, sorghum, buckwheat or their derived products'. Due to the limited availability of suitable occurrence data in food products, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) recommended that ongoing efforts should be made to collect analytical data on occurrence of TAs in relevant food commodities. In 2014 EFSA published a call for proposals to investigate the concentrations of TAs in cereal-based products from retail stores across different regions in Europe.

This report describes the outcome of project GP/EFSA/CONTAM/2014/01, 'Occurrence of Tropane Alkaloids in food' carried out in accordance with Article 36 of Regulation (EC) No 178/2002, which was designed to obtain representative data on the occurrence of TAs in Europe, using validated state-of-the-art analytical methods.

A literature review was performed to identify the most relevant TAs and food products. Literature on inherent TAs in foods as well as TAs from co-occurring weeds was searched and used. Very little information was available on occurrence of TAs other than atropine and scopolamine in food. It was therefore decided to measure as many TAs as possible for which analytical standards were available, including calystegines in food plants of the Solanaceae family.

All samples were analysed by liquid chromatography coupled to tandem mass spectrometry. Two methods were developed and validated in-house by all partners. The first method comprised 24 Datura TAs, and the limits of quantification (LOQ) for the various food groups were, depending on the type of TA, 0.5-5 µg/kg in single flours and cereal-based foods, dry (herbal) teas and in legumes and stir-fry mixes and 0.0067-0.0333 µg/L in tea infusion. The limits of detection (LOD) ranged from 0.05-2.5 µg/kg in single flours and cereal-based foods, dry (herbal) teas and in legumes and stir-fry mixes and from 0.0017-0.0133 µg/L in tea infusion. The second method comprised six calystegines and the LOQs in potatoes and aubergines were 1-2.5 mg/kg, while LODs ranged from 0.25-1 mg/kg. The results of the in-house validations showed that the methods were fit-for-purpose.

A total of 1709 samples of plant-derived products obtained from retail stores in nine EU countries were analysed for TAs. From these samples 27.4% were from organic production. Samples were collected between June 2015 and August 2016, in Czech Republic, France, Germany, Hungary, Italy, the Netherlands, Poland, Spain and the United Kingdom. Although the majority of the samples were produced in Europe, in particular for cereal-based products and herbal teas often no country of origin was reported. A total of 1305 samples, comprising 268 single component flours (buckwheat, millet, corn), 260 cereal-based foods for young children age 6-36 months (breakfast cereals, biscuits and other cereal-based foods), 219 breakfast cereals, 164 biscuits and pastry, 114 bread, 81 pasta, 121 dry (herbal) teas, 78 legumes and stir-fry mixes, were analysed for the presence of 24 TAs. A total of 404 samples, comprising 308 potato, 90 aubergines and six bell peppers were analysed for six calystegine alkaloids.

One or more TAs were detected in: 21.3% of the single component flours; 20.0% of the cereal-based foods for young children age 6-36 months; 6.8% of the breakfast cereals; 14.6% of biscuits and

pastry; 15.8% of the bread; none of the pasta; 70.2% of dry (herbal) tea; 26.2% of legumes, stir-fry mixes and oilseeds; 100% of the potatoes and 92.7% of aubergines. The highest mean concentration, 130.7 µg/kg, was detected in the food product group of cereal-based meals for children. Regarding individual samples the maximum concentration, 4357.6 µg/kg, was detected in a sample of dry herbal tea. The highest mean concentration for the sum of atropine and scopolamine, 13.4 µg/kg, in a specific food category was recorded for herbal teas. In individual samples, the maximum concentration for the sum of atropine and scopolamine, 428.5 µg/kg, was detected in a sample of dry herbal tea.

Of the 24 Datura TAs included in the analytical method, 19 were detected in one or more samples. Atropine was detected most often above the LOD (226 times), followed by scopolamine (172), pseudotropine (70) and tropine (54). TAs that were not at all detected included acetylscopolamine, anisodine, homatropine, a-hydroxymethylatropine and phenylacetoxytropane, while littorine, scopine and scopoline were reported only once. The typical patterns of TAs present differed between the food product groups. In single flours and cereal-based products that did not contain vegetables, the Datura-type TAs atropine and scopolamine were the major components. Mixed vegetable products and ready-to-eat meals for children containing vegetables as ingredients often contained the low molecular weight TAs tropine and pseudotropine as the major TAs. The herbal teas contained TAs from all types, most notably convolvine, atropine, scopolamine, tropine and pseudotropine. Overall, around 50% of TAs present in dry tea were transferred to the tea infusion when a standard protocol for tea preparation was applied. There was however substantial variability in the transfer efficiency for individual teas and TAs.

The mean content of calystegines in potatoes was 161.6 mg/kg fresh weight, the maximum concentration detected was 507.3 mg/kg. The mean content in aubergine was 21.1 mg/kg fresh weight, the maximum concentration detected was 181.5 mg/kg. In the limited number of bell peppers analysed only trace amounts of calystegines were detected (≤0.5 mg/kg).

Between potato, sweet potato, aubergine and bell pepper, the patterns and concentrations of calystegines were quite distinct. Calystegine A_3 was the most important TA in potatoes, followed by calystegine B_2. In aubergine calystegine B_2 was the predominant TA, followed by calystegine B_1 and A_3. Calystegines B_1 and B_2 are likely the major TAs present in sweet potato, while for bell peppers calystegine B_1 is likely to be the major TA present.

Table of contents

1. Introduction

1.1. Background and terms of reference as provided by EFSA

EFSA's Panel on contaminants in the food chain (CONTAM) recently assessed the risk for public and animal health related to the presence of tropane alkaloids in food and feed (EFSA, 2013).

Tropane alkaloids (TAs) are secondary metabolites which naturally occur in plants of several families including Brassicaceae, Solanaceae (e.g. mandrake, henbane, deadly nightshade, Jimson weed) and Erythroxylaceae (including coca). The tropane alkaloids are responsible for the toxic effects of some of these plants and are found in all parts of the plants. The group of tropane alkaloids comprises more than 200 compounds and the wide range of compounds occurring especially in the Solanaceae family arises from the esterification of tropine with a variety of acids. (-)-hyoscyamine and (-)-scopolamine are the main alkaloids found in tropane alkaloid producing plants.

Atropine, (-)-hyoscyamine and (-)-scopolamine are readily absorbed from the gastrointestinal tract, quickly and extensively distributed into tissues, and excreted predominantly in the urine. N-demethylation and Phase II conjugation of atropine, (-)-hyoscyamine and (-)-scopolamine are known metabolic pathways in humans. (-)-Hyoscyamine and (-)-scopolamine are antimuscarinic agents that are antagonists of the muscarinic acetylcholine receptors primarily present in the autonomic effector sites innervated by parasympathetic (cholinergic postganglionic) nerves but also in the central nervous system (CNS). The pharmacological effects of (-)-hyoscyamine and (-)-scopolamine occur within a short time after administration. The toxicological effects of tropane alkaloids in experimental animals relate to their pharmacological activity, particularly pupillary dilation and neurobehavioural effects. In humans, the predominant peripheral antimuscarinic effects are decreased production of secretions from the salivary, bronchial, and sweat glands, dilation of the pupils (mydriasis) and paralysis of accommodation, change in heart rate, inhibition of micturition, reduction in gastrointestinal tone and inhibition of gastric acid secretion.

In the adopted opinion by EFSA's CONTAM Panel (EFSA, 2013), only limited occurrence data was available, and a reliable exposure assessment could only be carried out for two tropane alkaloids ((-)-hyoscyamine and (-)-scopolamine)), covering only one food group and one age class. The CONTAM Panel therefore recommended the collection of more occurrence data for the various tropane alkaloids, i.e., anisodamine, apoatropine, aposcopolamine, calystegines, convolidine, convolvine, cuscohygrine, homatropine, littorine, phygrine, pseudotropine, scopoline, sectropane, tigloidine, 3α-tigloyloxytropane, tropane, tropine and tropinone; and not only for (-)-hyoscyamine and (-)-scopolamine.

Therefore, the European Food Safety Authority (EFSA) wished to outsource a study on the occurrence of tropane alkaloids in food for human consumption from different geographic regions in Europe, to serve as supporting information to the CONTAM panel for future exposure assessments for tropane alkaloids. The present call aims to identify which tropane alkaloids in addition to (-)-hyoscyamine and (-)-scopolamine are likely to occur in food as contaminants in Europe; to characterise the relevant tropane alkaloids that are occurring in food as contaminants and to measure their occurrence in food samples using a validated method.

This call for proposals aims to obtain representative occurrence data for tropane alkaloids in food. In particular to identify which tropane alkaloids in addition to (-)-hyoscyamine and (-)-scopolamine are likely to occur in food as contaminants in Europe; to characterise the relevant tropane alkaloids that are occurring in food as contaminants and to measure their occurrence in food samples using a validated method. This work will be undertaken in two main phases; consultation with and the agreement of EFSA at the end of Phase 1 being essential before moving to Phase 2.

The beneficiary shall perform the following tasks, in order to achieve the objectives:

Phase 1: to be completed within 4 months from the start of the project (kick-off meeting):

- Identify which tropane alkaloids, (in addition to (-)-hyoscyamine and (-)-scopolamine), should be measured and characterise and describe the relevance of the tropane alkaloids selected i.e. as food contaminants;
- Identify at least 5 relevant food matrices to be sampled;
- Describe in detail the sampling strategy to collect in total 1200-1500 samples according to and covering relevant food products and from at least 3 different European countries (preferably not from neighbouring countries) to be used;
- Describe the feasibility of measuring the selected tropane alkaloids according to existing analytical methods and available standards and identify the analytical method(s) to be used for the analysis of the selected tropane alkaloids (in addition to (-)-hyoscyamine and (-)-scopolamine) in food matrices;
- Describe in detail the plan for method development and validation of the selected tropane alkaloids (in addition to (-)-hyoscyamine and (-)-scopolamine) in food matrices.

Phase 2: to be completed within 14 months from the end of Phase 1:

- Develop and validate the analytical methodologies required to measure the selected tropane alkaloids (in addition to (-)-hyoscyamine and (-)-scopolamine) in food matrices;
- Perform the sample collection covering the relevant food groups as agreed under Phase 1;
- Analyse the selected tropane alkaloids in food samples, using a validated analytical method;
- Compile the occurrence dataset in the EFSA standard sample description format.

External scientific Interim and Final reports and a database will be prepared according to the time schedule reported in Section 1.4 of the present call for proposals.

The Final as well as the Interim Scientific reports shall be written in English and will follow the template structure provided by EFSA and the EFSA citation standards. The External Scientific Report shall contain the following information:

- The reasoning for the selection of the tropane alkaloids and the food products chosen;
- Justification of the choice of the used analytical method(s); the detailed description of the applied analytical method(s), including storing procedures for the samples, sample pre-treatment and sample preparation steps, instrumental determination of tropane alkaloids including calibrants and the instrumentation to be used, the quality control of the analytical method, an example on reporting of the analytical results;
- A validation report obtained from the validation of the method(s) for the analysis of tropane alkaloids in the food matrices included in the sampling plan. Similar matrices may be combined for the validation however reasoning must be provided. The validation report should also include the estimation of the measurement uncertainty. In addition, the validation report shall compare the performance of the method to those methods recently reported for tropane alkaloids in the literature;
- The description of the sampling procedure applied for a representative collection of 1200-1500 samples from different European countries that is in accordance with the Commission Regulation (EC) 401/2006 (EU, 2006), that includes: a justification of the choices and number of European countries where the food samples were collected, the types of food products sampled, the number of samples analysed for each food product and from each European country taking into consideration that the number of samples are representative per food product and the country, the sampling place and production country, if available, the size of the lot to be sampled;
- The results of the individual samples;

- Common statistical descriptors (e.g. mean, median, standard deviation) of the concentrations;
- An overall summary, conclusions and discussion of the whole project.

The database shall contain information on the concentrations of tropane alkaloids in the analysed samples, associated information describing the sample and the other sample description details specified in the most recent EFSA Guidance on standard sample description.

This grant was awarded by EFSA to:	Stichting Landbouwkundig Onderzoek DLO-RIKILT; Akkermaalsbos 2; 6708 WB Wageningen; the Netherlands
Beneficiary:	RIKILT Wageningen University & Research
Grant title:	Occurrence of tropane alkaloids in food
Grant number:	GA/EFSA/BIOCONTAM/2014/01 - GA 1

1.2. Interpretation of the terms of Reference

During the project time, the European Commission has implemented Commission Regulation (EU) 2016/239, setting a limit for tropane alkaloids (TAs) in 'Processed cereal-based foods and baby foods for infants and young children, containing millet, sorghum, buckwheat or their derived products' (EU, 2016). The results of the here presented study will be evaluated for compliance to this EU regulation.

1.3. Additional information

1.3.1. Introduction

TAs are secondary metabolites which naturally occur in plants of several families including Brassicaceae, Solanaceae (e.g. mandrake, henbane, deadly nightshade, Jimson weed) and Erythroxylaceae (Adamse et al., 2014). Although over 200 TAs are known, (-)-hyoscyamine and (-)-scopolamine are the main alkaloids found in TA-producing plants. TAs are antimuscarinic agents that are antagonists of the muscarinic acetylcholine receptors primarily present in the autonomic effector sites innervated by parasympathetic (cholinergic postganglionic) nerves but also in the central nervous system (CNS). In humans, the predominant peripheral antimuscarinic effects are: decreased production of secretions from the salivary, bronchial, and sweat glands, dilation of the pupils (mydriasis) and paralysis of accommodation, change in heart rate, inhibition of micturition, reduction in gastrointestinal tone and inhibition of gastric acid secretion. Intoxications in humans are rare but do occur, and are often the result of errors in preparing herbal preparations and tea (Adamse et al., 2014). TAs can, furthermore, contaminate food when weeds are co-harvested with the crop. Common practices for cleaning cereals are not always sufficient to remove the weed plant parts.

More recently, it has been disclosed that a group of very polar TAs, the calystegines, are found in various edible plants of the families of Solanaceae (e.g. *Solanum tuberosum* (potato), *Capsicum annuum* (peppers) and *Solanum melongenes* (aubergine (eggplant)) and Convolvulaceae (e.g. *Ipomorea batatas* (sweet potato)) (Dräger, 2004; Jockovic et al., 2013). About 14 calystegines are currently known but only calystegines A_3, B_2 and B_4 are reported to occur in potatoes and calystegine B_2 is dominant in aubergine, peppers and sweet potato. (Jockovic et al., 2013; Petersson et al., 2013). Calystegines are α-glucosidase inhibitors acting in the intestine. Human intoxications related to calystegines are not reported but it has been suggested, with no causative evidence, that crazy cow syndrome (*Solanum dimidiatum*) and maldronksiekte (*Solanum kwebense*) occurring in cows in South Africa are caused by calystegines (Griffiths et al., 2008).

There are several reports in the Rapid Alert System for Food and Feed (RASFF) (EU) on the contamination of food commodities with plant toxins, even though regulation of TAs in food is absent in the EU. In the recently adopted opinion by EFSA's CONTAM Panel (EFSA, 2013), only limited occurrence data was available, and a reliable exposure assessment could only be carried out for two TAs ((-)-hyoscyamine and (-)-scopolamine)), covering only one food group and one age class. The CONTAM Panel therefore recommended the collection of more occurrence data for various TAs, besides (-)-hyoscyamine and (-)-scopolamine.

1.3.2. Objectives

The main objective of this study is to provide representative data on the occurrence and levels of relevant TAs in food samples from different geographic regions in the EU. The quality of the delivered data must be suitable for use in future exposure assessments performed by the EFSA CONTAM panel.

The objective will be achieved by first identifying those TAs that are likely to occur as contaminants or as endogenous substances in relevant food groups in the EU (excluding recreational plants). TAs will be characterised in a literature study and their relevance as food contaminant will be described (when available, including expected concentrations). The relevant food commodities will be identified, with special emphasis to their relevance to different age groups, and described with the corresponding sampling strategy (including sampling protocol) for about 1500 samples in at least three different geographic regions in the EU. The feasibility of the requested analytical method, or methods, for the selected TAs, using available reference standards and analytical methods, will be described. The aim is to include all the relevant TAs in no more than two analytical methods. A detailed plan will describe the method(s) development and validation. Achievable levels of detection (LOD) and quantification (LOQ) will be based on the occurrence data available or will depend on the method(s). The aim will be to measure at the lowest possible range to increase the detection rate and thus the number of measurements with numerical occurrence data. The objective of phase two of the project is to develop and validate the analytical method(s), to collect ca. 1500 food samples in at least three geographic regions, to analyse the samples and to present the data to EFSA according to the EFSA standard sample description format.

2. Methodologies

2.1. Identification of relevant TAs and food products

Relevant TAs and food products will be identified from a literature study.

Relevant literature will be searched via ExLibris Metalib and SFX information services. The search hosts included are: OVID (which includes CAB Abstracts and full-text access to 117 CABI book titles, BIOSIS Previews, and Medline); Web of Science; Proquest Dialog (over 250 science databases including Current Contents, AGRICOLA, CSA Life Sciences Abstracts, SciSearch(R), AGRIS, Pascal, ELSEVIER BIOBASE); major 'grey literature' sources, and the specialist databases and the EFSA Inventory of Sources of Scientific Evidence Relevant to EFSA's Risk Assessment. The proposed search query is presented in Table A1 of Annex A.

Relevant TAs and food matrices will be identified as follows:

Step 1: Identification of relevant associated plants:

- Reported cases of intoxication in the EU are studied and associated plants will be identified;
- The EU RASFF system will be searched for alerts and notifications on TAs or the presence of the producing plants;
- Plant species that could potentially contaminate field crops in the EU will be identified.

Step 2: Identification of relevant food plants with inherent TAs:

- Food plants of the Solanaceae family with relevant for food consumption in the EU will be identified.

Step 2: Assessment of the TAs:

- TAs in the associated plants as well as in the food plants will be identified and described;
- Available toxicity data on the TAs are summarised.

Step 3: Identification of relevant TAs from associated plants and foods using the following criteria:

- The likelihood of relevant TAs containing plants contaminating food products in the EU;
- TA composition of these associated plants;
- TA composition of the food plants with inherent TAs;
- If available, toxicity data of individual TAs;
- Availability of the standard, based on the list presented in Appendix A.

Step 4: Identification of relevant food matrices using the following criteria:

- Foods associated with intoxications with TAs;
- Foods associated with the TA-containing weeds;
- Foods associated with holding inherent TAs;
- Volumes of consumption of these foods by the EU population (including vulnerable groups);
- Availability of these foods on the EU market.

Exclusions:

- Only literature in the English language will be used;
- TAs will be excluded from the study if no standard is available;
- Cocaine and other TAs used for recreational drugs are excluded.

2.2. Sampling plan, sample storage and sample treatment protocols

Draft sampling plan

The sampling plan will be designed to obtain as much information as possible regarding the occurrence of TAs in the different food products of interest, but also with the aim to obtain statistically representative data for use in risk assessment studies.

One of the main aims of the project is that the samples collected represent the situation in the EU with respect to the consumption habits in various EU regions. A first draft of the sampling plan was proposed at the start of the project and is presented in Appendix B. The four partners will sample three main EU regions; North Western EU (the United Kingdom, the Netherlands, Germany), Southern EU (Spain, France, Italy) and Eastern EU (the Czech Republic, Poland, Hungary). RIKILT will collect samples from retail stores in the Netherlands and Germany; IRTA will collect samples from Spain, France and Italy; UCT will collect samples from the Czech Republic, Poland and Hungary and FERA will collect samples within the United Kingdom. In total nine countries will be sampled by the members of the consortium. This means that the Scandinavian region is not covered. Nevertheless, the countries that are covered represent around 392 million inhabitants, which is approx. 77% of the current EU population. In principle, all identified food groups will be sampled in all the nine member states. The precise number and type of samples may vary per country and will depend on consumption patterns and on the outcomes of the literature study and additional sources of information available, as will be determined in the phase I study.

A limited survey has been carried out at the start of the project to identify the consumption of various foods in the EU that might be included in the study. The results are presented in Appendix C and show that the food categories of interest for TAs represent approximately 26.6% of the daily diet.

Size of the lot to be sampled and transport conditions

Sampling will be carried out taking into account the guidance for representative sampling as described in Regulation (EC) No 401/2006 (EU, 2006) and amended with Regulation (EU) No 519/2014 (EU, 2014)[1].

- Three consumer units per product per batch code will be collected, or at least 1 kg, unless the costs are prohibitive (e.g. for dry (herbal) tea samples). In that case three consumer units of smaller size will be bought;
- Products with limited shelf life, once collected in supermarkets and stores, will be immediately transferred to the laboratory, preferable under cold conditions;
- Samples from food plants of the *Solananceae* family will be transported under dark conditions.

Characteristics of the sample (land of origin of the product, when available), will be uniformly registered and these data will be stored, together with the analytical results, in an excel sheet based on the most recent EFSA Guidance on Standard Sample Description for Food and Feed (EFSA).

Storage upon arrival in the laboratory and sample preparation

All samples will be assigned a unique laboratory code upon arrival and will be registered in the LIMS system implemented by the laboratory. Photographs of the products will be taken to register all relevant information. Samples will be stored under the appropriate conditions. Samples of food plants of the *Solananceae* family must be stored under dark conditions and be processed within three days after arrival in the laboratory.

Samples will be homogenised to prepare an aggregate sample of at least 1 kg (in case of dry (herbal) tea samples this will be an aggregate sample of smaller size). Three replicate sub-samples will be prepared of approx. 50 g each. Cereal-based samples, together with the remaining aggregate sample, will be stored at ambient temperature, perishable samples, together with the remaining aggregate sample, will be stored at -20^{o}C until analysis. One of the three sub-samples will be used for analysis; the other two sub-samples might be used for repeating the analysis in case unexpected results are obtained from the first analysis.

2.3. Analysis of tropane alkaloids

Scope of the methods to be developed

Key principle in this project is to develop two complementary analytical methods that cover all relevant TAs (Datura TAs and calystegines) that are commercially available. The aim is that for both methods one generic extraction solvent can be used, preferably the acidified methanol/water mixture that has been shown to work well for the Datura TAs. The generic extract will be used to analyse the two groups of TAs by two separate analytical procedures.

Feasibility of analytical methods

Information available from the EFSA Scientific opinion on tropane alkaloids in food and feed indicates that two groups of TAs may be considered relevant, the Datura TAs and the calystegines (EFSA, 2013). The group of Datura TAs can be divided in three subgroups: the low molecular weight TAs (containing only the tropane ring), Convolvulaceae-type TAs (containing a tropane ring esterified to a benzoic acid derivative, which are typically found in the family of Convolvulaceae) and 'regular' Datura-type TAs (containing a tropane ring esterified to a phenylacetic acid derivative). Calystegines are a special group of the low molecular weight TAs, as they consist of a tropane ring containing at least 3 hydroxyl groups, yielding them very hydrophylic compounds. In plants producing Datura-type TAs the reported concentrations are typically in the range of 0.1 to 10 g/kg (EFSA, 2013). However, in food products, the concentrations of the Datura-type TAs as well as the other TA types are expected

[1] The legal acts as quoted in this publication refer, where applicable, to the latest amended version.

to be low, typically in the <1 to 100 μg/kg range, since they occur mainly as minor co-contamination in harvested crops. The expected concentrations of the calystegines in the selected *Solanacaea* food plants are relatively high, typically in the range of 1 to 100 mg/kg, since they are endogenous compounds (Andersson, 2002).

Analytical methods described in literature

Multi-analyte methods described in the literature for determination of Datura TAs and calystegines in food products are typically based on GC-MS(/MS) or LC-MS/MS detection. Regarding the analysis of Datura TAs, the number of published validated methods is quite small and the scope is mostly limited to atropine (hyoscyamine) and scopolamine. Methods in which a (much) wider scope of TAs is considered are primarily focussing on alkaloid profiling of various plant species including hydroponic cultures (Doncheva et al., 2006; El Bazaoui et al., 2011; Jousse et al., 2009). These methods are GC-MS based, have been optimised for the analysis of pure plant material and are not easily adapted to the analysis of food products. Several studies focussing on the *in vivo* metabolism of TAs have been published that use LC-MS/MS for the identification and semi-quantification of metabolites (Chen et al., 2006a; Chen et al., 2005a; Chen et al., 2005b). Compounds relevant for the current study that were included are: atropine, scopolamine, anisodamine, scopine, norscopine, norscopolamine, noratropine, aposcopolamine and 6β-hydroxytropine. Compounds were extracted from urine and purified by solid phase extraction (SPE). Compounds were separated using reversed phase chromatography using a C18 column run with a methanol/ammonium acetate/formic acid (pH 3.5) gradient. The most advanced method published thus far is the LC-MS/MS method developed by Jandrić *et al.* (2011) which comprises five TAs (hyoscyamine, scopolamine, homatropine, anisodamine, tropine) in cereal grains and seeds with favourable sensitivity (LOD: 0.7-0.8 μg/kg, LOQ: 2.2-4.9 μg/kg (Jandric et al., 2011). A QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) type of generic extraction was applied. Caligiani *et al.* (2011) developed a GC-SIM-MS method for the sensitive detection of hyoscyamine and scopolamine (LOD: 0.3-1 μg/kg and LOQ: 1-6 μg/kg) in buckwheat grains and products (Caligiani et al., 2011). To achieve the low limits of detection derivatisation and a multi-step sample preparation procedure was required.

The majority of analytical methods for calystegines is based on GC-MS analysis with pre-column derivatisation as originally been developed by Dräger (1995) (Dräger, 1995). Using the method of Dräger and applying sample clean-up over Dowex and Amberlite ion exchange columns, Bekkouche *et al.* (2001) developed a GC-MS method for 7 calystegines (A_3, A_5, B_1, B_2, B_3, B_4, C_1) with LODs: 1-2 μg/mL (corresponding to 0.1-0.2 mg/kg fresh weight) and LOQs: 3-6 μg/mL (corresponding to 0.3-0.6 mg/kg FW (Bekkouche et al., 2001). To date, the method for analysis of calystegines as described by Petersson *et al.* (2013) but developed by UCT, a partner in this project, is the only one described in literature using LC-MS/MS (Petersson et al., 2013). The method does not require derivatisation and the high detection sensitivity allows dilution of the extract prior to analysis, with LODs: 0.4-0.6 mg/kg FW and LOQs: 1-2 mg/kg.

Thus far, isotopically-labelled internal standards rarely have been incorporated in published methods for Datura TAs with the exception of Jandrić *et al.* (2011) and not at all for calystegines (Jandric et al., 2011). From the available literature it can be concluded that GC-MS/MS and LC-MS/MS methods have comparable sensitivity and that in principle all relevant TAs can be measured with both methods. The biggest advantage of the published LC-MS/MS methods is that simple and straightforward sample preparation procedures can be applied and that derivatisation of analytes is not necessary. For the purpose of this project, to analyse a variety of food products for a wide range of TAs, LC-MS/MS-based methods, therefore, offer the highest flexibility and efficiency.

Analytical method available for Datura TAs at the start of the project

RIKILT has a state-of-the art in-house validated method available for the detection at the relevant concentration range of a number of Datura-type TAs (atropine, scopolamine, anisodamine, anisodine, aposcopolamine and homatropine) in cereal-based food products (Mulder et al., 2015; Pereboom-de

Fauw and Mulder, 2012). At the start of the project is was envisioned that all Datura TAs can be extracted and detected based on the principle of the multi-method as summarised in Appendix D.1. In principle, a 4 g portion is extracted with 40 mL extraction solvent (methanol/water/formic acid, 60/40/0.4 (v/v/v)). After purification over a 30 kD ultrafilter an aliquot of typically 10 µL is injected in an LC-MS/MS system equipped with a Waters XBridge C18 column (150*3 mm, 5 µm). The mobile phase consists of A (6.5 mM ammonium hydroxide in water) and B (6.5 mM ammonium hydroxide in acetonitrile). The gradient starts at 10% B and is linearly raised to 60% B in 9 min. The column temperature is kept at 40°C and a flow rate of 0.4 mL/min is used. MS/MS detection using a Waters Ultima system is performed in positive electrospray ionisation mode using mass spectrometric conditions optimised for each TA. Due to the larger number of TAs to be included in the method, including a number of low molecular weight compounds, and the low detection levels required, it can be anticipated that the method needs to be upgraded by using UPLC chromatography in combination with sensitive, fast scanning LC-MS/MS equipment. It will also be investigated whether an acidic mobile phase will work equally well for the analysis of the Datura TAs included in the scope of this study.

Analytical method available for calystegines at the start of the project

At the start of the project only one method had been published that describes the analysis of calystegines A_3, B_2, and B_4 by LC-MS/MS (Petersson et al., 2013). This method may serve as starting point for the analysis of the six calystegines available for this study. The draft SOP is described in Appendix D.2. In principle, a 4 g portion is extracted with 40 mL extraction solvent (methanol/water/formic acid, 60/40/0.4 (v/v/v). After filtration, the extract is diluted ten times with acetonitrile and passed through a 0.22 µm polytetrafluoroethylene membrane filter. An aliquot of 2 µL was injected in the UPLC-MS/MS system equipped with an Atlantis HILIC column. A gradient of mobile phase A (0.020 M ammonium acetate, pH 5.3 in water) and B (acetonitrile) was applied at 40°C and flow rate of 0.5 mL/min. It will be investigated whether a more modern type of HILIC or Amide (UPLC) column, or perhaps a column with chiral stationary phase will work equally well (or better).

Required limits of quantification (LOQ)

Based on the average daily food consumption (Appendix C) it is estimated that the required level of quantification (LOQ) is in the range of 1-5 µg/kg for the Datura-type TAs and 1-5 mg/kg for the calystegines. In the RIKILT method for Datura-type TAs the LOQ for the 6 TAs (atropine, scopolamine, anisodamine, anisodine, aposcopolamine, homatropine) is 1 µg/kg, while in the UCT method for the calystegines A_3, B_2 and B_4 are in the range of 1-2 mg/kg. The LOQ for each substance will be determined during the in-house validation by each partner at the start of phase II of the project.

2.4. Validation plan and validation criteria

In the absence of a specific regulation or guidance document for the validation protocol of a method for the quantification of plant toxins in food products, the guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed SANCO/11945/2015 (EU_SANTE, 2015) will be used. The same applies for the performance characteristics, for which the performance characteristics for mycotoxins, as mentioned in Regulation (EC) No 519/2014 (EU, 2014), will be used.

The general requirements on performance characteristics derived from SANCO/11945/2015 for pesticides and Regulation (EU) No 401/2006 amended with Regulation (EU) No 519/2014 for mycotoxins are given below:

LOD	3 times S/N calculated from lowest matrix or extract spiked sample
LOQ	10 times S/N calculated from lowest matrix or extract spiked sample
Linearity	≥0.99 for matrix or extract matched calibration curve

Recovery	60 to 120% for Datura TAs; 70 to 110% for calystegines
Repeatability (RSD_r)	≤20%
Reproducibility (RSD_R)	2 × value derived from Horwitz Equation (modified by Thompson) for Datura TA; 2 × value derived from Horwitz Equation for calystegines
Measurement Uncertainty (U)	<50%

The validation of the methods will start with writing each proposed method in SOP format. The proposed protocol for the performance characteristics to be assessed in the in-house validation by RIKILT will include the limit of detection (LOD), limit of quantification (LOQ), linearity, recovery and repeatability (RSD_r) for each type of matrix included in the sampling plan.

Once successfully validated by RIKILT these methods will be in-house validated by each partner and must fulfil the validation criteria. Next step is a limited inter-laboratory study that will be organised by RIKILT to estimate the reproducibility (RSD_R) and the measurement uncertainty (U). All partners must fulfil acceptable performance quality scores in the limited inter-laboratory study.

In-house validation

In-house validation will concern:

- The Datura TAs and calystegines included in the methods;
- The food products selected for analysis of Datura TAs and/or calystegines;
- Estimated required limit of quantification (LOQ) will be in the range of 1-5 µg/kg for the Datura TAs and 1-5 mg/kg for the calystegines. The LOQ achieved for each substance will be determined during the in-house validation by each partner at the start of phase II of the project. Typically LOQ will be at 10 times the noise ratio and LOD at 3 times instrumental noise;
- Linearity over the working range will be assessed through matrix matched calibration standards for Datura TAs and through matrix matched extracts for calystegines;
- Recovery will determined by comparison the results of blank samples spiked before extraction with blank samples spiked after extraction;
- Repeatability (RSD_r) will be determined in spiked samples for each type of matrix in six-fold and at three different concentrations.

Limited inter-laboratory study

A limited inter-laboratory study among the four partners will be conducted to assess reproducibility (RSD_R) and measurement uncertainty (U). MMS and QC will be included in the limited inter-laboratory study. The results must fulfil the performance characteristics described above. A set of five spiked and/or naturally contaminated samples (one for each food product selected) will be prepared by RIKILT and shipped to the partners for analysis. The results will be processed by RIKILT. All partners must fulfil acceptable performance quality scores in the limited inter-laboratory study.

2.5. Analytical quality control

To prevent bias in trueness of reference standards and to ascertain comparability of the results, RIKILT will purchase all reference standards, prepared common spiking and working solutions, and distribute the standard solutions among the other partners.

To assure quality of the analyses relevant QC sample(-s), based on food products analysed, must be included in each analysis run. A quality control (QC) sample for each type of food product will be prepared by RIKILT from blank materials, and distributed among the partners. The QC samples will be prepared by spiking a homogenised blank sample of each food product with a mixture of TA standards. The samples will be filled out in polypropylene tubes and will be stored at the appropriate temperature

To assess stability of the compounds in the QC samples during the project, duplicate QC samples for each type of food product will be stored, depending on the product, at -80°C, -20°C, 4°C and at room temperature at RIKILT. These samples will be analysed at the end of the second reporting period and at the end of project.

For internal analytical quality control, MMS, relevant QC samples and at least one spiked blank sample (to assess recovery) will be included with each series of samples to be analysed during the survey and must meet the validation criteria. The adequate performance of the instruments must be verified at each run according to the requirements indicated before. Identification of the TAs in the samples will be carried out according to SANTE/11945/2015, matching retention time and a minimum of two transitions with correct ion ratios. All matrices included in the validation will also be analysed without spiking as well as reagent blanks in each run.

The on-going validation data on recovery and QC samples are thus collected and will allow to determine the within-laboratory reproducibility and measurement uncertainty.

2.6. Calculating analytical results

External calibration using matrix matched standards (MMS) will be applied for the quantification of Datura TAs. Isotopically-labelled internal standards of atropine and scopolamine will be added for correction of matrix effects.

External calibration using matrix matched extracts (MME) will be applied for the quantification of calystegines since it is not feasible to work with MMS, due to the limited availability of calystegine standards and the high amounts of standards needed (concentrations in the mg/kg range). Sample extracts for MME calibration will be prepared by extraction of samples that are blank or containing only low amounts of calystegines, using the generic extraction protocol and aliquots thereof will be spiked with the calystegine standards.

The results for Datura TAs will be calculated based on the matrix matched standard (MMS) calibration line included in the run and will be corrected for the recovery obtained in that run. The results for calystegines will be calculated based on the extract matched standard (MME) calibration line included in the run and will not be corrected for recovery. When the concentration of a TA detected in the sample falls outside the calibration range, the sample will be reanalysed with a proper dilution or extended calibration line. As an alternative, samples can quantified using standard addition to the sample at a relevant concentration level (e.g. 2-5 times the expected concentration in the sample).

Additionally, the results will only be allowed when the results of the quality control samples, recovery and QC sample, in the respective run meets the minimum quality requirements.

3. Results

3.1. Identification of relevant TAs and food products

The main results obtained from the literature study (Annex A), RASFF reports and the recommendations of the Standing Committee on Plants, Animals, Food and Feed (Standing_Committe_on_Plants_Animals_Food_and_feed, 2014) can be summarised as follows:

It can be concluded that information on the occurrence of TAs in food in the EU is scarce. Moreover, the available information is not always relevant to food, e.g. many reports concern occurrence of TAs in non-edible plant parts such as roots and flowers.

Most information is available on atropine and scopolamine and little information, which is often also very dated, is available on the other TAs occurring in foods. Likewise, knowledge on toxic effects of TAs, other than atropine and scopolamine, are scarce. This means that at this moment all TAs are considered toxic and it is advised to measure all TAs for which an analytical standard is available.

It is very difficult to predict which co-occurring weeds, and thus which TAs, will be present in the food in the EU. Areas where food is sourced can change quickly and are not only limited to the EU. Regarding contamination of cereal crops with weeds, the major weeds currently contaminating food crops in the EU are *Datura stramonium* and *Convolvulus arvensis*. The TAs tropine, tropinone, cuscohygrine, hygrine have been reported in *Convolvulus arvensis* and *C. sepium* (*Calystegia sepium*). Toxic weed plants from outside the EU likely to contaminate foods imported into the EU are *Solanum* species *S. ptycanthum*, *S. nigrum*, *S. viarum* and *S. torvum*. These plants are common in cultivated fields and have been harvested with peas, snap beans and soya beans (Binning, 1993; Crotser and Witt, 2000). Other solanums invasive and common in the USA and Asia are *S. viarum* and *S. torvum* (turkeyberry). It is therefore advised to analyse all foods for all TAs for which an analytical standard is available.

The EFSA Opinion on tropane alkaloids in 2013 indicates contamination of a high proportion of cereal-based food for infants and young children, and a lesser contamination of a high proportion of cereal-based food intended for both toddlers and infants. Other contamination has been revealed in food and feed based on oilseeds, especially sunflower seeds, and in millet and related plants such as sorghum.

From reports under RASFF the greatest incidence of contamination has concerned Datura seeds in millet (mainly organic and mainly originating in Austria) and Datura fruit (berries) in processed bean-based vegetables. Frozen green beans have also been contaminated with *Solanum* and poppy seeds with Hyoscyamus (henbane).

Considering the widespread contamination of herbal products with pyrrolizidine alkaloids (Mulder et al, 2015) and a single incidence of contamination of tea prepared from marshmallow root with *A. belladonna* root (Mulder et al, 2014) there might be a requirement to survey herbal teas from areas with potential tropane-containing plant growth.

Whilst there are many reports of contamination by Datura tropanes there are no reports of calystegine contamination of foods in which they do not occur naturally. There appears to be widespread and sometimes heavy growth of Convolvulus in cereal fields, but consumer exposure to calystegines will be greater and more sustained through consumption of potato products.

The primary recommendations are for a focus on:

- The review has highlighted the emerging potential for food contamination from non-food weed plants that are encroaching on field crops in Europe. *Convolvulus* species (bindweeds) present a particular problem that should be addressed in future projects.
- Analysis of all TAs for which a reliable analytical standard is available.

Foods to be analysed for inherent TAs

- Potato products of all types and cultivars should be analysed for calystegines as priority crop.
- Include aubergines in the survey to be analysed for calystegines based on consumption pattern in the EU.
- Future work should be extented to the occurrence of calystegines in *Capsicum annuum*, Solanum lycopersicum, *Brassica napa* and *B. oleracea*.
- It is recommended to also include emerging superfoods that do contain inherent TAs when appropriate, e.g. *B. oleracea* and goji berries.

Foods to be analysed for co-occurring TAs

Analyse for low molecular weight TAs, Convolvulaceae-type TAs, Datura-type TAs

- Processed cereal foods intended for babies, infants and toddlers, including a high proportion of organic products.
- Processed and unprocessed cereal foods for adults, including a high proportion of organic products, including some samples with poppy seeds and where identified some products containing millet.
- Processed foods and flours based on buckwheat, including a high proportion of organic products, including some samples with poppy seeds and where identified some products containing millet.
- Canned and frozen vegetables with a high proportion of green beans.
- Tea, specifically herbal teas.

Analyse for calystegines

- It is recommended to extend the calystegines survey to the field cereal products since some co-occurring weeds do also contain calystegines. However, concentration in the final product will be extremely low and may not be very relevant for exposure.

Considerations for sampling

- It is advised to include more than one harvest year.
- The sampling should include food from as many different sources as possible, including food sourced from outside the EU.

3.2. Proposed sampling plan

The draft sampling plan (Appendix B) was designed to obtain as much information as possible regarding the occurrence of TAs in the different food products of interest, but also with the aim to obtain statistically representative data for use in risk assessment studies. The sampling plan was based on the outcome of the literature study, the EFSA 2013 opinion on TAs in food and the original proposed plan and the advice of the Standing Committee on plants animals food and feed held in Brussels on July 1, 2014 (Standing_Committe_on_Plants_Animals_Food_and_feed, 2014).

From the literature study and additional sources it was recommended for the Datura TAs to focus on:

i) processed cereal foods intended for babies, infants and toddlers;

ii) processed and unprocessed cereal foods for adults;

iii) processed foods and flour based on buckwheat;

iv) canned and frozen vegetables with a high proportion of green beans;

v) herbal teas.

Focus in these products should be on:

i) organic production;

ii) for the cereals include some samples with poppy seeds;

iii) some products containing millet where possible.

Products most interesting regarding calystegines emerging from the literature study are:

i) potato products of all types;

ii) field cereal products.

Based on this input it was decided to amend the draft sampling plan (Appendix B) proposed during the kick-off meeting:

- To include the following food products: a) flour samples; b) canned and frozen vegetables with a high proportion of green beans; and c) herbal teas;
- To exclude the food products tomatoes and bell peppers;
- To collect a substantial number of food products form organic production (around 20%) in the country of origin of the institute;
- To collect food products of organic production only when readily available in retail shops in the neighbouring countries. Nevertheless, it is expected that at least 10% of the samples collected in these countries will be from organic production.

The proposed new sampling schedule is presented in Appendix E.

Countries

According to the sampling plan the four partners will collect samples in 9 countries in three main EU regions; North Western EU (United Kingdom, the Netherlands, Germany), Southern EU (Spain, France, Italy) and Eastern EU (Czech Republic, Poland, Hungary). RIKILT will collect samples from retail stores in the Netherlands and Germany; IRTA will collect samples from Spain, France and Italy; UCT will collect samples from Czech Republic, Poland and Hungary; FERA will collect samples within the United Kingdom. Regarding the focus on the geographic regions for sampling a slightly higher proportion of samples of a specific food product will be collected in countries where problems have been reported previously.

Sampling periods

The sampling has been divided into two sampling rounds (Appendix E.2), the first round from May till December 2015 and the second round from January to August 2016. Samples of flour (buckwheat and millet), cereal-based products (breakfast cereals, biscuits, bread and pasta), and samples of Solanaceae food plants (potatoes and aubergine) will be sampled during both rounds in the partners countries (UK, NL, ES, CZ). Samples to be collected in the other countries will be sampled only during one of the two rounds: DE, FR, HU and PO during the first round and IT during the second round. Samples of dry (herbal) teas and legumes will be sampled only in the partners countries and only during the second round of sampling. Overall, approximately 55% (820) of the samples will be taken during the first sampling round and the remaining 45% (680) of the samples during the second round.

3.3. Collection, transport and storage of the samples

The samples were collected in supermarkets, shops and other retail outlets. A limited number of samples was purchased from webshops. The sampling was conducted taking as guidance the methods of sampling for official control laboratories in Commission Regulation (EC) No 401/2006 (EU, 2006)[2].

As described in Commission Regulation (EC) No 401/2006 (EU, 2006), for each product three items with the same expiration date and the same lot number were collected. The combined amount of product collected should be sufficient to prepare an aggregate sample of at least 1 kg, with the exception of teas, for which it should be at least 100 g.

The sampling of the dry (herbal) tea products was performed taking as guidance epigraph E.4 of Commission Regulation (EC) No 401/2006 (EU, 2006), describing the sampling methods for spices.

[2] The legal acts quoted in this report refer, where applicable, to the latest amended version

Three incremental samples from the (sub-)lot were taken to form an aggregate. One package of tea (bags) had a sample size of approximately 30-60 g. The incremental samples were combined and homogenised to form a final aggregate of at least 100 g.

The purchased products were transported and stored at the usual temperature of storage of the product in the retail shop, e.g. at frozen condition (-20°C) for frozen products such as frozen beans and stir-fry mixes. Products with an extended shelf-life such as flours, breakfast cereals, biscuits and dry (herbal) teas were stored at a dry and dark place at room temperature or under cooled conditions such as ready-to-eat products.

All relevant information of the sample (as described on the product label) as well as the place and date of collection was recorded in the EFSA Standard Sample Description (SSD) form. The original packing and/or labels were kept as a back-up of the available product information. Alternatively, or additionally, scans and /or photos were taken of the sample for the same purpose.

3.4. Preparation of aggregate and sub-samples

The aggregate and sub-samples were prepared as soon as possible after collection, and always before the expiration date of the product. An aggregate sample of ca. 1 kg was prepared by combining equal amounts of three identical collected items, unless stated otherwise below.

Buckwheat, millet, breakfast cereals and biscuits. The purchased items were ground and mixed with a table milling apparatus (e.g. Retsch GM 200, Retsch, Haan, Germany). After homogenisation, aliquots (approx. 40 g) were transferred into polypropylene tubes of 50 mL. The respective three sub-samples were appropriately coded and stored at room temperature in a dry and dark place until analysis.

Bread. Bread and rolls were stored at -20°C for several hours until the products appeared lightly frozen (but not thoroughly or deep frozen). The appropriate number of slices or rolls were ground and mixed with a table milling apparatus (e.g. Retsch GM 200). After homogenisation, aliquots (approx. 40 g) were transferred into polypropylene tubes of 50 mL. The respective three sub-samples were appropriately coded and stored at -20°C until analysis.

Dry (herbal) teas. An aggregate sample of at least 100 g of each dry (herbal) tea was prepared by combining equal amounts of three identical collected items. One package of tea (bags) had a typical sample size of approximately 30-60 g. After removing the bags, the aggregate tea sample was ground and mixed with a table milling apparatus (e.g. Retsch GM 200). After homogenisation, aliquots (approx. 30 g) were transferred into polypropylene tubes of 50 mL. The respective three sub-samples and aggregate sample were appropriately coded and stored at room temperature in a dry and dark place until analysis.

Legumes and frozen stir-fry mixes. Pre-cooked stir-fry vegetable mixes were stored at -20°C after purchase. Portions were ground while being frozen with a table milling apparatus (e.g. Retsch GM 200). After homogenisation, aliquots (approx. 40 g) were transferred into polypropylene tubes of 50 mL. The respective three sub-samples were appropriately coded and stored at -20°C until analysis.

Potatoes. Potatoes were washed with tap water to remove soil or dirt when necessary. A representative set of potatoes (with respect to size and appearance) were selected for further processing. Large potatoes were cut in pieces before being ground and mixed with a table milling apparatus (e.g. Retsch GM 200). After homogenisation, aliquots (approx. 40 g) were transferred into polypropylene tubes of 50 mL. The respective three sub-samples were appropriately coded and stored at -20°C until analysis.

Aubergines. Aubergines were sliced and then ground and mixed with a table milling apparatus (e.g. Retsch GM 200). After homogenisation, aliquots (approx. 40 g) were transferred into polypropylene tubes of 50 mL. The respective three sub-samples were appropriately coded and stored at -20°C until analysis.

3.5. Quality control materials

Blank materials were prepared from buckwheat flour, breakfast cereals, biscuits, bread, green tea, potato and aubergine, purchased from local supermarkets, and were homogenised with the appropriate apparatus. An overview of the selected materials is given in Table 1 below. The materials did not contain TAs (<LOD). For each matrix, the same material was used for the preparation of quality control (QC) samples and for the preparation of stability samples. QC samples were prepared as sets of non-spiked (blank) and fortified at 10 µg/kg. With each analytical series one set of QC samples (blank + fortified) will be included, the matrix should match with the type of samples analysed in that particular series of samples.

Table 1: QC and stability samples prepared for the study[(a)]

Matrix	Product	No. of samples for QC	No. of samples for stability	Total
Buckwheat/millet	Buckwheat flour	30 blank + 30 spiked	30 spiked	90
Breakfast cereals	Mixed multigrain cereals for young children	30 blank + 30 spiked	30 spiked	90
Biscuits	Wholegrain cookies	30 blank + 30 spiked	30 spiked	90
Bread/pasta	Whole wheat rye bread	30 blank + 30 spiked	20 spiked	80
Dry (herbal) tea	Green tea	20 blank + 20 spiked	15 spiked	55
Potato	Main crop potato	30 incurred	10 incurred	40
Aubergine	Aubergine (Spanish)	25 incurred	10 incurred	35

(a): No QC and stability samples were prepared for legumes and frozen stir-fry mixes. These samples were analysed by means of standard addition to each sample.

Samples for stability tests of TAs under storage conditions were prepared in 6 sets of 5 samples each in September 2015. The stability samples were fortified at 10 µg/kg. Of buckwheat, breakfast cereals and biscuits two sets were stored at -20°C, two sets at 4°C and two sets were stored at room temperature. Of the bread samples two sets were stored at -80°C and two sets were stored at -20°C. Halfway the project, after 4 months (January 2016), for each matrix one set of 5 samples stored at the various temperatures, were analysed to assess the TA analyte stability in matrix under medium term storage conditions. The stability of TAs in matrix under long-term storage conditions at the various temperatures was checked at the end of the project, after 11 months (August 2016). At that time for each matrix the second set of 5 samples stored at the various temperatures was analysed.

For dry (herbal) teas three sets of 5 stability samples was prepared in February 2016. The samples were fortified at 10 µg/kg. One set was stored at -20°C, one set at 4°C and one set was stored at room temperature. The stability of TAs in dry (herbal) teas was assessed at the end of the project, after 6 months of storage (August 2016).

For stability tests of calystegines in potato and aubergine two sets of 5 stability samples were prepared in February 2016. The samples were incurred materials that were not additionally fortified. One set was stored at -80°C and one set was stored at -20°C. The stability of calystegines in potato and aubergine was assessed at the end of the project, after 6 months of storage (August 2016).

3.6. Selection of TA standards to be included in the survey

An inventory was made of the TAs mentioned in the EFSA 2013 Scientific opinion (EFSA, 2013) and the availability of TA analytical standards was investigated. In Table 2 (Datura TAs) and Table 3 (calystegines) the TAs identified by the EFSA Scientific opinion are tabulated. The Datura TAs can be divided in three subgroups: the low molecular weight TAs that contain only the tropane ring, Convolvulaceae-type TAs that contain a tropane ring esterified to a benzoic acid derivative, and the 'regular' Datura-type TAs that contain a tropane ring esterified to a phenylacetic acid derivative.

Calystegines are a special group of the low molecular weight TAs, as the tropane ring contains at least 3 hydroxyl groups. A large number of suppliers of chemical standards could be found on the internet offering one or more TAs. Some standards were only offered by a single company. Where an analytical standard was offered by more than one supplier the standard with the highest purity was ordered, or the standard offered for the lowest price/amount. The acquired standards are listed in Tables 2 and 3.

Table 2: Tropane alkaloids mentioned in scientific opinion of EFSA, and their availability as analytical standard

Compound type and name	CAS No.	MW (Da)	Mentioned in EFSA opinion	Available as standard	Selected Supplier[a]	Purity (%) according to supplier
Low molecular weight TAs						
Tropane	529-17-9	125.2	Y	N	-	-
Nortropinone	25602-68-0	125.2	N	Y	TCI	98
Nortropine	538-09-0	127.2	N	Y	TCI	98
Tropinone	532-24-1	139.2	Y	Y	TCI	98
Tropine	120-29-6	141.2	Y	Y	TCI	97
Pseudotropine	135-97-7	141.2	Y	Y	TRC	100
Physoperuvine	60723-27-5	141.2	Y	N[c]	ChemFaces	98[c]
Scopine	498-45-3	155.2	N	Y	TRC	98
Scopoline	487-27-4	155.2	Y	Y	Carbosynth	97
6-Hydroxytropinone	5932-53-6	155.2	N	Y	TCI	98
Tigloyltropine	495-83-0	223.3	Y	N[c]	ChemFaces	97[c]
Tigloidine	533-08-4	223.3	Y	N[c]	ChemFaces	97[c]
Convolvulaceae-type TAs						
Convolidine	63911-32-0	277.3	Y	Y	Latoxan	100
Convolvine	537-30-4	291.3	Y	Y	Latoxan	97
Fillalbine	4540-25-4	291.3	N	Y	Latoxan	97
Cocaine	50-36-2	303.4	Y	Y/N[d]	-	-
Convolamine	500-56-1	305.4	N	Y	Latoxan	97
Datura-type TAs						
3α-Phenylacetoxytropane	1690-22-8	259.3	N	Y	TRC	97
Apoatropine	500-55-0	271.3	Y	Y	Synchem UG	87.3
Noratropine	16839-98-8	275.3	N	Y	TRC	97
Homatropine	87-00-3	275.3	Y	Y	TCI	99
Aposcopolamine	535-26-2	285.3	N	Y	TRC	98
Atropine	51-55-8	289.3	Y	Y	TCI	99
(-)-Hyoscyamine	101-31-5	289.3	Y	Y/N[e]	TCI	99
(+)-Hyoscyamine	101-31-5	289.3	Y	N	-	-
Atropine-d_3[b]	1276197-36-4	292.3	N	Y	CDN Isotopes	99
Littorine	21956-47-8	289.3	Y	Y	TRC	91.93
Norscopolamine	4684-28-0	289.3	N	Y	TRC	98
Scopolamine	51-34-3	303.4	Y	Y	TCI	98
Scopolamine-d_3[b]	1279037-70-5	306.4	N	Y	CDN Isotopes	98.2
Anisodamine	55869-99-3	305.4	Y	Y	Phytolab	98.26
Anisodine	52646-92-1	319.4	N	Y	Phytolab	99.64
2α-Hydroxymethylatropine	2515-36-8	319.4	N	Y	TRC	98
O-Acetylscopolamine	na	345.3	N	Y	TRC	98
Others (no TAs)						
Cuscohygrine	454-14-8	224.3	Y	N[b]	Latoxan	100[c]
Phygrine	148139-97-3	280.3	Y	N	-	-

Y = yes, N = no.

(a): TCI Europe, Zwijndrecht, Belgium; TRC, Toronto Research Chemicals, Toronto, Canada; Chemfaces, Wuhan, PRC; Carbosynth, Compton, UK; Latoxan, Valence, France; Synchem UG, Felsberg-Altenberg, Germany; CDN Isotopes, Pointe Claire, Canada; Phytolab, Vestenbergsgreuth, Germany.
(b): Isotopically labelled standard to be used as internal standard.
(c): Identity of the standard offered by chemical supplier could not be confirmed by in-house verification with MS and/or 1H-NMR.
(d): Cocaine is available as a reference standard, but requires special permission. Not included in this study.
(e): (-)-Hyoscyamine is available as a reference standard, but coelutes with atropine (racemic hyoscyamine) in the analytical method. Atropine is used in this study.
Note: Several additionally available tropane alkaloids were purchased from ChemFaces, Wuhan, PRC, but the identity of none of these compounds could be confirmed by MS and/or ^{1}H NMR: 3-acetoxytropane (CAS 3423-26-5), cochlearine (CAS 52418-07-2), nortropanyl cinnamate (CAS 126394-79-4), tropanyl-3-hydroxy-4-methoxycinnamate (CAS 86702-58-1), tropanyl *trans*-cinnamate (CAS 35721-92-7), tropanyl phenylacetate (CAS 1690-22-8), valtropine (CAS 495-82-9).

The identity of each TA standard was checked by LC-MS/(MS) analysis. In case of doubt, ^{1}H NMR was used to verify the identity and purity of the compound (in particular for the calystegines). It turned out that, based on LC-MS analysis, the identity of several standards was evidently incorrect, e.g. because the standard produced a molecular ion different from the correct theoretical one, or the obtained MS/MS fragmentation spectra did not match with fragmentation patterns typical for tropane alkaloids. In some cases, standards sold under different names produced spectra having the same molecular ion, fragmentation spectra and identical retention times, indicating that the suppliers themselves not always check the identity of their analytical products. In particular the 'specialty' analytical TA standards supplied by ChemFaces (Wuhan ChemFaces Biochemical Co., Wuhan, People's Republic of China) were of very poor quality, in most cases not containing the supposed compound at all.

Regarding the calystegine standards, sets of up to 11 different calystegine standards are currently offered by ChemFaces (Wuhan, PRC), ALB Technology (Hong Kong, PRC), EMMX Biotechnology (Lake Forest, CA, USA) and Clearsynth (Mumbay, India). Several standards relevant for this study were ordered from ChemFaces (Calystegine B_1, B_2, B_3, B_4) and Clearsynth (calystegine B_1, B_4). However, ^{1}H NMR and LC-MS analysis revealed that none of these products did contain the supposed calystegines. NMR and LC-MS spectra obtained for B_1 and for B_4 from both companies were found to be identical, strongly suggesting that the origin of the standards offered by the two companies was the same. In the end only the calystegine standards offered by Carbosynth (Compton, UK) and Dextra Laboratories (Reading, UK) were found to be reliable, although according to ^{1}H NMR analysis the purity was not as high as indicated on the analysis certificates. Representative ^{1}H NMR spectra obtained for the calystegines are presented in Appendix F.

Table 3: Calystegine alkaloids mentioned in scientific opinion of EFSA, and their availability as analytical standard

Compound name	CAS No.	MW (Da)	Mentioned in EFSA opinion	Available as standard	Selected supplier[(a)]	Purity (%) according to supplier	Purity (%) according ^{1}H NMR analysis
Calystegine A_3	131580-36-4	159.2	Y	Y	Dextra Laboratories	97.7	69
Calystegine A_5	165905-26-0	159.2	Y	Y	Carbosynth	95	70[(b)]
Calystegine A_6	177794-04-6	159.2	Y	N	-	-	-
Calystegine A_7	197565-90-5	159.2	Y	N	-	-	-
Calystegine N_1	177794-03-5	174.2	Y	N	-	-	-
Calystegine B_1	127414-86-2	175.2	Y	Y	Carbosynth	95	84
Calystegine B_2	127414-85-1	175.2	Y	Y	Carbosynth	95	71
Calystegine B_3	178231-95-3	175.2	Y	Y	Dextra Laboratories	97	85
Calystegine B_4	184046-85-3	175.2	Y	Y	Carbosynth	95	95
Calystegine B_5	197565-91-6	175.2	Y	N	-	-	-

Compound name	CAS No.	MW (Da)	Mentioned in EFSA opinion	Available as standard	Selected supplier[(a)]	Purity (%) according to supplier	Purity (%) according ^{1}H NMR analysis
N-Methyl-calystegine B_2	-	188.2	Y	N	-	-	-
Calystegine C_1	-	191.2	Y	N	-	-	-
Calystegine C_2	190957-44-9	191.2	Y	N	-	-	-
N-Methyl-calystegine C_1	na	205.2	Y	N	-	-	-

Y = yes, N = no.
(a): Dextra Laboraties, Reading, UK; Carbosynth, Compton, UK.
(b): Also contains ca. 15% Calystegine A_3.
Note: Several calystegine standards were also purchased from ChemFaces, Wuhan, PRC (B_1, B_2, B_3, B_4) and from Clearsynth, Mumbai, India (B_1, B_4). However, the identity of none of these compounds could be confirmed by MS and ^{1}H NMR.

In total 26 Datura TAs, including 7 low molecular weight, 4 Convolvulaceae-type, 15 Datura-type including two isotopically labelled standards (atropine-d_3 and scopolamine-d_3), and 6 calystegine standards were obtained from commercial suppliers, of which the identity was positively verified.

3.7. Development of an LC-MS/MS method for Datura TAs

3.7.1. Optimization of MS/MS conditions

The analytical standards described in Table 2 were investigated by mass spectrometric analysis and fragmentation spectra at a range of collision energies (typically 10-40 eV) were obtained for each compound. Three MS/MS transitions characteristic for the standard and with sufficient sensitivity were selected for each standard and incorporated in the MRM method. MS/MS detection was performed in positive electrospray ionisation mode. In Appendix G.3 the selected fragments are tabulated, together with the fragmentation conditions optimised for the Waters Xevo TQ-S system. It should be noted that for instruments of other vendors different instrument settings may be required.

3.7.2. Development of an LC-MS/MS method for Datura TAs

The TAs available for this study cover a wide range with respect to molecular weight and polarity. Particularly the low molecular weight TAs were considered difficult to include in a multi-analyte method due to their relatively high polarity. Several TAs with higher molecular weights had been shown before to display suitable retention characteristics on typical C_{18}-based HPLC or UPLC columns (Mulder et al., 2015; Pereboom-de Fauw and Mulder, 2012). The effect of pH on the retention and peak shapes of the available TAs was therefore investigated in considerable detail using gradients in which aqueous buffer solutions of a wide pH range (3-11) were tried in combination with acetonitrile as organic modifier.

The pH was found to have a critical effect on the retention behaviour and peak shape of most Datura TAs, particularly for the low molecular weight TAs. At pH > 10, separation of some isobaric compounds (i.e. tropine and pseudo tropine and homatropine and noratropine) became problematic. At pH ≤ 9 retention times of many TAs started to shift towards shorter retention times and significant peak broadening was observed for several TAs. At low pH (i.e. using an aqueous formic acid/acetonitrile gradient) no retention was observed for the low molecular weight TAs. Optimal conditions were met for the majority of Datura TAs at a pH of 10. Only for nortropine no workable chromatographic conditions could be established. The compound eluted as a very broad peak, hampering a proper quantification and lowering its sensitivity. For this reason nortropine was excluded from further method development. Appendix G shows the LC-MS/MS chromatograms obtained for a mixed working standard solution containing 24 Datura TAs spiked to a blank extract of buckwheat and run using an ammonium carbonate buffer pH 10 / acetonitrile gradient. As can be seen from Appendix G.4-7 base line separation of all Datura TAs was possible with this gradient. Most TAs eluted as sharp peaks, but a few were somewhat broadened.

Under positive electrospray conditions the low molecular weight TAs produced fragment ions that in general were at least a factor 10 less sensitive that the fragment ions produced by the TAs with higher molecular weights. Consequently, this may result in higher LODs and LOQs for these low molecular weight compounds. The sensitivity for most of the Convolvulaceae-type and Datura-type TAs was good to excellent.

3.7.3. Development of an extraction procedure for Datura TAs

The extraction procedure available at RIKILT for the analysis of ergot and tropane alkaloids in cereal-based products was tried on the set of 24 TAs using blank buckwheat flour (Mulder et al., 2015; Pereboom-de Fauw and Mulder, 2012). In this procedure the TAs are extracted from the matrix with methanol/water/formic acid (60/40/0.4, v/v/v) in a solvent to matrix ratio of 10:1. Ultrafiltration through a 30 kD ultrafilter (Amicon Ultra-4, Millipore, Billerica, MA, USA) yields an extract that can directly be injected in to the LC-MS/MS system. The results were satisfactory for most of the Convolvulaceae-type and Datura-type TAs, but not for the low molecular weight TAs. Very strong suppression effects due to co-extracted matrix were observed, resulting in unacceptably high LODs and LOQs for these TAs.

Based on these results it was decided to investigate whether matrix effects could be reduced by the incorporation of a solid phase extraction (SPE) step in the procedure. Additional advantage of SPE is that a higher final concentration in the sample extract can be obtained, what is particularly important for the low molecular weight TAs, as these display lower intrinsic sensitivity by mass spectrometric detection. Strong cation exchange (SCE) SPE was selected as alkaloids are well retained on this type of SPE and interferences can often be removed by dedicated wash steps. Various conditions were tested (percentage organic in the extraction solvent, wash solutions to remove interferences, percentage ammonia in the final elution solvent) in order to optimise recoveries and reduce matrix effects. Optimal conditions were obtained when using methanol/water/formic acid (75/25/0.4, v/v/v) as extraction solvent and wash solvent and 2% ammonia (25%) in methanol as elution solvent. Suppression was reduced under these conditions, although suppression was still stronger for the low molecular weight TAs than for the other TAs.

The SPE clean-up procedure developed for cereal-based products was found to work for the extraction of TAs from dry (herbal) teas as well. However, matrix interferences were more prominent in dry teas and recoveries were generally lower than in cereal-based products. Tea infusions were prepared using a standardised protocol ISO 3103 (ISO, 1980). The SPE procedure for dry teas was slightly modified to accommodate tea infusions. To achieve sufficiently low detection limits a larger volume (37.5 ml) was applied to the cartridge. To avoid clogging of the SPE cartridges the tea infusions it was essential to centrifuge the infusions before application to the cartridge.

3.7.4. Final method for Datura TAs

Sample preparation for single component flours, cereal-based products, legumes and stir-fry mixes, and dry (herbal) teas

Frozen samples (bread) were allowed to thaw before processing.

Sample portions of 4 gram of single flours (buckwheat, millet), cereal-based products (breakfast cereals, pasta, bread, cookies), legumes and stir-fry mixes or dry (herbal) teas were transferred to polypropylene tubes of 50 mL and 40 µL of internal standard solution (atropine-d_3 and scopolamine-d_3 of 1000 ng/mL in methanol) was added. Forty mL of extraction solvent (methanol/water/formic acid, 75/25/0.4, v/v/v) was added to the tubes. The samples were extracted for 30 min on a rotary tumbler and then centrifuged for 15 min at 3500 rpm.

In case of single flours, cereal-based products and vegetable stir-fry mixes, 10 mL of clear extract was taken for further clean-up by SPE over an OASIS MCX 150 mg/6 cc (Waters, Milford, MA, USA) or alternatively, a StrataX 200 mg/6 mL (Phenomenex, Torrance, CA, USA). In case of dry (herbal) teas 5 mL of extract was used for clean-up by SPE. The cartridges were conditioned with 6 mL of methanol and equilibrated with 6 mL methanol/water/formic acid, 75/25/1 (v/v/v). The cartridges were loaded with 10 mL of extract (5 mL in case of dry (herbal) tea), washed with 6 mL methanol/water/formic acid, 75/25/1 (v/v/v) and dried under vacuum (using a vacuum manifold) for 5-10 min. TAs were eluted from the cartridges with 6 mL of methanol containing 0.5% ammonia (dry or added from 25% conc. ammonia solution). The eluates were evaporated under a nitrogen stream in a warmed water bath (50°C, TurboVap, Zymark, Uppsala, Sweden) and reconstituted in 500 µL water/methanol (90/10, v/v). The reconstituted sample extracts were filtered using 0.45 µm PTFE 500 µL filtervials (UniPrep, Whatman, Maidstone, UK).

Sample preparation for (herbal) tea infusions

(Herbal) tea infusions were prepared according to a standardised protocol ISO 3103 (ISO, 1980). In short, sample portions of 2 gram of dry, ground, homogenised (herbal) tea were transferred to paper tea filter bags (t-sac size 2, t-sac, Hannover, Germany), which was positioned in a beaker of 250 mL. 150 mL of boiling water was poured into the beaker, making sure that the tea bag is fully immersed. The sample was left to stand for 4.5 min, and subsequently, the tea bag was gently swirled around by hand in the beaker for 30 s. The tea bag was removed from the beaker and before disposal most of the adhering infusion was gently pressed out into the beaker. An aliquot of 37.5 mL tea infusion was transferred to a polypropylene tube of 50 mL and 75 µL of formic acid was added. The tube was centrifuged for 15 min at 3500 rpm and all of the supernatant was used for SPE clean-up.

For clean-up by SPE, an OASIS MCX 150 mg/6 cc (Waters, Milford, MA, USA) was used. The cartridges were conditioned with 6 mL of methanol and equilibrated with 6 mL 1% formic acid in water. The cartridges were loaded with 37.5 mL of tea infusion, washed with 6 mL methanol/water/formic acid, 75/25/1 (v/v/v) and dried under vacuum (using a vacuum manifold) for 5-10 min. TAs were eluted from the cartridges with 6 mL of methanol containing 0.5% ammonia (dry or added from 25% conc. ammonia solution). Further steps in the sample preparation were identical as described above for cereal-based products and dry (herbal) teas.

LC-MS/MS analysis (all matrices)

Chromatographic separation was achieved on a 150 x 2.1 mm, 1.7 µm particle size Waters UPLC BEH C18 analytical column (Waters, Milford, MA, USA). Eluent A was prepared from 100% water containing 10 mM ammonium carbonate ($(NH_4)_2CO_3$) buffer, adjusted with concentrated ammonia (25%) to pH 10.0. Eluent B consisted of 100% acetonitrile. A gradient elution was performed as follows: 0.0-2.0 min: isocratic at 100% A/0% B, 2.0-12.0 min: linear gradient to 60% A/40% B, 12.0-12.2 min: linear gradient to 100% A/0% B, 12.2-15.0 min: isocratic at 100% A/0% B. A flow rate of 400 µL/min was applied and 2 µL was injected. The column temperature was maintained at 50°C.

TAs were analysed in positive electrospray ionisation mode (ESI+). Up to three multiple reaction monitoring (MRM) transitions were measured per analyte (Appendix G.3).

3.7.5. In-house validation for Datura TAs

Single flours and cereal-based products

Validation of single flours and cereal-based products was performed by spiking three different samples of breakfast cereals, one sample of biscuits, one sample of bread and one sample of buckwheat flour. Validation of dry (herbal) teas was performed by spiking three different samples (green tea, peppermint tea, mixed herbal tea). Spiking was done in six-fold at three different levels (1, 5, 25 µg/kg). Linearity over the working range (0-50 µg/kg) was assessed through the incorporation of 8 matrix matched calibration standards (0, 0.5, 1, 2.5, 5, 10, 25, 50 µg/kg). Recovery was determined by including two blank samples in each validation experiment, of which the final extracts were spiked with the corresponding amount of TA standards at 10 µg/kg.

The limits of detection (LOD) and limits of quantification (LOQ) determined during the RIKILT in-house validation of the method are presented in Table 4. The results obtained for recovery (10 µg/kg) and accuracy at low (1 µg/kg), intermediate (5 µg/kg) and high (25 µg/kg) concentration are presented in Table 5. See also Appendix H. LODs and LOQs for the individual TAs were very similar for the 6 cereal (based) products tested and no significant differences were observed regarding matrix suppression or matrix interferences for the products tested. Therefore, a single LOD and LOQ value could be derived for each TA covering all types of cereal (based) products. LOQs were set equal to the lowest spiking level or calibration point for which acceptable accuracy data (RSD <30% for between-day repeatability) could be obtained or extrapolated. For the Convolvulaceae-type and Datura-type TAs this was typically at the lowest calibration point (0.5 µg/kg). For the low molecular weight TAs the LOQs were typically around 2.5 µg/kg. LODs were established based on the average performance observed during validation but also during the analysis of the various samples series. The requirement for the LOD values was that both product to ion transitions were observed with a S/N ratio of at least 10. Due to the high sensitivity of the Convolvulaceae-type and Datura-type TA LODs achieved were quite low and typically estimated to be around 0.1 µg/kg. For most low molecular weight TAs the estimated LODs were in the range 0.5-1.0 µg/kg.

Table 4: Limits of Detection (LOD) and Limits of Quantification (LOQ) for the TAs incorporated in the Datura TAs method for single flours and cereal-based products

Compound name	RIKILT		IRTA		FERA		UCT	
	LOD[(a)] (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
Low molecular weight TAs								
6-Hydroxytropinone	0.5	5.0	1.0	5.0	0.5	2.5	0.5	1.0
Nortropinone	0.5	2.5	0.5	5.0	0.5	5.0	0.5	1.0
Pseudotropine	0.5	2.5	0.5	5.0	0.5	5.0	0.5	1.0
Scopine	0.5	2.5	0.5	5.0	0.5	2.5	0.5	1.0
Scopoline	0.5	2.5	0.5	5.0	0.5	5.0	0.5	1.0
Tropine	0.5	2.5	1.0	5.0	0.5	2.5	0.5	1.0
Tropinone	0.5	2.5	1.0	5.0	0.5	2.5	0.25	1.0
Convolvulaceae-type TAs								
Convolamine	0.1	0.5	0.25	1.0	0.1	0.5	0.05	0.5
Convolidine	0.1	0.5	0.5	2.5	0.25	1.0	0.05	0.5
Convolvine	0.1	1.0	0.25	1.0	0.25	1.0	0.5	1.0
Fillalbine	0.1	0.5	0.25	1.0	0.1	0.5	0.1	0.5
Datura-type TAs								
O-Acetylscopolamine	0.1	0.5	0.5	2.5	0.1	0.5	0.05	0.5
Anisodamine	0.1	0.5	0.25	1.0	0.1	0.5	0.05	0.5
Anisodine	0.1	0.5	0.25	1.0	0.1	0.5	0.05	0.5
Apoatropine	0.1	1.0	0.25	1.0	0.1	0.5	0.05	0.5
Aposcopolamine	0.1	0.5	0.25	1.0	0.1	0.5	0.05	0.5
Atropine	0.05	0.5	0.2	1.0	0.05	0.5	0.05	0.5
Homatropine	0.1	0.5	0.25	1.0	0.1	0.5	0.5	1.0
2α-Hydroxymethyl atropine	0.1	0.5	0.25	1.0	0.1	0.5	0.1	0.5
Littorine	0.1	0.5	0.25	1.0	0.1	0.5	0.05	0.5
Noratropine	0.1	0.5	0.25	1.0	0.1	0.5	0.5	1.0
Norscopolamine	0.1	0.5	0.25	1.0	0.1	0.5	0.05	0.5
Phenylacetoxytropane	0.25	0.5	0.25	1.0	0.1	0.5	0.1	0.5
Scopolamine	0.05	0.5	0.2	1.0	0.05	0.5	0.05	0.5

(a): LOD: S/N = 3 for both selected fragments. LOQ: Lowest calibration level for which acceptable repeatability was obtained.

In Tables 4 and 5 the in-house validation results, with respect to LODs, LOQs and recoveries, obtained by IRTA, FERA and UCT are also included. Full details can be found in Appendix I, J and K, respectively. As can be seen from Table 4, LODs and LOQs differed somewhat between the various laboratories. This is for a large part caused by the fact that different LC-MS/MS instruments were used by the partners. Achieved LODs and LOQs by RIKILT, FERA and UCT were in general fairly similar to each other (deviating not more than by a factor of 2), but the LODs and LOQs reported by IRTA generally were a factor of 2-5 higher due to a less sensitive instrument.

As presented in Table 5, recovery data were quite comparable between laboratories. Acceptable recoveries between 70% and 120% were obtained for almost all compounds by the partners for the 4 different cereal matrices (single flours, breakfast cereals, biscuits and bread). Repeatability (Appendix H.2, I.5, J.4 and K.5) was acceptable (≤20%) for most compounds at the concentrations tested, although some TAs proved more difficult than others. Increased variability in the results was reported by some of the partners for convolvine, convolamine and acetylscopolamine, as well as for the low molecular weight TAs at the lower concentration levels.

Table 5: Average recovery of individual TAs at 10 µg/kg and relative standard deviation of the recovery in single flours and cereal-based products (breakfast cereals, bread, biscuits and buckwheat flour) during in-house validation

Compound name	RIKILT (n = 6)		IRTA (n = 4)		FERA (n = 4)		UCT (n = 4)	
	Recovery (%)	RSD recovery (%)	Recovery (%)	RSD recovery (%)	Recovery (%)	RSD recovery (%)	Recovery (%)	RSD recovery (%)
Low molecular weight TAs								
6-Hydroxytropinone	61	20	83	16	86	7	94	19
Nortropinone	78	20	79	13	82	16	87	20
Pseudotropine	86	15	82	29	87	4	82	5
Scopine	77	17	100	14	87	3	84	5
Scopoline	80	10	91	13	80	8	107	9
Tropine	84	14	94	33	88	5	105	15
Tropinone	64	16	99	34	59	28	102	13
Convolvulaceae-type TAs								
Convolamine	80	17	72	11	114	20	92	22
Convolidine	81	18	99	16	88	15	82	13
Convolvine	80	11	81	10	133	21	83	11
Fillalbine	89	12	90	2	89	12	99	22
Datura-type TAs								
O-Acetylscopolamine	85	17	76	41	16[b]	39[b]	106	8
Anisodamine	105	9	86	8	94	10	101	20
Anisodine	105	6	84	6	100	12	99	12
Apoatropine	63	33	76	9	99	22	85	17
Aposcopolamine	71	19	73	14	118	15	88	24
Atropine[a]	98	7	93	7	95	16	93	14
Homatropine	99	5	79	9	99	12	98	3
2α-Hydroxymethyl atropine	95	9	80	8	93	5	98	20
Littorine	96	8	81	6	126	25	99	10
Noratropine	67	22	83	7	93	17	102	9
Norscopolamine	101	5	86	10	102	19	94	12
Phenylacetoxytropane	72	22	80	8	91	28	90	23
Scopolamine [a]	103	6	96	6	105	9	92	17

(a): Internal standard corrected.
(b): Excluding breakfast cereals (recovery: 88%).

Dry (herbal) teas

In-house validation of dry (herbal) teas was performed at RIKILT by spiking three different samples (green tea, peppermint tea, mixed herbal tea). Spiking was done in six-fold at three different levels (1, 5, 25 µg/kg). Linearity over the working range (0-50 µg/kg) was assessed through the incorporation of 8 matrix matched calibration standards (0, 0.5, 1, 2.5, 5, 10, 25, 50 µg/kg). Recovery was determined by including two blank samples in each validation experiment, of which the final extracts were spiked with the corresponding amount of TA standards at 10 µg/kg. At the partners laboratories a single day validation was performed using green tea that was spiked in three-fold at three different levels (1, 5, 25 µg/kg) (Tables 6 and 7).

Table 6: Limits of Detection (LOD) and Limits of Quantification (LOQ) for the TAs incorporated in the Datura TA method for dry (herbal) teas

Compound name	RIKILT		IRTA		FERA		UCT	
	LOD[(a)] (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
Low molecular weight TAs								
6-Hydroxytropinone	2.5	5.0	2.5	5.0	1.0	5.0	0.5	1.0
Nortropinone	2.5	5.0	2.5	5.0	2.5	5.0	0.5	1.0
Pseudotropine	2.5	5.0	2.5	5.0	2.5	5.0	0.5	1.0
Scopine	2.5	5.0	2.5	5.0	1.0	5.0	0.5	1.0
Scopoline	2.5	5.0	2.5	5.0	1.0	5.0	0.5	1.0
Tropine	2.5	5.0	2.5	5.0	2.5	5.0	0.5	1.0
Tropinone	1.0	2.5	1.0	5.0	1.0	5.0	0.25	1.0
Convolvulaceae-type TAs								
Convolamine	0.25	1.0	0.5	1.0	0.5	1.0	0.1	0.5
Convolidine	0.25	1.0	0.5	1.0	0.5	1.0	0.1	0.5
Convolvine	0.25	1.0	0.5	1.0	0.5	1.0	0.5	1.0
Fillalbine	0.25	1.0	0.5	1.0	0.5	1.0	0.1	0.5
Datura-type TAs								
O-Acetylscopolamine	0.1	0.5	1.0	5.0	0.25	0.5	0.1	0.5
Anisodamine	0.1	0.5	0.25	1.0	0.25	0.5	0.1	0.5
Anisodine	0.1	0.5	0.25	1.0	0.25	0.5	0.1	0.5
Apoatropine	0.1	0.5	0.5	2.5	0.25	0.5	0.1	0.5
Aposcopolamine	0.1	0.5	0.25	1.0	0.25	0.5	0.1	0.5
Atropine	0.1	0.5	0.2	1.0	0.1	0.5	0.05	0.5
Homatropine	0.1	0.5	0.25	1.0	0.25	0.5	0.5	1.0
2α-Hydroxymethyl atropine	0.1	0.5	0.25	1.0	0.25	0.5	0.1	0.5
Littorine	0.1	0.5	0.25	1.0	0.25	0.5	0.1	0.5
Noratropine	0.25	1.0	0.25	1.0	0.25	0.5	0.5	1.0
Norscopolamine	0.25	1.0	0.25	1.0	0.25	0.5	0.1	0.5
Phenylacetoxytropane	0.25	0.5	0.25	1.0	0.25	0.5	0.1	0.5
Scopolamine	0.1	0.5	0.2	1.0	0.1	0.5	0.05	0.5

(a): LOD: S/N = 3 for both selected fragments. LOQ: Lowest calibration level for which acceptable repeatability was obtained.

Dry (herbal) teas proved a more difficult matrix than cereal-based products. Recoveries obtained during in-house validation at RIKILT were lower than for cereal-based products, even when only 5 mL of extract was purified instead of 10 mL (Table 7). Recoveries were in the order of 40-60% for most TAs, but for some it was only 20-40%. The variation between the different types of tea validated (green tea, peppermint tea, mixed herbal tea) was also higher, resulting in relatively large standard deviations. Due to the lower recoveries and reduced sample intake the LOQs and LODs for a number of TAs were somewhat higher than for cereals (Table 6). The partners obtained good recoveries for the majority of TAs (Table 7) in green tea. Repeatability was also acceptable (Appendix H.3, I.6, J.5, and K.6). However, the rather large variability in the obtained recoveries between the institutes and between the type of samples negatively effects the reliability of the results, particularly in case of positive samples. The method can be used as a screening approach, but in order to reliably quantify TAs in positive samples it is necessary to conduct a standard addition approach, in which the positive sample is additionally spiked with a (mixture of) TAs at a suitable level (e.g. 2-5 times the indicative concentration).

Table 7: Average recovery of individual TAs at 10 µg/kg and relative standard deviation of the recovery in dry (herbal) teas (matrices: green tea, peppermint tea, mixed herbal tea) obtained during in-house validation

Compound name	RIKILT(a)		IRTA(b)	FERA(b)	UCT(b)
	Recovery (%)	RSD recovery (%)	Recovery (%)	Recovery (%)	Recovery (%)
Low molecular weight TAs					
6-Hydroxytropinone	46	29	107	46	68
Nortropinone	55	36	66	36	72
Pseudotropine	21	54	38	80	56
Scopine	20	50	72	74	66
Scopoline	36	55	92	75	83
Tropine	41	43	98	82	85
Tropinone	59(d)	12(d)	106	38	66
Convolvulaceae-type TAs					
Convolamine	49	13	105	32	151
Convolidine	65	12	93	58	99
Convolvine	55	20	99	40	127
Fillalbine	59	9	94	48	135
Datura-type TAs					
O-Acetylscopolamine	30	24	- (e)	20	53
Anisodamine	58	21	100	83	95
Anisodine	45	53	78	84	90
Apoatropine	47	10	29	38	124
Aposcopolamine	52	23	74	58	118
Atropine(c)	64	15	96	89	112
Homatropine	61	11	90	69	108
2α-Hydroxymethyl atropine	51	29	108	76	101
Littorine	65	8	90	67	108
Noratropine	73	8	113	75	94
Norscopolamine	62	26	99	88	89
Phenylacetoxytropane	56	6	80	53	113
Scopolamine(c)	51	48	96	89	117

(a): Three-day validation with three different teas (green tea, peppermint tea, mixed herbal tea).
(b): Single-day validation using green tea.
(c): Internal standard corrected.
(d): Excluding peppermint tea.
(e): Not determined.

Herbal tea infusions

Validation of the procedure for the preparation of herbal tea infusions and analysis of the infusions was only conducted at RIKILT. (Herbal) tea infusions were prepared according to a standardised protocol ISO 3103 (ISO, 1980). In this preparation 2 g of dry tea is extracted with 150 mL of boiling water. The validation focussed on the extraction of the TAs from the tea infusion. However, the efficiency of extraction of TAs from the solid tea during tea preparation was also taken into account. Because the dilution during tea preparation equals a factor of 75, the validation levels were adjusted accordingly. As can been seen from Table 8, low LODs and LOQs could be achieved for many of the Convolvulaceae-type and Datura-type TAs. For the low MW TAs achieved LODs and LOQs were higher, but this is fully in line with the results obtained for the dry teas. Recoveries from tea infusion varied between 70 and 100% with acceptable repeatability for most TAs, except for a few low MW TAs. The efficiency of extraction of TAs from dry tea during tea preparation was between 60 and 85% for most TAs (Table 8). Exceptions are the Convolvulaceae-type TAs which were extracted with somewhat lower (50-60%) efficiency and apoatropine with an extraction efficiency of 43%. The latter compound is a relatively lipophilic substance that possibly is not extracted well with (hot) water. The low MW TAs are extracted from the tea with good efficiency. This is a good indication that evaporation from the hot infusion is probably limited or negligible (the low MW TAs may be expected to be relatively volatile, which potentially could lead to losses during tea preparation).

Table 8: Limits of Detection (LOD) and Limits of Quantification (LOQ) for the TAs incorporated in the Datura TA method for (herbal) tea infusions and average recovery of individual TAs at 0.1333 µg/L in (herbal) tea infusions (green tea, peppermint tea, mixed herbal tea) obtained during in-house validation at RIKILT[(a)]

Compound name	RIKILT		RIKILT		RIKILT	
	LOD[(a)] (µg/L)	LOQ (µg/L)	Recovery (%)	RSD recovery (%)[(b)]	Efficiency tea preparation (%)	RSD tea preparation (%)[(b)]
Low molecular weight TAs						
6-Hydroxytropinone	0.0133	0.0333	77	22	72	40
Nortropinone	0.0133	0.0333	104	21	110	26
Pseudotropine	0.0133	0.0333	69	44	83	4
Scopine	0.0133	0.0333	51	44	71	22
Scopoline	0.0133	0.0333	64	33	84	7
Tropine	0.0133	0.0333	88	16	76	8
Tropinone	0.0133	0.0333	72	19	83	4
Convolvulaceae-type TAs						
Convolamine	0.0017	0.0067	84	8	53	17
Convolidine	0.0017	0.0067	77	24	54	20
Convolvine	0.0033	0.0133	70	7	49	18
Fillalbine	0.0017	0.0067	89	14	57	16
Datura-type TAs						
O-Acetylscopolamine	0.0017	0.0067	91	2	70	24
Anisodamine	0.0017	0.0067	100	14	84	6
Anisodine	0.0017	0.0067	102	15	86	12
Apoatropine	0.0017	0.0067	75	8	43	25
Aposcopolamine	0.0017	0.0067	82	7	61	24
Atropine[(a)]	0.0017	0.0067	92	6	71	9
Homatropine	0.0017	0.0067	98	12	70	14
2α-Hydroxymethyl atropine	0.0017	0.0067	97	10	74	10
Littorine	0.0017	0.0067	98	10	73	13
Noratropine	0.0033	0.0067	95	16	70	16
Norscopolamine	0.0033	0.0133	103	14	82	13
Phenylacetoxytropane	0.0017	0.0067	87	6	59	16
Scopolamine[(a)]	0.0017	0.0067	90	6	84	9

(a): LOD: S/N = 3 for both selected fragments; LOQ: Lowest calibration level for which acceptable repeatability was obtained. n.a.: no acceptable results obtained.
(b): n = 3.
(c): Internal standard corrected.

Legumes, beans and stir-fry mixes

No specific sample preparation and validation protocol was developed for samples of the category of legumes, beans and stir-fry mixes, because the number of samples collected in this category was relatively small. All samples were individually quantified by means of standard addition to the sample. Based on the standard addition sample the LODs and LOQs were estimated (see Table 9). The estimated LODs and LOQs were very similar to those derived for single flours and cereal-based products.

Table 9: Limits of Detection (LOD) and Limits of Quantification (LOQ) for the TAs incorporated in the Datura TA method for legumes and stir-fry mixes[(a)]

Compound name	RIKILT		IRTA		FERA		UCT	
	LOD[(a)] (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
Low molecular weight TAs								
6-Hydroxytropinone	0.5	2.5	1.0	5.0	0.5	2.5	0.5	1.0
Nortropinone	0.5	2.5	0.5	5.0	0.5	5.0	0.5	1.0
Pseudotropine	0.5	2.5	0.5	5.0	0.5	5.0	0.5	1.0
Scopine	0.5	2.5	0.5	5.0	0.5	2.5	0.5	1.0
Scopoline	0.5	2.5	0.5	5.0	0.5	5.0	0.5	1.0
Tropine	0.5	2.5	1.0	5.0	0.5	2.5	0.5	1.0
Tropinone	0.5	2.5	1.0	5.0	0.5	2.5	0.5	1.0
Convolvulaceae-type TAs								
Convolamine	0.25	1.0	0.25	1.0	0.1	0.5	0.1	0.5
Convolidine	0.25	1.0	0.5	2.5	0.25	1.0	0.1	0.5
Convolvine	0.25	1.0	0.25	1.0	0.25	1.0	0.5	1.0
Fillalbine	0.25	1.0	0.25	1.0	0.1	0.5	0.1	0.5
Datura-type TAs								
O-Acetylscopolamine	0.1	0.5	0.5	2.5	0.1	0.5	0.1	0.5
Anisodamine	0.1	0.5	0.25	1.0	0.1	0.5	0.1	0.5
Anisodine	0.1	0.5	0.25	1.0	0.1	0.5	0.1	0.5
Apoatropine	0.1	0.5	0.25	1.0	0.1	0.5	0.1	0.5
Aposcopolamine	0.1	0.5	0.25	1.0	0.1	0.5	0.1	0.5
Atropine	0.1	0.5	0.2	1.0	0.05	0.5	0.05	0.5
Homatropine	0.1	0.5	0.25	1.0	0.1	0.5	0.5	1.0
2α-Hydroxymethyl atropine	0.1	0.5	0.25	1.0	0.1	0.5	0.1	0.5
Littorine	0.1	0.5	0.25	1.0	0.1	0.5	0.1	0.5
Noratropine	0.25	1.0	0.25	1.0	0.1	0.5	0.5	1.0
Norscopolamine	0.25	1.0	0.25	1.0	0.1	0.5	0.1	0.5
Phenylacetoxytropane	0.1	0.5	0.25	1.0	0.1	0.5	0.1	0.5
Scopolamine	0.1	0.5	0.2	1.0	0.05	0.5	0.05	0.5

(a): LOD: estimation based on S/N = 3 for both selected fragments in the fortified samples; LOQ: estimation, based on S/N = 10 for both selected fragments in the fortified samples.

3.8. Development of an LC-MS/MS method for calystegines

As described in section 3.6, in total six calystegine analytical standards could be obtained that were of sufficient purity to be incorporated in the method for calystegine alkaloids.

3.8.1. Optimization of MS/MS conditions

The analytical standards described in Table 3 were investigated by mass spectrometric analysis and fragmentation spectra at a range of collision energies (typically 10-40 eV) were obtained for each compound. Three MS/MS transitions characteristic for the standard and with sufficient sensitivity were selected for each standard and incorporated in the MRM method. MS/MS detection was performed in positive electrospray ionisation mode. In Appendix L.3 the selected fragments are tabulated, together with the fragmentation conditions optimised for the Waters Xevo TQ-S system. It should be noted that for instruments of other vendors different instrument settings may be required.

3.8.2. Development of an LC-MS/MS method for calystegines

Due to the complications encountered in acquiring the correct calystegine standards, development of a suitable analytical method was delayed. Exploratory work has been conducted using 4 calystegines (A_3, A_5, B_2, B_3). Test experiments, using an UPLC amide column (150 x 2.1 mm, 1.7 µm particles) in combination with HILIC like-chromatographic conditions, showed sufficient retention but poor peak shapes and poor separation between the isomeric pairs. When an UPLC HILIC column (150 x 2.1 mm, 1.7 µm particles) was used, peak shapes were somewhat better, but full separation between the isomeric pairs was still difficult to achieve.

Chiral HPLC columns can offer a different kind of interaction and induce separation between isomeric compounds than regular columns. Chiral columns containing protein-based stationary phases (i.e. chiral-AGP) or vancomycin-based stationary phases (i.e. Chirobiotic V and Chirobiotic T) are the most interesting because they can operate under regular reversed phase conditions that are fully compatible with ESI-MS/MS detection. Three different chiral columns were tested using a range of chromatographic conditions (pH, buffer strength, organic modifier). Best results were obtained with the Astec Chirobiotic V column (Sigma-Aldrich), that was capable of separating calystegine A_3 and A_5 as well as all four isomeric calystegine B standards. An example chromatogram using this column in combination with an ammonium acetate buffer pH 7/methanol gradient is shown in Appendix L.4.

3.8.3. Final method for calystegines

Extraction procedure

Frozen potato and aubergine samples were left to thaw overnight in a refrigerator.

A sample portion of 4 gram of potato or aubergine was transferred to a polypropylene tube of 50 mL and 37 mL of extraction solvent (acetonitrile/water/formic acid 50/50/0.2, v/v/v) was added to the tube. The sample was extracted for 30 min on a rotary tumbler and then centrifuged for 15 min at 3500 rpm. Fifty µL of the extract was transferred to a 0.45 µm PTFE 500 µL filter vial (UniPrep, Whatman, Maidstone, UK). 450 µL of 5 mM ammonium acetate pH 7 buffer was added to the filter vial and the contents were mixed and the vial closed.

LC-MS/MS analysis

Chromatographic separation was achieved on a 150 x 2.1 mm, 5 µm particle size Astec Chirobiotic V column (Sigma-Aldrich, Zwijndrecht, the Netherlands). Eluent A was prepared from 100% water containing 5 mM ammonium acetate (NH_4OAc) buffer, adjusted with concentrated ammonia (25%) to pH 7.0. Eluent B consisted of 100% methanol. A gradient elution was performed as follows: 0.0-8.0 min: isocratic at 100% A/0% B, 8.0-8.5 min: linear gradient to 50% A/50% B, 8.5-11.5 min: isocratic at 50% A/50% B, 11.5-12.0 min linear gradient to 100% A/0% B, 12.0-15.0 min: isocratic at 100% A/0% B. A flow rate of 300 µL/min was applied and 10 µL was injected. The column temperature was maintained at 35°C (Appendix L).

Calystegine alkaloids were analysed in positive electrospray ionisation mode (ESI+). Up to four multiple reaction monitoring (MRM) transitions were measured per analyte (Appendix L.3). Example chromatograms, including examples of potato and aubergine are shown in Appendix L.4-6).

3.8.4. In-house validation for calystegines

For potato and aubergine no naturally blank materials were available. Therefore it was decided to use naturally incurred materials and check the method for repeatability and extraction efficiency by means of these materials.
In Table 10 the LODs and LOQs obtained for the calystegines are tabulated. See also Appendix M, N.4 and O.5. Somewhat lower LODs and LOQs were obtained for the four calystegine B isomers than for the two calystegine A isomers, due to a higher sensitivity on the mass spectrometer. FERA did not implement the method in their laboratory. The samples collected in the UK were sent to RIKILT for analysis.

Table 10: Limits of Detection (LOD) and Limits of Quantification (LOQ) for calystegines potato and aubergine[(a)]

Compound name	RIKILT		IRTA		UCT	
	LOD (mg/kg)	LOQ (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)
Calystegine A_3	0.5	1.0	1.0	2.5	0.5	1.0
Calystegine A_5	1.0	2.5	1.0	2.5	0.5	2.5
Calystegine B_1	0.25	1.0	1.0	2.5	0.25	1.0
Calystegine B_2	0.25	1.0	1.0	2.5	0.25	1.0
Calystegine B_3	0.25	1.0	1.0	2.5	0.25	1.0
Calystegine B_4	0.25	1.0	1.0	2.5	0.25	1.0

(a): LOD: estimation based on S/N = 3 for both selected fragments in the fortified samples; LOQ: estimation, based on S/N = 10 for both selected fragments in the fortified samples.

The extraction efficiency was determined by extracting the samples twice. The results for potato and aubergine are shown in Tables 11 and 12, respectively. Good recoveries (between 83 and 98%) were obtained for the calystegines. On the basis of these results it was decided to report the results without correction for the recovery.

Table 11: Recovery (extraction efficiency) and relative standard deviation of the recovery in potato, obtained during in-house validation[(a)]

Compound name	RIKILT (n = 5)		IRTA (n = 1)	UCT (n= 3)	
	Recovery (%)	RSD (%)	Recovery (%)	Recovery (%)	RSD (%)
Calystegine A_3	93.8	3.9	91.2	86.7	3.2
Calystegine A_5	100[(b)]	-	-	-	-
Calystegine B_1	89.0[(c)]	-	-	-	-
Calystegine B_2	91.7	3.3	91.7	92.3	4.2
Calystegine B_3	-	-	-	-	-
Calystegine B_4	92.7	4.2	92.9	-	-

- = no data
(a): Based on incurred materials.
(b): n = 3.
(c): n = 1.

Table 12: Recovery (extraction efficiency) and relative standard deviation of the recovery in aubergine, obtained during in-house validation[(a)]

Compound name	RIKILT (n = 3)		IRTA (n = 1)	UCT (n = 1)
	Recovery (%)	RSD (%)	Recovery (%)	Recovery (%)
Calystegine A_3	98.0[(b)]	-	83.2	-
Calystegine A_5	-	-	-	-
Calystegine B_1	89.8	0.8	86.2	-
Calystegine B_2	90.4	0.7	85.6	85.0
Calystegine B_3	-	-	-	-
Calystegine B_4	-	-	-	-

- = no data
(a): Based on incurred materials.
(b): n = 1.

3.9. Quality control

3.9.1. Mid-term and long-term stability of samples

For the long-term (12 months) and/or mid-term (4 months) stability tests for storage at room temperature, 4°C, -20°C or -80°C, stability samples (containing 5 subsamples each) for each matrix were either prepared at the beginning of the project (buckwheat, breakfast cereals, biscuits and bread) or halfway the project (herbal tea, potato and aubergine) (see section 3.5). At the indicated time of storage, one set of stability samples for each matrix stored at room temperature, 4°C, -20°C or -80°C was taken and analysed for their TAs (Table 13) or calystegine (Table 14) content.

As seen from Table 13 the samples spiked with TAs were stable (recovery > 90%) under the storage conditions over the whole period. Only some decline was noticed for TAs in buckwheat flour after 12 months of storage at room temperature. This decline could be linked to a low recovery of apoatropine (23%), aposcopolamine (26%) and O-acetylscopolamine (38%), while the other TAs were not affected. Various levels of degradation (recoveries between 35 and 80%) were also observed for the same compounds in buckwheat flour samples stored for 12 months at 4°C and for the samples stored for 4 months at room temperature. Degradation was only observed in buckwheat flour but not in the other matrices and the reason for this instability remains unknown.

Table 13: Mid-term (4 months) and long-term (12 months) stability results for samples spiked with Datura TAs and stored at room temperature, 4°C, -20°C or -80°C[(a)]

Matrix	Spike level (µg/kg)	Datura TAs, RT		Datura TAs, 4°C		Datura TAs, -20°C		Datura TAs, -80°C
		Recovery	RSD	Recovery	RSD	Recovery	RSD	RSD
Mid-term (4 months)								
Buckwheat flour	10	94.8%	8.2%	99.8%	5.2%	-	5.5%	-
Breakfast cereals	10	97.1%	3.9%	95.5%	4.9%	-	10.1%	-
Biscuits	10	96.0%	7.5%	97.0%	5.3%	-	3.8%	-
Bread	10	-	-	-	-	97.1%	6.4%	7.7%
Herbal tea[(b)]	10	97.7%	28%	-	-	-	16.9%	-
Long-term (12 months)								
Buckwheat flour	10	86.6%	14.3%	95.7%	8.8%	-	7.8%	-
Breakfast cereals	10	93.5%	7.8%	99.7%	5.3%	-	9.0%	-
Biscuits	10	95.7%	7.0%	97.4%	5.7%	-	7.2%	-
Bread	10	-	-	-	-	97.0%	7.0%	6.5%

RT = room temperature.
- = not analysed.
(a): n = 5.
(b): For herbal tea only samples for mid-term stability had been prepared.

Stability samples for potato and for aubergine were prepared halfway the project and these were analysed after 6 months of storage at -20°C or -80°C (Table 14). The data suggest a slight increase in the content of calystegines in samples stored at -20°C compared to samples stored at -80°C. It is not fully clear whether this increase is due to instrumental variation or that this is an indication that changes in the calystegine content in stored potato and aubergine samples can occur, even when the samples are kept frozen at -20°C.

Table 14: Medium-term (6 months) stability results for calystegines in incurred potato and aubergine samples stored at -20°C or -80°C[(a)]

Matrix	Calystegine A_3		Calystegine B_1		Calystegine B_2		Calystegine B_4	
	Recovery, -20°C[(b)]	RSD	Recovery, -20°C[(b)]	RSD	Recovery, -20°C[(b)]	RSD	Recovery, -20°C[(b)]	RSD
Potato	115%	17%	-	-	119%	19%	118%	18%
Aubergine	-	-	116%	12%	175%	10%	-	-

- = no data.
(a): n = 5.
(b): Difference in amount extracted from samples stored at 20°C compared to samples stored at -80°.

3.9.2. Quality control data obtained during the study

The method performance with regard to routine analysis was monitored by the partners during the project by the analysis of QC samples included in each series. Most important parameter monitored was the method recovery for the TAs, which should preferably be in the range of 60-120%, although results outside this range do not necessary mean that the quality of the measurements is compromised. A lower than usual recovery may still be sufficient to guarantee the LOD and LOQ obtained during in-house validation of the method (Tables 4 and 6). A higher than usual recovery for most TAs (higher than 120%) may indicate a possible error regarding the preparation of the QC extract. The results are shown below in Figure 1 (single component flour buckwheat), Figure 2 (bread), Figure 3 (breakfast cereals), Figure 4 (biscuits) and Figure 5 (herbal tea) for the 24 Datura TAs. No QC samples were monitored for the analysis of legumes and stir-fry mixes. The quality of measurement of these samples was secured by means of standard addition to each sample. This was also the case for the measurement of herbal tea infusions. All infusions were measured and quantified by means of standard addition to the infusion. Overall, due to the availability of isotopically labelled internal standards, the quality of measurement was best for atropine and scopolamine. Mean recoveries close to 100% with RSD < 15% were obtained for these compounds irrespecitive of the matrix.

Single component flours

The single component flour samples were analysed by the partners in 12 separate runs between November 30, 2015 and July 29, 2016 (Figure 1). The recovery values for the buckwheat QC samples spiked at 10 µg/kg indicate that for the majority of compounds acceptable recoveries between 60 and 120% were obtained (Figure 1). No obvious trends could be detected, although a somewhat higher variability appears to be present for the series of results reported early in the project, perhaps reflecting the somewhat limited experience with the method at that time. The mean recovery obtained for the compounds over all series was 92% (SD: 10%). The lowest mean recovery was observed for acetylscopolamine (69 ± 24%) and the highest mean recovery was obtained for scopolamine (104 ± 13%, internal standard corrected). Overall, the results obtained were in accordance with the validation parameters obtained previously (see Section 3.7.5).

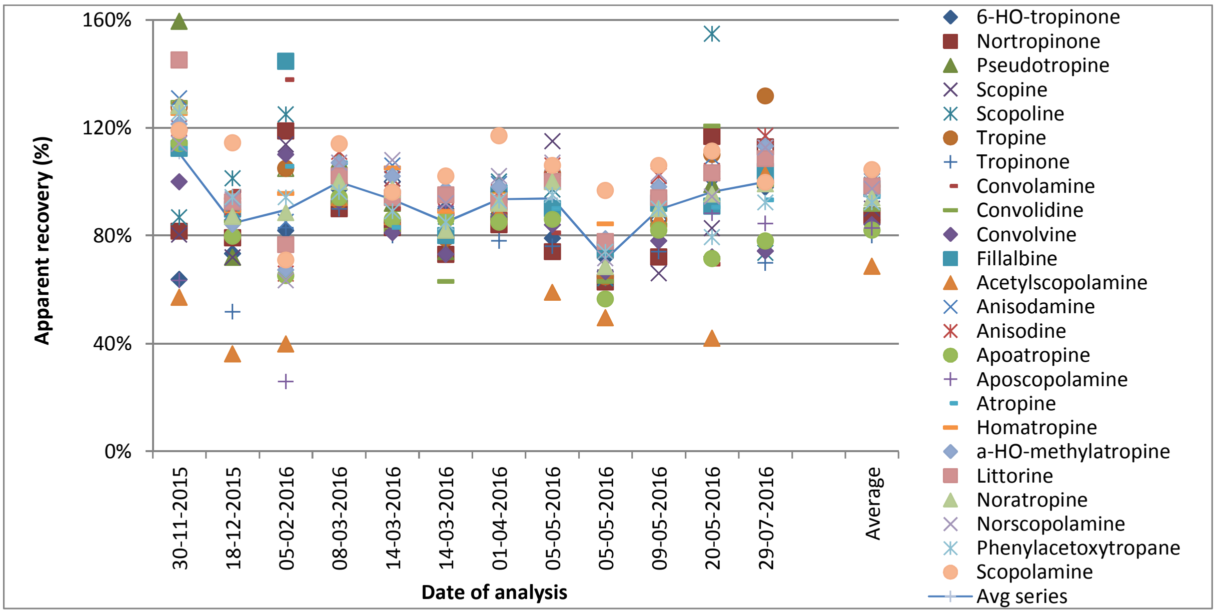


Figure 1: Overview of the apparent recovery obtained for 24 TA in single component flours, using a buckwheat sample spiked at 10 µg/kg as QC sample. Each spot represents the individual recovery per series of analysis by the partners

Cereal-based products

The bread and pasta samples were analysed by the partners in 8 separate runs between December 2, 2015 and June 8, 2016. The recovery values for the bread QC samples spiked at 10 µg/kg indicate that for the majority of compounds acceptable recoveries between 60 and 120% were obtained (Figure 2). No obvious trends could be detected. One series of samples (13-04-2016) had a higher than usual recovery for most of the TAs. Possibly an error has occurred during the preparation of the sample. Excluding the run of 13-04-2016, the overall mean recovery obtained for the compounds was 83% (SD: 10%). The lowest mean recovery was observed for tropinone (44 ± 22%) and the highest mean recovery was obtained for scopolamine (102 ± 12%, internal standard corrected). Overall, the results obtained were in accordance with the validation parameters obtained previously (see Section 3.7.5).

Breakfast cereals and part of the cereal-based products for children (breakfast cereals and cereal-based meals) were analysed by the partners in 13 separate runs between December 9, 2015 and July 30, 2016. The recovery values for the breakfast cereal QC samples spiked at 10 µg/kg indicate that for the majority of compounds acceptable recoveries between 60 and 120% were obtained (Figure 3). No obvious trends could be detected. One series of samples (04-04-2016) had a higher than usual recovery for most of the TAs. Possibly an error has occurred during the preparation of the sample. Excluding the run of 04-04-2016, the mean recovery obtained for the compounds overall was 80% (SD: 9%). The lowest mean recovery was observed for acetylscopolamine (60 ± 27%) and the highest mean recovery was obtained for scopolamine (98 ± 15%, internal standard corrected). Overall, the results obtained were in accordance with the validation parameters obtained previously (see Section 3.7.5).

Biscuits and pastry and part of the cereal-based products for children (cookies for children) were analysed by the partners in 11 separate runs between November 25, 2015 and June 5, 2016. The recovery values for the biscuit QC samples spiked at 10 µg/kg indicate that for the majority of compounds acceptable recoveries between 60 and 120% were obtained (Figure 4). No obvious trends could be detected. The mean recovery obtained for the compounds overall was 81% (SD: 8%). The lowest mean recovery was observed for tropinone (44 ± 26%) and the highest mean recovery was obtained for scopolamine (101 ± 10%, internal standard corrected). Overall, the results obtained were in accordance with the validation parameters obtained previously (see Section 3.7.5).

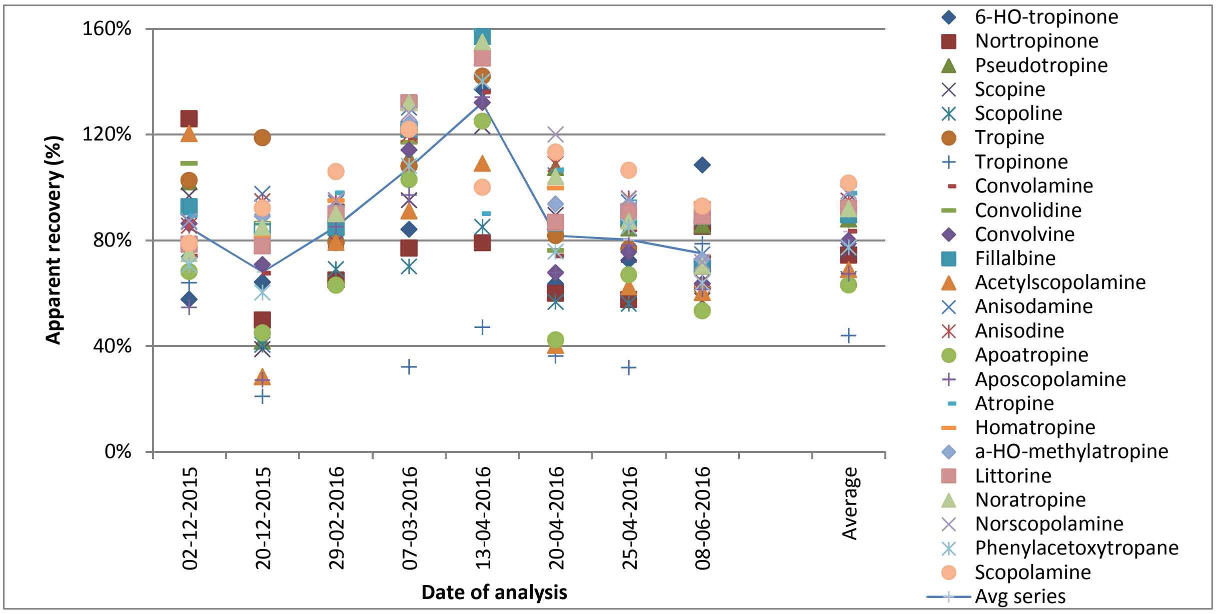


Figure 2: Overview of the apparent recovery obtained for 24 TA in bread and pasta samples, using a bread sample spiked at 10 µg/kg as QC sample. Each spot represents the individual recovery per series of analysis by the partners

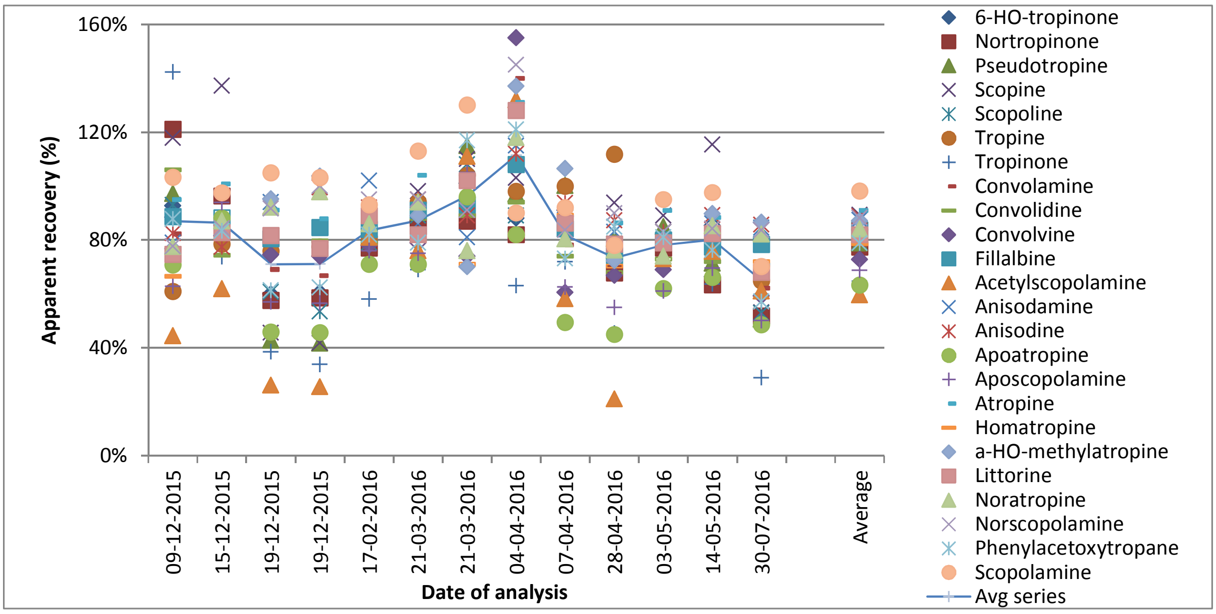


Figure 3: Overview of the apparent recovery obtained for 24 TA in breakfast cereals and cereal-based products for young children, using a breakfast cereal sample spiked at 10 µg/kg as QC sample. Each spot represents the individual recovery per series of analysis by the partners

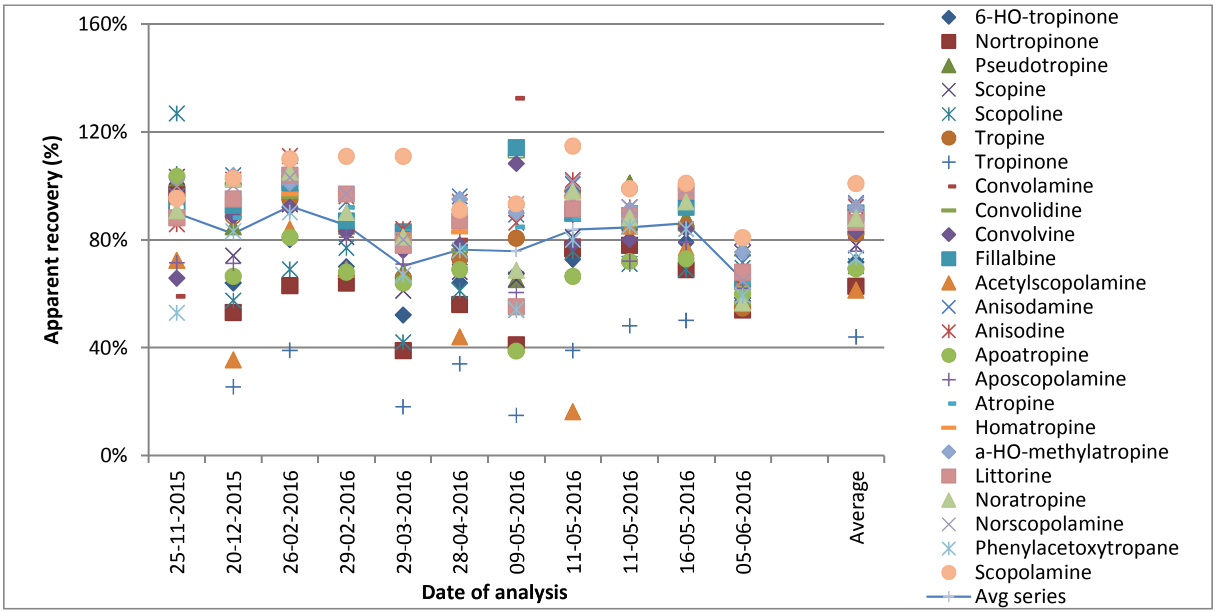


Figure 4: Overview of the apparent recovery obtained for 24 TA in biscuits and pastry and cereal-based products for young children, using a biscuit sample spiked at 10 µg/kg as QC sample. Each spot represents the individual recovery per series of analysis by the partners

Herbal teas

Herbal dry teas were analysed in 7 separate runs between May 13, 2016 and July 30, 2016. The recovery values for the green tea QC samples spiked at 10 µg/kg indicate that although for the majority of compounds acceptable recoveries between 60 and 120% were obtained, variation between runs was considerable, reflecting the more difficult nature of this type of matrix (Figure 5). No obvious trends could be detected. The mean recovery obtained for the compounds overall was 78% (SD: 18%). The lowest mean recovery was observed for tropinone (50 ± 17%) and the highest mean recovery was obtained for atropine (96 ± 13%, internal standard corrected). Overall, the results obtained were in line with the validation parameters obtained previously (see Section 3.7.5). Because of the wider spread in recovery and repeatability data all samples were analysed with standard addition to guarantee a good quality of the results.

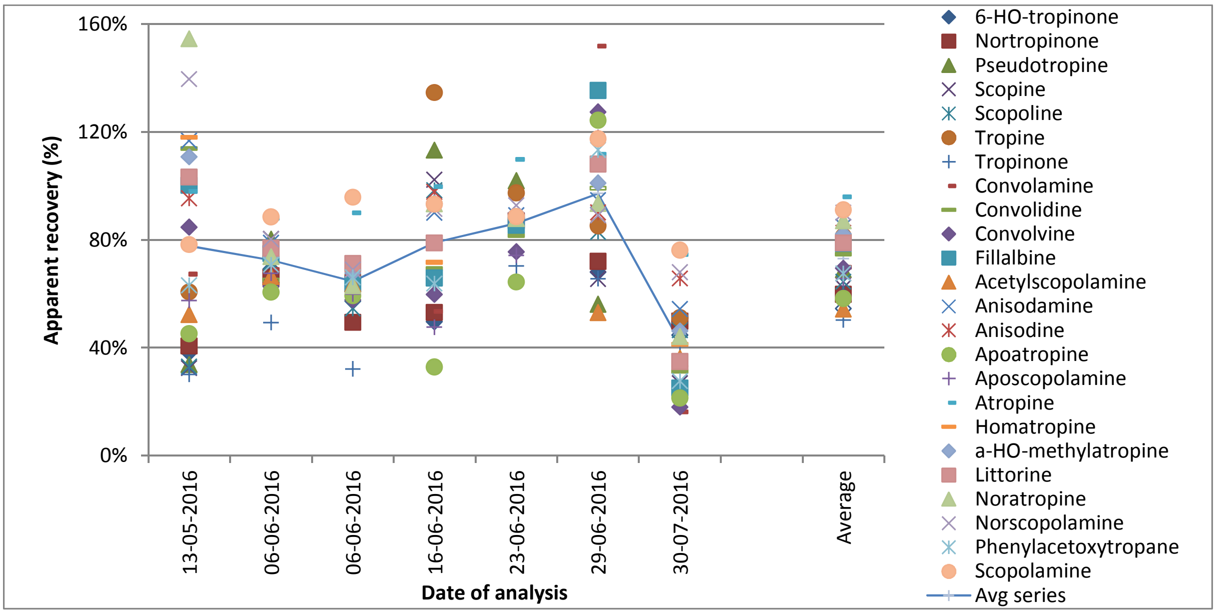


Figure 5: Overview of the apparent recovery obtained for 24 TA in dry herbal tea samples, using a dry green tea sample spiked at 10 µg/kg as QC sample. Each spot represents the individual recovery per series of analysis by the partners

3.10. Sampling

3.10.1. Single component flours, cereal-based foods, dry (herbal) teas, legumes, stir-fry mixes and oilseeds

A total of 1305 samples of single flours, cereal-based foods and other products available at retail stores were taken between June 2015 and August 2016. A summary of the collected samples is presented in Table 15, showing the planned and the actual number of samples. For all three major categories more samples were collected than originally planned. In total 390 samples of organic production were collected, which is 30% of the total number of samples. Originally approximately 25% of organic samples was envisioned. The percentage of organic samples was relatively high for the category of single component flours (more than 50%). For the other categories the percentage organic was close to the expected percentage of 25%.

Table 15: Overview of single component flours, cereal-based foods, dry (herbal) teas, legumes, stir-fry mixes and oilseeds available at retail stores collected for analysis on TA

Food catergory	Target number of samples	Samples collected and analysed	% Of target number of samples	Production		% Organic
				Traditional	Organic	
Single component flours	**220**	**268**	**122%**	**124**	**144**	**54%**
Buckwheat	113	113	100%	58	55	49%
Millet & sorghum	107	102	95%	36	66	65%
Corn & others	-	53	-	30	23	43%
Cereals available at retail stores	**820**	**838**	**102%**	**641**	**197**	**24%**
Bread and pasta	**205**	**195**	**95%**	**157**	**38**	**19%**
Bread	125	114	91%	96	18	16%
Pasta	80	81	101%	61	20	25%
Breakfast cereals	**205**	**219**	**107%**	**168**	**51**	**23%**
Biscuits and pastry	**175**	**164**	**94%**	**137**	**27**	**16%**
Biscuits	175	150	86%	125	25	17%
Pastry	-	14	-	12	2	14%
Cereal-based foods for children	**235**	**260**	**111%**	**179**	**81**	**31%**
Breakfast cereals	135	135	100%	97	38	28%
Cookies	100	107	107%	73	34	32%
Pasta and cereal-based meals	-	18	-	9	9	50%
Other products available at retail stores	**160**	**199**	**124%**	**150**	**49**	**25%**
Dry (herbal) teas	**100**	**121**	**121%**	**86**	**35**	**29%**
Legumes, stir-fry mixes, oil seeds	**60**	**78**	**130%**	**64**	**14**	**18%**
Legumes, stir-fry mixes	60	65	108%	54	11	17%
Oil seeds	-	13	-	10	3	23%
Total	**1200**	**1305**	**109%**	**915**	**390**	**30%**

- = no samples taken.

Figure 6 shows an overview of the number of food products collected for TA analysis. Samples were collected between June 2015 and June 2016 in two major sampling rounds. In the first sampling round (June to December 2015) a total of 687 samples were collected and during the second sampling round (January to June 2016) another 618 products. Except for the bread products that have a very short shelf-life, the majority of products had a relatively long shelf-life, varying from 6-12 months for biscuits and 1-2 years for flours, breakfast cereals and frozen products to 2-5 years for pasta, dry (herbal) teas and dry beans. Products sampled cover a span of several years, covering different seasons and production years.

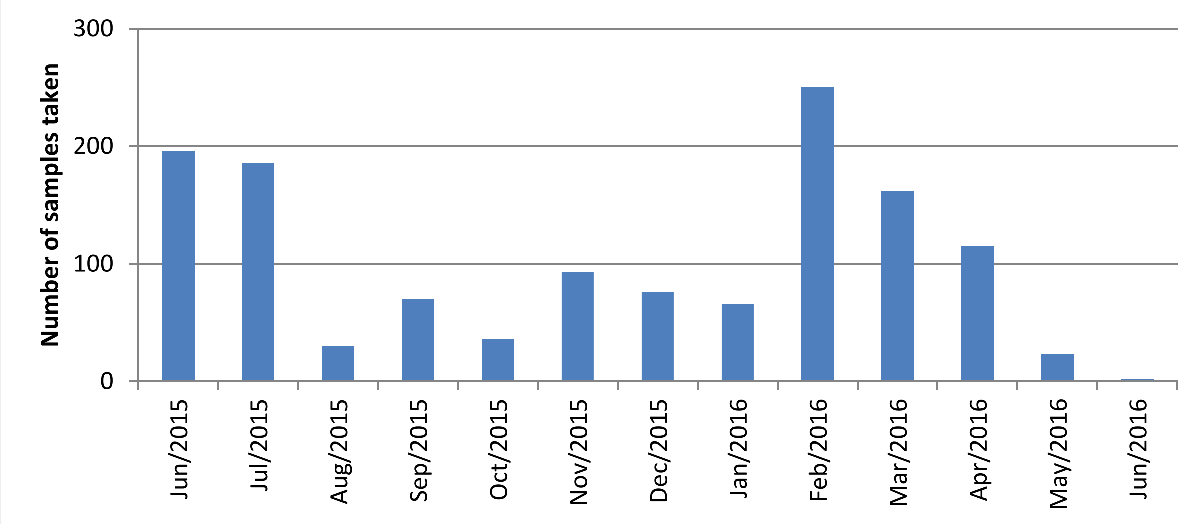


Figure 6: Overview of the monthly number of single component flours, cereal-based foods, dry (herbal) teas, legumes, stir-fry mixes and oilseeds collected for TA analysis

An overview of the country of production of the sampled products is shown in Table 16. For just over half of the samples (51.6%) no country of origin was indicated on the product. For approximately 10% the origin was indicated as the European Union and for 1.8% as non-EU. The percentage of samples with unknown country of origin was highest for herbal teas (75%), biscuits and pastry (69%) and breakfast cereals (66%). These are product categories that typically consist of processed foods containing many ingredients or of blended materials, coming from different origins. In contrast the percentage of 'unknowns' is only 24% for the category of single component flours.

For 474 samples (36%) a single country of origin was indicated. Of these countries the United Kingdom was the most important producer (8.7%), followed by Italy (4.0%), Germany (3.6%) and Spain (3.4%) Including the products labelled 'non-EU', 97 samples (7.4%) came from countries outside the EU. A relatively large part of these samples were single component flours (buckwheat and millet) produced in China, Ukraine or the US. It can be concluded that for the food products carrying information on the country or region of origin, the larger part has been produced within Europe.

Table 16: Overview of country of origin of the food samples collected and analysed for tropane alkaloids

Country of production	Total number of samples	Single flours	Bread and pasta	Cereal-based products	Biscuits and pastry	Foods for children	Dry (herbal) teas	Legumes and vegetables	% Of total
Argentina	1	-	-	-	-	-	-	1	0.1%
Australia	3	1	1	-	-	-	-	1	0.2%
Austria	10	3	-	1	-	6	-	-	0.8%
Belgium	10	-	7	-	-	3	-	-	0.8%
Bolivia	1	1	-	-	-	-	-	-	0.1%
Canada	5		-	-	-	-	-	5	0.4%
China	17	14	-	-	-	-	1	2	1.3%
Croatia	3	-	-	-	-	3			0.2%
Czech Republic	25	10	5	3	2	1	2	2	1.9%
Eastern Europe	2	2	-	-	-	-	-	-	0.2%
Egypt	1	-	-	-	-	-	1	-	0.1%
Ethiopia	1	-	-	1	-	-	-	-	0.1%
European Union	133	35	13	15	7	50	5	8	10.2%

Country of production	Total number of samples	Single flours	Bread and pasta	Cereal-based products	Biscuits and pastry	Foods for children	Dry (herbal) teas	Legumes and vegetables	% Of total
France	29	12	-	2	6	9	-	-	2.2%
Germany	47	12	9	8	5	11	2	-	3.6%
Hungary	9	1	3	4	-	1	-	-	0.7%
India	3	3	-	-	-	-	-	-	0.2%
Ireland	3	1	-	-	-	1	-	1	0.2%
Israel	2	-	1	-	-	1	-	-	0.2%
Italy	52	15	23	4	-	10	-	-	4.0%
Japan	1	-	1	-	-	-	-	-	0.1%
Lithuania	6	6	-	-	-	-	-	-	0.5%
Malta	1	-	1	-	-	-	-	-	0.1%
Mexico	1	-	-	-	-	-	-	1	0.1%
the Netherlands	22	2	8	-	2	4	-	6	1.7%
Non-EU	23	19	-	-	-	-	4	-	1.8%
Poland	19	17	1	-	-	-	1	-	1.5%
Russia	3	3	-	-	-	-	-	-	0.2%
Slovak Republic	2	1	1	-	-	-	-	-	0.2%
South Africa	4	-	-	-	-	-	4	-	0.3%
Spain	45	5	15	1	9	6	5	4	3.4%
Switzerland	4	-	-	-	-	4	-	-	0.3%
Turkey	3	-	1	-	1	-	-	1	0.2%
Ukraine	17	17	-	-	-	-	-	-	1.3%
United Kingdom	113	17	18	33	19	15	5	6	8.7%
United States	11	7	-	2	-	-	-	2	0.8%
Unknown	673	64	87	145	113	135	91	38	51.6%
Total	**1305**	**268**	**195**	**219**	**164**	**260**	**121**	**78**	

- = no samples taken.

Single component flours

In this survey 268 samples of single component grain products (flours, grains, peeled, groats, grits, flakes, puffed) were sampled (Table 17). The majority of samples collected were the pseudograins buckwheat and millet/sorghum. A smaller proportion of corn (polenta) and other grains (wheat, spelt) samples was collected as well. The areas of production were often Eastern Europe and South Europe (Table 16), but China was also a relatively important production country for buckwheat. More than half of the collected samples were from organic production. One of the reasons for this is that buckwheat and millet were often more readily available through specialised health stores which often offer organically grown food only. However, organically grown products were also available from general stores in the UK, the Netherlands, Germany and Czech Republic but to a lesser extent in France, Hungary and Poland.

Table 17: Single component flours collected per type and country

Sampling country	Buckwheat		Millet		Corn		Others		Total		Total
	Trad[(a)]	Org[(a)]	Trad	Org	Trad	Org	Trad	Org	Trad	Org	
Czech Republic	18	7	13	7	1	1	-	-	32	15	47
France	4	1	-	5	-	-	-	-	4	6	10
Germany	2	6	1	8	2	4	-	-	5	18	23
Hungary	10	-	9	2	1	-	1	-	21	2	23
Italy	1	2	-	4	6	-	-	-	7	6	13
the Netherlands	1	14	1	11	3	5	-	1	5	31	36
Poland	10	-	2	2	-	-	-	-	12	2	14
Spain	3	16	1	19	4	-	-	-	8	35	43
United Kingdom	9	9	9	8	7	4	5	8	30	29	59
Total	**58**	**55**	**36**	**66**	**24**	**14**	**6**	**9**	**124**	**144**	**268**
% Organic	49%		65%		37%		60%		54 %		

- = no samples taken.
(a): Trad = traditional production; Org = organic production.

Cereal-based products

In Table 18 an overview is presented of the different types of cereal-based products sampled in this survey. Averaged over the five categories of cereal-based products 20% was of organic origin. The percentage of organic samples is somewhat higher than average for the pasta and breakfast cereals and somewhat lower for bread, biscuits and pastry. For the large proportion (60%) of the cereal samples the country of origin of the product is not known (Table 16). Of the samples with known origin, only a very small number of samples (0.9%) came from outside the EU. Within the EU, the United Kingdom was the largest producer (12%), followed by Italy (4.7%), Spain (4.3%) and Germany (3.8%) (Table 16).

Table 18: Cereal-based products collected per type and country

Sampling country	Bread		Pasta		Breakfast cereals		Biscuits		Pastry		Total		Total
	Trad[(a)]	Org[(a)]	Trad	Org	Trad	Org	Trad	Org	Trad	Org	Trad	Org	
Czech Republic	10	4	5	1	23	6	15	5	-	-	53	16	69
France	9	-	8	2	20	-	14	-	2	-	53	2	55
Germany	6	-	1	4	10	8	10	1	-	-	27	13	40
Hungary	9	-	9	-	14	2	10	-	-	-	42	2	44
Italy	8	1	6	-	18	3	15	-	-	-	47	4	51
the Netherlands	10	3	1	-	12	9	14	4	1	-	38	16	54
Poland	7	-	8	-	15	-	10	-	-	-	40	0	40
Spain	15	2	16	9	30	10	29	9	2	-	92	30	122
United Kingdom	22	8	7	4	26	13	8	6	7	2	70	33	103
Total	**96**	**18**	**61**	**20**	**168**	**51**	**125**	**25**	**12**	**2**	**462**	**116**	**578**
% Organic	16%		25%		23%		17%		14%		20%		

- = no samples taken.
(a): Trad = traditional production; Org = organic production.

Cereal-based products for young children

Cereals and cookies were the two main categories of food products that were sampled that are specifically produced to be consumed by toddlers and young children. Table 19 gives an overview of the number of products sampled per country. Some samples of pasta and cereal-based ready-to-eat meals containing vegetables were collected as well in the United Kingdom. Approximately 30% of the samples were of organic production. The country of origin was not indicated on 52% of the products, while the others all came from within the EU (Table 16).

The cereal products for children consisted of 111 cereal products that need to be fortified with milk before consumption and of 22 products that need to be prepared with water (for 2 products the way of preparation was not known). Typically these products are produced for an specific age group, varying between 6 and 36 months. The number of different cereals present in the products could vary between one and eight types. Wheat, rye, oats and rice were the most common cereals used, but products could also contain (smaller) amounts of buckwheat, millet, barley, corn, spelt and sorghum. The cereal-based vegetable meals were typically products containing spaghetti or macaroni as cereal base, together with a mix of cooked vegetables, often including tomato, onion, bell pepper, carrot and courgette.

Table 19: Cereal-based products for children per type and country

Sampling country	Cereals		Cookies and rusks		Pasta		Cereal-based meals		Total		Total
	Trad[(a)]	Org[(a)]	Trad	Org	Trad	Org	Trad	Org	Trad	Org	
Czech Republic	8	-	11	5	-	-	-	-	19	5	24
France	20	-	-	-	-	-	-	-	20	0	20
Germany	4	5	6	1	-	-	-	-	10	6	16
Hungary	5	-	6	4	-	-	-	-	11	4	15
Italy	4	6	8	2	-	-	-	-	12	8	20
the Netherlands	9	7	10	5	-	-	-	-	19	12	31
Poland	7	-	6	2	-	-	-	-	13	2	15
Spain	21	7	19	8	-	-	-	-	40	15	55
United Kingdom	19	13	7	7	1	3	8	6	35	29	64
Total	**97**	**38**	**73**	**34**	**1**	**3**	**8**	**6**	**179**	**81**	**260**
% Organic	28%		32%		75%		43%		31%		

- = no samples taken.
(a): Trad = traditional production; Org = organic production.

Dry (herbal) teas

A total of 121 dry (herbal) teas products were collected, predominantly in the partners countries. Of these samples 65% was classified as mixed herbal tea, although some teas only contained one herbal (e.g. anise, linden or nettle tea). This category also contained six teas for children, two of which contained an extract of fennel. In Table 20, rooibos, peppermint and camomile herbal teas are specified individually, as well as the samples of regular black and green tea prepared from *Camellia sinensis*. Around 30% of the samples were of organic production. For the large proportion of the products (75%) the country of production was not indicated (Table 16).

Table 20: Dry (herbal) teas collected per type and country

Sampling country	Black/green		Rooibos		Peppermint		Camomile		Mixed herbal		Total		Total
	Trad[(a)]	Org[(a)]	Trad	Org	Trad	Org	Trad	Org	Trad	Org	Trad	Org	
Czech Republic	-	-	-	-	-	-	-	-	15	5	15	5	20
Germany	-	-	-	-	-	-	-	-	8[b]	6	8	6	14
the Netherlands	1	-	-	-	1	2	-	-	8	6	10	8	18
Spain	-	-	-	-	5	1	6	3	19	6[c]	30	10	40
United Kingdom	8	1	3	2	5	-	4	-	3	3	23	6	29
Total	**9**	**1**	**3**	**2**	**11**	**3**	**10**	**3**	**53[(b)]**	**26[(c)]**	**86**	**35**	**121**
% Organic	10%		40%		21%		23%		33%		29%		

- = no samples taken.
(a): Trad = traditional production; Org = organic production.
(b): Including 4 samples of mixed herbal tea for children.
(c): Including 2 samples of mixed herbal tea for children.

Legumes, stir-fry mixes and oilseeds

The category with the smallest number of samples collected consisted of a variety of products, such as beans and peas, lentils, oilseeds and stir-fry mixes (Table 21). Products were only sampled in a limited number of countries, e.g. beans in Czech Republic, Spain and the United Kingdom, while stir-fry mixes were collected in the Netherlands and the United Kingdom. Oilseeds were only collected in the United Kingdom. The mixed vegetables category included six bell pepper samples. The other mixed vegetable products contained, in different combinations and amounts, a wide variety of vegetables and legumes including bell pepper, potato, courgette, onion, cauliflower, cabbage, peas, green beans, broccoli and carrot.

Table 21: Legumes, stir-fry mixes and oilseeds per type and country

Sampling country	Beans & peas		Lentils		(Mixed) vegetables		Oilseeds		Total		Total
	Trad[(a)]	Org[(a)]	Trad	Org	Trad	Org	Trad	Org	Trad	Org	
Czech Republic	6	1	5	2	-	-	-	-	11	3	14
Italy	-	-	-	-	1	-	-	-	1	0	1
the Netherlands	-	-	-	-	11	3	-	-	11	3	14
Spain	16	2	4	3	-	-	-	-	20	5	25
United Kingdom	6	-	-	-	5	-	10	3	21	3	24
Total	**28**	**3**	**9**	**5**	**17**	**3**	**10**	**3**	**64**	**14**	**78**
% Organic	10%		36%		15%		23%		18%		

- = no samples taken.
(a): Trad = traditional production; Org = organic production.

3.10.2. Food from the Solanaceae family

A total of 404 samples were collected of food plants from the Solanaceae family in this survey from retail stores. A summary of the collected samples is presented in Table 22. Almost 50% more samples of potato were collected than the number originally planned. The number of aubergine collected was equal to the number planned. Overall 19% of the potato and aubergine samples was from organic production.

Table 22: Overview of collected samples of food plants from the Solanaceae family available at retail stores

Food category	Target number of samples	Samples collected and analysed	% Of target number of samples	Production		% Organic
				Traditional	Organic	
Potatoes	**210**	**308**	**147%**	**252**	**56**	**18%**
Potatoes, fresh	210	297	141%	241	56	19%
Potatoes, processed	-	11	-	11	-	0%
Aubergine & bell pepper	**90**	**96**	**107%**	**74**	**22**	**23%**
Aubergine	90	90	100%	71	19	21%
Bell pepper	-	6	-	3	3	50%
Total Solanaceae food plants	**300**	**404**	**135%**	**326**	**78**	**19%**

- = no samples taken.

In Figure 7 the monthly number of samples of the Solanaceae family collected by the partners is shown. In 2015, during the first sampling round, 239 samples were collected, while in 2016, during the second sampling round, another 165 products were purchased. Potato samples were purchased either as packed material (paper or plastic bags) or as loose potatoes from bulk, while aubergines and bell peppers are almost always purchased per piece from the bulk available in the shop. Potatoes can be stored for up to a year under the right conditions while aubergines and bell peppers have a short shelf-life.

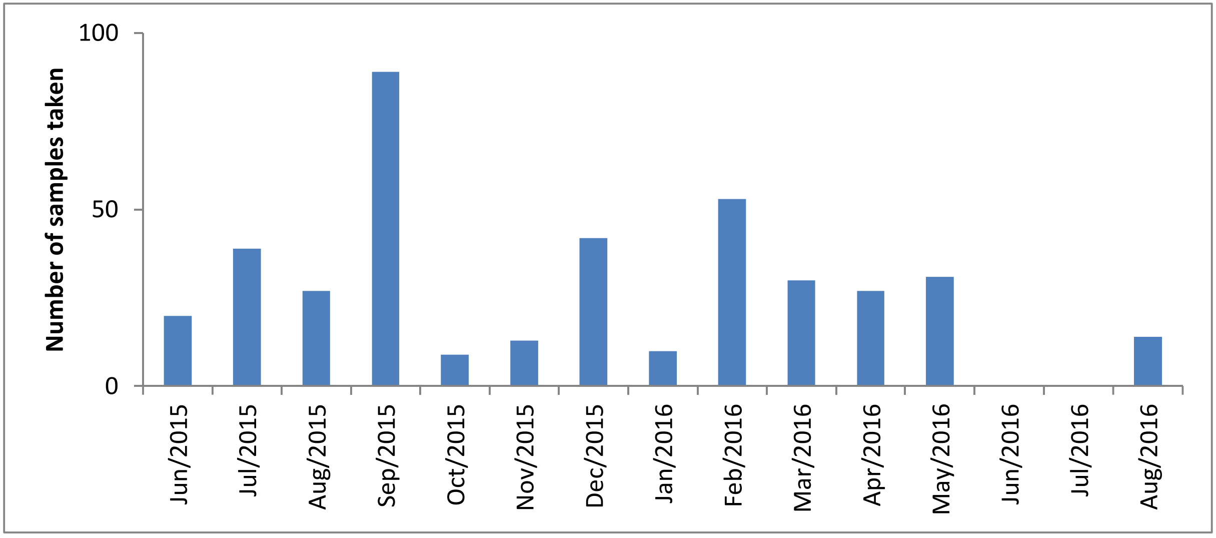


Figure 7: Overview of the monthly number of the Solanaceae family collected for calystegines analysis

The country of origin of the samples from the Solanaceae family is shown in Table 23. Overall, for the majority of samples (80.7%) a country of origin was indicated on the product. It should be noted, however, that for more than half of the aubergine and bell pepper samples no country of production was indicated on the label while for more than 90% of the potato samples the country of origin was specified. With respect to potatoes the major production countries were the Netherlands (29%), followed the UK (16%), France (13%) and Spain (8%). A small proportion of potatoes (5.2%) originated from outside the EU, mostly potato samples from Israel. All of the aubergines and bell peppers with known origin were produced in Europe, of which Spain (29%) and the Netherlands (16%) were the main suppliers. Aubergines and bell peppers are produced in Europe around the year. Potatoes are mostly harvested in summer and early autumn in North-West Europe, and the harvesting season is in spring in Southern Europe.

Table 23: Country of origin of Solanaceae food samples collected and analysed for calystegines

Country of production	Total number of samples	Potatoes, fresh and processed	Aubergine, bell peppers	% Of total
Belgium	3	3	-	0.7%
Cyprus	1	1	-	0.2%
Czech Republic	14	14	-	3.5%
Egypt	4	4	-	1.0%
European Union	2	2	-	0.5%
France	39	39	-	9.7%
Germany	16	16	-	4.0%
Honduras	1	1	-	0.2%
Hungary	7	7	-	1.7%
Israel	10	10	-	2.5%
Italy	17	11	6	4.2%
Malta	3	3	-	0.7%

Country of production	Total number of samples	Potatoes, fresh and processed	Aubergine, bell peppers	% Of total
the Netherlands	104	90	14	25.7%
Poland	4	4	-	1.0%
Portugal	1	1	-	0.2%
Spain	50	24	26	12.4%
United Kingdom	49	48	1	12.1%
United States	1	1	-	0.2%
Unknown	78	29	49	19.3%
Total	**404**	**308**	**96**	

- = no samples taken.

In the food group of potatoes a total of 297 samples of fresh potatoes and 11 samples of processed potato samples were taken from retail stores (Table 24). Fresh potatoes and aubergines were sampled in all nine countries. Bell peppers were only sampled in the Netherlands. About 19% of the Solanaceae samples were of organic origin. A large variety of potato cultivars was collected, predominantly in the Netherlands.

Table 24: Potatoes and aubergines per type and country

Sampling country	Potatoes, fresh		Potatoes, processed		Aubergine		Bell pepper		Total		Total
	Trad[(a)]	Org[(a)]	Trad	Org	Trad	Org	Trad	Org	Trad	Org	
Czech Republic	25	5	-	-	10	5	-	-	35	10	45
France	5	1	4	-	5	-	-	-	14	1	15
Germany	8	-	2	-	4	1	-	-	14	1	15
Hungary	10	-	-	-	8	1	-	-	18	1	19
Italy	9	1	-	-	4	1	-	-	13	2	15
the Netherlands	107	29	3	-	12	3	3	3	125	35	160
Poland	10	-	-	-	1		-	-	11	0	11
Spain	33	10	2	-	13	7	-	-	48	17	65
United Kingdom	34	10	-	-	14	1	-	-	48	11	59
Total	**241**	**56**	**11**	**0**	**71**	**19**	**3**	**3**	**326**	**78**	**404**
% Organic	**19%**		**0%**		**21%**		**50%**		**19 %**		

- = no samples taken.
(a): Trad = traditional production; Org = organic production.

3.11. Occurrence of Datura TAs in single component flours, cereal-based food products and other products available at retail stores

A total of 1305 samples was analysed for tropane alkaloids. Results are presented in Table 25. Of all samples, 22.5% contained one or more Datura TAs above the LOD. A relatively large part of the generated positive results fall in the range between LOD and LOQ. The mean concentration of Datura TAs in all samples was 12.9 µg/kg with a range of <LOD in pasta to 130.7 µg/kg in cereals-based meals for children. Maximum concentration of Datura TAs ranged in the various product categories from <LOD in pasta to 4357.6 µg/kg in a sample of dry herbal tea.

One or more TAs were detected in 21.3% of the single component flours with the highest contamination incidence and levels in sorghum and millet samples.

Table 25: Results (concentrations are the sum of Datura TAs present in the sample) obtained for single flours, cereal-based products and other products for the analysis of tropane alkaloids

Food category	Samples collected and analysed	All TAs[(a)]			Atropine + scopolamine		
		% of samples > LOD[(b)]	Mean conc. (µg/kg)	Max conc. (µg/kg)	% of samples > LOD[(b)]	Mean conc. (µg/kg)	Max conc. (µg/kg)
Single component flours	**268**	21.3%	3.11	361.2	20.1%	2.87	334.8
Buckwheat	113	9.7%	2.69	258.1	9.7%	2.58	246.8
Millet & sorghum	102	23.5%	5.15	361.2	22.5%	4.67	334.8
Corn & others	53	20.8%	0.07	2.2	18.9%	0.03	0.8
Cereals available at retail stores	**838**	14.0%	0.30	111.8	12.5%	0.25	108.5
Bread and pasta	**195**	9.2%	0.06	4.2	7.7%	0.04	4.2
Bread	114	15.8%	0.10	4.2	13.2%	0.07	4.2
Pasta	81	0.0%	0.00	0.0	0.0%	0.00	0.0
Breakfast cereals	**219**	6.8%	0.63	111.8	5.9%	0.59	108.5
Biscuits and pastry	**164**	14.6%	0.14	12.0	13.4%	0.06	2.3
Biscuits	150	14.7%	0.14	12.0	13.3%	0.05	2.3
Pastry	14	14.3%	0.15	1.9	14.3%	0.15	1.9
Cereal-based foods for children	**260**	20.0%	9.49	859.5	14.2%	0.09	4.2
Breakfast cereals	135	13.3%	0.16	4.5	12.6%	0.13	4.2
Cookies	107	13.1%	0.85	86.2	11.2%	0.03	0.8
Pasta and cereal-based meals	18	55.6%	130.7	859.5	22.2%	0.20	1.5
Other products available at retail stores	**199**	52.3%	73.35	4357.6	39.7%	8.15	428.5
Dry (herbal) teas	**121**	70.2%	71.38	4357.6	63.6%	13.40	428.5
Legumes, stir-fry mixes, oil seeds	**78**	24.4%	76.42	2215.9	2.6%	0.00	0.2
Legumes, stir-fry mixes	65	26.2%	91.70	2215.9	0.0%	0.00	0.0
Oil seeds	13	15.4%	0.02	0.2	15.4%	0.02	0.2
Total	**1305**	22.5%	12.90	4357.6	18.5%	1.96	428.5

(a): Analysis of 24 TAs, including atropine and scopolamine.
(b): Products containing at least one TA above the LOD. LOD differs between components, matrices and laboratories.

A total of 14% of the category 'cereals available at retail stores' was contaminated with Datura TAs. Highest incidence was in the category of bread, 15.8%, and lowest for pasta (none of the samples positive). Highest mean and maximum concentrations were detected in breakfast cereals (0.63 µg/kg and 111.8 µg/kg, respectively).

TAs were detected in 20.0% of the cereal-based foods for young children age 6-36 months. The category pasta and cereal-based meals (18 samples) contributed most to the contamination, both in incidence, 55.6% of the samples positive, and in mean (130.7 µg/kg) and maximum (859.5 µg/kg) concentration. 13% of both breakfast cereals and cookies for children were contaminated with Datura-type TAs. Contamination with TAs of samples in the categories 'cookies for children' and 'biscuits and pastry' were comparable in incidence, 13.1% and 14.6% respectively. However, both mean and maximum concentrations were higher in the category 'cookies for children' (0.85 µg/kg and 86.2 µg/kg, respectively) than for the category 'biscuits and pastry' (0.14 µg/kg and 12.0 µg/kg, respectively).

Of the 121 samples dry (herbal) tea 70.2% was positive for one or more TA. TAs were detected in 26.2% of legumes and stir-fry mixes. Substantial amounts of TAs were occasionally detected in these product groups. The maximum TA concentration detected in herbal teas was 4357.6 µg/kg and for legumes and stir-fry mixes this was 2215.9 µg/kg.

Single component flours

In Figure 8 the relative occurrence of individual Datura TAs and their mean and maximum concentration in flours is shown. The individual sample results can be found in Appendix P. Atropine and scopolamine were the TAs most often found and in highest concentrations. One sample of buckwheat and one sample of millet exceeded 100 µg/kg for the sum of atropine and scopolamine, while 10 samples contained more than 10 µg/kg. Atropine and scopolamine together accounted for over 92% of the mean TA content of the single flours. However, also other Datura-type TAs (anisodamine, apoatropine, aposcopolamine, noratropine and norscopolamine) and several low molecular weight TAs (pseudotropine, scopine, scopoline, tropine, tropinone) were occasionally present.

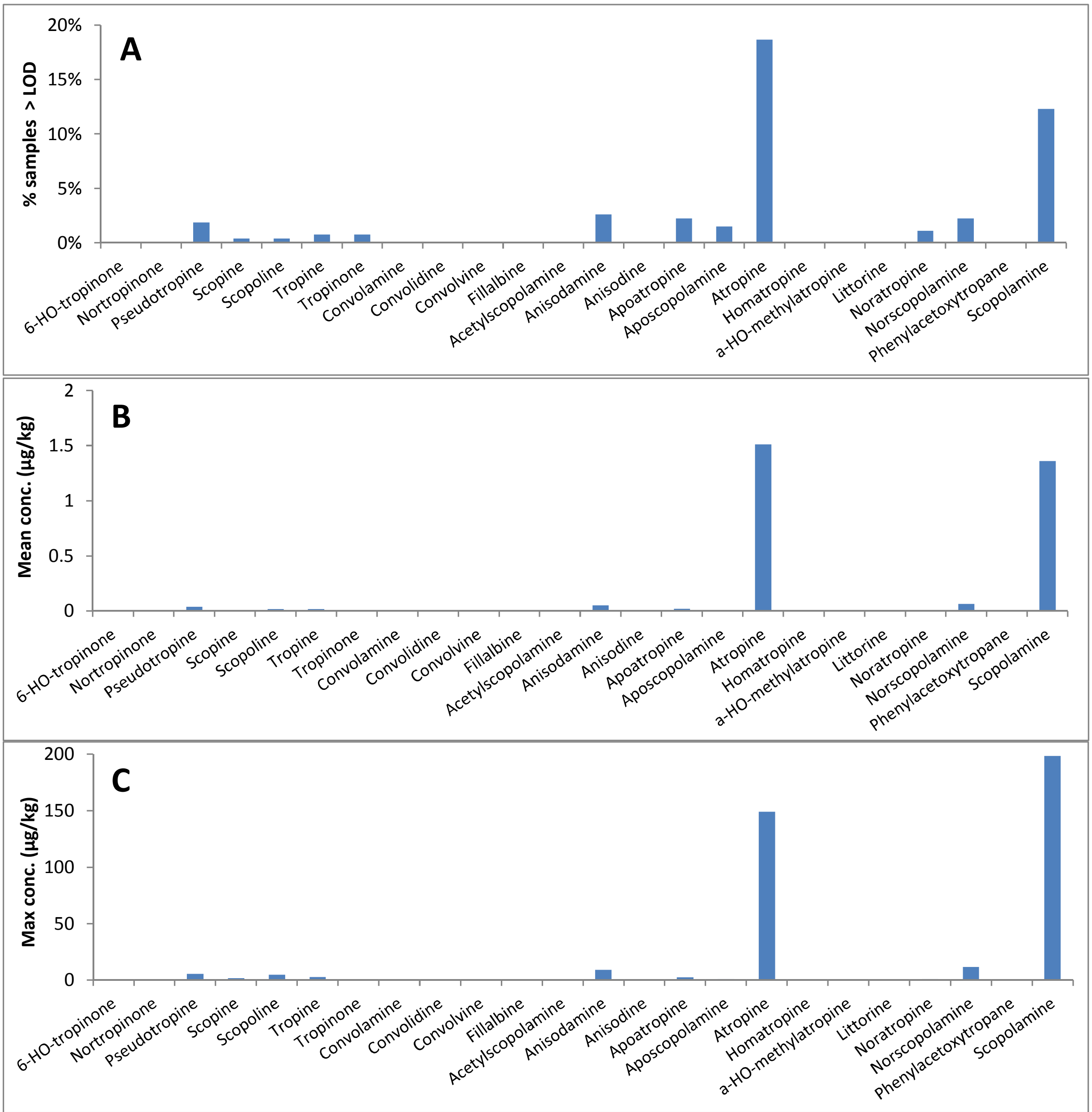


Figure 8: The percentage of 268 single component flours containing an individual Datura TA above the LOD (A), the mean concentration (B) and maximum concentration of the individual TAs in the analysed samples (C)

Cereal-based products

In Figure 9 the relative occurrence of individual Datura TAs and their mean and maximum concentration in the combined category of cereal-based products (bread, pasta, breakfast cereals, biscuits and pastry) is shown. The individual sample results can be found in Appendix Q (bread and pasta), R (breakfast cereals) and S (biscuits and pastry).

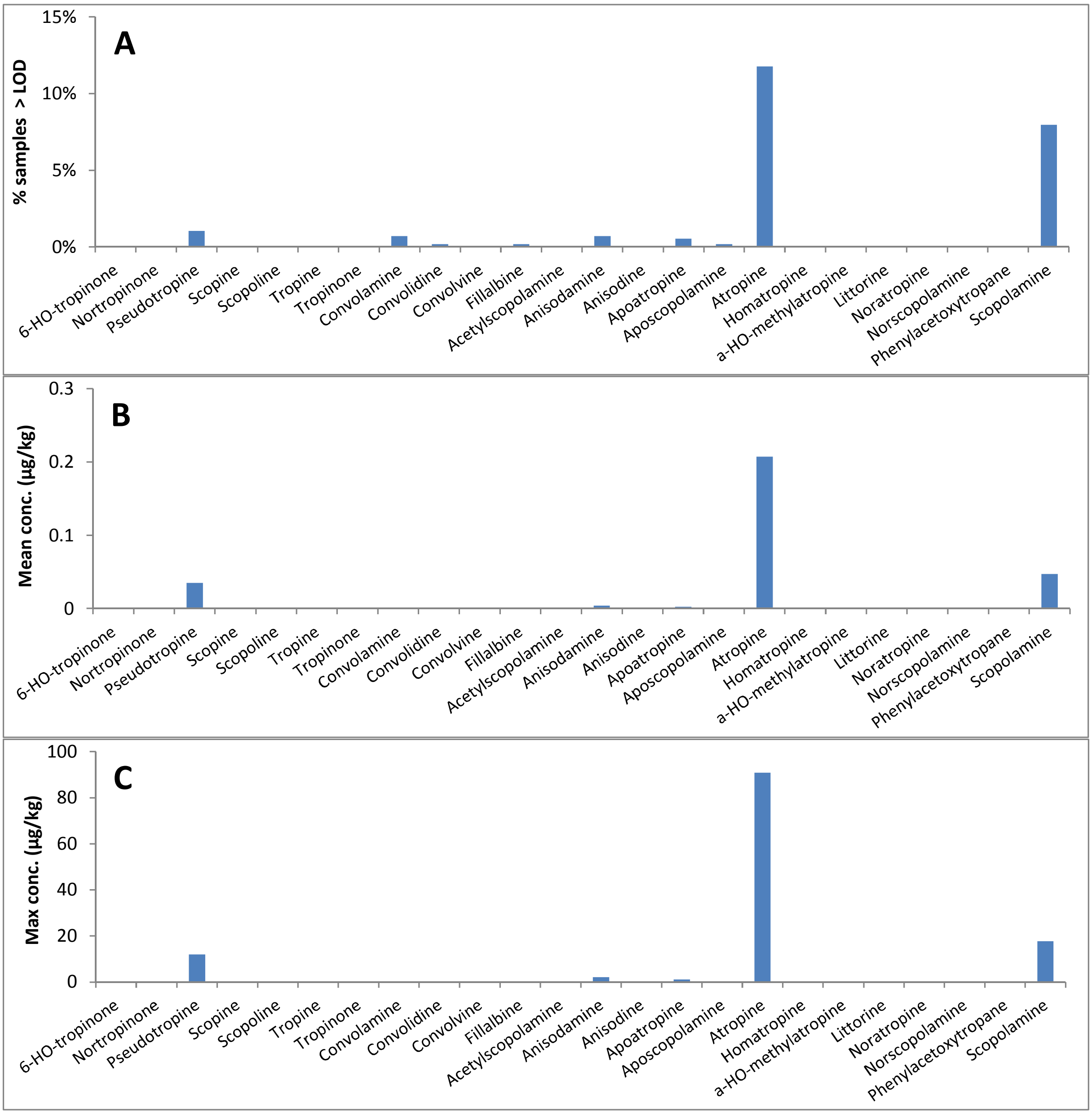


Figure 9: The percentage of 578 cereal-based products containing an individual Datura TA above the LOD (A), the mean concentration (B) and maximum concentration of the individual TAs in the analysed samples (C)

As for the single flours, atropine and scopolamine were the TAs most often found and in highest concentrations. One sample of breakfast cereals exceeded 100 µg/kg for the sum of atropine and scopolamine, while this was also the only sample containing more than 10 µg/kg, an indication that contamination with higher levels of TAs is rather rare for cereal-based products. Atropine and scopolamine accounted for 83% of the TA found in cereal-based products. Other Datura-type TAs were occasionally present in lower concentrations, such as anisodamine, apoatropine and aposcopolamine. Furthermore, pseudotropine and convolamine were occasionally present in cereal-based products.

Cereal-based foods for young children

In Figure 10 the relative occurrence of individual Datura TAs and their mean and maximum concentration in cereal-based foods for young children is shown. The individual sample results can be found in Appendix T (cereals for children), U (biscuits for children) and V (ready-to-eat meals for children).

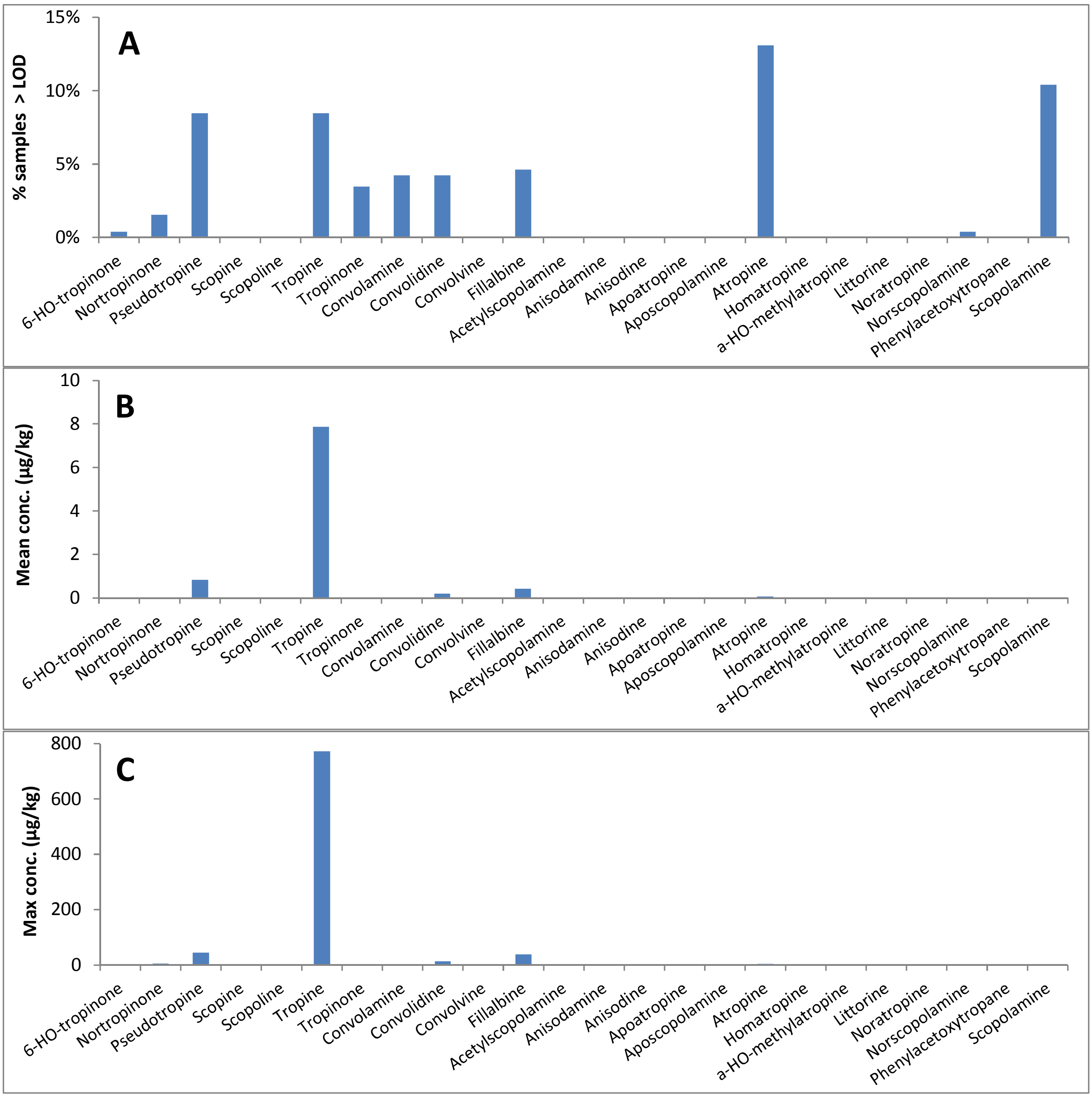


Figure 10: The percentage of 260 cereal-based foods for young children containing an individual Datura TA above the LOD (A), the mean concentration (B) and maximum concentration of the individual Datura TAs in the analysed samples (C)

The pattern of Datura TAs present in 'cereal-based foods for young children' differs from those in the 'single compound flours' and the 'cereal-based products'. Remarkable is that although the incidence of atropine and scopolamine is comparable to that in cereal-based products, the mean concentration is low. Eight samples contained more than 1 µg/kg for the sum of atropine and scopolamine (Table 26). Commission Regulation (EU) 2016/239 of 19 February 2016 has set a maximum level (ML) for the presence of tropane alkaloids in certain cereal-based foods for infants and young children (EU, 2016). The regulation specifically mentions processed cereal-based foods and baby foods for infants and young children, containing millet, sorghum, buckwheat or their derived products. The ML is set at 1.0 µg/kg for atropine and 1.0 µg/kg for scopolamine. An ML for the sum of atropine and scopolamine is not given. Of the 8 samples of cereal-based foods for young children, 6 exceeded for either atropine or scopolamine the ML of 1 µg/kg (Table 26). The 3 samples that contained the highest amounts of atropine and/or scopolamine were all collected in 2015, well before the starting date of regulation EU 2016/239. The 3 other samples were collected in March 2016, just after the starting date of the regulation. Although these samples exceeded the ML for atropine, the sum of atropine and scopolamine was well below 2 µg/kg.

Table 26: Cereal-based foods for young children containing more than 1 µg/kg for the sum of atropine and scopolamine

Sample code	Description	Atropine (µg/kg)	Scopolamine (µg/kg)	Sum (µg/kg)	Remarks
RIK BCC9	Cereals for children	0.19	1.86	2.05	Sampled in 2015
IRTA_TA_0952	Cereals for children	1.82	0.64	2.46	Sampled in 2015
IRTA_TA_0964	Cereals for children	0.47	0.56	1.03	Atropine and scopolamine individually below 1 µg/kg
VV 866/15	Cereals for children	0.90	0.18	1.08	Atropine and scopolamine individually below 1 µg/kg
S15-100580	Cereals for children	3.73	0.51	4.24	Sampled in 2015
S16-003166	Cereals for children	1.28	0.17	1.45	Atropine above 1 µg/kg, sum below 2 µg/kg
S15-100702	Ready-to-eat meal for children	1.14	0.26	1.40	Atropine above 1 µg/kg, sum below 2 µg/kg
S16-003181	Ready-to-eat meal for children	1.25	0.26	1.51	Atropine above 1 µg/kg, sum below 2 µg/kg

Besides atropine and scopolamine, other Datura TAs were found in the cereal products for children as well, and in higher amounts (Figure 10). The low molecular weight TAs tropine and pseudotropine were the most prominent TAs, but also the Convolvulaceae-type TAs convolamine, convolidine and fillalbine were detected. The presence of these TAs was largely confined to the ready-to-eat meals for children. These products contained besides cereal-based ingredients also a variety of vegetable ingredients. As will be discussed below for the vegetable products including stir-fry mixes, these low molecular weight and Convolvulaceae-type TAs can be associated with vegetable components in the meals, in particular with bell peppers.

Dry (herbal) tea and tea infusions

In Figure 11 the relative occurrence of individual Datura TAs and their mean and maximum concentration in dry (herbal) teas is shown. The individual sample results can be found in Appendix W.

Atropine (60%) and scopolamine (53%) were the Datura-type TAs most often found in dry (herbal) teas. A number of other Datura TAs were present, but with lower incidence rates of 1-20%: the low molecular weight TAs pseudotropine, tropine and tropinone; the Convolvulaceae-type TAs convolamine, convolidine, convolvine and fillalbine and the Datura-type TAs anisodamine, apoatropine, aposcopolamine, noratropine and norscopolamine.

Convolvine is the TA contributing (72%) most to the mean total concentration in herbal teas. Atropine, scopolamine and tropine contribute a further 13.5% to the mean total concentration. Together these four TAs accounted for 95% of the total TA content in tea. In five samples convolvine was present in a concentration exceeding 100 µg/kg, with one sample containing 4074 µg/kg. Four different samples contained 100 µg/kg or more for the sum of atropine and scopolamine. The fact that TAs of three different types (low molecular weight, Convolvulaceae-type and Datura-type) substantially contribute can be seen as an indication that several sources of TA-containing plants may be relevant regarding contamination of herbal teas.

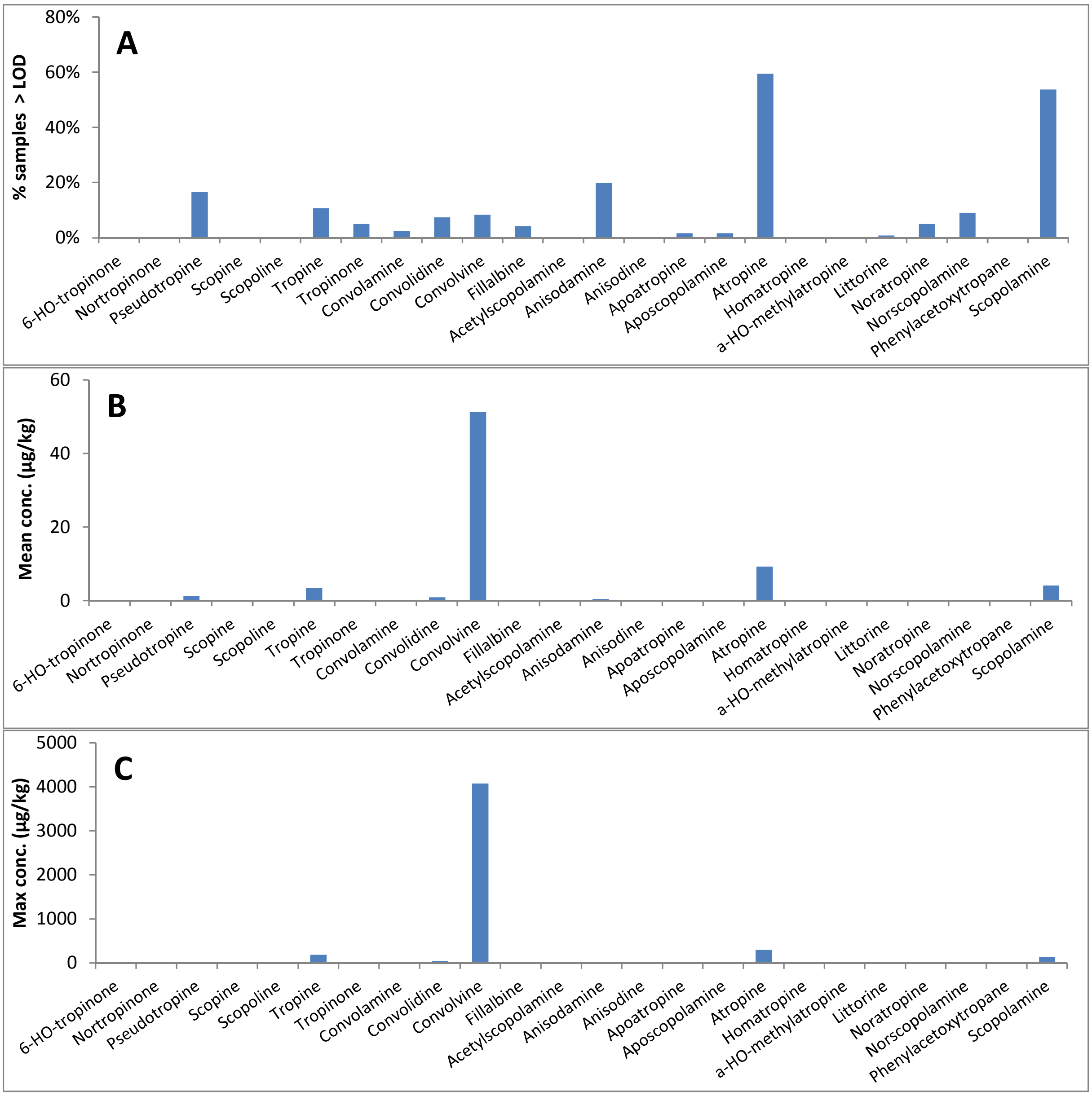


Figure 11: The percentage of 121 dry (herbal) teas containing an individual TA above the LOD (A), the mean concentration (B) and the maximum concentration of individual TAs in the analysed dry (herbal) teas (C)

The efficiency of the transfer of TAs from herbal tea to the tea infusion was studied by using a selection of contaminated dry herbal teas. Twenty herbal teas were selected that contained the highest TA content and in which at least one TA was present at 10 µg/kg. Tea infusions were prepared using a standardised protocol (ISO 3103, ISO 1980) (Section 3.7.4). The results obtained for the tea infusions were compared with those obtained for the corresponding dry teas, to calculate the extraction efficiency of the boiling water. A relatively wide variation was observed for the obtained results (Figure 12). Nevertheless it can be concluded that in general the extraction from the dry tea is less than 100% (or more precise: less than 100% compared to the extraction procedure used for dry tea). On average a transfer efficiency of 47% was obtained for the 20 teas included in the study. Atropine and scopolamine were present in almost all of these samples and for these two compounds the transfer efficiency was 54% and 42%, respectively. The average transfer of convolvine was only 23%. During validation of the method (Section 3.7.5) a somewhat higher average (70%) extraction efficiency was derived for the TAs, including atropine (71%), scopolamine (84%) and convolvine (49%). It should be noted that validation was performed with tea samples that were spiked with TA standards to the dry material before tea preparation. With real samples, boiling water may not be as effective to extract TAs from within the incurred material itself as to extract TAs spiked to the exterior of the leaves.

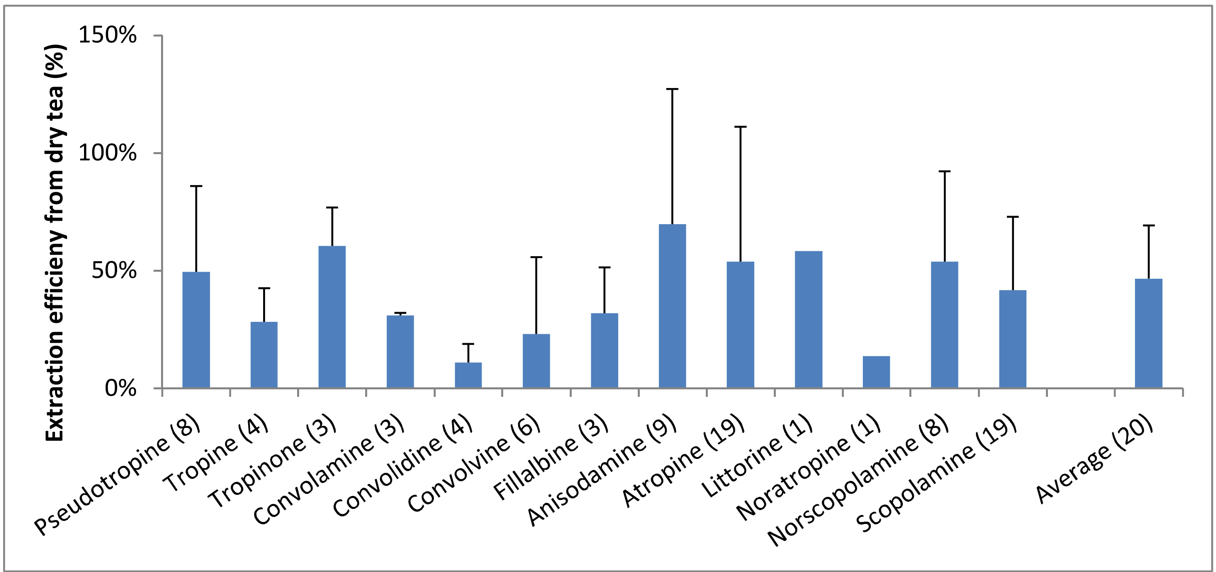


Figure 12: The extraction efficiency of TAs from dry tea when preparing tea infusion. Twenty dry herbal teas contaminated with different composition and levels of TAs were processed according to a standardised protocol (ISO 3103, 1980). For each TA the number of results is given and the error bars represent the relative standard deviation in the results

Legumes, stir-fry mixes and oilseeds

In Figure 13 the relative occurrence of individual Datura TAs and their mean and maximum concentration legumes, stir-fry mixes and oilseeds is shown. The individual sample results can be found in Appendix X.

The incidence of the low molecular weight TAs 6-hydroxytropinone, nortropinone, pseudotropine, tropine and tropinone as well as the Convolvulaceae-type TAs convolamine, convolidine, convolvine and fillalbine was high in this food category. The positive results were, however, all related to the stir-fry mixes and the bell peppers, while the legumes (green beans, peas, linseed) and oilseeds were essentially free of TAs.

Tropine was the TA present at the highest mean as well as maximum concentration. Also pseudotropine was present at significant levels and together these two TAs accounted for approx. 95% of the total TA content of this food group. Atropine and scopolamine were only found at trace amounts in a small number of samples, perhaps an indication that the risk of contamination is relatively low. The TA pattern in the stir-fry mixes was very similar to that in the bell peppers as well as to that of the ready-to-eat meals for children as described above. There is a strong correlation between the presence of bell pepper in the vegetable mixes and the ready-to-eat meals for children and the presence of the low molecular weight and Convolvulaceae-type TAs. Nevertheless, it cannot be excluded that other common vegetable ingredients, such as tomato, broccoli, cauliflower or cabbage, that potentially could produce TAs, contribute to the TA content of these food products. The levels detected in the 6 bell pepper samples as such were not high enough to explain the levels found in these composite food products.

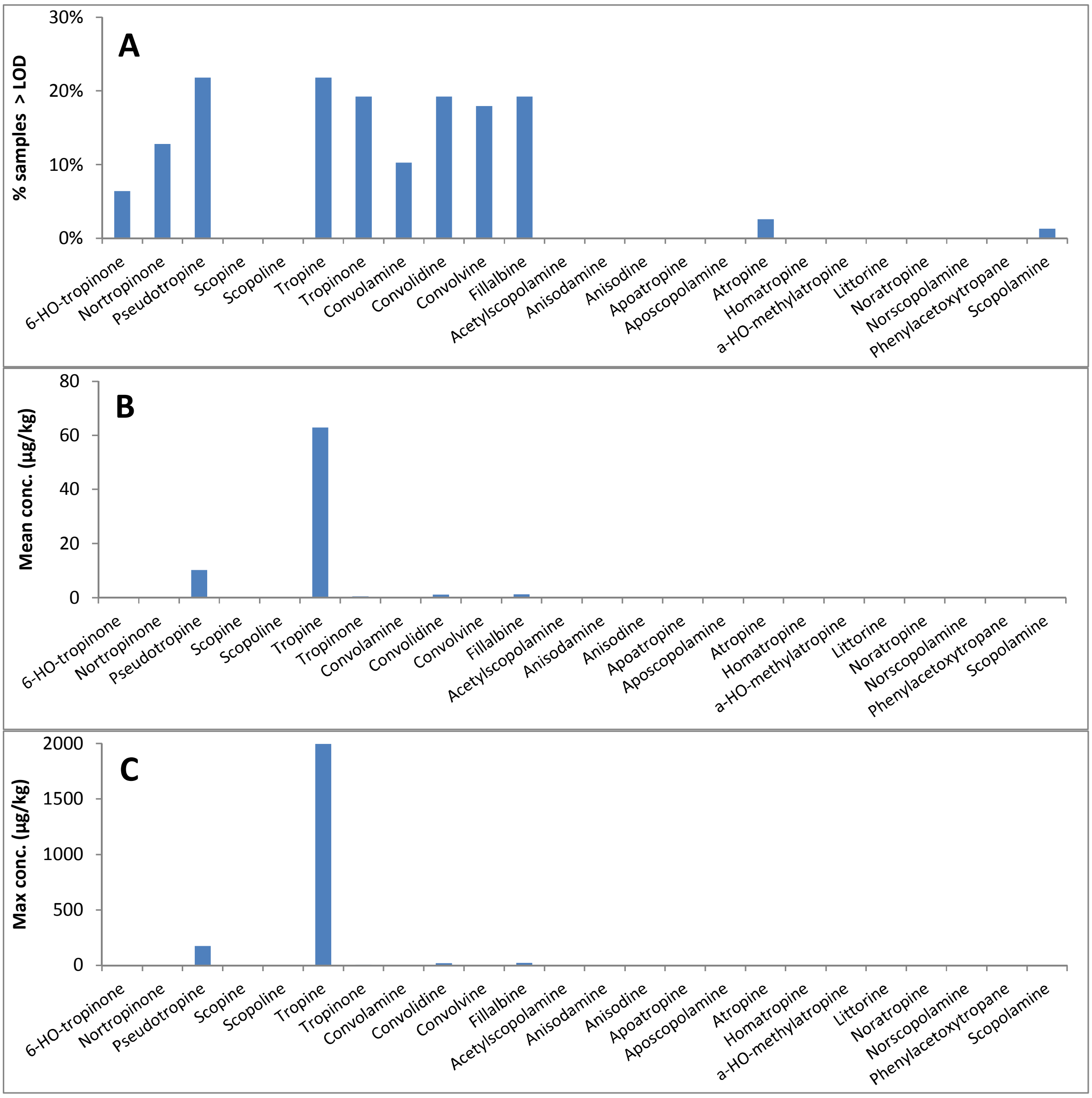


Figure 13: The percentage of 78 products of legumes, stir-fry mixes and oilseeds containing an individual TA above the LOD (A), the mean concentration (B) and the maximum concentration of the individual TAs in the analysed samples (C)

3.12. Occurrence of calystegines in food plants from the Solanaceae family available at retail stores

The results obtained for the samples of the Solanaceae family are shown in Table 27. A total of 404 samples was collected and analysed for six calystegines. Of all samples 98.3% contained calystegines above the LOD. Calystegines were detected in all potato samples, in 96.7% of the aubergine samples and in 33% of the bell pepper samples.

The mean concentration of calystegines in the fresh potato samples was 164.0 mg/kg with a maximum of 507.3 mg/kg. The mean and maximum concentrations of the samples of processed potatoes were somewhat lower at 95.6 mg/kg and 207.7 mg/kg, respectively.

The mean calystegine concentration in the aubergine samples was 21.1 mg/kg with a maximum of 181.5 mg/kg.

Calystegines in the 6 bell pepper samples were present only at very low levels, at a mean concentration of 0.2 mg/kg and a maximum of 0.5 mg/kg.

Table 27: Results obtained for potato, aubergine and bell pepper samples for the analysis of calystegines

Food category	Samples collected and analysed	% Of samples > LOD	Mean conc. (mg/kg)	Max conc. (mg/kg)
Potatoes	**308**	**100.0%**	**161.6**	**507.3**
Potatoes, fresh	297	100.0%	164.0	507.3
Potatoes, processed	11	100.0%	95.6	207.7
Aubergine & bell pepper	**96**	**92.7%**	**19.8**	**181.5**
Aubergine	90	96.7%	21.1	181.5
Bell pepper	6	33.3%	0.2	0.5
Total Solanaceae food plants	**404**	**98.3%**	**127.9**	**507.3**

Potatoes

In Figure 14 the relative occurrence of individual calystegines and their mean and maximum concentration in potatoes is shown. The results for individual samples are shown in Appenxdix Y. Calystegine A_3, calystegine B_2 and calystegine B_4 were the calystegines predominantly detected. Calystegine A_5 and calystegine B_3 were occasionally present. Calystegine A_3 was detected at the highest mean and maximum concentration, followed by calystegine B_2 and calystegine B_4.

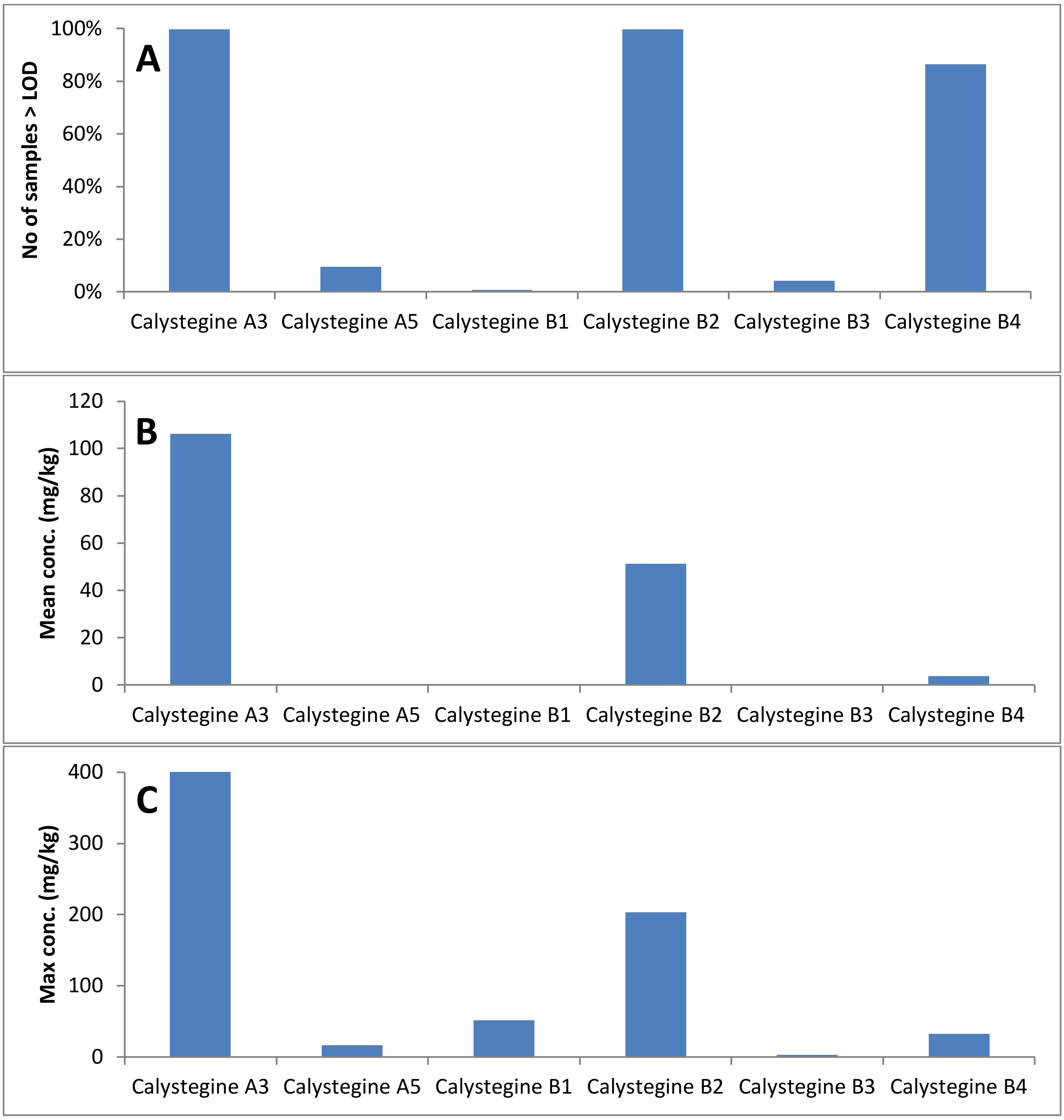


Figure 14: The percentage of 308 products of potatoes containing an individual calystegine above the LOD (A), the mean concentration (B) and maximum concentration of the individual calystegines in the analysed samples (C)

Two samples of sweet potato (*Ipomoea batatas*) were analysed as well (Appendix Y). These samples displayed a different calystegine pattern from that of regular potato (*Solanum tuberosum*) samples. Calystegines B_1 and B_2 were the major calystegines in sweet potato, while calystegines A_3, A_5 and B_4 were present at much lower levels.

Aubergine and bell pepper

In Figure 15 the relative occurrence of individual calystegines and their mean and maximum concentration in aubergine is shown. The results for individual samples are shown in Appendix Z.

Calystegine A_3, calystegine B_1 and calystegine B_2 were detected most frequently, while calystegine A_5 was present at low incidence. Calystegine B_2 was detected at the highest mean concentration in aubergine. The mean concentration of calystegine B_1 and calystegine A_3 was lower.

The pattern of calystegines in aubergine clearly differs from those present in potato as well as in sweet potato. Furthermore, the mean concentrations present in aubergine are substantially lower than in potato, although occasionally aubergine may contain concentrations similar to that in potato.

Bell peppers did contain only traces of calystegine B_1 ($\leq$ 0.5 mg/kg) and are not included in Figure 15.

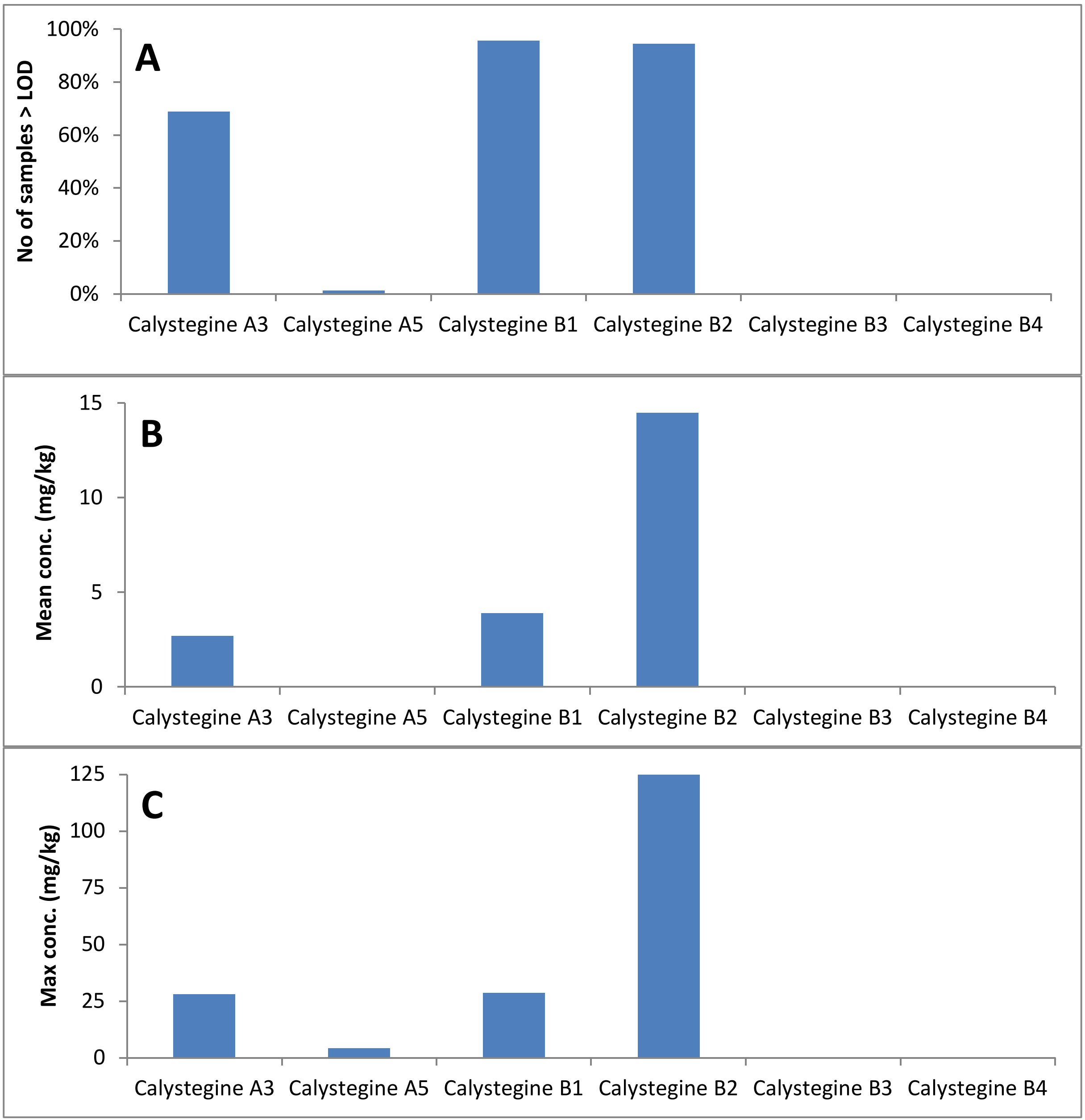


Figure 15: The percentage of 90 samples of aubergines containing an individual calystegine above the LOD (A), the mean concentration (B) and maximum concentration of the individual calystegines in the analysed aubergine samples (C)

4. Conclusions

- From the literature study it was recommended for the Datura tropane alkaloids (TAs) to focus on: i) processed cereal foods intended for babies, infants and toddlers; ii) processed and unprocessed cereal foods for adults; iii) processed foods and flour based on buckwheat and millet, iv) canned and frozen vegetables with a high proportion of green beans and v) dry (herbal) teas.
- The aim of this study to assess the occurrence of TAs in single component flours, cereal-based foods for young children age 6-36 months (cereal-based foods, breakfast cereals, biscuits, and other cereal-based foods), cereal-based foods (bread, pasta, breakfast cereals, biscuits, cake and pastry), dry (herbal) teas, frozen beans and stir-fry mixes, potatoes and aubergines across different regions in Europe, has been successfully achieved.
- One analytical method, based on multi-analyte LC-MS/MS, has been satisfactory validated to detect and accurately quantify 24 TAs in cereal-based food samples, dry (herbal) teas and frozen beans and stir-fry mixes. A second analytical method based on multi-analyte LC-MS/MS has been satisfactory validated to detect and accurately quantify six calystegines in potato and aubergine samples.
- Quality control data showed adequate performance of the analytical methods for single component flours, cereal-based foods, and to a lesser extent, herbal teas, as well as sufficient stability of the QC samples.
- A total of 1709 samples have been collected from retail stores in nine different European countries. A total of 1305 samples (268 single component flours, 260 cereal-based foods for young children age 6-36 months, 219 breakfast cereals, 164 biscuits and pastry, 114 bread, 81 pasta, 121 dry (herbal) teas and 78 legumes and stir-fry mixes) have been analysed for the presence of 24 Datura TAs and a total of 404 samples (308 potato and potato products, 90 aubergines and six bell peppers) have been analysed for six calystegines.
- One or more Datura TAs were detected in: 21.3% of the single component flours; 20.0% of the cereal-based foods for young children age 6-36 months; 6.8% of the breakfast cereals; 14.6% of biscuits and pastry; in 15.8% of the bread; in none of the pasta; in 70.2% of dry (herbal) teas; 26.2% of legumes and stir-fry mixes; one or more calystegines were detected in 100% of the potatoes and 92.7% of aubergines.
- The highest mean Datura TA concentration in a specific food category, 130.7 µg/kg, was recorded for cereals-based meals for children, the maximum Datura TA concentration, 4357.6 µg/kg, was detected in a dry herbal tea sample. The highest mean concentration in a specific food category for the sum of atropine and scopolamine, 13.4 µg/kg, was recorded for herbal teas. The maximum concentration for the sum of atropine and scopolamine, 428.5 µg/kg, was detected in a sample of dry herbal tea.
- Of the 24 Datura TAs included in the analytical method, 19 were detected in one or more samples. Atropine was detected most often above the LOD (226 times), followed by scopolamine (172), pseudotropine (70) and tropine (54). TAs that were not at all detected included acetylscopolamine, anisodine, homatropine, a-hydroxymethylatropine and phenylacetoxytropane, while littorine, scopine and scopoline were reported only once.
- The typical patterns of TAs present differed between the food categories. In single flours and cereal-based products that did not contain vegetables, the Datura-type TAs atropine and scopolamine were the major components. Mixed vegetable products and ready-to-eat meals for children containing vegetables as ingredients often contained the low molecular weight TAs tropine and pseudotropine as the major TAs. The herbal teas contained TAs from all types, most notably atropine, scopolamine, convolvine, tropine and pseudotropine.

- Overall, around 50% of TAs present in dry tea were transferred to the tea infusion when a standard protocol for tea preparation was applied. There was however substantial variability in the transfer efficiency for individual teas and TAs. For atropine and scopolamine the transfer rate was approximately 50% as well.
- Mean content of calystegines in potatoes was 161.6 mg/kg fresh weight, the maximum concentration detected was 507.3 mg/kg. The mean content in aubergine was 21.1 mg/kg fresh weight, the maximum concentration detected was 181.5 mg/kg. In the limited number of bell peppers analysed only trace amounts of calystegines were detected (≤0.5 mg/kg).
- Between potato, sweet potato, aubergine and bell pepper the patterns and concentrations of calystegines were quite distinct. Calystegine A_3 was the most important TA in potatoes, followed by calystegine B_2. In aubergine calystegine B_2 was the predominant TA, followed by calystegine B_1 and A_3. Calystegines B_1 and B_2 are likely the major TAs present in sweet potato, while for bell peppers this may be calystegine B_1.

5. Recommendations

- The literature review has highlighted the emerging potential for food contamination from non-food weed plants that are encroaching on field crops in Europe. *Convolvulus* species (bindweeds) present a particular problem that should be addressed in future projects.
- Future work should be extended to the occurrence of calystegines and Datura TAs in other food products that are consumed in high amounts such as *Capsicum annuum* (bell pepper), *Solanum esculentum* (tomato), *Brassica napa* (turnip) and *B. oleracea* (broccoli, cauliflower, Brussels sprouts).
- It is advised to sample food crops from several harvest years and to include food products from as many different sources as possible, including food sourced from outside the EU to assess yearly variation in food crops and regional differences.
- It is recommended to also analyse emerging 'superfoods' that do contain inherent TAs, e.g. *B. oleracea* and goji berries.
- Availability of certified analytical standards and reference materials should be improved and in particular the availability of isotopically labelled internal standards would greatly assist in a reliable quantification of TAs in the relevant matrices.
- More information is needed on the toxicity of calystegines in order to interpret the relevance of the occurrence data.
- More information is needed on the occurrence of TAs in dry (herbal) teas and the transfer to tea infusions, specifically for children. The sources and routes of contamination are still largely unknown.

References

Adams M, Wiedenmann M, Tittel G and Bauer R, 2006. HPLC-MS trace analysis of atropine in *Lycium barbarum* berries. Phytochemical Analysis, 17, 279-283.

Adamse P, van Egmond HP, Noordam MY, Mulder PPJ and de Nijs M, 2014. Tropane alkaloids in food: Poisoning incidents. Quality Assurance and Safety of Crops & Foods, 6, 15-24.

AFSSA, 2008. Avis de l'Agence française de securité sanitaire des aliments relative à la presence d'alcaloïdes (atropine et scopolamine) en tant que substances indésirables dans la farine de sarrasin destinée à la consommation humaine et à la pertinence du seuil de gestion provisoire proposé par la DGCCRF. Saisine n° 2008-SA-0221. 11 pp. https://www.anses.fr/sites/default/files/documents/RCCP2008sa0221.pdf.

AgroAtlas_Project, 2009. Interactive agricultural Ecological atlas of Russia and neighbouring countries. Ecomin plants and their diseases, pests and weeds. http://www.agroatlas.ru/en/content/weeds/.

Ali-Melkkilä T, Kanto J and Lisalo E, 1993. Pharmacokinetics and related pharmacodynamics of anticholinergic drugs. Acta Anaesthesiologica Scandinavica, 37, 633-642.

Andersson HC, 2002. Calystegine alkaloids in solanaceaous food plants. ThemaNord, 315, 1-55.

Arcuri PA and Gautieri RF, 1973. Morphine-induced fetal malformations. 3. Possible mechanisms of action. Journal of pharmaceutical sciences, 62, 1626-1634.

Arraez-Roman D, Zurek G, Bassmann C, Segura-Carretero A and Fernandez-Gutierrez A, 2008. Characterization of *Atropa belladonna* L. compounds by Capillary Electrophoresis-Electrospray Ionization-Time of Flight-Mass Spectrometry and Capillary Electrophoresiselectrospray Ionization-Ion Trap-Mass Spectrometry. Electrophoresis, 29, 2112-2116.

Asano N, 2000. Water soluble nortropane alkaloids in crude drugs, edible fruits and vegetables: biological activities and therapeutic applications. Mechanisms of Ageing and Development, 116, 155-156.

Asano N, Kato A, Kizu H, Matsui K, Watson AA and Nash RJ, 1996. Calystegine B_4, a novel trehalase inhibitor from *Scopolia japonica*. Carbohydrate Research, 293, 195-204.

Asano N, Kato A, Matsui K, Watson AA, Nash RJ, Molyneux RJ, Hackett L, Topping J and Winchester B, 1997a. The effects of calystegines isolated from edible fruits and vegetables on mammalian liver glycosidases. Glycobiology, 7, 1085-1088.

Asano N, Kato A, Miyauchi M, Kizu H, Tomimori T, Matsui K, Nash RJ and Molyneux RJ, 1997b. Specific alpha-galactosidase inhibitors, N-methylcalystegines - Structure/activity relationships of calystegines from *Lycium chinense*. European Journal of Biochemistry, 248, 296-303.

Asano N, Kato A, Oseki K, Kizu H and Matsui K, 1995. Calystegins of *Physalis alkekengi* var f*rancheti* (Solanaceae) - Structure determination and their glycosidase inhibitory activities. European Journal of Biochemistry, 229, 369-376.

Asano N, Oseki K, Tomioka E, Kizu H and Matsui K, 1994. N-Containing sugars from *Morus alba* and their glycosidase inhibitory activities. Carbohydrate Research, 259, 243-255.

Asano N, Yamashita T, Yasuda K, Ikeda K, Kizu H, Kameda Y, Kato A, Nash RJ, Lee HS and Ryu KS, 2001a. Polyhydroxylated alkaloids isolated from mulberry trees (*Morus alba* L.) and silkworms (*Bombyx mori* L.). Journal of Agricultural and Food Chemistry, 49, 4208-4213.

Asano N, Yokoyama K, Sakurai M, Ikeda K, Kizu H, Kato A, Arisawa M, Hoke D, Dräger B, Watson AA and Nash RJ, 2001b. Dihydroxynortropane alkaloids from calystegine-producing plants. Phytochemistry, 57, 721-726.

Ashtiania F and Sefidkonb F, 2011. Tropane alkaloids of *Atropa belladonna* L. and *Atropa acuminata* Royle ex Miers plants. Journal of Medicinal Plants Research, 5, 6515-6522.

Austin DF, 2000. Bindweed (*Convolvulus arvensis,* Convolvulaceae) in North America - From medicine to menace. Journal of the Torrey Botanical Society, 127, 172-177.

Azemi ME, Mosaddegh M, Cheraghali AM, Namjooyan F and Dräger B, 2006. Isolation and identification of calystegines in root cultures of four *Physalis* species. Iranian Journal of Pharmaceutical Research, 1, 69-72.

Bajpai S, BhaskaraRao AV, Muthukumaran M and Nagalakshmamma K, 2012. History and active pharmacokinetic principles of mulberry: a review. OSR Journal of Pharmacy, 3, 13-16.

Basey K, Mcgaw BA and Woolley JG, 1992. Phygrine, an alkaloid from *Physalis* species. Phytochemistry, 31, 4173-4176.

Bekkouche K, Daali Y, Cherkaoui S, Veuthey JL and Christen P, 2001. Calystegine distribution in some solanaceous species. Phytochemistry, 58, 455-462.

Beresford PJ and Woolley JG, 1974. Biosynthesis of tigloidine in *Physalis peruviana*. Phytochemistry, 13, 2143-2144.

Berkov S, Zayed R and Doncheva T, 2006. Alkaloid patterns in some varieties of *Datura stramonium*. Fitoterapia, 77, 179-182.

BfR, 2006. Poisoning reported by physicians. BfR information sheet http://www.bfr.bund.de/cm/364/cases_of_poisoning_reported_by_physicians_2006.pdf.

Binning K, 1993. How Toxic is Eastern Black Nightshade? http://fyi.uwex.edu/weedsci/1993/10/04/1187/.

Birnbaum C, 2006. NOBANIS – Invasive alien species fact sheet – *Bunias orientalis* – From: Online Database of the European Network on Invasive Alien Species – NOBANIS www.nobanis.org.

Boumba VA, Mitselou A and Vougiouklakis T, 2004. Fatal poisoning from ingestion of *Datura stramonium* seeds. Veterinary and Human Toxicology, 46, 81-82.

Bovell-Benjamin AC, 2007. Sweet potato: A review of its past, present, and future role in human nutrition. Advances in Food Nutrition Research, 52, 1-59.

Boyd JW, Murray DS and Tyrl RJ, 1984. Silverleaf nightshade, *Solanum elaeagnifolium*, origin, distribution, and relation to man. Economic Botany, 38, 210-217.

Brock A, Herzfeld T, Paschke R, Koch M and Dräger B, 2006. Brassicaceae contain nortropane alkaloids a. Phytochemistry, 67, 2050-2057.

Brunton LL, Chabner BA and Knollman BC, 2011. Goodman and Gilman's The Pharmacological Basis of Therapeutics, Twelfth Edition 12th McGraw Hill Medical, p. 2084.

Caligiani A, Palla G, Bonzanini F, Bianchi A and Bruni R, 2011. A validated GC-MS method for the detection of tropane alkaloids in buckwheat (*Fagopyron esculentum* L.) fruits, flours and commercial foods. Food Chemistry, 127, 204-209.

Chen HX, Chen Y, Du P, Han FM, Wang H and Zhang HS, 2006a. Sensitive and specific Liquid Chromatographic-Tandem Mass Spectrometric assay for atropine and its eleven metabolites in rat urine. Journal of Pharmaceutical and Biomedical Analysis, 40, 142-150.

Chen HX, Du P, Han FM and Chen Y, 2006b. Analysis of anisodine and its metabolites in homogenized liver of rat by liquid chromatography-tandem mass spectrometry. Chinese Journal of Analytical Chemistry, 34, 1729-1732.

Chen HX, Du P, Han FM and Chen Y, 2006c. [Analysis of anisodine and its metabolites in rat plasma by liquid chromatography-tandem mass spectrometry]. Yao Xue Xue Bao, 41, 518-521.

Chen HX, Wang H, Yong Y and Zhang HS, 2005a. Liquid Chromatography-Tandem Mass Spectrometry analysis of anisodamine and its phase I and II metabolites in rat urine. Journal of Chromatography B, 824, 21-29.

Chen Y, Chen HX, Du P, Han FM and Zhang HS, 2005b. Analysis of anisodine and identification of twenty of its metabolites in rat urine by Liquid Chromatography-Tandem Mass Spectrometry. Chromatographia, 62, 563-569.

Crotser MP and Witt WW, 2000. Effect of *Glycine max* canopy characteristics, *G. max* interference, and weed-free period on *Solanum ptycanthum* growth. Weed Science, 48, 20-26.

DAISIE, DAISIE http://www.europe-aliens.org/speciesFactsheet.do?speciesId=15406#.

Das M and Barua N, 2013. Pharmacological activities of *Solanum melongena* Linn. (Brinjal plant). International Journal of Green Pharmacy, 74, 274-277.

De Simone R, Margarucci L and De Feo V, 2008. Tropane alkaloids: An overview. Pharmacologyonline, 1, 70-89.

Dhar AK and Bhat BK, 1982. Ontogenic variability in alkaloid synthesis and other morphological characters in 5 genotypes of *Belladonna*. Journal of Natural Products, 45, 525-531.

Doncheva T, Berkov S and Philipov S, 2006. Comparative study of the alkaloids in tribe Datureae and their chemosystematic significance. Biochemical Systematics and Ecology, 34, 478-488.

Dräger B, 1995. Identification and quantification of calystegines, polyhydroxyl nortropane alkaloids. Phytochemical Analysis, 6, 31-37.

Dräger B, 2004. Chemistry and biology of calystegines. Natural Product Reports, 21, 211-223.

Dräger B, Funck C, Hohler A, Mrachatz G, Nahrstedt A, Portsteffen A, Schaal A and Schmidt R, 1994. Calystegines as a new group of tropane alkaloids in Solanaceae. Plant Cell Tissue and Organ Culture, 38, 235-240.

Dräger B, van Almsick A and Mrachatz G, 1995. Distribution of calystegines in several Solanaceae. Planta Medica, 61, 577-579.

EFSA, How to submit data to EFSA data collection. http://www.efsa.europa.eu/en/datex/datexsubmitdata.htm.

EFSA, 2013. Scientific Opinion on tropane alkaloids in food and feed. EFSA Panel on Contaminants in the Food Chain. EFSA Journal, 11(10): 3386, p. 113.

EFSA, 2015. The EFSA Comprehensive European Food Consumption Database. http://www.efsa.europa.eu/en/food-consumption/comprehensive-database Accessed 04.05.16.

Eich E, 2008. Solanaceae and Convolvulaceae: Secondary Metabolites - Biosynthesis, Chemotaxonomy, Biological and Economic Significance (A Handbook). Springer-Verlag Berlin Heidelberg.

El-Gengaihi SE, Hassan EE, Hamed MA, Zahran HG and Mohammed MA, 2013. Chemical composition and biological evaluation of *Physalis peruviana* root as hepato-renal protective agent. Journal of Dietary Supplements, 10, 39-53.

El Bazaoui A, Bellimam MA and Soulaymani A, 2011. Nine new tropane alkaloids from *Datura stramonium* L. identified by GC/MS. Fitoterapia, 82, 193-197.

EMEA, 1997. Committee for Veterinary Medicinal Products. Butylscopolaminium bromide. Summary Report. http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500011136.pdf.

EMEA, 1998. *Atropa belladonna* summary report. Committee for Veterinary medicinal products. EMEA/MRL/540/98-Final. http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500010748.pdf

EMPPO, 2006. Pest risk analysis for *Solanum elaeagnifolium* (Cav.) http://www.eppo.int/QUARANTINE/Pest_Risk_Analysis/PRAdocs_plants/06-12702_PRA_Solanum_elaeagnifolium_final.doc

EU, 2006. Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of the European Communities, L70, 12-34.

EU, 2014. Commission Regulation (EU) No 519/2014 of 16 May 2014 amending Regulation (EC) No 401/2006 as regards methods of sampling of large lots, spices and food supplements, performance criteria for T-2, HT-2 toxin and citrinin and screening methods of analysis. Official Journal of the European Communities, L147, 29-43.

EU, 2015. RASFF – the Rapid Alert System for Food and Feed. https://webgate.ec.europa.eu/rasff-window/portal/?event=SearchForm&cleanSearch=1.

EU, 2016. Commission Regulation (EU) 2016/239 amending Regulation (EC) No 1881/2006 as regards maximum levels of tropane alkaloids in certain cereal-based foods for infant and young children. Official Journal of the European Union, L45, 3-5.

EU_SANTE, 2015. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. http://ec.europa.eu/food/plant/docs/plant_pesticides_mrl_guidelines_wrkdoc_11945_en.pdf, p. 46.

Evans WC and Somanabandhu A, 1974. Cuscohygrine - Constituent of roots of some British Convolvulaceae. Phytochemistry, 13, 519-520.

FAO_WHO, 2015. https://extranet.who.int/sree/Reports?op=vs&path=/WHO_HQ_Reports/G7/PROD/EXT/CIFOCOSS_Country&userid=G7_ro&password=inetsoft123. http://www.who.int/foodsafety/databases/en/.

Finland_Times, 2013. Sale of toxic Valio product also banned: 3 more poisoned by Rainbow toxic vegetables. http://www.finlandtimes.fi/national/2013/05/24/1155/Sale-of-toxic-Valio-product-also-banned.

Fretz R, Schmid D, Brueller W, Girsch L, Pichler AM, Riediger K, Safer M and Allerberger F, 2007. Food poisoning due to Jimson weed mimicking *Bacillus cereus* food intoxication in Austria, 2006. International Journal of Infectious Diseases, 11, 557-558.

Friedman M, Roitman JN and Kozukue N, 2003. Glycoalkaloid and calystegine contents of eight potato cultivars. Journal of Agricultural and Food Chemistry, 51, 2964-2973.

Gaillard Y and Pepin G, 1999. Poisoning by plant material: Review of human cases and analytical determination of main toxins by High-Performance Liquid Chromatography-(Tandem) Mass Spectrometry. Journal of Chromatography B, 733, 181-229.

Galizia E, 1983. Clinical curio hallucinations in elderly tea drinkers. British Medical Journal, 287, 979.

Genaust H, 1996. Etymologisches Wörterbuch der Botanischen Pflanzen-Namen, 3. Auflage. Basel: Birkhäuser Verlag.[quoted in Potterat 2010].

Glaizal M, Schmitt C, Tichadou L, Sapori JM, Hayek-Lanthois M and de Haro L, 2013. Food poisonings related to organic buckwheat contaminated with *Datura* sp.: A poison control and toxicovigilance center activity. Presse Medicale, 42, 1412-1415.

Glick D and Glaubach S, 1941. The occurrence and distribution of atropinesterase, and the specificity of tropinesterases. Journal of General Physiology, 25, 197-205.

Gmira N, Douira A and Bouhache M, 1998. Ecological grouping of *Solanum elaeagnifolium*: a principal weed in the irrigated Tadla plain (central Morocco). Weed Research, 38, 87-94.

Goldmann A, Milat ML, Ducrot PH, Lallemand JY, Maille M, Lepingle A, Charpin I and Tepfer D, 1990. Tropane derivatives from *Calystegia sepium*. Phytochemistry, 29, 2125-2127.

Griffin WJ and Lin GD, 2000. Chemotaxonomy and geographical distribution of tropane alkaloids. Phytochemistry, 53, 623-637.

Griffiths DW, Shepherd T and Stewart D, 2008. Comparison of the calystegine composition and content of potato sprouts and tubers from *Solanum tuberosum* group Phureja and *Solanum tuberosum* group Tuberosum. Journal of Agricultural and Food Chemistry, 56, 5197-5204.

Hanf M, 1983. The arable weeds of Europe, with their seedlings and seeds. BASF Aktiengesellschaft, D-6700 Ludwigshafen.

Hanus LO, Rezanka T, Spizek J and Dembitsky VM, 2005. Substances isolated from *Mandragora* species. Phytochemistry, 66, 2408-2417.

Harsh ML, 1989. Tropane alkaloids from *Lycium barbarum* Linn *in vivo* and *in vitro*. Current Science, 58, 817-818.

Hashimoto T, Hayashi A, Amano Y, Kohno J, Iwanari H, Usuda S and Yamada Y, 1991. Hyoscyamine 6-beta-hydroxylase, an enzyme involved in tropane alkaloid biosynthesis, is localized at the pericycle of the root. Journal of Biological Chemistry, 266, 4648-4653.

Ionkova I, Witte L and Alfermann AW, 1994. Spectrum of tropane alkaloids in transformed roots of *Datura innoxia* and *Hyoscyamus* X *gyorffyi* cultivated *in vitro*. Planta Medica, 60, 382-384.

ISO, 1980. ISO 3103 - Tea – Preparation of liquor for use in sensory tests. ISO standards.

Jackson BP and Berry MI, 1973. Hydroxytropane tiglates in roots of *Mandragora* species. Phytochemistry, 12, 1165-1166.

Jandric Z, Rathor MN, Svarc-Gajic J, Maestroni BM, Sasanya JJ, Djurica R and Cannavan A, 2011. Development of a Liquid Chromatography-Tandem Mass Spectrometric method for the simultaneous determination of tropane alkaloids and glycoalkaloids in crops. Food Additives and Contaminants Part A, 28, 1205-1219.

Jaspersen-Schib R, Theus L, Guirguis-Oeschger M, Gossweiler B and Meier-Abt PJ, 1996. Serious plant poisonings in Switzerland 1966-1994. Case analysis from the Swiss Toxicology Information Center. Schweiz Schweizerische medizinische Wochenschrift, 126, 1085-1098.

Jenett-Siems K and Eich E, 1994. Hygrines and tropane alkaloids, alkaloids of considerable importance for the chemotaxonomy of the Convolvulaceae. European Journal of Pharmaceutical Sciences, 2, 122.

Jockovic N, Fischer W, Brandsch M, Brandt W and Dräger B, 2013. Inhibition of human intestinal alpha-glucosidases by calystegines. Journal of Agricultural and Food Chemistry, 61, 5550-5557.

Jousse C, Vu TD, Tran TLM, Boitel-Conti M, Pilard S, Mathiron D, Hehn A, Bourgaud F and Gontier E, 2009. Tropane alkaloid profiling of hydroponic *Datura innoxia* Mill. plants inoculated with *Agrobacterium rhizogenes*. Phytochemical Analysis, 21, 118-127.

Kaçan K, Tursun N and Uludağ A, 2013. Determination of weed emergence in sunflower. Proceedings of the Joint workshop of the EWRS working groups: novel and sustainable weed management in arid and semi-arid agro ecosystems and weed mapping. 29th September – 03rd October 2013

Mediterranean Agronomic Institute of Chania Crete, Greece. http://www.ewrs.org/doc/EWRS_Chania_Proceedings_2013.pdf.

Kato A, Asano N, Kizu H, Matsui K, Suzuki S and Arisawa M, 1997. Calystegine alkaloids from *Duboisia leichhardtii*. Phytochemistry, 45, 425-429.

Keiner R and Dräger B, 2000. Calystegine distribution in potato (*Solanum tuberosum*) tubers and plants. Plant Science, 150, 171-179.

Klenow S, Latté KP, Wegewitz U, Dusemund B, Pöting A, Appel KE, Großklaus R, Schumann R and Lampen A, 2012. Risikobewertung von Pflanzen und pflanzlichen Zubereitungen. Part B, Chapter 1: *Lychium barbarum* L. (Gojibeeren). BfR Wissenschaft, 19-39.

Kubwabo C, Rollmann B and Tilquin B, 1993. Analysis of alkaloids from *Physalis peruviana* by capillary GC, capillary GC-MS, and GC-FTIR. Planta Medica, 59, 161-163.

Lange A and Toft P, 1990. Poisoning with nightshade, *Atropa belladonna*. Ugeskr Laeger, 152, 1096.

Le_Parisien, 2010. Rappel de conserves de haricots verts des marques U et Notre Jardin. http://www.leparisien.fr/laparisienne/cuisine/rappel-de-conserves-de-haricots-verts-des-marques-u-et-notre-jardin-05-05-2010-911937.php#xtref=http%3A%2F%2Fwww.bing.com%2Fsearch%3Fq%3Dhttp%253A%252F%252Fwww.sante-sports.gouv.fr%252Frappel-de-produits-presence-de-datura-stramonium-dans-des-conserves-de-haricots-verts-des-marques-%24qs%3Dn%24form%3DQBRE%24pq%3Dhttp%253A%252F%252Fwww.sante-sports.gouv.fr%252Frappel-de-produits-presence-de-datura-%24sc%3D0-70%24sp%3D-1%24sk%3D%24cvid%3D0697C58ACD584591A744F4953258CBCD.

Lewis HW and Elvin-Lewis MPF, 1977. Tropane alkaloids as undesirable substances in animal feed. Medical Botany. Wiley & Sons, New York, pp. 419-425

Li R, Reed DW, Liu E, Nowak J, Pelcher LE, Page JE and Covello PS, 2006. Functional genomic analysis of alkaloid biosynthesis in *Hyoscyamus niger* reveals a cytochrome P450 involved in littorine rearrangement. Chemistry & Biology, 13, 513-520.

Mandal S, Naqvi AA and Thakur RS, 1991. Analysis of some tropane alkaloids in plants by mixed-column high-performance liquid-chromatography. Journal of Chromatography, 547, 468-471.

Mekki M, 2007. Biology, distribution and impacts of silverleaf nightshade (*Solanum elaeagnifolium* Cav.). EPPO Bulletin, 37, 114-118.

Mirzaev YR and Aripova SF, 1998. Neuro- and psychopharmacological investigation of the alkaloids convolvine and atropine. Chemistry of Natural Compounds, 34, 56-58.

Molyneux RJ, Pan YT, Goldmann A, Tepfer DA and Elbein AD, 1993. Calystegins, a novel class of alkaloid glycosidase inhibitors. Archives of Biochemistry and Biophysics, 304, 81-88.

Mulder PPJ, Pereboom-de Fauw DP, Hoogenboom RL, de Stoppelaar J and de Nijs M, 2015. Tropane and ergot alkaloids in grain-based products for infants and young children in the Netherlands in 2011-2014. Food Additives and Contaminants Part B 8, 284-290.

Mulder PPJ, von Holst C, Nivarlet N and van Egmond HP, 2014. Intra- and inter-laboratory validation of a dipstick immunoassay for the detection of tropane alkaloids hyoscyamine and scopolamine in animal feed. Food Additives and Contaminants Part A, 31, 1165-1176.

Mulder PPJ López Sánchez P, Castellari M, Preiss-Weigert A, These A, 2015. Occurrence of Pyrrolizidine Alkaloids in food. EFSA supporting publication 2015:EN-859, 114 pp.

Nakanishi F, Sasaki K and Shimomura K, 1998. Isolation and identification of littorine from hairy roots of *Atropa belladonna*. Plant Cell Reports, 18, 249-251.

Nash RJ, Rothschild M, Porter EA, Watson AA, Waigh RD and Waterman PG, 1993. Calystegines in *Solanum* and *Datura* species and the deaths-head hawk-moth (*Acherontia atropus*). Phytochemistry, 34, 1281-1283.

Nash RJ, Watson AA, Asano N, Winchester B, Molyneux RJ and Stegelmeier BL, 1998. Calystegine alkaloids in the potato and other food plants. Abstracts of Papers of the American Chemical Society, 216, U73-U73.

Oksman_Caldentey KM, Vuorela H, Strauss A and Hiltunen R, 1987. Variation in the tropane alkaloid content of *Hyoscyamus muticus p*lants and cell-culture clones. Planta Medica, 349-354.

Oshiro N, Kuniyoshi K, Nakamura A, Araki Y, Tamanaha K and Inafuku Y, 2008. A case of food poisoning due to ingestion of eggplant, *Solanum melongena*, grafted on devil's trumpet, *Datura metel*. Journal of the Food Hygienic Society of Japan, 49, 376-379.

Papoutsis I, Nikolaou P, Athanaselis S, Stefanidou M, Pistos C, Spiliopoulou C and Maravelias C, 2010. Mass intoxication with *Datura innoxia*-case series and confirmation by analytical toxicology. Clinical Toxicology, 48, 143-145.

Pereboom-de Fauw DP and Mulder PPJ, 2012. Animal feed – Determination of ergot alkaloids and tropane alkaloids LC-MS/MS. SOP-A-1070, Version 4. 18 April 2012. RIKILT Standard Opeartion Procedure, 1-.

Perharič L, 2005. Mass tropane alkaloid poisoning due to buckwheat flour contamination. Clinical Toxicology, 43, 413.

Perharič L, Kozelj G, Druzina B and Stanovnik L, 2013. Risk assessment of buckwheat flour contaminated by thorn-apple (*Datura stramonium* L.) alkaloids: a case study from Slovenia. Food Additives and Contaminants Part A, 30, 321-330.

Petersson EV, Arif U, Schulzova V, Krtkova V, Hajslova J, Meijer J, Andersson HC, Jonsson L and Sitbon F, 2013. Glycoalkaloid and calystegine levels in table potato cultivars subjected to wounding, light, and heat treatments. Journal of Agricultural and Food Chemistry, 61, 5893-5902.

PFAF, 2015. Plants for a Future. http://www.pfaf.org/.

Philipov S, Berkov S and Doncheva T, 2007. GC-MS survey of *Datura stramonium* alkaloids. Comptes rendus de L'Académie bulgare des Sciences, 60, 239-250.

Piccillo GA, Mondati EG and Moro PA, 2002. Six clinical cases of *Mandragora autumnalis* poisoning: Diagnosis and treatment. European Journal of Emergency Medicine, 9, 342-347.

Pieper R, Kroger S, Weigend M, Hanschen F, Kroh L and Zentek J, 2010. Hoary alyssum (*Berteroa incana*) in hay: a "new" potential health hazard for horses. Tieraerztliche Praxis Ausgabe Grosstiere Nutztiere, 38, 171-176.

Pietsch J, Koch I, Hermanns-Clausen M, Huller G, Wagner R and Dressler J, 2008. Pediatric plant exposures in Germany, 1998-2004. Clinical Toxicology, 46, 686-691.

Plenert B, Prasa D, Hentschel H and Deters M, 2012. Plant exposures reported to the poisons information centre Erfurt from 2001-2010. Planta Medica, 78, 401-408.

Pomilio AB, Falzoni EM and Vitale AA, 2008. Toxic chemical compounds of the Solanaceae. Nat Prod Commun, 3, 593-628.

Potterat O, 2010. Goji (*Lycium barbarum* and *L. chinense*): Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. Planta Medica, 76, 7-19.

Putcha L, Cintron NM, Tietze KJ, Hunter RP, Parise CM, Baker HE and Vanderploeg JM, 1991. Salivary pharmacodynamics of scopolamine. Clinical Pharmacology & Therapeutics, 49, 124-124.

Rancic D and Spasic A, 2009. Identification of atropine and scopolamine by HPLC in buckwheat flour contamination with *Datura stramonium* seeds. Toxicology Letters, 189, S234-S234.

Renner UD, Oertel R and Kirch W, 2005. Pharmacokinetics and pharmacodynamics in clinical use of scopolamine. Therapeutic Drug Monitoring, 27, 655-665.

Routledge PA and Spriggs TLB, 1989. Atropine as possible contaminant of comfrey tea. Lancet, 1, 963-964.

Russo R and Reggiani R, 2012. Antinutritive compounds in twelve *Camelina sativa g*enotypes. American Journal of Plant Sciences, 3, 1408-1412.

Sahai M and Ray AB, 1980. Secotropane alkaloids of *Physalis peruviana*. Journal of Organic Chemistry, 45, 3265-3268.

Schimming T, Jenett-Siems K, Mann P, Tofern-Reblin B, Milson J, Johnson RW, Deroin T, Austin DF and Eich E, 2005. Calystegines as chemotaxonomic markers in the Convolvulaceae. Phytochemistry, 66, 469-480.

Schneider F, Lutun P, Kintz P, Astruc D, Flesch F and Tempe JD, 1996. Plasma and urine concentrations of atropine after the ingestion of cooked deadly nightshade berries. Journal of Toxicology Clinical Toxicology, 34, 113-117.

Scholz H, Kascha S and Zingerle H, 1980. Atropin-vergiftung durch "gesundheitstee". Fortschritte der Medizin, 98, 1525-1526.

Schroeder D, Muellerschaerer H and Stinson CSA, 1993. A European weed survey in 10 major crop systems to identify targets for biological-control. Weed Research, 33, 449-458.

Shukla YN, Bhakuni RS, Singh DP, Jain SP and Khanuja SPS, 2006. Chemical and biological studies on the genus *Convolvulus* - A review. Journal of Medicinal and Aromatic Plant Sciences, 28, 392-413.

Simola LK, Nieminen S, Huhtikangas A, Ylinen M, Naaranlahti T and Lounasmaa M, 1988. Tropane alkaloids from *Atropa belladonna*, Part II. Interaction of origin, age, and environment in alkaloid production of callus cultures. Journal of Natural Products, 51, 234-242.

Singh R, Bagachi A, Semwal A, Kaur S and Bharadwa A, 2013. Traditional uses, phytochemistry and pharmacology of *Morus alba* Linn.: A review. Journal of Medicinal Plants Research, 7, 461-469.

Singhal BK, Khan MA, Dhar A, Baqual FM and Bindroo BB, 2010. Approaches to industrial exploitation of mulberry (*Mulberry* sp.) fruits. Journal of Fruit and Ornamental Plant Research, 18, 83-99.

SNE, 2014. http://www.anid.pt/attachments/article/45/alkaloids.pdf.

Standing_Committe_on_Plants_Animals_Food_and_feed, 2014. Summary report of the Standing Committe on Plants, Animals, Food and Feed held in Brussels on 1 July 2014. Section Toxicological Safety on the Food Chain. http://ec.europa.eu/food/food/chemicalsafety/contaminants/index_en.htm Plant toxins and certain mycotoxins.

Taleb A and Bouhache M, 2006. Etat actuel de nos connaissances sur les plantes envahissantes au Maroc. In. Brunel, S. Ed. Invasive plants in Mediterranean type regions of the world. Council of Europe Publishing. Strasbourg. 428 pp 99-107.

Termala AM, Pohjalainen T and Hoppu K, 2014. *Datura* contamination of a large batch of frozen vegetables-some poisonings and a big hassle. Clinical Toxicology, 52, 384-384.

Todd FG, Stermitz FR, Schultheis P, Knight AP and Traubdargatz J, 1995. Tropane alkaloids and toxicity of *Convolvulus arvensis*. Phytochemistry, 39, 301-303.

Trabattoni G, Visintini D, Terzano GM and Lechi A, 1984. Accidental poisoning with deadly nightshade berries - A case report. Human Toxicology, 3, 513-516.

Tsiligianni C, 2009. A two cases clinical report of mandragora poisoning in primary care in Crete, Greece: Two case report. BioMed Central, P. 3.

USDA_ARS, 2006. US Department of Agriculture Agricultural Research Service http://www.ars.usda.gov/main/main.htm.

van Riel AJHP, Meulenbelt J and de Vries I, 2014. Anticholinergic poisoning after drinking contaminated herbal tea. Clinical Toxicology, 52, 383-383.

van_der_Heide RM, 1988. Poisoning with 'Hustentee' [in Dutch]. Nederlands Tijdschrift voor Geneeskunde, 132, 1993.

Vichova P and Jahodar L, 2003. Plant poisonings in children in the Czech Republic, 1996-2001. Human & Experimental Toxicology, 22, 467-472.

Wada S, Shimizudani T, Yamada H, Oguri K and Yoshimura H, 1994. Sulfotransferase-dependent dehydration of atropine and scopolamine in guinea pig. Xenobiotica, 24, 853-861.

Watson AA, Davies DR, Asano N, Winchester B, Kato A, Molyneux RJ, Stegelmeier BL and Nash RJ, 2000. Calystegine alkaloids in the potato and other food plants. Natural and Selected Synthetic Toxins, 745, 129-139.

Watson AA, Fleet GW, Asano N, Molyneux RJ and Nash RJ, 2001. Polyhydroxylated alkaloids - Natural occurrence and therapeutic applications. Phytochemistry, 56, 265-295.

Watson ES, Murphy JC, Elsohly HN, Elsohly MA and Turner CE, 1983. Effects of the administration of coca alkaloids on the primary immune-responses of mice - Interaction with delta-9-tetrahydrocannabinol and ethanol. Toxicology and Applied Pharmacology, 71, 1-13.

Williams G and Hunyadi K, 1987. Dictionary of weeds of Eastern Europe: Their common names and importance in Latin, Albanian, Bulgarian, Czech, German, English, Greek, Hungarian, Polish, Romanian, Russian, Serbo-Croatia and Slovak. Elsevier. Amsterdam.

Williams GH, 1982. Elseviers's dictionary of weeds of Western Europe. Elsevier Scientific Publishing Company Amsterdam.

Zhang L, Yang B, Lu BB, Kai GY, Wang ZN, Xia Y, Ding RX, Zhang HM, Sun XF, Chen WS and Tang KX, 2007. Tropane alkaloids production in transgenic *Hyoscyamus niger* hairy root cultures over-expressing putrescine N-methyltransferase is methyl jasmonate-dependent. Planta, 225, 887-896.

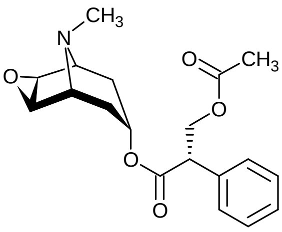
Zhao GH, Kan JQ, Li ZX and Chen ZD, 2005. Characterization and immunostimulatory activity of an (1 -> 6)-α-D-glucan from the root of *Ipomoea batatas*. International Immunopharmacology, 5, 1436-1445.

Abbreviations

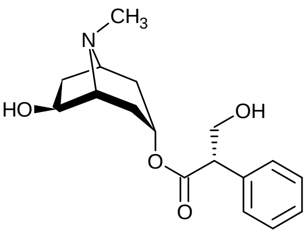
AChE	Acetylcholineesterase
ANS	Autonomic nervous system
ARfD	Acute reference dose
CNS	Central nervous system
CONTAM	EFSA Panel on Contaminants in the Food Chain
CZ	Czech Republic
DE	Germany
DW	Dry weight
EFSA	European Food Safety Authority
EMEA	European Medicines Agency
ES	Spain
ESI	Electrospray ionisation
ESI+	Positive electrospray ionisation
EU	European Union
eV	Electron volt
FAO	Food and Agriculture Organisation
FR	France
GC-MS	Gas chromatography-mass spectrometry
HU	Hungary
IRTA	Institute for Reseach and Technology in Food and Agriculture
IS	Internal standard
ISO	International Organization for Standardization
IT	Italy
LB	Lower bound
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
ML	Maximum level
MME	Matrix-matched extract
MMRS	Matrix-matched recovery standard
MMS	Matrix-matched standard
MRM	Multiple reaction monitoring
MS/MS	Tandem mass spectrometry
m/z	Mass over charge ratio
NL	the Netherlands
PL	Poland
PNS	Peripheral nervous system
QC	Quality control
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RASFF	Rapid Alert System for Food and Feed
RSD	Relative standard deviation
RT	Retention time or room temperature
S/N	Signal to noise (ratio)
SNE	Specialised Nutrition Europe
TA	Tropane Alkaloid
UB	Upper bound
UCT	University of Chemistry and Technology
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

Appendix A – Chemical structures of tropane alkaloids (TAs)

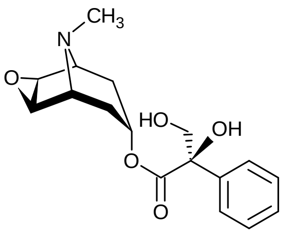
Appendix A.1 Datura-type TAs



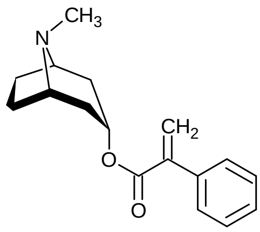
O-Acetylscopolamine
$C_{19}H_{23}NO_5$
Mass: 345.158 Da
[M+H]+: 346.165 Da



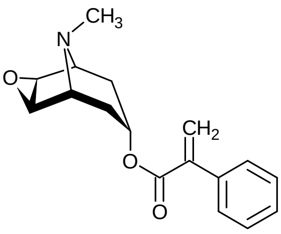
Anisodamine
6β-Hydroxyhyoscyamine
$C_{17}H_{23}NO_4$
Mass: 305.163 Da
[M+H]+: 306.170 Da



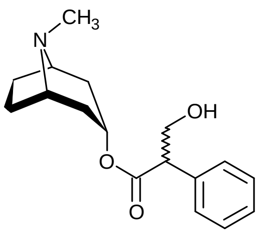
Anisodine
α-Hydroxyscopolamine
$C_{17}H_{21}NO_5$
Mass: 319.142 Da
[M+H]+: 320.149 Da



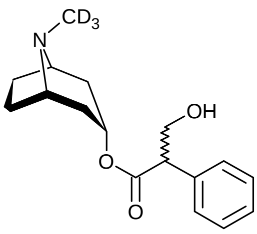
Apoatropine
$C_{17}H_{21}NO_2$
Mass: 271.157 Da
[M+H]+: 272.165 Da



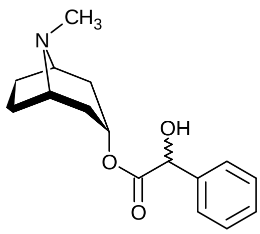
Aposcopolamine
Apohyoscine
$C_{17}H_{19}NO_3$
Mass: 285.136 Da
[M+H]+: 286.144 Da



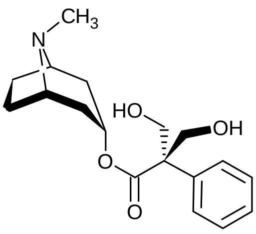
Atropine
(+/-)-Hyoscyamine
$C_{17}H_{23}NO_3$
Mass: 289.168 Da
[M+H]+: 290.175 Da



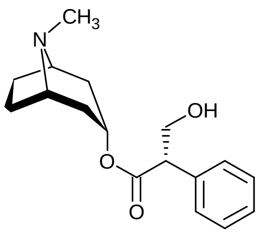
Atropine-d$_3$
$C_{17}H_{20}D_3NO_3$
Mass: 292.187 Da
[M+H]+: 293.194 Da



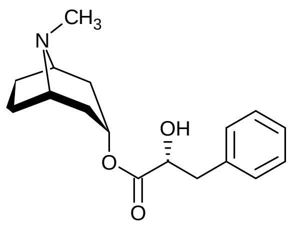
(+/-)-Homatropine
$C_{16}H_{21}NO_3$
Mass: 275.152 Da
[M+H]+: 276.159 Da



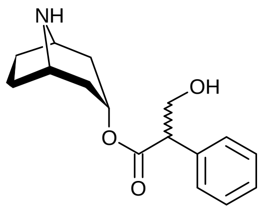
2-Hydroxymethyl atropine
$C_{17}H_{21}NO_5$
Mass: 319.142 Da
[M+H]+: 320.149 Da



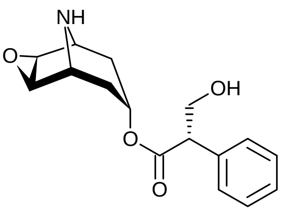
(-)-Hyoscyamine
$C_{17}H_{23}NO_3$
Mass: 289.168 Da
[M+H]+: 290.175 Da



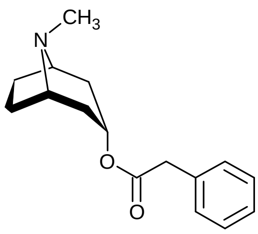
(-)-Littorine
$C_{17}H_{23}NO_3$
Mass: 289.168 Da
[M+H]+: 290.175 Da



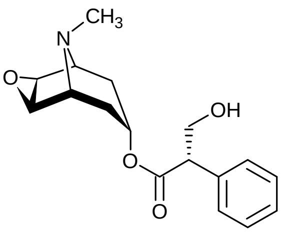
(+/-)-Noratropine
$C_{16}H_{21}NO_3$
Mass: 275.152 Da
[M+H]+: 276.159 Da



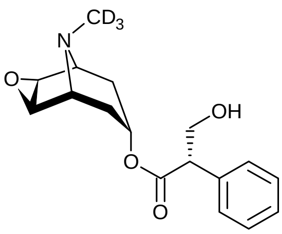
(-)-Norscopolamine
$C_{16}H_{19}NO_4$
Mass: 289.131 Da
[M+H]+: 290.139 Da



3α-Phenylacetoxy tropane
$C_{16}H_{21}NO_2$
Mass: 259.157 Da
[M+H]+: 260.165 Da

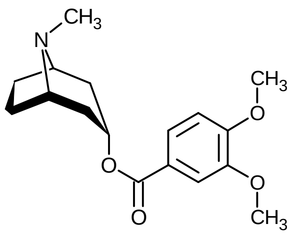


(-)-Scopolamine
Hyoscine
$C_{17}H_{21}NO_4$
Mass: 303.147 Da
[M+H]+: 304.154 Da

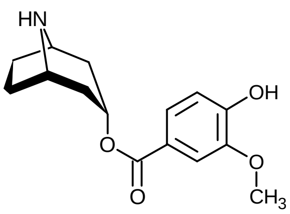


(-)-Scopolamine-d$_3$
$C_{17}H_{18}D_3NO_4$
Mass: 306.166 Da
[M+H]+: 307.173 Da

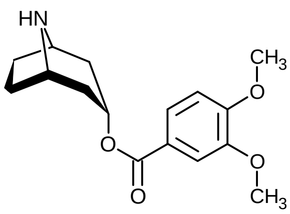
Appendix A.2 Convolvulaceae-type TAs



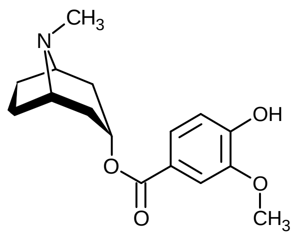
Convolamine
$C_{17}H_{23}NO_4$
Mass: 305.163 Da
[M+H]+: 306.167 Da



Convolidine
$C_{15}H_{19}NO_4$
Mass: 277.131 Da
[M+H]+: 278.139 Da

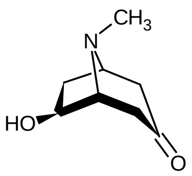


Convolvine
$C_{16}H_{21}NO_4$
Mass: 291.147 Da
[M+H]+: 292.154 Da

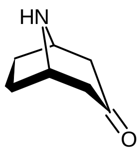


Fillalbine
$C_{16}H_{21}NO_4$
Mass: 291.147 Da
[M+H]+: 292.154 Da

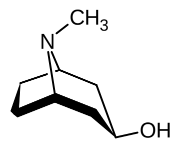
Appendix A.3 Low molecular weight TAs



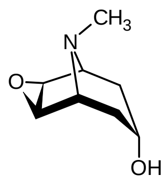
6-Hydroxytropinone
$C_8H_{13}NO_2$
Mass: 155.095 Da
[M+H]+: 156.102 Da



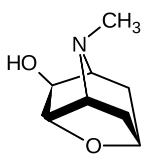
Nortropinone
$C_7H_{11}NO$
Mass: 125.084 Da
[M+H]+: 126.091 Da



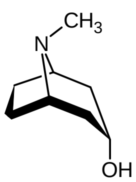
Pseudotropine
$C_8H_{15}NO$
Mass: 141.115 Da
[M+H]+: 142.123 Da



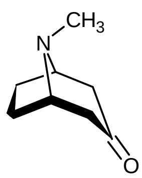
Scopine
$C_8H_{13}NO_2$
Mass: 155.095 Da
[M+H]+: 156.102 Da



Scopoline
$C_8H_{13}NO_2$
Mass: 155.095 Da
[M+H]+: 156.102 Da

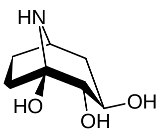


Tropine
$C_8H_{15}NO$
Mass: 141.115 Da
[M+H]+: 142.123 Da

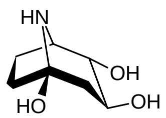


Tropinone
$C_8H_{13}NO$
Mass: 139.100 Da
[M+H]+: 140.107 Da

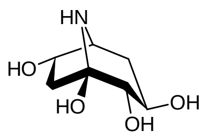
Appendix A.4 Calystegine TAs



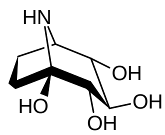
Calystegine A3
$C_7H_{13}NO_3$
Mass: 159.090 Da
[M+H]+: 160.097 Da



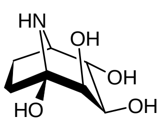
Calystegine A5
$C_7H_{13}NO_3$
Mass: 159.090 Da
[M+H]+: 160.097 Da



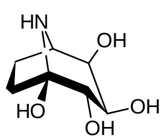
Calystegine B1
$C_7H_{13}NO_4$
Mass: 175.084 Da
[M+H]+: 176.092 Da



Calystegine B2
$C_7H_{13}NO_4$
Mass: 175.084 Da
[M+H]+: 176.092 Da



Calystegine B3
$C_7H_{13}NO_4$
Mass: 175.084 Da
[M+H]+: 176.092 Da



Calystegine B4
$C_7H_{13}NO_4$
Mass: 175.084 Da
[M+H]+: 176.092 Da

Appendix B – First draft sampling plan proposed at the start of the project

Food category	Total	UK	NL	DE	ES	FR	IT	CZ	HU	PO
Cereals	**900**	**170**	**95**	**45**	**185**	**90**	**75**	**110**	**65**	**65**
Breakfast cereals	**200**	40	25	10	40	20	15	30	10	10
Bread/pasta	**300**	50	30	15	65	30	30	40	20	20
Biscuits, cake other cereal-based foods	**200**	40	20	10	45	20	15	20	15	15
Cereal-based food for young children (age 6-36 months)	**200**	40	20	10	35	20	15	20	20	20
Food plants from the Solanaceae family	**600**	**105**	**105**	**30**	**90**	**60**	**50**	**90**	**35**	**35**
Potato	**250**	45	45	10	20	20	20	50	20	20
Aubergine	**100**	20	15	5	20	10	10	10	5	5
Tomato	**150**	25	25	10	30	20	10	20	5	5
Sweet pepper	**100**	15	20	5	20	10	10	10	5	5
Total	**1500**	**275**	**200**	**75**	**275**	**150**	**125**	**200**	**100**	**100**
Total per region (NW/S/E Europe)			**550**			**550**			**400**	

Appendix C – Consumption (g/day) of cereals, cereals-based products and food plants from the family of the Solanaceae in the selected countries in Europe, grouped according to the WHO database (2012)

Food category \ Country	FR, UK (G07)	DE, ES,PL (G08)	IT (G10)	NL (G11)	CZ, HU (G15)	Average	% Of total diet[(a)]
Roots and tubers raw or boiled	203.8	216.7	173.5	235.8	193.4	204.7	8.2%
Roots and tubers processed	10.4	4.8	3.2	8.2	9.5	7.2	0.3%
Cereal grains & flours	216.7	245.0	260.7	183.6	262.3	233.7	9.3%
Further processed cereals and by-products	30.1	15.5	23.0	12.7	19.4	20.1	0.8%
Fruiting vegetables, cucurbits	19.6	34.2	36.1	12.5	34.4	27.4	1.1%
Fruiting vegetables (other than cucurbits) and mushrooms	52.5	61.0	86.0	38.0	73.9	62.3	2.5%
Food for infants and small children	98.9	110.4	129.5	120.6	87.4	109.3	4.4%
Total	**632.0**	**687.6**	**712.0**	**611.5**	**680.4**	**664.7**	**26.6%**

(a): The average fresh weight of the daily total diet per adult person in the EU is approximately 2500 g.

Appendix D – Draft SOPs available for the detection of tropane alkaloids in food by LC-MS/MS at the start of the project

Appendix D.1 Draft SOP available for the detection of Datura-type TAs in food by LC-MS/MS

Adapted from: RIKILT SOP A1070 (Pereboom-de Fauw and Mulder, 2012).

Preparation of sample extract:

- Weigh 4 ± 0.05 g sample in to a 50 mL polypropylene tube with screw cap.
- Add 40 mL extraction solvent: 0.4% formic acid in methanol/water (60:40) (v/v), and shake vigorously.
- Extract by using a rotary tumbler for 30 min.
- Centrifuge at 3300 g for 15 min at room temperature.
- Transfer 2 mL of the supernatant to the ultrafilter tubes.
- Centrifuge the ultrafilter tubes (Millipore, Amicon Ultra-4, Ultracel 30kD) during 15 minutes at 3300 g at room temperature.
- Transfer the filtrate to a HPLC vial.

LC-MS/MS analysis:

- Chromatographic separation: Waters: Xbridge C18, 5 µm, 150*3 mm column.
- Mobile phase: gradient of water/acetonitrile with 6 mM ammonia.
    - During the run the acetonitrile proportion is raised from 10 to 35% in 2 min.
    - Linearly increased to 90% in 10 minutes.
    - Total run time 17 min.
    - Flow rate: 400 µL/min.
    - Column temperature: 40^{o}C.
    - Injection volume: 10 µL.
- MS/MS fragmentation conditions using positive electrospray ionization.
- Target LODs: 0.1-0.5 µg/kg.
- Target LOQs: 0.3-1 µg/kg.

Appendix D.2 Draft SOP available for the detection of calystegines in food by LC-MS/MS

Adapted from: Petersson et al., (2013) (Petersson et al., 2013).

Preparation of sample extract:

- Weigh 5 ± 0.05 g sample in to a 50 mL polypropylene tube with screw cap.
- Add 80 mL extraction solvent: methanol / water 1:1 v/v. Shake vigorously.
- Extract by using a rotary tumbler for 30 min.
- Filter over buchner filter in 100 mL volumetric flask.
- Fill up to 100 mL with extraction solvent.
- Dilute filtrate 50-100 times with 90% acetonitrile in water.
- Filter using 0.22 µm polytetrafluoroethylene membrane.
- Transfer the filtrate to a HPLC vial.

LC-MS/MS analysis:

- Chromatographic separation: e.g. (Waters) Acquity Ultra-Performance LC system: Atlantis HILIC column 100mm * 3 id, 3 µm column.
- Injection volume: 2 µL.
- Mobile phase:
  - Mobile phase A: 0.020 M ammonium acetate, pH 5.3 in milliQ water.
  - Mobile phase B: acetonitrile.
  - Flow rate 0.45 mL/min.
  - Column temperature: 35°C.
  - Gradient:
    - 0.5 min held: 10% mobile phase A and 90% acetonitrile.
    - in 4.5 min to: 60% mobile phase A and 40% acetonitrile.
    - 1 min held: 60% mobile phase A and 40% acetonitrile.
- MS/MS fragmentation conditions:
  - Positive electrospray ionization.
  - Needle voltage 4300V; curtain gas 275 kPa, nebulizer and turbo gas 410 kPa. temperature of turbo gas 600°C.
- Target LODs: 0.4-0.6 mg/kg.
- Target LOQs: 1-2 mg/kg.

Appendix E – Sampling plan

Appendix E.1 Overall sampling plan

Food category	Total	UK		NL		DE	ES		FR	IT	CZ		HU	PO
		Trad[(a)]	Org[(a)]	Trad	Org	Trad	Trad	Org	Trad	Trad	Trad	Org	Trad	Trad
Single component flours available at retail stores	**220**	**30**	**10**	**20**	**5**	**10**	**30**	**10**	**10**	**10**	**35**	**10**	**20**	20
Buckwheat	**113**	15	5	10	3	5	15	5	5	5	20	5	10	10
Millet	**107**	15	5	10	2	5	15	5	5	5	15	5	10	10
Cereals products available at retail stores	**820**	**105**	**40**	**65**	**25**	**50**	**135**	**45**	**75**	**75**	**75**	**20**	**55**	**55**
Breakfast cereals	**205**	25	10	15	5	15	30	10	20	20	20	5	15	15
Bread/pasta	**205**	25	10	15	5	10	35	10	20	20	20	5	15	15
Biscuits, cake other cereal-based foods	**175**	25	10	15	5	10	30	10	15	15	15	5	10	10
Cereal-based food for young children (age 6-36 months)	**235**	30	10	20	10	15	40	15	20	20	20	5	15	15
Other products available at retail stores	**160**	**23**	**7**	**23**	**7**	**-**	**50**	**15**	**-**	**-**	**27**	**8**	**-**	**-**
Dry (herbal) teas	**100**	15	5	15	5	-	30	10	-	-	15	5	-	-
Legumes, frozen beans and stir-fry mixes	**60**	8	2	8	2	-	20	5	-	-	12	3	-	
Food plants from the Solanaceae family	**300**	**45**	**15**	**40**	**15**	**15**	**50**	**15**	**15**	**15**	**35**	**10**	**15**	**15**
Potato	**210**	35	10	30	10	10	35	10	10	10	25	5	10	10
Aubergine	**90**	10	5	10	5	5	15	5	5	5	10	5	5	5
Total	**1500**	**203**	**72**	**148**	**52**	**75**	**265**	**85**	**100**	**100**	**172**	**48**	**90**	**90**
Total per country		**275**		**200**		**75**	**350**		**100**	**100**	**220**		**90**	**90**
Total per region (NW/S/E Europe)		**550**					**550**				**400**			

- = no samples taken.

(a): Trad = traditional production; Org = organic production.

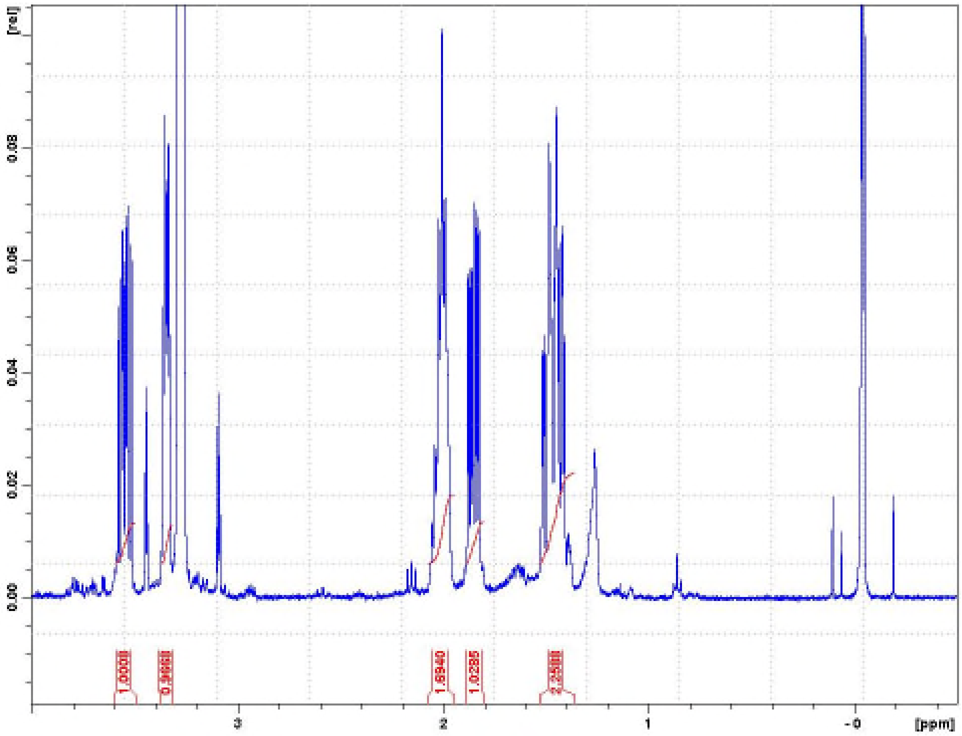
Appendix E.2 Sampling plan per sampling round[(a)]

Food category	Total	Total	Total	UK		NL		DE	ES		FR	IT	CZ		HU	PO
		1st	2nd	1st	2nd	1st	2nd	1st	1st	2nd	1st	2nd	1st	2nd	1st	1st
Single component flours available at retail stores	**220**	**143**	**77**	**20**	**20**	**13**	**12**	**10**	**20**	**20**	**10**	**10**	**26**	**19**	**20**	**20**
Buckwheat	**113**	72	41	10	10	7	6	5	10	10	5	5	13	12	10	10
Millet	**107**	71	36	10	10	6	6	5	10	10	5	5	13	7	10	10
Cereals available at retail stores	**820**	**495**	**325**	**75**	**70**	**45**	**45**	**50**	**90**	**90**	**75**	**75**	**50**	**45**	**55**	**55**
Breakfast cereals	**205**	126	79	18	17	10	10	15	20	20	20	20	13	12	15	15
Bread/pasta	**205**	123	82	18	17	10	10	10	22	23	20	20	13	12	15	15
Biscuits, cake other cereal-based foods	**175**	104	71	18	17	10	10	10	20	20	15	15	11	9	10	10
Cereal-based food for young children (age 6- 36 months)	**235**	142	93	21	19	15	15	15	28	27	20	20	13	12	15	15
Other products available at retail stores	**160**	**-**	**160**	**-**	**30**	**-**	**30**	**-**	**-**	**65**	**-**	**-**	**-**	**35**	**-**	**-**
Dry (herbal) teas	**100**	-	100	-	20	-	20	-	-	40	-	-	-	20	-	-
Legumes, frozen beans and stir-fry mixes	**60**	-	60	-	10	-	10	-	-	25	-	-	-	15	-	-
Food plants from the Solanaceae family	**300**	**181**	**119**	**30**	**30**	**27**	**28**	**15**	**40**	**25**	**15**	**15**	**24**	**21**	**15**	**15**
Potato	**210**	127	83	23	22	20	20	10	28	17	10	10	16	14	10	10
Aubergine	**90**	54	36	7	8	7	8	5	12	8	5	5	8	7	5	5
Total	**1500**	**819**	**681**	**125**	**150**	**85**	**115**	**75**	**150**	**200**	**100**	**100**	**100**	**120**	**90**	**90**
Total per country				**275**		**200**		**75**	**350**		**100**	**100**	**220**		**90**	**90**
Total per region (NW/S/E Europe)				**550**					**550**				**400**			

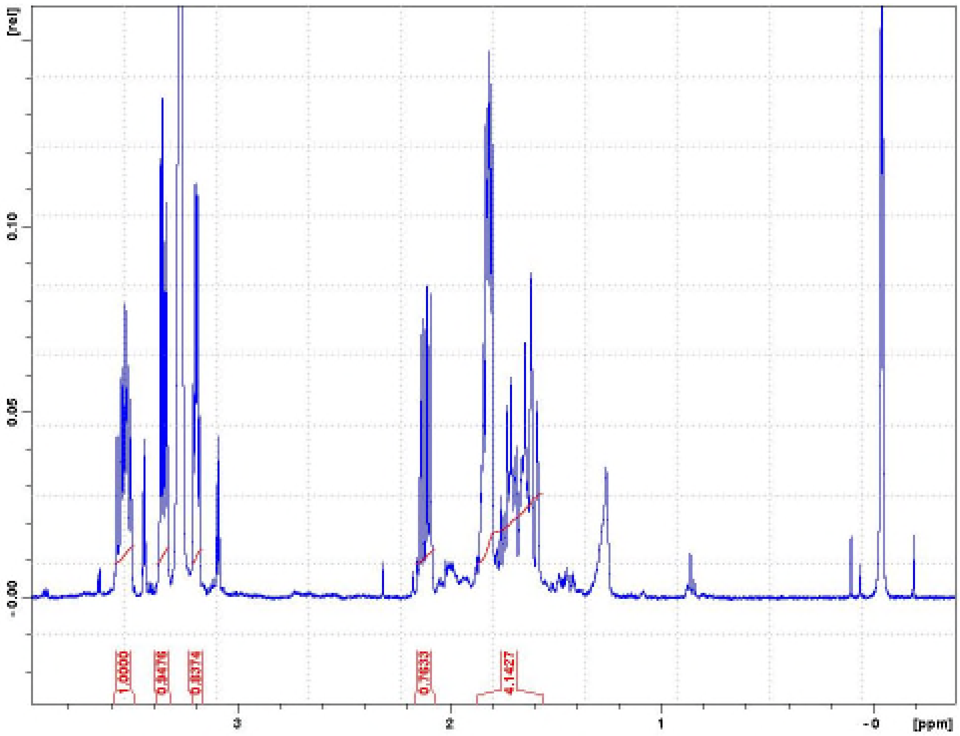
\- = no samples taken.
(a): 1st sampling round: May-December 2015. 2nd sampling round: January-August 2016.

Appendix F – [1]H NMR spectra obtained for calystegine TA standards

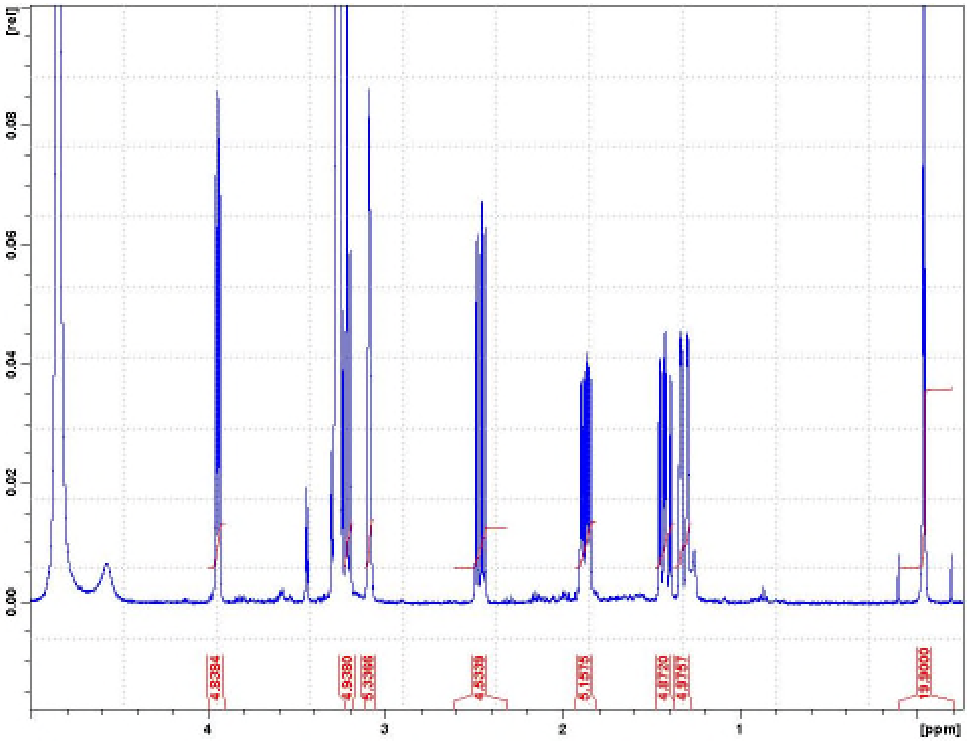
Appendix F.1. [1]H NMR spectrum of calystegine A_3 (5.1 mM in methanol-d_4)



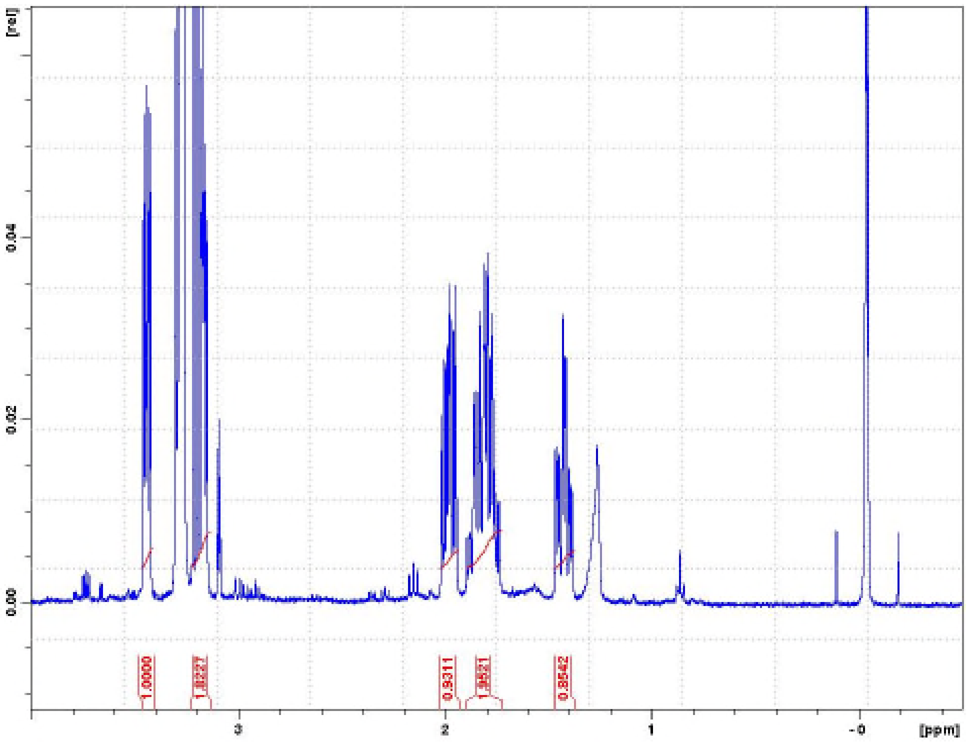
Appendix F.2 [1]H NMR spectrum of calystegine A_5 (4.4 mM in methanol-d_4)



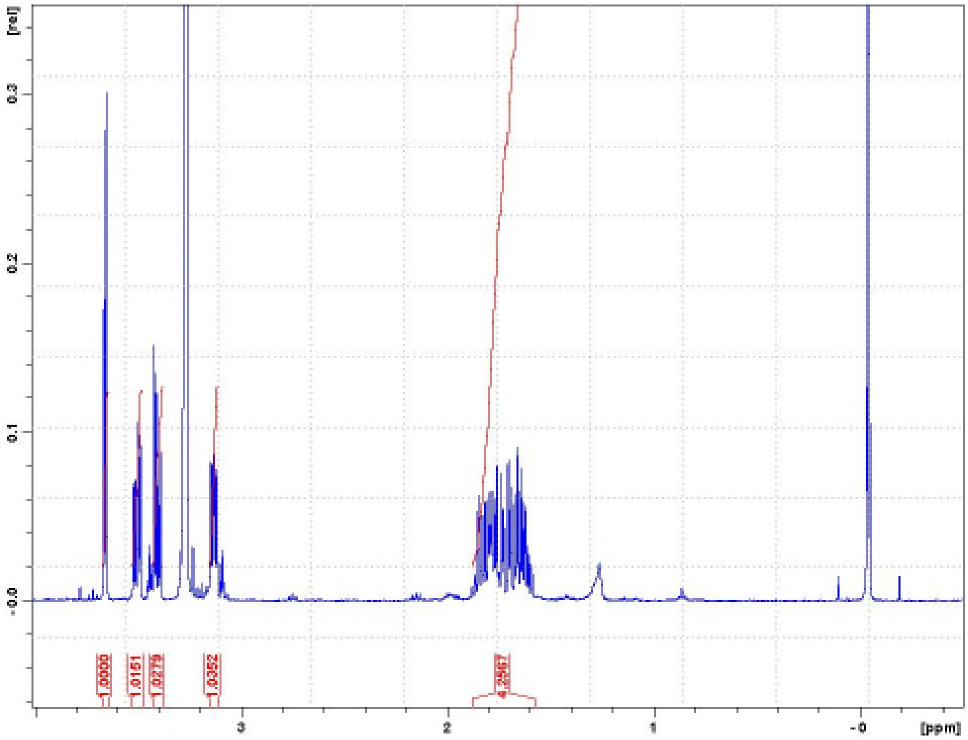
Appendix F.3 ^{1}H NMR spectrum of calystegine B_1 (4.8 mM in methanol-d_4)



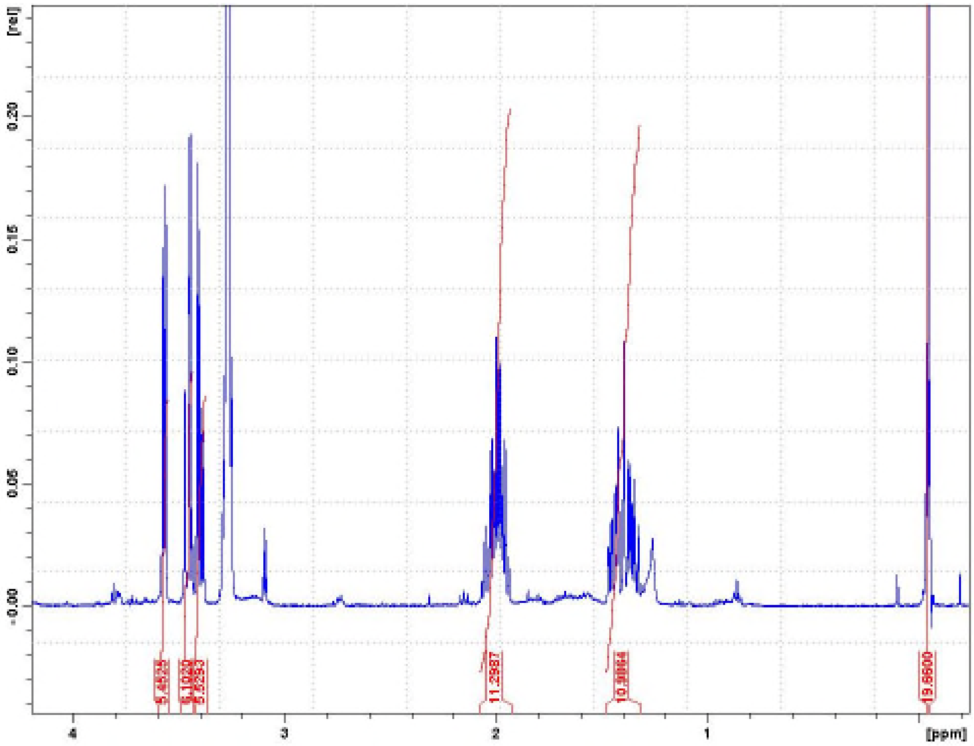
Appendix F.4 ^{1}H NMR spectrum of calystegine B_2 (4.1 mM in methanol-d_4)



Appendix F.5 ^{1}H NMR spectrum of calystegine B_3 (5.1 mM in methanol-d_4)



Appendix F.6 ^{1}H NMR spectrum of calystegine B_4 (5.45 mM in methanol-d_4)



Appendix G – RIKILT LC-MS/MS conditions for Datura TAs

Samples were analysed using an Acquity UPLC system coupled to a Xevo TQ-S tandem quadrupole mass spectrometer (Waters).

Appendix G.1 Chromatography

UPLC Method:

- Column: Waters UPLC BEH C18 150 x 2.1 mm, 1.7 µm (Waters 186002353).
- Column temperature: 50°C.
- Injection volume: 2 µL.
- Mobile phase A: 10 mM ammonium carbonate in water pH 10.
- Mobile phase B: Acetonitrile.
- Gradient (linear) timetable:

Time (min)	Mobile phase A	Mobile phase B
0.0	100%	0%
2.0	100%	0%
12.0	40%	60%
12.2	100%	0%
15.0	100%	0%

- Total run time is 15 min.

Appendix G.2 MS Tune settings

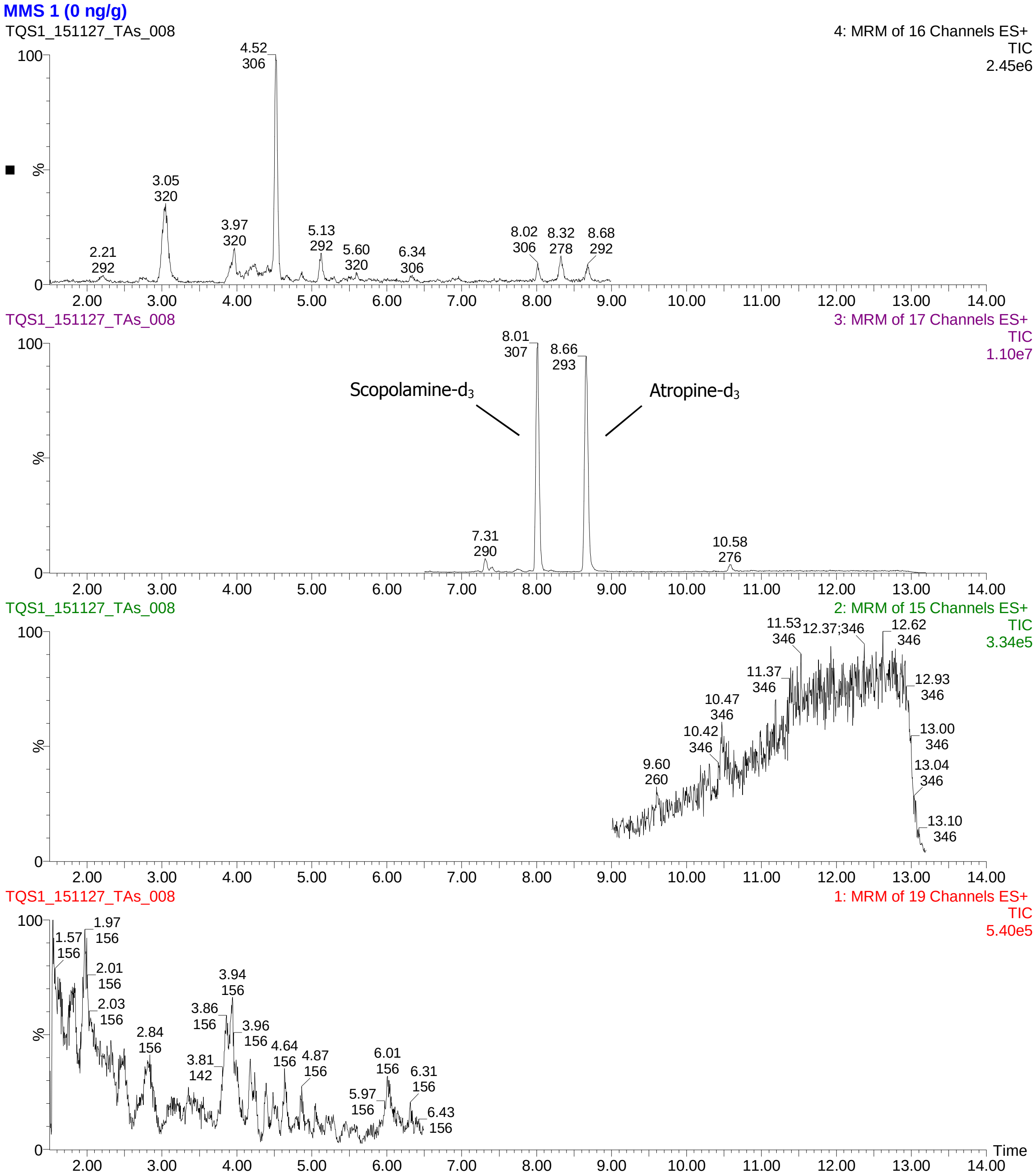
- Ionisation mode: positive electrospray (ESI+).
- Capillary voltage: 3 kV.
- Desolvation temperature: 600°C.
- Desolvation gas flow rate: 800 L/h.
- Nebuliser gas flow: 7 bar.
- Source temperature: 150°C.
- Cone gas flow rate: 100 L/h.
- Collision gas: argon.
- Collision gas flow rate: 0.18 mL/min (4.2 mbar).

Appendix G.3 MRM settings optimised for Datura TAs

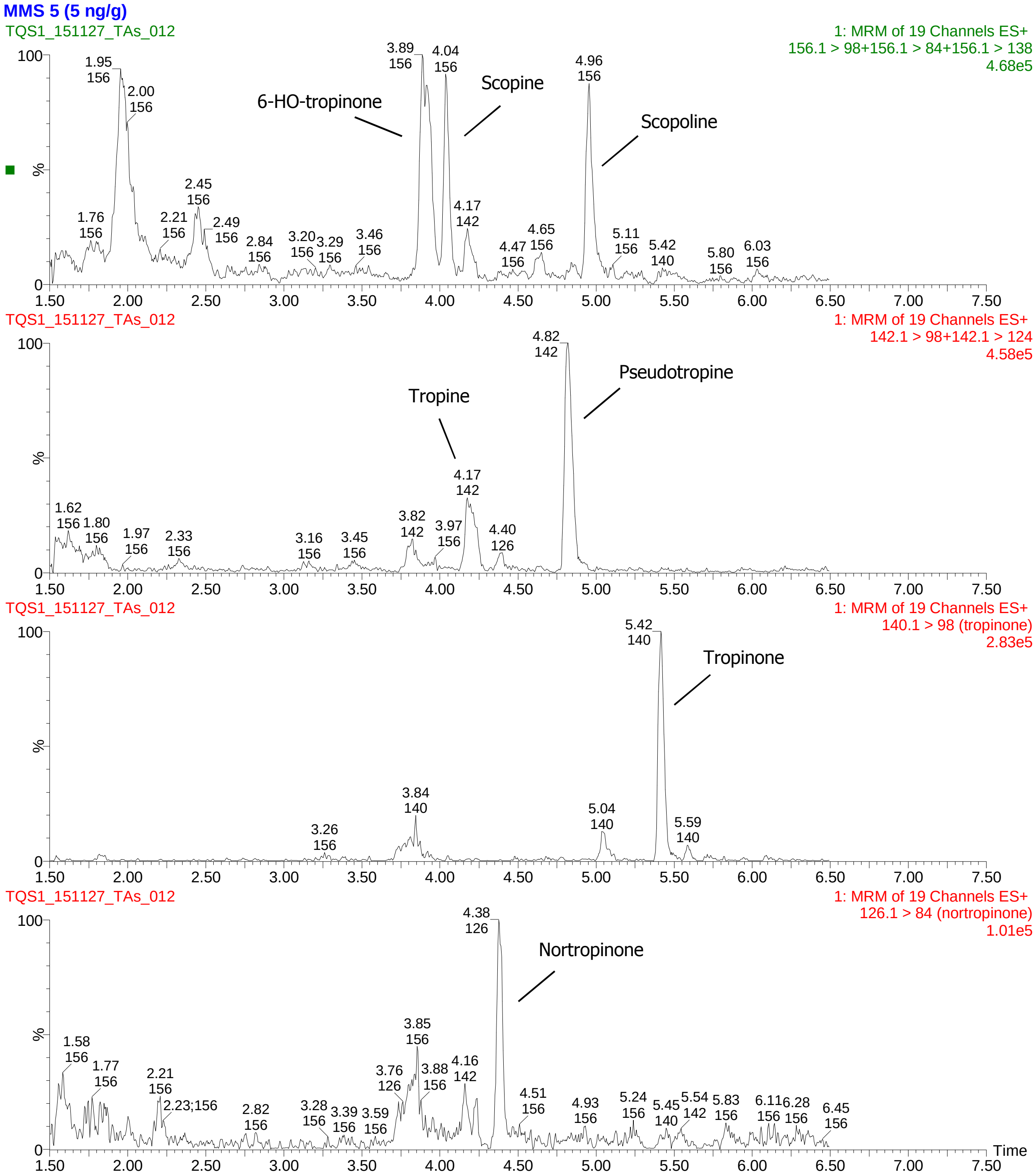
Compound name	Precursor mass (Da)	Fragment 1 (Da)	Collision energy 1 (eV)	Fragment 2 (Da)	Collision energy 2 (eV)	Fragment 2 (Da)	Collision energy 2 (eV)	Retention time (min)
Low molecular weight TAs								
6-Hydroxytropinone	156.1	**98.0**	**15**	81.0	20	58.0	20	3.90
Nortropinone	126.1	**84.0**	**15**	68.0	15	67.0	20	4.40
Pseudotropine	142.1	**124.0**	**15**	**96.0**	**20**	67.0	20	4.85
Scopine	156.1	98.0	15	**84.0**	**15**	73.0	15	4.05
Scopoline	156.1	**138.0**	**15**	110.0	20	81.0	20	4.95
Tropine	142.1	**98.0**	**20**	93.0	20	58.0	20	4.20
Tropinone	140.1	**98.0**	**20**	82.0	15	57.0	25	5.45
Convulvalaceae-type TAs								
Convolamine	306.2	**124.0**	**25**	93.0	25	91.0	35	9.79
Convolidine	278.2	151.0	30	110.0	20	**93.0**	**20**	5.35
Convolvine	292.2	165.0	30	110.0	20	**93.0**	**20**	8.65
Fillalbine	292.2	**124.0**	**20**	93.0	25	91.0	35	6.35
Datura-type TAs								
O-Acetylscopolamine	346.2	286.0	20	**138.0**	**20**	103.0	20	9.85
Anisodamine (6β-hydroxyhyoscyamine)	306.2	**140.0**	**20**	122.0	25	91.0	35	7.30
Anisodine	320.2	**156.0**	**20**	**138.0**	**20**	91.0	35	7.30
Apoatropine	272.2	**124.0**	**20**	93.0	25	91.0	35	11.40
Aposcopolamine	286.2	**138.0**	**20**	110.0	20	103.0	20	10.35
Atropine	290.2	**124.0**	**20**	93.0	25	91.0	35	8.70
Atropine-d_3	293.2	**127.0**	**20**	93.0	25	91.0	35	8.65
Homatropine	276.2	142.0	25	**124.0**	**20**	93.0	25	8.45
2α-Hydroxymethyl atropine	320.2	**124.0**	**25**	93.0	35	91.0	35	7.75
Littorine	290.2	142.0	25	**124.0**	**20**	93.0	25	9.20
Noratropine	276.2	110.0	20	**93.0**	**20**	91.0	30	7.70
Norscopolamine	290.2	**142.0**	**20**	124.0	15	121.0	20	7.25
Phenylacetoxytropane	260.2	**124.0**	**20**	93.0	25	91.0	35	10.45
Scopolamine	304.2	156.0	25	**138.0**	**20**	103.0	35	8.00
Scopolamine-d_3	307.2	156.0	25	**141.0**	**20**	103.0	35	8.00

In bold = most intense fragment.

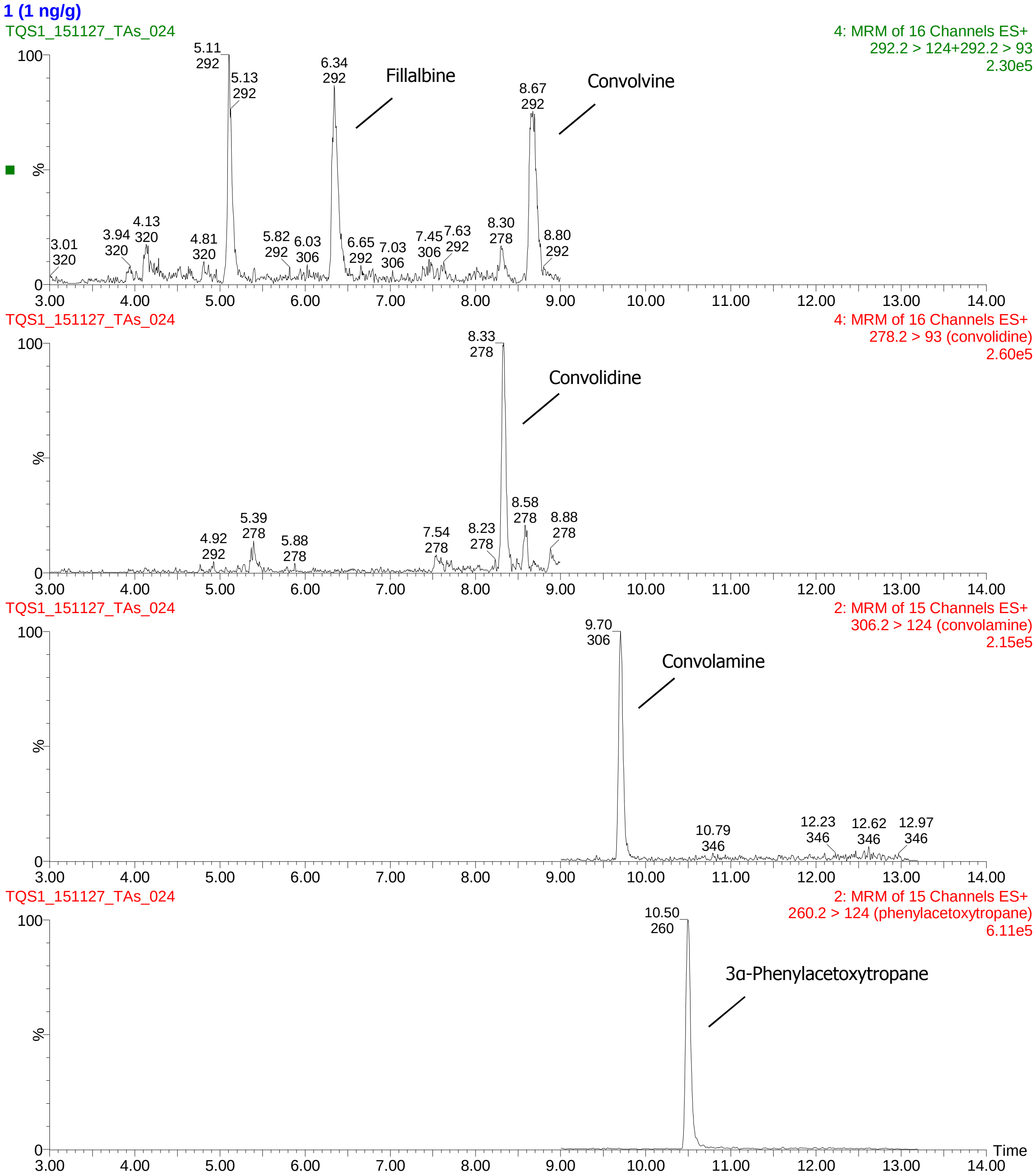
Appendix G.4 LC-MS/MS MRM chromatograms for blank buckwheat flour, spiked with internal standards atropine-d_3 and scopolamine-d_3 at 10 µg/kg



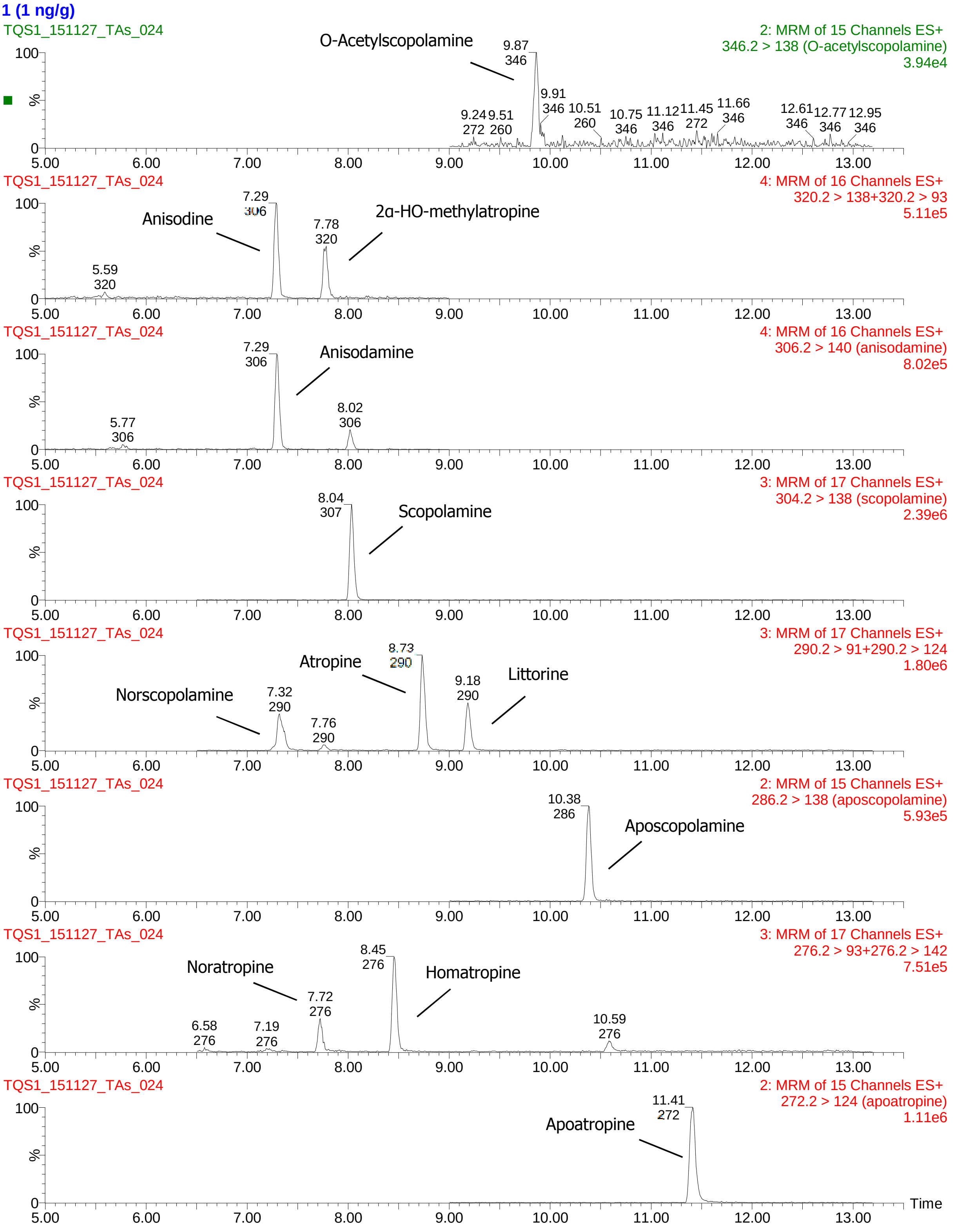
Appendix G.5 LC-MS/MS MRM chromatograms for blank buckwheat flour, spiked with low molecular weight TAs at 5 µg/kg



Appendix G.6 LC-MS/MS MRM chromatograms for blank buckwheat flour, spiked with convolvulaceae-type TAs at 1 µg/kg



Appendix G.7 LC-MS/MS MRM chromatograms for blank buckwheat flour, spiked with Datura-type TAs at 1 µg/kg



Appendix H – RIKILT in-house validation results for Datura TAs

Appendix H.1 Limits of Detection (LOD) and Limits of Quantification (LOQ) for the TAs incorporated in the Datura TA method obtained during in-house validation at RIKILT

Compound name	Single flours and cereal-based products		Dry (herbal) tea		Herbal tea infusion		Legumes, frozen beans and stir-fry mixes	
	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/l)	LOQ (µg/l)	LOD[(a)] (µg/kg)	LOQ[(a)] (µg/kg)
Low molecular weight TAs								
6-Hydroxytropinone	0.5	5.0	2.5	5.0	0.0133	0.0333	0.5	5.0
Nortropinone	0.5	2.5	2.5	5.0	0.0133	0.0333	0.5	2.5
Pseudotropine	1.0	2.5	2.5	5.0	0.0133	0.0333	1.0	2.5
Scopine	0.5	2.5	2.5	5.0	0.0133	0.0333	0.5	2.5
Scopoline	0.5	2.5	2.5	5.0	0.0133	0.0333	0.5	2.5
Tropine	0.5	2.5	2.5	5.0	0.0133	0.0333	0.5	2.5
Tropinone	0.5	2.5	1.0	2.5	0.0133	0.0333	0.5	2.5
Convolvulaceae-type TAs								
Convolamine	0.1	0.5	0.25	1.0	0.0017	0.0067	0.1	0.5
Convolidine	0.1	0.5	0.25	1.0	0.0017	0.0067	0.1	0.5
Convolvine	0.1	1.0	0.25	1.0	0.0033	0.0133	0.1	1.0
Fillalbine	0.1	0.5	0.25	1.0	0.0017	0.0067	0.1	0.5
Datura-type TAs								
O-Acetylscopolamine	0.1	0.5	0.1	0.5	0.0017	0.0067	0.1	0.5
Anisodamine	0.1	0.5	0.1	0.5	0.0017	0.0067	0.1	0.5
Anisodine	0.1	0.5	0.1	0.5	0.0017	0.0067	0.1	0.5
Apoatropine	0.1	1.0	0.1	0.5	0.0017	0.0067	0.1	1.0
Aposcopolamine	0.1	0.5	0.1	0.5	0.0017	0.0067	0.1	0.5
Atropine	0.05	0.5	0.1	0.5	0.0017	0.0067	0.05	0.5
Homatropine	0.1	0.5	0.1	0.5	0.0017	0.0067	0.1	0.5
2α-Hydroxymethyl atropine	0.1	0.5	0.1	0.5	0.0017	0.0067	0.1	0.5
Littorine	0.1	0.5	0.1	0.5	0.0017	0.0067	0.1	0.5
Noratropine	0.1	0.5	0.25	1.0	0.0033	0.0067	0.1	0.5
Norscopolamine	0.1	0.5	0.25	1.0	0.0033	0.0133	0.1	0.5
Phenylacetoxytropane	0.25	0.5	0.1	0.5	0.0017	0.0067	0.25	0.5
Scopolamine	0.05	0.5	0.1	0.5	0.0017	0.0067	0.05	0.5

(a): Estimation based on standard addition to the individual samples.

Appendix H.2 Average recovery (n = 6) of individual TAs and repeatability (n = 6) at three concentration levels in cereal-based foods (breakfast cereals, bread, biscuits and buckwheat flour) obtained during in-house validation at RIKILT

Compound name	Recovery (10 µg/kg) (%)	RSD recovery (10 µg/kg) (%)	Repeatability (%)		
			1 µg/kg	5 µg/kg	25 µg/kg
Low molecular weight TAs					
6-Hydroxytropinone	61	20	n.a.	50	36
Nortropinone	78	20	n.a.	14	11
Pseudotropine	86	15	n.a.	13	15
Scopine	77	17	n.a.	19	23
Scopoline	80	10	n.a.	12	11
Tropine	84	14	n.a.	5	10
Tropinone	64	16	n.a.	22	5
Convolvulaceae-type TA					
Convolamine	80	17	19	7	6
Convolidine	81	18	15	9	10
Convolvine	80	11	34	23	26
Fillalbine	89	12	14	4	5
Datura-type TAs					
O-Acetylscopolamine	85	17	21	9	6
Anisodamine	105	9	12	5	4
Anisodine	105	6	9	4	5
Apoatropine	63	33	33	12	12
Aposcopolamine	71	19	23	12	8
Atropine[(a)]	98	7	10	3	2
Homatropine	99	5	12	5	4
2α-Hydroxymethyl atropine[(b)]	95	9			
Littorine	96	8	19	5	3
Noratropine	67	22	17	8	6
Norscopolamine	101	5	10	5	5
Phenylacetoxytropane[(b)]	72	22			
Scopolamine[(a)]	103	6	13	9	3

n.a. = No acceptable results obtained.
(a): Internal standard corrected.
(b): Not included in the original validation.

Appendix H.3 Average recovery (n = 3) of individual TAs and repeatability (n = 6) at three concentration levels in dry (herbal) teas (green tea, peppermint tea, mixed herbal tea) obtained during in-house validation at RIKILT

Compound name	Recovery (10 µg/kg) (%)	RSD recovery (10 µg/kg) (%)	Repeatability (%)		
			1 µg/kg	5 µg/kg	25 µg/kg
Low molecular weight TAs					
6-Hydroxytropinone	46	29	n.a.	6	19
Nortropinone	55	36	n.a.	11	21
Pseudotropine	21	54	n.a.	2	25
Scopine	20	50	n.a.	7	16
Scopoline	36	55	n.a.	9	15
Tropine	41	43	n.a.	11	23
Tropinone	59[(b)]	12[(b)]	n.a.	6	21
Convolvulaceae-type TAs					
Convolamine	49	13	12	16	9
Convolidine	65	12	6	19	18
Convolvine	55	20	11	21	17
Fillalbine	59	9	10	16	13
Datura-type TAs					
O-Acetylscopolamine	30	24	9[(c)]	4	16
Anisodamine	58	21	24	6	18
Anisodine	45	53	35	11	15
Apoatropine	47	10	6	23	12
Aposcopolamine	52	23	7	12	13
Atropine[(a)]	64	15	5	3	4
Homatropine	61	11	11	10	16
2α-Hydroxymethyl atropine	51	29	28	8	23
Littorine	65	8	9	13	14
Noratropine	73	8	6	15	17
Norscopolamine	62	26	36	1	16
Phenylacetoxytropane	56	6	6	17	7
Scopolamine[(a)]	51	48	4	3	4

n.a. = No acceptable results obtained.
(a): Internal standard corrected.
(b): Excluding peppermint tea.
(c): Excluding mixed herbal tea.

Appendix H.4 Average recovery (n = 3) of individual TAs and repeatability (n = 6) at three concentration levels in (herbal) tea infusions (green tea, peppermint tea, mixed herbal tea) obtained during in-house validation at RIKILT

Compound name	Recovery (10 µg/kg) (%)	RSD recovery (10 µg/kg) (%)	Repeatability (%)		
			0.0133 µg/l	0.0667 µg/l	0.3333 µg/l
Low molecular weight TAs					
6-Hydroxytropinone	77	22	n.a.	23	8
Nortropinone	104	21	n.a.	15	9
Pseudotropine	69	44	n.a.	19	7
Scopine	51	44	n.a.	19	9
Scopoline	64	33	n.a.	18	8
Tropine	88	16	n.a.	10	7
Tropinone	72	19	n.a.	8	8
Convolvulaceae-type TAs					
Convolamine	84	8	10	8	5
Convolidine	77	24	22	17	12
Convolvine	70	7	19	9	7
Fillalbine	89	14	7	10	5
Datura-type TAs					
O-Acetylscopolamine	91	2	7	7	4
Anisodamine	100	14	5	7	4
Anisodine	102	15	7	9	4
Apoatropine	75	8	8	9	7
Aposcopolamine	82	7	5	8	5
Atropine[(a)]	92	6	9	3	2
Homatropine	98	12	5	8	5
2α-Hydroxymethyl atropine	97	10	7	5	5
Littorine	98	10	6	6	3
Noratropine	95	16	10	10	5
Norscopolamine	103	14	9	7	4
Phenylacetoxytropane	87	6	5	8	5
Scopolamine[(a)]	90	6	2	5	2

n.a. = No acceptable results obtained.
(a): Internal standard corrected.

Appendix I – IRTA in-house validation results for Datura TAs

Samples were analysed using a Waters Acquity UPLC coupled to a Waters TQ-D tandem mass spectrometer (Waters).

Appendix I.1 Chromatography

UPLC conditions:

- Column: Waters UPLC BEH C18 100 x 1.0 mm, 1.7 µm (Waters 186002346).
- Column temperature: 40°C.
- Mobile phase A: 6.65 mM ammonia in water.
- Mobile phase B: 1.30 mM ammonia in acetonitrile.
- Flow: 0.15 ml/min.
- Injection volume: 2 µL.
- Gradient (linear):

Time (min)	Mobile phase A	Mobile phase B
0.0	100%	0%
0.5	100%	0%
12.0	45%	55%
12.5	100%	0%
14.0	100%	0%

- Total run time: 14 min.

Appendix I.2 MS Tune settings:

- Ionisation mode: positive electrospray (ESI+)
- Source temperature: 135°C.
- Desolvation gas temperature: 400°C.
- Desolvation gas flow: 400 L/h.
- Cone gas flow: 25 L/h.
- Cone voltage: 30 V.
- Capillary voltage: 3.0 kV.
- Argon collision gas pressure: 4.0 mbar.

Appendix I.3 MRM conditions optimised for Datura TAs

Compound name	Precursor mass (Da)	Fragment 1 (Da)	Collision energy 1 (eV)	Fragment 2 (Da)	Collision energy 2 (eV)	Retention time (min)
Low molecular weight TAs						
6-OH-tropinone	155.9	81.0	20	**98.0**	**20**	2.2
Nortropinone	126.1	67.2	20	**84.1**	**16**	2.7
Pseudotropine	142.1	**96.1**	**22**	124.1	20	3.7
Scopine	155.9	73.1	18	**84.1**	**15**	2.4
Scopoline	156.0	110.0	24	**138.0**	**22**	3.1
Tropine	142.1	93.0	24	**98.1**	**24**	3.7
Tropinone	140.1	82.1	20	**98.1**	**20**	3.6
Convulvalaceae-type TAs						
Convolamine	306.0	93.1	33	**124.1**	**25**	9.5
Convolidine	277.9	**93.0**	**26**	110.1	20	3.3
Convolvine	291.9	**93.1**	**26**	165.0	30	9.3
Fillalbin	291.9	93.1	30	**124.1**	**24**	4.0
Datura-type TAs						
Acetylscopolamine	346.0	131.0	20	**138.1**	**25**	8.6
Anisodamine	305.9	122.0	32	**140.1**	**26**	6.1
Anisodine	320.0	138.0	22	**156.0**	**18**	5.7
Apoatropine	272.0	93.0	25	**124.0**	**20**	12.1
Aposcopolamine	285.9	103.1	28	**138.1**	**20**	9.1
Atropine	289.9	93.0	32	**124.1**	**25**	8.4
Atropine-d_3	293.2	93.0	25	**127.0**	**25**	8.4
Homatropine	276.0	**124.0**	**25**	142.1	30	7.9
a-HO-methylatropine	320.2	93.0	35	**124.0**	**25**	7.1
Littorine	290.0	93.1	35	**124.1**	**25**	8.9
Noratropine	276.0	77.1	55	**110.0**	**22**	8.1
Norscopolamine	289.9	124.1	18	**142.0**	**16**	5.8
Phenylacetoxytropane	260.2	91.0	35	**124.0**	**20**	11
Scopolamine	303.9	**138.0**	**24**	156.0	20	6.6
Scopolamine-d_3	307.2	**141.0**	**24**	159.0	25	6.6

In bold = quantification fragment.

Appendix I.4 Limits of Detection (LOD) and Limits of Quantification (LOQ) for the TAs incorporated in the Datura TA method obtained during in-house validation at IRTA

Compound name	Single flours and cereal-based products		Dry herbal tea		Legumes, frozen beans and stir-fry mixes	
	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD(a) (µg/kg)	LOQ(a) (µg/kg)
Low molecular weight TAs						
6-Hydroxytropinone	1.0	5.0	1.0	5.0	1.0	5.0
Nortropinone	0.5	2.5	1.0	5.0	1.0	5.0
Pseudotropine	0.5	2.5	1.0	5.0	1.0	5.0
Scopine	0.5	2.5	1.0	5.0	1.0	5.0
Scopoline	0.5	2.5	1.0	5.0	1.0	5.0
Tropine	1.0	5.0	1.0	5.0	1.0	5.0
Tropinone	1.0	5.0	1.0	2.5	1.0	2.5
Convolvulaceae-type TAs						
Convolamine	0.25	1.0	0.25	1.0	0.25	1.0
Convolidine	0.5	2.5	0.25	1.0	0.5	1.0
Convolvine	0.25	1.0	0.25	1.0	0.25	1.0
Fillalbine	0.25	1.0	0.25	1.0	0.25	1.0
Datura-type TAs						
O-Acetylscopolamine	0.5	2.5	1.0	5.0	0.5	5.0
Anisodamine	0.25	1.0	0.25	1.0	0.25	1.0
Anisodine	0.25	1.0	0.25	1.0	0.25	1.0
Apoatropine	0.25	1.0	0.5	1.0	0.25	1.0
Aposcopolamine	0.25	1.0	0.25	1.0	0.25	1.0
Atropine	0.2	1.0	0.2	1.0	0.2	1.0
Homatropine	0.25	1.0	0.25	1.0	0.25	1.0
2α-Hydroxymethyl atropine	0.25	1.0	0.25	1.0	0.25	1.0
Littorine	0.25	1.0	0.25	1.0	0.25	1.0
Noratropine	0.25	1.0	0.25	1.0	0.25	1.0
Norscopolamine	0.25	1.0	0.25	1.0	0.25	1.0
Phenylacetoxytropane	0.25	1.0	0.25	1.0	0.25	1.0
Scopolamine	0.2	1.0	0.2	1.0	0.2	1.0

(a): Estimation based on standard addition to the individual samples.

Appendix I.5 Average recovery (n = 4) of individual TAs and repeatability (n = 6) at three concentration levels in cereal-based foods (breakfast cereals, breakfast cereals for children, bread, biscuits) obtained during in-house validation at IRTA

Compound name	Recovery (10 µg/kg) (%)	RSD recovery (10 µg/kg) (%)	Repeataility (%)		
			1 µg/kg	5 µg/kg	25 µg/kg
Low molecular weight TAs					
6-Hydroxytropinone	83	16	n.a.	24	24
Nortropinone	79	13	n.a.	32	31
Pseudotropine	82	29	n.a.	8	20
Scopine	100	14	n.a.	9	6
Scopoline	91	13	n.a.	11	8
Tropine	94	33	n.a.	9	7
Tropinone	99	34	n.a.	7	8
Convolvulaceae-type TAs					
Convolamine	72	11	21	24	10
Convolidine	99	16	n.a.	16	23
Convolvine	81	10	11	27	n.a.
Fillalbine	90	2	17	10	6
Datura-type TAs					
O-Acetylscopolamine	76	41	34	9	7
Anisodamine	86	8	20	12	10
Anisodine	84	6	14	7	5
Apoatropine	76	9	16	7	15
Aposcopolamine	73	14	20	33	32
Atropine[(a)]	93	7	7	5	4
Homatropine	79	9	12	4	9
2α-Hydroxymethyl atropine	80	8	24	13	16
Littorine	81	6	36	15	13
Noratropine	83	7	26	4	7
Norscopolamine	86	10	13	5	2
Phenylacetoxytropane	80	8	25	7	18
Scopolamine[(a)]	96	6	14	7	5

n.a. = No acceptable results obtained.
(a): Internal standard corrected.

Appendix I.6 Recovery of individual TAs and repeatability (n = 3) at three concentration levels in dry green tea obtained during in-house validation at IRTA

Compound name	Recovery (10 µg/kg) (%)	Repeatability (%)		
		1 µg/kg	5 µg/kg	25 µg/kg
Low molecular weight TAs				
6-Hydroxytropinone	107	n.a.	10	6
Nortropinone	66	n.a.	16	4
Pseudotropine	38	n.a.	18	13
Scopine	72	n.a.	14	6
Scopoline	92	n.a.	20	13
Tropine	98	n.a.	6	18
Tropinone	106	29	7	12
Convolvulaceae-type TAs				
Convolamine	105	3	6	2
Convolidine	93	18	4	3
Convolvine	99	2	10	6
Fillalbine	94	6	9	5
Datura-type TAs				
O-Acetylscopolamine	-(b)	-(b)	-(b)	-(b)
Anisodamine	100	4	5	7
Anisodine	78	15	2	4
Apoatropine	29	n.a.	8	18
Aposcopolamine	74	15	9	17
Atropine(a)	96	5	6	3
Homatropine	90	19	14	6
2α-Hydroxymethyl atropine	108	13	7	6
Littorine	90	2	2	5
Noratropine	113	18	12	8
Norscopolamine	99	10	5	5
Phenylacetoxytropane	80	8	6	14
Scopolamine(a)	96	9	6	4

n.a. = No acceptable results obtained.
(a): Internal standard corrected.
(b): Not determined.

Appendix J – FERA in-house validation results for Datura TAs

Samples were analysed using an Acquity UPLC system coupled to a Xevo TQ-S tandem quadrupole mass spectrometer (Waters).

Appendix J.1 Chromatography

UPLC conditions:

- Column: Waters UPLC BEH C18 150 x 2.1 mm, 1.7 µm (Waters 186002353).
- Column temperature: 50^{o}C.
- Injection volume: 2 µL.
- Mobile phase A: 10 mM ammonium carbonate in water.
- Mobile phase B: Acetonitrile.
- Gradient (linear) timetable:

Time (min)	Mobile phase A	Mobile phase B
0.0	100%	0%
2.0	100%	0%
12.0	50%	50%
12.2	0%	100%
16.0	0%	100%
16.2	100%	0%
20.0	100%	0%

The gradient profile differs from that in the RIKILT SOP by the addition of the flush step at 100% B.

- Total run time is 20 min.

Appendix J.2 MS Tune settings:

- Ionisation mode: positive electrospray (ESI+).
- Capillary voltage: 1 kV.
- Desolvation temperature: 500^{o}C.
- Desolvation gas flow rate: 1000 L/h.
- Nebuliser gas flow: 7 bar.
- Source temperature: 150^{o}C.
- Cone gas flow rate: 100 L/h.
- Collision gas flow rate: 0.15 mL/min (3.5 mbar).

- MRM conditions: Same as SOP RIKILT (see Appendix G.3).

Appendix J.3 Limits of Detection (LOD) and Limits of Quantification (LOQ) for the TAs incorporated in the Datura TA method obtained during in-house validation at FERA

Compound name	Single flours and cereal-based products		Dry herbal tea		Legumes, frozen beans and stir-fry mixes	
	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD[(a)] (µg/kg)	LOQ[(a)] (µg/kg)
Low molecular weight TAs						
6-Hydroxytropinone	0.25	1.0	0.25	1.0	0.5	2.5
Nortropinone	2.5	5.0	2.5	5.0	0.5	2.5
Pseudotropine	2.5	5.0	2.5	5.0	0.5	2.5
Scopine	0.5	2.5	0.5	2.5	0.5	2.5
Scopoline	2.5	5.0	2.5	5.0	0.5	2.5
Tropine	0.25	1.0	0.25	1.0	0.5	2.5
Tropinone	0.25	1.0	0.25	1.0	0.5	2.5
Convolvulaceae-type TAs						
Convolamine	0.25	1.0	0.25	1.0	0.1	0.5
Convolidine	0.25	1.0	0.25	1.0	0.5	1.0
Convolvine	0.25	1.0	0.25	1.0	0.25	1.0
Fillalbine	0.1	0.5	0.1	0.5	0.25	1.0
Datura-type TAs						
O-Acetylscopolamine	0.1	0.5	0.1	0.5	0.1	0.5
Anisodamine	0.1	0.5	0.1	0.5	0.1	0.5
Anisodine	0.1	0.5	0.1	0.5	0.1	0.5
Apoatropine	0.1	0.5	0.1	0.5	0.1	0.5
Aposcopolamine	0.1	0.5	0.1	0.5	0.25	1.0
Atropine	0.05	0.5	0.05	0.5	0.05	0.5
Homatropine	0.1	0.5	0.1	0.5	0.25	1.0
2α-Hydroxymethyl atropine	0.1	0.5	0.1	0.5	0.1	0.5
Littorine	0.25	0.5	0.25	0.5	0.25	1.0
Noratropine	0.1	0.5	0.1	0.5	0.25	1.0
Norscopolamine	0.1	0.5	0.1	0.5	0.25	1.0
Phenylacetoxytropane	0.1	0.5	0.1	0.5	0.2	1.0
Scopolamine	0.05	0.5	0.05	0.5	0.05	0.5

(a): Estimation based on standard addition to the individual samples.

Appendix J.4 Average recovery (n = 4) of individual TAs and repeatability (n = 6) at three concentration levels in cereal-based foods (breakfast cereals, breakfast cereals for children, bread, biscuits) obtained during in-house validation at FERA

Compound name	Recovery (10 µg/kg) (%)	RSD recovery (10 µg/kg) (%)	Repeatability (%)		
			1 µg/kg	10 µg/kg	25 µg/kg
Low molecular weight TAs					
6-Hydroxytropinone	86	7	6	7	4
Nortropinone	82	16	n.a.	28	15
Pseudotropine	87	4	n.a.	4	4
Scopine	87	3	5	6	3
Scopoline	80	8	n.a.	18	8
Tropine	88	5	3	6	5
Tropinone	59	28	8	5	13
Convolvulaceae-type TAs					
Convolamine	114	20	42	27	30
Convolidine	88	15	23	18	42
Convolvine	133	21	5	4	5
Fillalbine	89	12	6	4	3
Datura-type TAs					
O-Acetylscopolamine	16[(b)]	39[(b)]	9	12	29
Anisodamine	94	10	7	6	5
Anisodine	100	12	9	5	14
Apoatropine	99	22	6	26	16
Aposcopolamine	118	15	14	21	15
Atropine[(a)]	95	16	6	7	23
Homatropine	99	12	13	13	12
2α-Hydroxymethyl atropine	93	5	7	5	5
Littorine	126	25	11	14	21
Noratropine	93	17	10	4	4
Norscopolamine	102	19	12	3	19
Phenylacetoxytropane	91	28	11	18	11
Scopolamine[(a)]	105	9	4	5	9

n.a. = No acceptable results obtained.
(a): Internal standard corrected.
(b): Excluding breakfast cereals (recovery: 88%).

Appendix J.5 Recovery of individual TAs and repeatability (n = 6) at three concentration levels in dry green tea obtained during in-house validation at FERA

Compound name	Recovery (10 µg/kg) (%)	Repeatability (%)		
		1 µg/kg	5 µg/kg	25 µg/kg
Low molecular weight TAs				
6-Hydroxytropinone	46	5	19	3
Nortropinone	36	9	10	6
Pseudotropine	80	3	3	1
Scopine	74	4	3	2
Scopoline	75	7	3	2
Tropine	82	4	2	1
Tropinone	38	6	7	5
Convolvulaceae-type TAs				
Convolamine	32	3	7	6
Convolidine	58	3	5	4
Convolvine	40	2	4	4
Fillalbine	48	2	5	5
Datura-type TAs				
O-Acetylscopolamine	20	16	12	9
Anisodamine	83	1	2	3
Anisodine	84	3	1	3
Apoatropine	38	3	6	5
Aposcopolamine	58	1	8	4
Atropine[(a)]	89	4	7	9
Homatropine	69	2	5	5
2α-Hydroxymethyl atropine	76	1	3	4
Littorine	67	1	5	5
Noratropine	75	2	2	3
Norscopolamine	88	3	1	2
Phenylacetoxytropane	53	2	5	5
Scopolamine[(a)]	89	4	6	7

(a): Internal standard corrected.

Appendix K – UCT in-house validation results for Datura TAs

Samples were analysed using a Agilent Technologies 1290 Infinity II HPLC system coupled to an Agilent Technologies 6495 LC-MS/MS (Agilent Technologies).

Appendix K.1 Chromatography

UPLC conditions:

- Column: Waters UPLC BEH C18 150 x 2.1 mm, 1.7 µm (Waters 186002353).
- Column temperature: 50°C.
- Mobile phase A: 6.65 mM ammonia in water.
- Mobile phase B: 1.30 mM ammonia in acetonitrile.
- Flow: 0.4 ml/min.
- Injection volume: 2 µL.
- Gradient (linear):

Time (min)	Mobile phase A	Mobile phase B
0.0	100%	0%
2.0	100%	0%
12.0	30%	70%
12.2	0%	100%
14.0	0%	100%
14.1	100%	0%
17.0	100%	0%

- Total run time: 17 min.

Appendix K.2 MS Tune settings

- Ionisation mode: positive electrospray (ESI+).
- Gas Temp: 200°C.
- Gas Flow: 12 l/min.
- Nebulizer: 20 psi.
- Sheath Gas Temp: 400°C.
- Sheat Gas Flow: 11 l/min.
- Capillary: 3500 V.
- Nozzle Voltage: 500 V.

iFunnel parameters:

- High Pressure RF: 200 V.
- Low Pressure RF: 100 V.

MassHunter Workstation Software:

- LC/MS Data Acquisition version B.08.00.
- Qualitative Analysis version B.07.00.
- Quantitative Analysis version B.07.00.
- Optimizer version B.08.00.
- Source and iFunnel Optimizer version B.08.00.

Appendix K.3 MS/MS conditions used in MRM method for Datura TAs

Compound name	Precursor mass (Da)	Fragment 1 (Da)	Collision energy 1 (eV)	Fragment 2 (Da)	Collision energy 2 (eV)	Fragment 3 (Da)	Collision energy 3 (eV)	Retention time (min)
Low molecular weight TAs								
6-OH-tropinone	156.1	58.0	44	81.0	20	98.0	20	4.1
Nortropinone	126.1	67.0	28	68.0	15	84.0	16	4.61
Pseudotropine	142.1	67.0	36	96.0	24	124.0	20	5.60
Scopine	156.1	73.0	15	84.0	15	98.0	15	4.19
Scopoline	156.1	81.0	20	110.0	24	138.0	20	5.22
Tropine	142.1	58.0	32	93.0	20	98.0	28	5.81
Tropinone	140.1	57.0	36	82.0	20	98.0	20	5.49
Convulvalaceae-type TAs								
Convolamine	306.2	91.0	48	93.0	36	124.0	24	10.17
Convolidine	278.2	93.0	24	110.0	24	151.0	28	5.11
Convolvine	292.2	93.0	20	110.0	24	165.0	30	9.80
Fillalbin	292.2	91.0	48	93.0	32	124.0	24	5.73
Datura-type TAs								
Acetylscopolamine	346.2	103.0	32	138.0	20	43.0	64	9.46
Anisodamine	306.2	91.0	35	122.0	32	140.0	28	7.31
Anisodine	320.2	91.0	40	138.0	24	156.0	16	7.11
Apoatropine	272.2	91.0	35	93.0	25	124.0	20	12.06
Aposcopolamine	286.2	103.0	40	110.0	20	138.0	12	9.94
Atropine	290.2	91.0	48	93.0	36	124.0	24	9.34
Atropine-d_3	293.2	93.0	25	127.0	20			9.34
Homatropine	276.2	93.0	36	124.0	24	142.0	28	9.05
a-HO-methylatropine	320.2	91.0	35	93.0	35	124.0	25	8.26
Littorine	290.2	93.0	25	124.0	24	142.0	36	9.76
Noratropine	276.2	91.0	40	93.0	28	110.0	24	8.80
Norscopolamine	290.2	121.0	20	124.0	16	142.0	16	7.13
Phenylacetoxytropane	260.2	91.0	35	93.0	25	124.0	20	11.33
Scopolamine	304.2	103.0	44	138.0	20	156.0	16	7.80
Scopolamine-d_3	307.2	141.0	24	159.0	25			7.80

Appendix K.4 Limits of Detection (LOD) and Limits of Quantification (LOQ) for the TAs incorporated in the Datura TA method obtained during in-house validation at UCT

Compound name	Single flours and cereal-based products		Dry herbal tea		Legumes, frozen beans and stir-fry mixes	
	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD[(a)] (µg/kg)	LOQ[(a)] (µg/kg)
Low molecular weight TAs						
6-Hydroxytropinone	0.5	1.0	0.5	1.0	0.5	1.0
Nortropinone	0.5	1.0	0.5	1.0	0.5	1.0
Pseudotropine	0.5	1.0	0.5	1.0	0.5	1.0
Scopine	0.5	1.0	0.5	1.0	0.5	1.0
Scopoline	0.5	1.0	0.5	1.0	0.5	1.0
Tropine	0.5	1.0	0.5	1.0	0.5	1.0
Tropinone	0.1	0.5	0.25	1.0	0.1	0.5
Convolvulaceae-type TAs						
Convolamine	0.05	0.5	0.1	0.5	0.05	0.5
Convolidine	0.05	0.5	0.1	0.5	0.05	0.5
Convolvine	0.5	1.0	0.5	1.0	0.5	1.0
Fillalbine	0.1	0.5	0.1	0.5	0.1	0.5
Datura-type TAs						
O-Acetylscopolamine	0.05	0.5	0.1	0.5	0.05	0.5
Anisodamine	0.05	0.5	0.1	0.5	0.05	0.5
Anisodine	0.05	0.5	0.1	0.5	0.05	0.5
Apoatropine	0.05	0.5	0.1	0.5	0.05	0.5
Aposcopolamine	0.05	0.5	0.1	0.5	0.05	0.5
Atropine	0.05	0.5	0.05	0.5	0.05	0.5
Homatropine	0.5	1.0	0.5	1.0	0.5	1.0
2α-Hydroxymethyl atropine	0.1	0.5	0.1	0.5	0.1	0.5
Littorine	0.05	0.5	0.1	0.5	0.05	0.5
Noratropine	0.5	1.0	0.5	1.0	0.5	1.0
Norscopolamine	0.05	0.5	0.1	0.5	0.05	0.5
Phenylacetoxytropane	0.1	0.5	0.1	0.5	0.1	0.5
Scopolamine	0.05	0.5	0.05	0.5	0.05	0.5

(a): Estimation based on standard addition to the individual samples.

Appendix K.5 Average recovery (n = 4) of individual TAs and average repeatability (n = 3) at three concentration levels in cereal-based foods (breakfast cereals, breakfast cereals for children, bread, biscuits) obtained during in-house validation at UCT

Compound name	Recovery (10 µg/kg) (%)	RSD recovery (10 µg/kg) (%)	Repeatability (%)		
			1 µg/kg	5 µg/kg	25 µg/kg
Low molecular weight TAs					
6-Hydroxytropinone	94	19	10	8	3
Nortropinone	87	20	9	5	3
Pseudotropine	82	5	10	8	4
Scopine	84	5	9	7	4
Scopoline	107	9	10	8	4
Tropine	105	15	11	6	3
Tropinone	102	13	9	7	3
Convolvulaceae-type TAs					
Convolamine	92	22	9	6	2
Convolidine	82	13	9	8	5
Convolvine	83	11	9	8	5
Fillalbine	99	22	9	6	4
Datura-type TAs					
O-Acetylscopolamine	106	8			
Anisodamine	101	20	9	7	5
Anisodine	99	12	9	7	5
Apoatropine	85	17	9	8	3
Aposcopolamine	88	24	8	4	3
Atropine[(a)]	93	14	9	6	3
Homatropine	98	3	9	6	3
2α-Hydroxymethyl atropine	98	20	9	6	4
Littorine	99	10	7	5	2
Noratropine	102	9	9	5	2
Norscopolamine	94	12	8	6	4
Phenylacetoxytropane	90	23	8	5	3
Scopolamine[(a)]	92	17	7	5	3

n.a. = No acceptable results obtained.
(a): Internal standard corrected.

Appendix K.6 Recovery of individual TAs and repeatability (n = 6) at three concentration levels in dry green tea obtained during in-house validation at UCT

Compound name	Recovery (10 µg/kg) (%)	Repeatability (%)		
		1 µg/kg	5 µg/kg	25 µg/kg
Low molecular weight TAs				
6-Hydroxytropinone	68	10	9	6
Nortropinone	72	9	15	3
Pseudotropine	56	11	12	5
Scopine	66	11	13	5
Scopoline	83	10	17	4
Tropine	85	11	10	2
Tropinone	66	13	7	4
Convolvulaceae-type TAs				
Convolamine	151	8	17	3
Convolidine	99	10	9	1
Convolvine	127	9	13	2
Fillalbine	135	11	13	3
Datura-type TAs				
O-Acetylscopolamine	53	11	12	3
Anisodamine	95	8	8	2
Anisodine	90	6	10	1
Apoatropine	124	10	19	1
Aposcopolamine	118	10	15	4
Atropine[(a)]	112	7	10	3
Homatropine	108	11	10	3
2α-Hydroxymethyl atropine	101	12	9	4
Littorine	108	7	10	2
Noratropine	94	11	7	3
Norscopolamine	89	11	9	3
Phenylacetoxytropane	113	13	15	1
Scopolamine[(a)]	117	11	7	5

(a): Internal standard corrected.

Appendix L – RIKILT LC-MS/MS conditions for calystegine TAs

Samples were analysed using an Acquity UPLC system coupled to a Xevo TQ-S tandem quadrupole mass spectrometer (Waters).

Appendix L.1 Chromatography

HPLC Method:

- Column: Chirobiotic V column (150 x 2.1 mm, 5 µm, 100Å) (Astec, Sigma-Aldrich).
- Column temperature: 35°C.
- Injection volume: 10 µL.
- Mobile phase A: 5 mM ammonium acetate in water pH 7.0.
- Mobile phase B: Methanol.
- Gradient (linear) timetable:

Time (min)	Mobile phase A	Mobile phase B
0.0	100%	0%
8.0	100%	0%
8.5	50%	50%
11.5	50%	50%
12.0	100%	0%
15.0	100%	0%

- Total run time is 15 min.

Appendix L.2 MS Tune settings

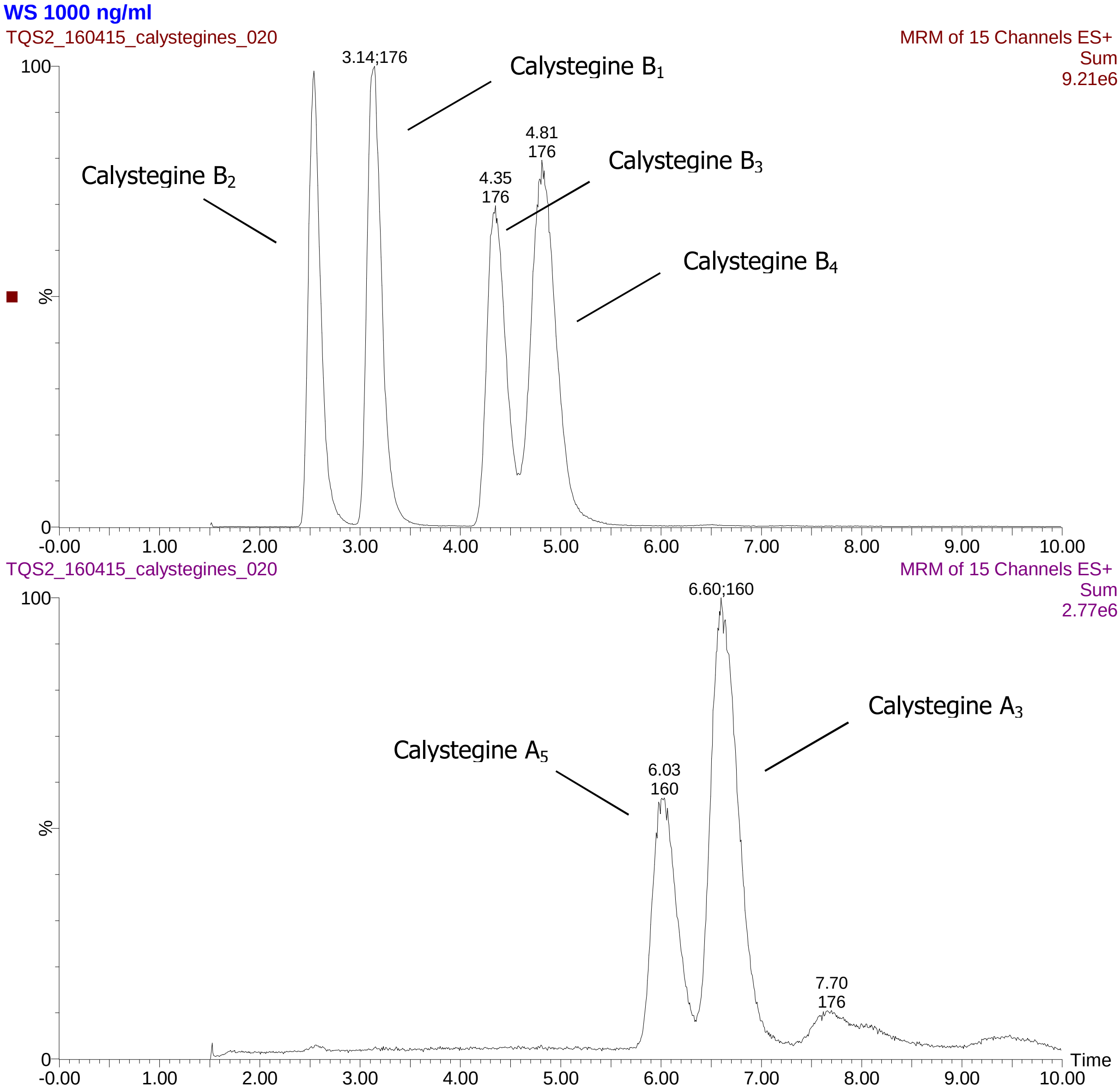
- Ionisation mode: positive electrospray (ESI+).
- Capillary voltage: 3 kV.
- Desolvation temperature: 600°C.
- Desolvation gas flow rate: 800 L/h.
- Nebuliser gas flow: 7 bar.
- Source temperature: 150°C.
- Cone gas flow rate: 100 L/h.
- Collision gas: argon.
- Collision gas flow rate: 0.18 mL/min (4.2 mbar).

Appendix L.3 MRM conditions optimised for calystegine alkaloids

Compound name	Precursor mass (Da)	Fragment 1 (Da)	Collision energy 1 (eV)	Fragment 2 (Da)	Collision energy 2 (eV)	Fragment 3 (Da)	Collision energy 3 (eV)	Fragment 4 (Da)	Collision energy 4 (eV)	Retention time (min)
Calystegine A_3	160.1	**142.0**	**10**	125.0	15	98.2	15	79.2	20	6.70
Calystegine A_5	160.1	142.0	10	124.0	10	**81.2**	**15**	79.2	20	5.90
Calystegine B_1	176.1	**140.0**	**10**	123.0	15	112.0	15	95.2	15	2.90
Calystegine B_2	176.1	158.0	10	**140.0**	**10**	97.2	15	95.2	15	2.40
Calystegine B_3	176.1	158.0	10	116.0	15	**98.2**	**15**	67.2	20	4.20
Calystegine B_4	176.1	140.0	10	123.0	15	**112.0**	**20**	98.2	15	4.70

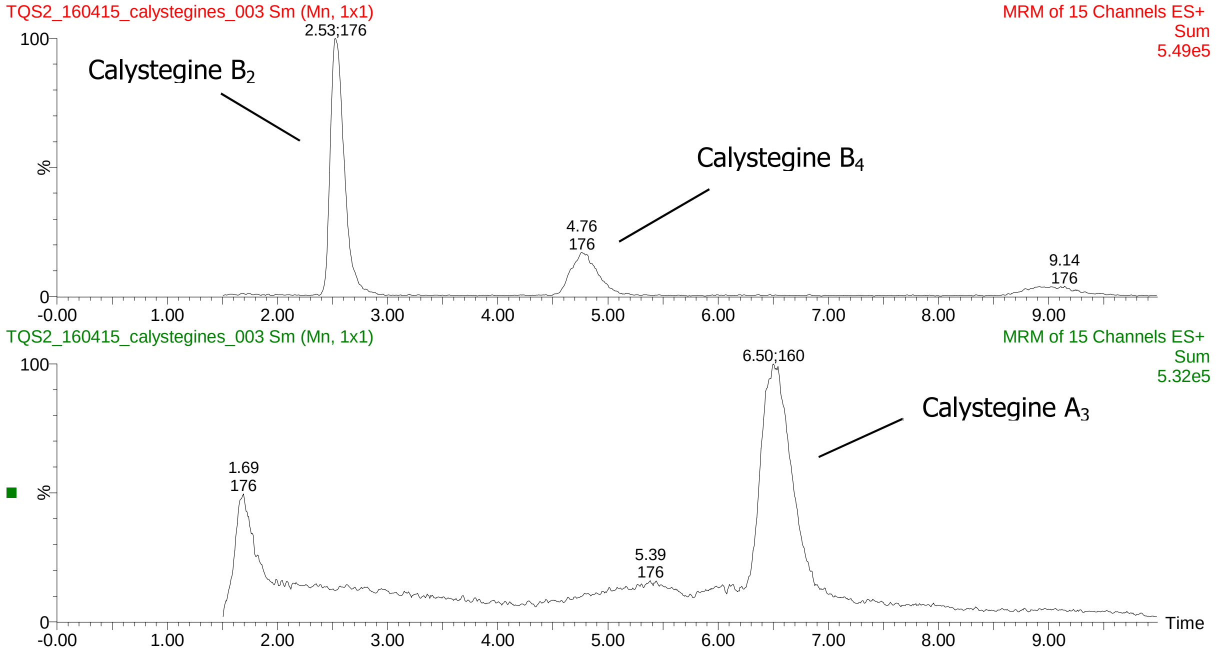
In bold = most intense fragment.

Appendix L.4 LC-MS/MS MRM chromatograms using a Chirobiotic V column, with an ammonium acetate pH 7/methanol gradient. A mixed working standard of 1000 ng/mL containing all 6 calystegines is shown as an example



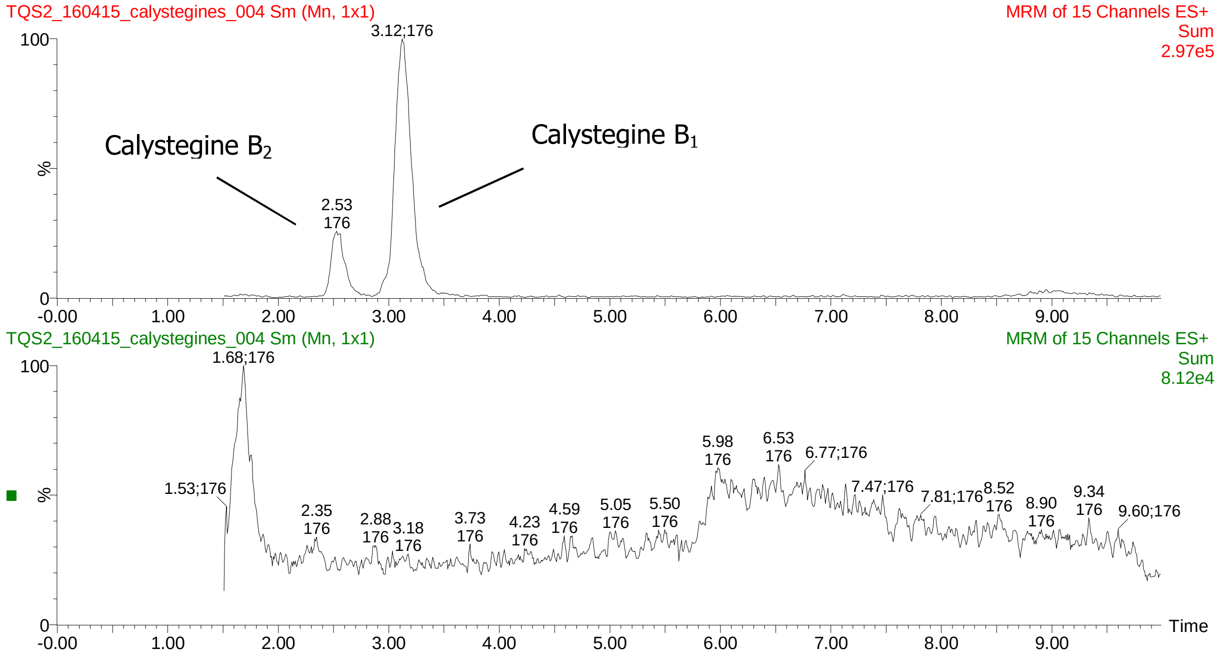
Appendix L.5 LC-MS/MS MRM chromatograms for calystegines in potato. Potato extract P1 is shown as an example, containing 62.4 mg/kg calystegine A_3, 35.4 mg/kg calystegine B_2 and 1.4 mg/kg calystegine B_4.

P1



Appendix L.6 LC-MS/MS MRM chromatograms for calystegines in aubergine. Extract A5 is shown as an example, containing 5.3 mg/kg calystegine B_1 and 3.1 mg/kg calystegine B_2.

A5



Appendix M – RIKILT in-house validation results for calystegine TAs

Limits of Detection (LOD), Limits of Quantification (LOQ), recovery and repeatability for the calystegines incorporated in the calystegine alkaloids method obtained during in-house validation at RIKILT. Five naturally contaminated potato and three naturally contaminated aubergine samples were used for validation

Compound name	RIKILT		Potato (n = 5)			Aubergine (n = 3)		
	LOD (mg/kg)	LOQ (mg/kg)	Recovery (%)[(a)]	RSD Recovery (%)	Repeat-ability (n = 6) (%)	Recovery (%)[(a)]	RSD Recovery (%)	Repeat-ability (n =6) (%)
Calystegine A_3	0.5	1.0	93.8	3.9	5.1	98[(c)]	-	-
Calystegine A_5	1.0	2.5	100[(b)]	-	9.6	-[(d)]	-	-
Calystegine B_1	0.25	1.0	89.0[(c)]	-	5.1	89.8	0.8	3.8
Calystegine B_2	0.25	1.0	91.7	3.3	4.2	90.4	0.7	4.9
Calystegine B_3	0.25	1.0	-[(d)]	-	-	-[(d)]	-	-
Calystegine B_4	0.25	1.0	92.7	4.2	4.8	-[(d)]	-	-

\- = no data.
(a): Recovery is defined as the extraction efficiency.
(b): n = 3.
(c): n = 1.
(d): None of the incurred materials contained measurable amounts of the analyte.

Appendix N – IRTA in-house validation results for calystegine TAs

Samples were analysed using an Acquity UPLC system coupled to a Xevo TQ-D tandem quadrupole mass spectrometer (Waters).

Appendix N.1 Chromatography

HPLC Method:

- Column: Chirobiotic V column (150 x 2.1 mm, 5 µm, 100Å) (Astec, Sigma-Aldrich).
- Column temperature: 35°C.
- Injection volume: 10 µL.
- Mobile phase A: 5 mM ammonium acetate in water pH 7.0.
- Mobile phase B: Methanol.
- Gradient (linear) timetable:

Time (min)	Mobile phase A	Mobile phase B
0.0	100%	0%
8.0	100%	0%
8.5	50%	50%
11.5	50%	50%
12.0	100%	0%
15.0	100%	0%

- Total run time is 15 min.

Appendix N.2 MS Tune settings

- Ionisation mode: positive electrospray (ESI+).
- Source temperature: 135°C.
- Desolvation gas temperature: 40°C.
- Desolvation gas flow: 400 L/h.
- Cone gas flow: 25 L/h.
- Cone voltage: 30 V.
- Capillary voltage: 3.0 kV.
- Argon collision gas pressure: 4.0 mbar.

Appendix N.3 MRM conditions optimised for calystegine alkaloids

Compound name	Precursor mass (Da)	Fragment 1 (Da)	Collision energy 1 (eV)	Fragment 2 (Da)	Collision energy 2 (eV)	Fragment 3 (Da)	Collision energy 3 (eV)
Calystegine A_3	160.1	142.0	10	125.0	15	98.2	15
Calystegine A_5	160.1	142.0	10	124.0	10	81.2	15
Calystegine B_1	176.1	140.0	10	123.0	15	112.0	15
Calystegine B_2	176.1	158.0	10	140.0	10	97.2	15
Calystegine B_3	176.1	158.0	10	116.0	15	98.2	15
Calystegine B_4	176.1	140.0	10	123.0	15	112.0	20

Appendix N.4 Limits of Detection (LOD), Limits of Quantification (LOQ), recovery and repeatability for the calystegines incorporated in the calystegine alkaloids method obtained during in-house validation at IRTA. One naturally contaminated potato and one naturally contaminated aubergine sample was used for validation

Compound name	RIKILT		Potato (n =1)		Aubergine (n = 1)	
	LOD (mg/kg)	LOQ (mg/kg)	Recovery (%)[(a)]	Repeatability (n = 6) (%)	Recovery (%)[(a)]	Repeatability (n =6) (%)
Calystegine A_3	1.0	2.5	91.2	3.3	83.2	19.7
Calystegine A_5	1.0	2.5	-	-	-	-
Calystegine B_1	1.0	2.5	-	-	86.2	4.3
Calystegine B_2	1.0	2.5	91.7	3.9	85.6	8.1
Calystegine B_3	1.0	2.5	-	-	-	-
Calystegine B_4	1.0	2.5	92.9	17.4	-	-

- = no data.
(a): Recovery is defined as the extraction efficiency.

Appendix O – UCT in-house validation results for calystegine TAs

Samples were analysed using an UltiMate® 3000 Binary Rapid Separation LC system (Thermo Scientific) coupled to a Q Exactive TM Plus Mass spectrometer (Thermo Scientific)

Appendix O.1 Chromatography

HPLC Method:

- Column: Chirobiotic V column (150 x 2.1 mm, 5 µm, 100Å) (Astec, Sigma-Aldrich).
- Column temperature: 35°C.
- Injection volume: 10 µL.
- Mobile phase A: 5 mM ammonium acetate in water pH 7.0.
- Mobile phase B: Methanol.
- Gradient (linear) timetable:

Time (min)	Mobile phase A	Mobile phase B
0.0	100%	0%
8.0	100%	0%
8.5	50%	50%
11.5	50%	50%
12.0	100%	0%
15.0	100%	0%

- Total run time is 15 min.

Appendix O.2 MS Tune settings

- Ionisation mode: positive electrospray (ESI+).
- Capillary temperature: 350°C.
- Auxiliary gas flow: 7 arbitary units.
- Sheat gas flow: 35 arbitary units.
- Spray voltage: 3.5 kV.
- Capillary voltage: 60 V.

Full MS conditions:

- Resolution: 70 000 FWHM; 1.5 Hz.
- Mass range: 120-1000 *m/z.*

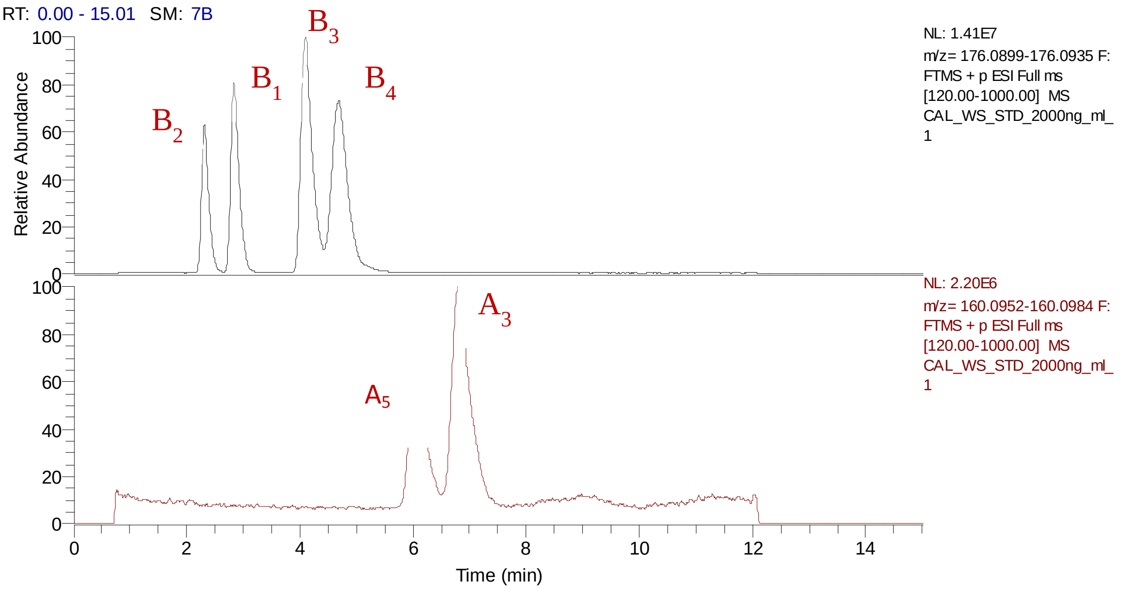
Product reaction monitoring MS2 conditions:

- Resolution: 17 500 FWHM; 12 Hz.
- Mass range: 50 *m/z*.

Appendix O.3 MRM conditions optimised for calystegine alkaloids

Compound name	Formula	Precursor mass [M+H]$^+$	Fragment 1 (Da)	Fragment 2 (Da)	Fragment 3 (Da)	Retention time (min)
Calystegine A_3	$C_7H_{13}NO_4$	160.0968	124.0757	142.0863	100.0757	2.31
Calystegine A_5	$C_7H_{13}NO_4$	160.0968	142.0863	125.0597	98.0600	2.83
Calystegine B_1	$C_7H_{13}NO_5$	176.0917	140.0706	158.0812	116.0706	4.11
Calystegine B_2	$C_7H_{13}NO_5$	176.0917	140.0706	158.0812	112.0393	4.69
Calystegine B_3	$C_7H_{13}NO_5$	176.0917	158.0812	116.0706	98.0600	6.02
Calystegine B_4	$C_7H_{13}NO_5$	176.0917	112.0393	140.0706	123.0442	6.80

Appendix O.4 LC-MS chromatograms using a Chirobiotic V column, with an ammonium acetate pH 7/methanol gradient. A mixed working standard of 2000 ng/mL containing all 6 calystegines is shown as an example



Appendix O.5 Limits of Detection (LOD), Limits of Quantification (LOQ), recovery and repeatability for the calystegines incorporated in the calystegine alkaloids method obtained during in-house validation at UCT. Three naturally contaminated potatoes and one naturally contaminated aubergine sample were used for validation.

Compound name	RIKILT		Potato (n =3)			Aubergine (n = 1)	
	LOD (mg/kg)	LOQ (mg/kg)	Recovery (%)[(a)]	RSD recovery (%)	Repeatability (n = 6) (%)	Recovery (%)[(a)]	Repeatability (n =6) (%)
Calystegine A_3	0.25	1.0	86.7	3.2	5.7	-	-
Calystegine A_5	0.25	1.0	-	-	-	-	-
Calystegine B_1	0.25	1.0	-	-	-	-	-
Calystegine B_2	0.25	1.0	92.3	4.2	6.3	85.0	6.0
Calystegine B_3	0.25	1.0	-	-	-	-	-
Calystegine B_4	0.25	1.0	-	-	-	-	-

- = no data.
(a): Recovery is defined as the extraction efficiency.

Appendix P – TA concentrations (µg/kg) in positive single component flours

Laboratory sample code	Country of sampling	Product	6-HO-tropinone	Nortropinone	Pseudotropine	Scopine	Scopoline	Tropine	Tropinone	Convolamine	Convolidine	Convolvine	Fillalbine
IRTA_TA_0002	ES	Buckwheat flour	<LOD	<LOD	<LOD	<LOD	<LOD	2.56	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0007	ES	Buckwheat flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0011	ES	Buckwheat flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0503	ES	Buckwheat flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0507	ES	Buckwheat flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0901	FR	Buckwheat flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0903	FR	Buckwheat flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 846/15	CZ	Buckwheat grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 240/16	CZ	Buckwheat groats	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 1153/15	HU	Buckwheat grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003148	UK	Buckwheat flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK MS4	NL	Sorghum flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.64	<LOD	<LOD	<LOD	<LOD
RIK MS11	NL	Millet flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK MS102	DE	Millet grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK MS103	DE	Millet grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK MS105	DE	Millet grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK MS106	DE	Millet grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK MS107	DE	Millet groats	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK MS109	DE	Millet grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0012	ES	Millet flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0013	ES	Millet flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0019	ES	Millet grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0509	ES	Millet grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0513	ES	Millet grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0905	FR	Millet flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_1208	IT	Millet flour	<LOD	<LOD	5.62	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 860/15	CZ	Millet grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 861/15	CZ	Millet flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 244/16	CZ	Millet flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix P, cont'd. TA concentrations (µg/kg) in positive single component flours

Laboratory sample code	Country of sampling	Product	Anisodamine	Apoatropine	Aposcopolamine	Atropine	Homatropine	Littorine	Noratropine	Norscopolamine	Scopolamine
IRTA_TA_0002	ES	Buckwheat flour	3.14	1.73	0.49	149.0	<LOD	<LOD	1.00	2.32	97.82
IRTA_TA_0007	ES	Buckwheat flour	<LOD	<LOD	<LOD	2.31	<LOD	<LOD	<LOD	<LOD	1.43
IRTA_TA_0011	ES	Buckwheat flour	<LOD	<LOD	<LOD	8.06	<LOD	<LOD	<LOD	<LOD	3.58
IRTA_TA_0503	ES	Buckwheat flour	<LOD	<LOD	<LOD	2.27	<LOD	<LOD	<LOD	<LOD	1.66
IRTA_TA_0507	ES	Buckwheat flour	<LOD	<LOD	<LOD	1.32	<LOD	<LOD	<LOD	<LOD	0.90
IRTA_TA_0901	FR	Buckwheat flour	<LOD	<LOD	<LOD	0.42	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0903	FR	Buckwheat flour	<LOD	<LOD	<LOD	0.40	<LOD	<LOD	<LOD	<LOD	0.28
VV 846/15	CZ	Buckwheat grain	<LOD	<LOD	<LOD	0.06	<LOD	<LOD	<LOD	<LOD	<LOD
VV 240/16	CZ	Buckwheat groat	0.21	0.16	<LOD	18.22	<LOD	<LOD	<LOD	0.71	3.03
VV 1153/15	HU	Buckwheat grain	<LOD	<LOD	<LOD	0.24	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003148	UK	Buckwheat flour	<LOD	<LOD	<LOD	0.56	<LOD	<LOD	<LOD	<LOD	0.09
RIK MS4	NL	Sorghum flour	0.15	<LOD	<LOD	1.13	<LOD	<LOD	<LOD	0.10	0.70
RIK MS11	NL	Millet flour	<LOD	<LOD	<LOD	0.31	<LOD	<LOD	<LOD	<LOD	0.14
RIK MS102	DE	Millet grain	<LOD	<LOD	<LOD	0.24	<LOD	<LOD	<LOD	<LOD	<LOD
RIK MS103	DE	Millet grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.09
RIK MS105	DE	Millet grain	<LOD	<LOD	<LOD	0.30	<LOD	<LOD	<LOD	<LOD	0.14
RIK MS106	DE	Millet grain	<LOD	<LOD	<LOD	0.25	<LOD	<LOD	<LOD	<LOD	0.14
RIK MS107	DE	Millet groats	<LOD	<LOD	<LOD	0.55	<LOD	<LOD	<LOD	<LOD	0.29
RIK MS109	DE	Millet grain	<LOD	<LOD	<LOD	0.33	<LOD	<LOD	<LOD	<LOD	0.16
IRTA_TA_0012	ES	Millet flour	0.25	<LOD	<LOD	9.40	<LOD	<LOD	<LOD	<LOD	6.84
IRTA_TA_0013	ES	Millet flour	<LOD	<LOD	<LOD	1.07	<LOD	<LOD	<LOD	<LOD	0.44
IRTA_TA_0019	ES	Millet grain	<LOD	<LOD	<LOD	0.37	<LOD	<LOD	<LOD	<LOD	0.22
IRTA_TA_0509	ES	Millet grain	<LOD	<LOD	<LOD	10.1	<LOD	<LOD	<LOD	1.48	9.69
IRTA_TA_0513	ES	Millet grain	<LOD	<LOD	<LOD	6.22	<LOD	<LOD	<LOD	<LOD	5.58
IRTA_TA_0905	FR	Millet flour	<LOD	2.36	0.99	30.78	<LOD	<LOD	<LOD	<LOD	15.33
IRTA_TA_1208	IT	Millet flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 860/15	CZ	Millet grain	<LOD	<LOD	<LOD	0.06	<LOD	<LOD	<LOD	<LOD	<LOD
VV 861/15	CZ	Millet flour	<LOD	<LOD	<LOD	0.10	<LOD	<LOD	<LOD	<LOD	0.10
VV 244/16	CZ	Millet flour	<LOD	<LOD	<LOD	1.00	<LOD	<LOD	<LOD	<LOD	0.39

Appendix P, cont'd. TA concentrations (µg/kg) in positive single component flours

Laboratory sample code	Country of sampling	Product	6-HO-tropinone	Nortropinone	Pseudotropine	Scopine	Scopoline	Tropine	Tropinone	Convolamine	Convolidine	Convolvine	Fillalbine
VV 248/16	CZ	Millet groats	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 249/16	CZ	Millet grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 252/16	CZ	Millet grain	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 1147/15	HU	Millet grain	<LOD	<LOD	1.86	1.59	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100572	UK	Sorghum flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003153	UK	Millet grain	<LOD	<LOD	0.80	<LOD	4.72	2.26	0.78	<LOD	<LOD	<LOD	<LOD
RIK CO1	NL	Corn starch	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK CO2	NL	Corn flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK CO5	NL	Corn flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK CO6	NL	Cornmeal	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK CO7	NL	Corn flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK CO106	DE	Cornmeal	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 1219/16	HU	Cornmeal	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100709	UK	Cornmeal	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003305	UK	Cornmeal	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BW7	NL	Other milling products	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003151	UK	Other milling products	<LOD	<LOD	2.17	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix P, cont'd. TA concentrations (µg/kg) in positive single component flours

Laboratory sample code	Country of sampling	Product	Anisodamine	Apoatropine	Aposcopolamine	Atropine	Homatropine	Littorine	Noratropine	Norscopolamine	Scopolamine
VV 248/16	CZ	Millet groats	0.09	0.06	<LOD	12.17	<LOD	<LOD	<LOD	<LOD	2.66
VV 249/16	CZ	Millet grain	<LOD	<LOD	<LOD	0.06	<LOD	<LOD	<LOD	<LOD	<LOD
VV 252/16	CZ	Millet grain	<LOD	<LOD	<LOD	0.05	<LOD	<LOD	<LOD	<LOD	<LOD
VV 1147/15	HU	Millet grain	9.07	0.67	0.81	136.3	<LOD	<LOD	0.79	11.64	198.5
S15-100572	UK	Sorghum flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.45
S16-003153	UK	Millet grain	0.74	0.49	0.25	9.79	<LOD	<LOD	0.12	0.73	12.87
RIK CO1	NL	Corn starch	<LOD	<LOD	<LOD	0.08	<LOD	<LOD	<LOD	<LOD	<LOD
RIK CO2	NL	Corn flour	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.08
RIK CO5	NL	Corn flour	<LOD	<LOD	<LOD	0.21	<LOD	<LOD	<LOD	<LOD	<LOD
RIK CO6	NL	Cornmeal	<LOD	<LOD	<LOD	0.05	<LOD	<LOD	<LOD	<LOD	<LOD
RIK CO7	NL	Corn flour	<LOD	<LOD	<LOD	0.06	<LOD	<LOD	<LOD	<LOD	<LOD
RIK CO106	DE	Cornmeal	<LOD	<LOD	<LOD	0.13	<LOD	<LOD	<LOD	<LOD	<LOD
VV 1219/16	HU	Cornmeal	<LOD	<LOD	<LOD	0.07	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100709	UK	Cornmeal	<LOD	<LOD	<LOD	0.22	<LOD	<LOD	<LOD	<LOD	0.06
S16-003305	UK	Cornmeal	<LOD	<LOD	<LOD	0.63	<LOD	<LOD	<LOD	<LOD	0.16
RIK BW7	NL	Other milling products	<LOD	<LOD	<LOD	0.07	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003151	UK	Other milling products	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix Q – TA concentrations (µg/kg) in positive bread and pasta samples

Laboratory sample code	Country of sampling	Product	6-HO-tropinone	Nortropinone	Pseudotropine	Scopine	Scopoline	Tropine	Tropinone	Convolamine	Convolidine	Convolvine	Fillalbine
RIK BR2	NL	Wheat rolls	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.16	<LOD	<LOD
RIK BR10	NL	Spelt bread	<LOD	<LOD	3.43	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0114	ES	Wheat bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0636	ES	Corn bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_1000	FR	Wheat toast bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_1002	FR	Wheat toast bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 801/15	CZ	Wheat toast bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 201/16	CZ	Wheat toast bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 924/15	HU	Wheat toast bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 927/15	HU	Wheat toast bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100622	UK	Multigrain bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003214	UK	Corn bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003219	UK	Crackers	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003220	UK	Crackers	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003221	UK	Multigrain toast bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003225	UK	Wheat bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003228	UK	Wheat bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003230	UK	Wheat bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix Q, cont'd. TA concentrations (μg/kg) in positive bread and pasta samples

Laboratory sample code	Country of sampling	Product	Anisodamine	Apoatropine	Aposcopolamine	Atropine	Homatropine	Littorine	Noratropine	Norscopolamine	Scopolamine
RIK BR2	NL	Wheat rolls	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BR10	NL	Spelt bread	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0114	ES	Wheat bread	<LOD	<LOD	<LOD	0.90	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0636	ES	Corn bread	<LOD	<LOD	<LOD	3.80	<LOD	<LOD	<LOD	<LOD	0.36
IRTA_TA_1000	FR	Wheat toast bread	<LOD	<LOD	<LOD	0.37	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_1002	FR	Wheat toast bread	<LOD	<LOD	<LOD	0.62	<LOD	<LOD	<LOD	<LOD	<LOD
VV 801/15	CZ	Wheat toast bread	<LOD	<LOD	<LOD	0.05	<LOD	<LOD	<LOD	<LOD	<LOD
VV 201/16	CZ	Wheat toast bread	<LOD	<LOD	<LOD	0.63	<LOD	<LOD	<LOD	<LOD	0.26
VV 924/15	HU	Wheat toast bread	<LOD	<LOD	<LOD	0.13	<LOD	<LOD	<LOD	<LOD	<LOD
VV 927/15	HU	Wheat toast bread	<LOD	<LOD	<LOD	0.09	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100622	UK	Multigrain bread	<LOD	<LOD	<LOD	0.05	<LOD	<LOD	<LOD	<LOD	0.05
S16-003214	UK	Corn bread	<LOD	<LOD	<LOD	0.07	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003219	UK	Crackers	<LOD	<LOD	<LOD	0.05	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003220	UK	Crackers	<LOD	<LOD	<LOD	0.11	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003221	UK	Multigrain toast bread	<LOD	<LOD	<LOD	0.07	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003225	UK	Wheat bread	<LOD	<LOD	<LOD	0.13	<LOD	<LOD	<LOD	<LOD	0.09
S16-003228	UK	Wheat bread	0.13	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003230	UK	Wheat bread	<LOD	<LOD	<LOD	0.12	<LOD	<LOD	<LOD	<LOD	0.15

Appendix R – TA concentrations (µg/kg) in positive breakfast cereals

Laboratory sample code	Country of sampling	Product	6-HO-tropinone	Nortropinone	Pseudotropine	Scopine	Scopoline	Tropine	Tropinone	Convolamine	Convolidine	Convolvine	Fillalbine
RIK BC10	NL	Muesli	<LOD	<LOD	4.32	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BC108	DE	Muesli	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BC109	DE	Muesli	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0577	ES	Corn flakes	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.34
IRTA_TA_1370	IT	Maize, popped	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 851/15	CZ	Mixed cereal flakes	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 878/15	CZ	Cereal bar	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 266/16	CZ	Corn flakes	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 269/16	CZ	Cereal bar	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 314/16	CZ	Corn flakes	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 949/15	HU	Mixed breakfast cereals	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 951/15	HU	Mixed breakfast cereals	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 955/15	HU	Millet flakes	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100597	UK	Wheat flakes	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100607	UK	Muesli	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix R, cont'd. TA concentrations (µg/kg) in positive breakfast cereals

Laboratory sample code	Country of sampling	Product	Anisodamine	Apoatropine	Aposcopolamine	Atropine	Homatropine	Littorine	Noratropine	Norscopolamine	Scopolamine
RIK BC10	NL	Muesli	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BC108	DE	Muesli	<LOD	<LOD	<LOD	0.48	<LOD	<LOD	<LOD	<LOD	0.20
RIK BC109	DE	Muesli	<LOD	<LOD	<LOD	0.06	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0577	ES	Corn flakes	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_1370	IT	Maize, popped	<LOD	<LOD	<LOD	2.81	<LOD	<LOD	<LOD	<LOD	0.98
VV 851/15	CZ	Mixed cereal flakes	<LOD	<LOD	<LOD	0.06	<LOD	<LOD	<LOD	<LOD	<LOD
VV 878/15	CZ	Cereal bar	<LOD	<LOD	<LOD	0.27	<LOD	<LOD	<LOD	<LOD	0.08
VV 266/16	CZ	Corn flakes	<LOD	<LOD	<LOD	0.47	<LOD	<LOD	<LOD	<LOD	0.06
VV 269/16	CZ	Cereal bar	2.07	1.10	0.19	90.83	<LOD	<LOD	<LOD	<LOD	17.64
VV 314/16	CZ	Corn flakes	0.12	0.09	<LOD	6.79	<LOD	<LOD	<LOD	<LOD	2.01
VV 949/15	HU	Mixed breakfast cereals	<LOD	<LOD	<LOD	0.34	<LOD	<LOD	<LOD	<LOD	0.05
VV 951/15	HU	Mixed breakfast cereals	<LOD	<LOD	<LOD	0.05	<LOD	<LOD	<LOD	<LOD	<LOD
VV 955/15	HU	Millet flakes	0.07	0.15	<LOD	2.29	<LOD	<LOD	<LOD	<LOD	1.64
S15-100597	UK	Wheat flakes	<LOD	<LOD	<LOD	0.67	<LOD	<LOD	<LOD	<LOD	0.38
S15-100607	UK	Muesli	<LOD	<LOD	<LOD	0.32	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix S – TA concentrations (µg/kg) in positive biscuits and pastry

Laboratory sample code	Country of sampling	Product	6-HO-tropinone	Nortropinone	Pseudotropine	Scopine	Scopoline	Tropine	Tropinone	Convolamine	Convolidine	Convolvine	Fillalbine
IRTA_TA_0621	ES	Pastry	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100636	UK	Pancakes	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BI4	NL	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BI12	NL	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BI13	NL	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BI105	DE	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BI111	DE	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0128	ES	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.28	<LOD	<LOD	<LOD
IRTA_TA_0131	ES	Biscuits	<LOD	<LOD	0.63	<LOD	<LOD	<LOD	<LOD	0.29	<LOD	<LOD	<LOD
IRTA_TA_0135	ES	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0624	ES	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0626	ES	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0629	ES	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_1014	FR	Biscuits	<LOD	<LOD	11.98	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_1024	FR	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 902/15	CZ	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 279/16	CZ	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 970/15	HU	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 972/15	HU	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 1122/15	PL	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 1124/15	PL	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 1131/15	PL	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003206	UK	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003217	UK	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix S, cont'd. TA concentrations (µg/kg) in positive biscuits and pastry

Laboratory sample code	Country of sampling	Product	Anisodamine	Apoatropine	Aposcopolamine	Atropine	Homatropine	Littorine	Noratropine	Norscopolamine	Scopolamine
IRTA_TA_0621	ES	Pastry	<LOD	<LOD	<LOD	0.21	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100636	UK	Pancakes	<LOD	<LOD	<LOD	1.23	<LOD	<LOD	<LOD	<LOD	0.65
RIK BI4	NL	Biscuits	<LOD	<LOD	<LOD	0.51	<LOD	<LOD	<LOD	<LOD	0.07
RIK BI12	NL	Biscuits	<LOD	<LOD	<LOD	0.09	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BI13	NL	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.08
RIK BI105	DE	Biscuits	<LOD	<LOD	<LOD	0.07	<LOD	<LOD	<LOD	<LOD	0.07
RIK BI111	DE	Biscuits	<LOD	<LOD	<LOD	0.06	<LOD	<LOD	<LOD	<LOD	0.16
IRTA_TA_0128	ES	Biscuits	<LOD	<LOD	<LOD	0.29	<LOD	<LOD	<LOD	<LOD	0.20
IRTA_TA_0131	ES	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0135	ES	Biscuits	<LOD	<LOD	<LOD	0.60	<LOD	<LOD	<LOD	<LOD	0.34
IRTA_TA_0624	ES	Biscuits	<LOD	<LOD	<LOD	0.56	<LOD	<LOD	<LOD	<LOD	0.27
IRTA_TA_0626	ES	Biscuits	<LOD	<LOD	<LOD	1.85	<LOD	<LOD	<LOD	<LOD	0.48
IRTA_TA_0629	ES	Biscuits	<LOD	<LOD	<LOD	0.40	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_1014	FR	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_1024	FR	Biscuits	<LOD	<LOD	<LOD	0.45	<LOD	<LOD	<LOD	<LOD	<LOD
VV 902/15	CZ	Biscuits	<LOD	<LOD	<LOD	0.05	<LOD	<LOD	<LOD	<LOD	0.13
VV 279/16	CZ	Biscuits	<LOD	<LOD	<LOD	0.07	<LOD	<LOD	<LOD	<LOD	<LOD
VV 970/15	HU	Biscuits	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.12
VV 972/15	HU	Biscuits	<LOD	<LOD	<LOD	0.13	<LOD	<LOD	<LOD	<LOD	0.15
VV 1122/15	PL	Biscuits	<LOD	<LOD	<LOD	0.07	<LOD	<LOD	<LOD	<LOD	0.13
VV 1124/15	PL	Biscuits	<LOD	<LOD	<LOD	0.14	<LOD	<LOD	<LOD	<LOD	0.12
VV 1131/15	PL	Biscuits	<LOD	<LOD	<LOD	0.07	<LOD	<LOD	<LOD	<LOD	0.11
S16-003206	UK	Biscuits	<LOD	<LOD	<LOD	0.16	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003217	UK	Biscuits	<LOD	<LOD	<LOD	0.14	<LOD	<LOD	<LOD	<LOD	0.05

Appendix T – TA concentrations (µg/kg) in positive cereals for children

Laboratory sample code	Country of sampling	Product	6-HO-tropinone	Nortropinone	Pseudotropine	Scopine	Scopoline	Tropine	Tropinone	Convolamine	Convolidine	Convolvine	Fillalbine
RIK BCC7	NL	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BCC9	NL	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BCC13	NL	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	1.76	<LOD	<LOD	<LOD	<LOD	0.12
RIK BCC16	NL	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BCC109	DE	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0068	ES	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0952	FR	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	2.01	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0964	FR	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 866/15	CZ	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 254/16	CZ	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 255/16	CZ	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	0.87	<LOD	<LOD	<LOD	<LOD	<LOD
VV 1089/15	PL	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 1090/15	PL	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100580	UK	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100703	UK	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003166	UK	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003293	UK	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003294	UK	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix T, cont'd. TA concentrations (µg/kg) in cereals for children

Laboratory sample code	Country of sampling	Product	Anisodamine	Apoatropine	Aposcopolamine	Atropine	Homatropine	Littorine	Noratropine	Norscopolamine	Scopolamine
RIK BCC7	NL	Cereals for children	<LOD	<LOD	<LOD	0.14	<LOD	<LOD	<LOD	<LOD	0.18
RIK BCC9	NL	Cereals for children	<LOD	<LOD	<LOD	0.19	<LOD	<LOD	<LOD	<LOD	1.86
RIK BCC13	NL	Cereals for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BCC16	NL	Cereals for children	<LOD	<LOD	<LOD	0.11	<LOD	<LOD	<LOD	<LOD	0.78
RIK BCC109	DE	Cereals for children	<LOD	<LOD	<LOD	0.07	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0068	ES	Cereals for children	<LOD	<LOD	<LOD	0.20	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0952	FR	Cereals for children	<LOD	<LOD	<LOD	1.82	<LOD	<LOD	<LOD	<LOD	0.64
IRTA_TA_0964	FR	Cereals for children	<LOD	<LOD	<LOD	0.47	<LOD	<LOD	<LOD	<LOD	0.56
VV 866/15	CZ	Cereals for children	<LOD	<LOD	<LOD	0.90	<LOD	<LOD	<LOD	<LOD	0.18
VV 254/16	CZ	Cereals for children	<LOD	<LOD	<LOD	0.48	<LOD	<LOD	<LOD	<LOD	0.13
VV 255/16	CZ	Cereals for children	<LOD	<LOD	<LOD	0.61	<LOD	<LOD	<LOD	<LOD	0.27
VV 1089/15	PL	Cereals for children	<LOD	<LOD	<LOD	0.44	<LOD	<LOD	<LOD	<LOD	0.12
VV 1090/15	PL	Cereals for children	<LOD	<LOD	<LOD	0.05	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100580	UK	Cereals for children	<LOD	<LOD	<LOD	3.73	<LOD	<LOD	<LOD	<LOD	0.51
S15-100703	UK	Cereals for children	<LOD	<LOD	<LOD	0.16	<LOD	<LOD	<LOD	<LOD	0.09
S16-003166	UK	Cereals for children	<LOD	<LOD	<LOD	1.28	<LOD	<LOD	<LOD	<LOD	0.17
S16-003293	UK	Cereals for children	<LOD	<LOD	<LOD	0.07	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003294	UK	Cereals for children	<LOD	<LOD	<LOD	0.05	<LOD	<LOD	<LOD	0.12	0.88

Appendix U – TA concentrations (µg/kg) in positive biscuits for children

Laboratory sample code	Country of sampling	Product	6-HO-tropinone	Nortropinone	Pseudotropine	Scopine	Scopoline	Tropine	Tropinone	Convolamine	Convolidine	Convolvine	Fillalbine
RIK BIC1	NL	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BIC2	NL	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BIC5	NL	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BIC6	NL	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BIC12	NL	Biscuits for children	<LOD	<LOD	1.11	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BIC102	DE	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0551	ES	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_1310	IT	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100586	UK	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003162	UK	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003184	UK	Biscuits for children	1.49	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003185	UK	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003186	UK	Biscuits for children	<LOD	<LOD	14.67	<LOD	<LOD	48.21	1.40	0.17	8.82	<LOD	12.40
S16-003233	UK	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix U, cont'd. TA concentrations (µg/kg) in biscuits for children

Laboratory sample code	Country of sampling	Product	Anisodamine	Apoatropine	Aposcopolamine	Atropine	Homatropine	Littorine	Noratropine	Norscopolamine	Scopolamine
RIK BIC1	NL	Biscuits for children	<LOD	<LOD	<LOD	0.05	<LOD	<LOD	<LOD	<LOD	0.05
RIK BIC2	NL	Biscuits for children	<LOD	<LOD	<LOD	0.27	<LOD	<LOD	<LOD	<LOD	0.06
RIK BIC5	NL	Biscuits for children	<LOD	<LOD	<LOD	0.05	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BIC6	NL	Biscuits for children	<LOD	<LOD	<LOD	0.09	<LOD	<LOD	<LOD	<LOD	0.05
RIK BIC12	NL	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK BIC102	DE	Biscuits for children	<LOD	<LOD	<LOD	0.07	<LOD	<LOD	<LOD	<LOD	0.05
IRTA_TA_0551	ES	Biscuits for children	<LOD	<LOD	<LOD	0.84	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_1310	IT	Biscuits for children	<LOD	<LOD	<LOD	0.52	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100586	UK	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.18
S16-003162	UK	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.09
S16-003184	UK	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003185	UK	Biscuits for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.11
S16-003186	UK	Biscuits for children	<LOD	<LOD	<LOD	0.45	<LOD	<LOD	<LOD	<LOD	0.10
S16-003233	UK	Biscuits for children	<LOD	<LOD	<LOD	0.10	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix V – TA concentrations (µg/kg) in positive ready-to-eat meals for children

Laboratory sample code	Country of sampling	Product	6-HO-tropinone	Nortropinone	Pseudotropine	Scopine	Scopoline	Tropine	Tropinone	Convolamine	Convolidine	Convolvine	Fillalbine
S15-100583	UK	Ready-to-eat meal for children	<LOD	<LOD	44.06	<LOD	<LOD	515.6	2.13	1.30	12.24	<LOD	28.32
S15-100585	UK	Ready-to-eat meal for children	<LOD	<LOD	5.97	<LOD	<LOD	0.52	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100587	UK	Ready-to-eat meal for children	<LOD	<LOD	18.37	<LOD	<LOD	0.98	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100702	UK	Ready-to-eat meal for children	<LOD	<LOD	25.8	<LOD	<LOD	492.0	1.66	1.03	8.84	<LOD	38.6
S15-100705	UK	Ready-to-eat meal for children	<LOD	1.64	8.31	<LOD	<LOD	1.31	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100706	UK	Ready-to-eat meal for children	<LOD	<LOD	12.82	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100707	UK	Ready-to-eat meal for children	<LOD	4.53	29.6	<LOD	<LOD	2.51	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003179	UK	Ready-to-eat meal for children	<LOD	<LOD	8.67	<LOD	<LOD	109.3	<LOD	0.15	3.57	<LOD	0.79
S16-003181	UK	Ready-to-eat meal for children	<LOD	<LOD	41.40	<LOD	<LOD	772.8	2.43	1.13	13.41	<LOD	26.83
S16-003292	UK	Ready-to-eat meal for children	<LOD	<LOD	5.51	<LOD	<LOD	97.78	0.60	0.17	3.39	<LOD	3.28

Appendix V, cont'd. TA concentrations (µg/kg) in ready-to-eat meals for children

Laboratory sample code	Country of sampling	Product	Anisodamine	Apoatropine	Aposcopolamine	Atropine	Homatropine	Littorine	Noratropine	Norscopolamine	Scopolamine
S15-100583	UK	Ready-to-eat meal for children	<LOD	<LOD	<LOD	0.42	<LOD	<LOD	<LOD	<LOD	0.13
S15-100585	UK	Ready-to-eat meal for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100587	UK	Ready-to-eat meal for children	<LOD	<LOD	<LOD	0.10	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100702	UK	Ready-to-eat meal for children	<LOD	<LOD	<LOD	1.14	<LOD	<LOD	<LOD	<LOD	0.26
S15-100705	UK	Ready-to-eat meal for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100706	UK	Ready-to-eat meal for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S15-100707	UK	Ready-to-eat meal for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003179	UK	Ready-to-eat meal for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003181	UK	Ready-to-eat meal for children	<LOD	<LOD	<LOD	1.25	<LOD	<LOD	<LOD	<LOD	0.26
S16-003292	UK	Ready-to-eat meal for children	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix W – TA concentrations (µg/kg) in positive herbal teas

Laboratory sample code	Country of sampling	Product	6-HO-tropinone	Nortropinone	Pseudotropine	Scopine	Scopoline	Tropine	Tropinone	Convolamine	Convolidine	Convolvine	Fillalbine
RIK T1	NL	Mixed herbal tea	<LOD	<LOD	4.08	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T2	NL	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T3	NL	Peppermint tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T4	NL	Peppermint tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T5	NL	Nettle herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T6	NL	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T7	NL	Herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T8	NL	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T9	NL	Peppermint tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T10	NL	Fennel tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T13	NL	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T14	NL	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.44	10.64	<LOD
RIK T16	NL	Nettle tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T17	NL	Mixed herbal tea	<LOD	<LOD	8.23	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T102	DE	Fennel tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T103	DE	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T104	DE	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T105	DE	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T108	DE	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T110	DE	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T112	DE	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T113	DE	Fennel tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T114	DE	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0700	ES	Peppermint tea	<LOD	<LOD	9.79	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0702	ES	Black tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0703	ES	Camomile tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.10	<LOD
IRTA_TA_0704	ES	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0705	ES	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0706	ES	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix W, cont'd. TA concentrations (µg/kg) in positive herbal teas

Laboratory sample code	Country of sampling	Product	Anisodamine	Apoatropine	Aposcopolamine	Atropine	Homatropine	Littorine	Noratropine	Norscopolamine	Scopolamine
RIK T1	NL	Mixed herbal tea	0.96	<LOD	<LOD	21.72	<LOD	<LOD	<LOD	0.50	12.75
RIK T2	NL	Mixed herbal tea	0.27	<LOD	<LOD	1.41	<LOD	<LOD	<LOD	<LOD	0.96
RIK T3	NL	Peppermint tea	0.26	<LOD	<LOD	2.20	<LOD	<LOD	<LOD	<LOD	3.77
RIK T4	NL	Peppermint tea	<LOD	<LOD	<LOD	0.43	<LOD	<LOD	<LOD	<LOD	4.19
RIK T5	NL	Nettle herbal tea	<LOD	<LOD	<LOD	4.89	<LOD	<LOD	<LOD	<LOD	1.25
RIK T6	NL	Mixed herbal tea	<LOD	<LOD	<LOD	4.92	<LOD	<LOD	<LOD	<LOD	2.67
RIK T7	NL	Herbal tea	<LOD	<LOD	<LOD	0.10	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T8	NL	Mixed herbal tea	<LOD	<LOD	<LOD	0.16	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T9	NL	Peppermint tea	<LOD	<LOD	<LOD	0.10	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T10	NL	Fennel tea	<LOD	<LOD	<LOD	0.93	<LOD	<LOD	<LOD	<LOD	0.36
RIK T13	NL	Mixed herbal tea	<LOD	<LOD	<LOD	5.20	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T14	NL	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T16	NL	Nettle tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.58
RIK T17	NL	Mixed herbal tea	<LOD	<LOD	<LOD	2.97	<LOD	<LOD	<LOD	<LOD	1.48
RIK T102	DE	Fennel tea	1.77	<LOD	<LOD	81.64	<LOD	<LOD	0.42	0.66	18.02
RIK T103	DE	Mixed herbal tea	0.31	<LOD	<LOD	18.30	<LOD	<LOD	<LOD	<LOD	1.26
RIK T104	DE	Mixed herbal tea	<LOD	<LOD	<LOD	2.00	<LOD	<LOD	<LOD	<LOD	1.83
RIK T105	DE	Mixed herbal tea	<LOD	<LOD	<LOD	0.35	<LOD	<LOD	<LOD	<LOD	0.28
RIK T108	DE	Mixed herbal tea	<LOD	<LOD	<LOD	0.13	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T110	DE	Mixed herbal tea	<LOD	<LOD	<LOD	0.39	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T112	DE	Mixed herbal tea	0.19	<LOD	<LOD	2.71	<LOD	<LOD	<LOD	<LOD	0.82
RIK T113	DE	Fennel tea	7.80	<LOD	<LOD	101.6	<LOD	<LOD	0.79	0.76	43.46
RIK T114	DE	Mixed herbal tea	<LOD	<LOD	<LOD	0.65	<LOD	<LOD	<LOD	<LOD	0.44
IRTA_TA_0700	ES	Peppermint tea	3.27	<LOD	<LOD	34.99	<LOD	<LOD	<LOD	<LOD	25.64
IRTA_TA_0702	ES	Black tea	<LOD	<LOD	<LOD	0.30	<LOD	<LOD	<LOD	<LOD	0.90
IRTA_TA_0703	ES	Camomile tea	<LOD	<LOD	<LOD	0.40	<LOD	<LOD	<LOD	<LOD	1.20
IRTA_TA_0704	ES	Mixed herbal tea	0.70	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.90
IRTA_TA_0705	ES	Mixed herbal tea	1.76	<LOD	<LOD	<LOD	<0.25	0.34	<LOD	0.93	30.45
IRTA_TA_0706	ES	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.60

Appendix W, cont'd. TA concentrations (µg/kg) in positive herbal teas

Laboratory sample code	Country of sampling	Product	6-HO-tropinone	Nortropinone	Pseudotropine	Scopine	Scopoline	Tropine	Tropinone	Convolamine	Convolidine	Convolvine	Fillalbine
IRTA_TA_0707	ES	Herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0708	ES	Camomile tea	<LOD	<LOD	14.85	<LOD	<LOD	<LOD	<LOD	<LOD	11.45	<LOD	<LOD
IRTA_TA_0709	ES	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0710	ES	Herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0711	ES	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0712	ES	Herbal tea	<LOD	<LOD	3.94	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0713	ES	Mixed herbal tea	<LOD	<LOD	3.14	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0714	ES	Peppermint tea	<LOD	<LOD	14.70	<LOD	<LOD	10.08	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0715	ES	Peppermint tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0716	ES	Camomile tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0718	ES	Peppermint tea	<LOD	<LOD	14.19	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0721	ES	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0723	ES	Peppermint tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0725	ES	Mixed herbal tea	<LOD	<LOD	10.63	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0726	ES	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0727	ES	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0728	ES	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0729	ES	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0737	ES	Herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.20
VV 903/15	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	5.98	<LOD
VV 904/15	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	18.94	<LOD	<LOD	0.73	427.7	<LOD
VV 905/15	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 906/15	CZ	Mixed herbal tea	<LOD	<LOD	1.77	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 907/15	CZ	Mixed herbal tea	<LOD	<LOD	13.40	<LOD	<LOD	69.76	2.39	1.35	47.36	814.3	3.81
VV 908/15	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	6.52	<LOD	<LOD	1.98	35.21	<LOD
VV 909/15	CZ	Mixed herbal tea	<LOD	<LOD	7.16	<LOD	<LOD	56.22	1.66	0.96	9.16	575.7	2.03
VV 910/15	CZ	Mixed herbal tea	<LOD	<LOD	1.45	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 911/15	CZ	Mixed herbal tea	<LOD	<LOD	19.42	<LOD	<LOD	185.8	9.00	5.46	29.64	4074.0	5.03
VV 290/16	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix W, cont'd. TA concentrations (µg/kg) in positive herbal teas

Laboratory sample code	Country of sampling	Product	Anisodamine	Apoatropine	Aposcopolamine	Atropine	Homatropine	Littorine	Noratropine	Norscopolamine	Scopolamine
IRTA_TA_0707	ES	Herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,70
IRTA_TA_0708	ES	Camomile tea	<LOD	<LOD	<LOD	1.67	<LOD	<LOD	<LOD	<LOD	0.92
IRTA_TA_0709	ES	Mixed herbal tea	2.27	<LOD	<LOD	72.94	<LOD	<LOD	0.53	0.44	20.70
IRTA_TA_0710	ES	Herbal tea	<LOD	<LOD	<LOD	2.70	<LOD	<LOD	<LOD	<LOD	0.80
IRTA_TA_0711	ES	Mixed herbal tea	<LOD	<LOD	<LOD	5.30	<LOD	<LOD	<LOD	<LOD	4.20
IRTA_TA_0712	ES	Herbal tea	0.98	<LOD	<LOD	17.14	<LOD	<LOD	<LOD	<LOD	7.75
IRTA_TA_0713	ES	Mixed herbal tea	0.36	<LOD	<LOD	3.63	<LOD	<LOD	<LOD	<LOD	1.07
IRTA_TA_0714	ES	Peppermint tea	2.04	<LOD	<LOD	30.00	<LOD	<LOD	<LOD	1.40	18.89
IRTA_TA_0715	ES	Peppermint tea	<LOD	<LOD	<LOD	0.60	<LOD	<LOD	<LOD	<LOD	3.20
IRTA_TA_0716	ES	Camomile tea	0.43	<LOD	<LOD	3.74	<LOD	<LOD	<LOD	<LOD	2.94
IRTA_TA_0718	ES	Peppermint tea	6.84	<LOD	<LOD	64.91	<LOD	<LOD	<LOD	3.35	33.20
IRTA_TA_0721	ES	Mixed herbal tea	<LOD	<LOD	<LOD	2.50	<LOD	<LOD	<LOD	<LOD	1.00
IRTA_TA_0723	ES	Peppermint tea	<LOD	<LOD	<LOD	5.40	<LOD	<LOD	<LOD	<LOD	2.10
IRTA_TA_0725	ES	Mixed herbal tea	10.61	<LOD	0.96	294.8	<LOD	<LOD	5.09	15.08	133.7
IRTA_TA_0726	ES	Mixed herbal tea	<LOD	<LOD	<LOD	0.50	<LOD	<LOD	<LOD	<LOD	0.70
IRTA_TA_0727	ES	Mixed herbal tea	<LOD	<LOD	<LOD	12.25	<LOD	<LOD	<LOD	<LOD	2.09
IRTA_TA_0728	ES	Mixed herbal tea	<LOD	<LOD	<LOD	8.90	<LOD	<LOD	<LOD	<LOD	4.10
IRTA_TA_0729	ES	Mixed herbal tea	<LOD	<LOD	<LOD	1.80	<LOD	<LOD	<LOD	<LOD	4.10
IRTA_TA_0737	ES	Herbal tea	<LOD	<LOD	<LOD	0.30	<LOD	<LOD	<LOD	<LOD	<LOD
VV 903/15	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	1.62	<LOD	<LOD	<LOD	<LOD	0.40
VV 904/15	CZ	Mixed herbal tea	0.22	<LOD	<LOD	2.91	<LOD	<LOD	<LOD	<LOD	2.74
VV 905/15	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	0.30	<LOD	<LOD	<LOD	<LOD	0.46
VV 906/15	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 907/15	CZ	Mixed herbal tea	0.80	<LOD	<LOD	21.33	<LOD	<LOD	<LOD	<LOD	4.99
VV 908/15	CZ	Mixed herbal tea	0.46	<LOD	<LOD	3.85	<LOD	<LOD	<LOD	<LOD	0.78
VV 909/15	CZ	Mixed herbal tea	0.25	<LOD	<LOD	8.34	<LOD	<LOD	<LOD	<LOD	0.31
VV 910/15	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 911/15	CZ	Mixed herbal tea	0.79	<LOD	<LOD	24.43	<LOD	<LOD	<LOD	<LOD	3.81
VV 290/16	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	0.80	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix W, cont'd. TA concentrations (µg/kg) in positive herbal teas

Laboratory sample code	Country of sampling	Product	6-HO-tropinone	Nortropinone	Pseudotropine	Scopine	Scopoline	Tropine	Tropinone	Convolamine	Convolidine	Convolvine	Fillalbine
VV 291/16	CZ	Mixed herbal tea	<LOD	<LOD	3.99	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 292/16	CZ	Mixed herbal tea	<LOD	<LOD	1.13	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 293/16	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	19.66	<LOD	<LOD	6.56	239.4	1.96
VV 294/16	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 295/16	CZ	Mixed herbal tea	<LOD	<LOD	4.19	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 297/16	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 298/16	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.70	<LOD	<LOD	<LOD	<LOD
S16-003154	UK	Nettle tea	<LOD	<LOD	5.49	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003156	UK	Peppermint tea	<LOD	<LOD	9.26	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003231	UK	Camomile tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003238	UK	Peppermint tea	<LOD	<LOD	<LOD	<LOD	<LOD	12.50	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003242	UK	Nettle tea	<LOD	<LOD	<LOD	<LOD	<LOD	14.00	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003243	UK	Peppermint tea	<LOD	<LOD	<LOD	<LOD	<LOD	3.43	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003244	UK	Camomile tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.78	<LOD	<LOD	<LOD	<LOD
S16-003246	UK	Peppermint tea	<LOD	<LOD	<LOD	<LOD	<LOD	9.62	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003249	UK	Black tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003251	UK	Rooibos tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003252	UK	Herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	2.82	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003253	UK	Nettle tea	<LOD	<LOD	3.29	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003254	UK	Peppermint tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003255	UK	Camomile tea	<LOD	<LOD	<LOD	<LOD	<LOD	3.97	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003311	UK	Rooibos tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003312	UK	Camomile tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.96	<LOD	<LOD	<LOD	<LOD
S16-015464	UK	Camomile tea	<LOD	<LOD	<LOD	<LOD	<LOD	9.27	<LOD	<LOD	0.78	19.1	<LOD
RIK T101	DE	Fennel tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T106	DE	Fennel tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0732	ES	Mixed herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix W, cont'd. TA concentrations (µg/kg) in positive herbal teas

Laboratory sample code	Country of sampling	Product	Anisodamine	Apoatropine	Aposcopolamine	Atropine	Homatropine	Littorine	Noratropine	Norscopolamine	Scopolamine
VV 291/16	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	0.71	<LOD	<LOD	<LOD	<LOD	0.24
VV 292/16	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	5.95	<LOD	<LOD	<LOD	<LOD	0.98
VV 293/16	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	0.17	<LOD	<LOD	<LOD	<LOD	1.98
VV 294/16	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	2.81	<LOD	<LOD	<LOD	<LOD	1.28
VV 295/16	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	1.79	<LOD	<LOD	<LOD	<LOD	0.45
VV 297/16	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	21.02	<LOD	<LOD	<LOD	<LOD	4.88
VV 298/16	CZ	Mixed herbal tea	<LOD	<LOD	<LOD	0.72	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003154	UK	Nettle tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003156	UK	Peppermint tea	<LOD	<LOD	<LOD	0.95	<LOD	<LOD	<LOD	<LOD	1.35
S16-003231	UK	Camomile tea	<LOD	<LOD	<LOD	0.84	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003238	UK	Peppermint tea	<LOD	<LOD	<LOD	1.54	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003242	UK	Nettle tea	<LOD	<LOD	<LOD	2.26	<LOD	<LOD	<LOD	<LOD	0.74
S16-003243	UK	Peppermint tea	3.33	0.32	0.28	50.2	<LOD	<LOD	0.41	0.98	34.10
S16-003244	UK	Camomile tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.17
S16-003246	UK	Peppermint tea	2.48	0.14	<LOD	129.0	<LOD	<LOD	0.43	0.3	33.70
S16-003249	UK	Black tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.15
S16-003251	UK	Rooibos tea	<LOD	<LOD	<LOD	2.1	<LOD	<LOD	<LOD	<LOD	0.37
S16-003252	UK	Herbal tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003253	UK	Nettle tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003254	UK	Peppermint tea	<LOD	<LOD	<LOD	0.38	<LOD	<LOD	<LOD	<LOD	1.02
S16-003255	UK	Camomile tea	<LOD	<LOD	<LOD	2.44	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003311	UK	Rooibos tea	<LOD	<LOD	<LOD	1.55	<LOD	<LOD	<LOD	<LOD	0.33
S16-003312	UK	Camomile tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-015464	UK	Camomile tea	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK T101	DE	Fennel tea	<LOD	<LOD	<LOD	1.23	<LOD	<LOD	<LOD	<LOD	0.22
RIK T106	DE	Fennel tea	<LOD	<LOD	<LOD	0.12	<LOD	<LOD	<LOD	<LOD	<LOD
IRTA_TA_0732	ES	Mixed herbal tea	<LOD	<LOD	<LOD	0.30	<LOD	<LOD	<LOD	<LOD	0.40

Appendix X – TA concentrations (µg/kg) in positive legumes, stir-fry mixes and oilseeds

Laboratory sample code	Country of sampling	Product	6-HO-tropinone	Nortropinone	Pseudo-tropine	Scopine	Scopoline	Tropine	Tropinone	Convolamine	Convolidine	Convolvine	Fillalbine
RIK FV1	NL	Vegetable stir-fry mix	<LOD	<LOD	158.7	<LOD	<LOD	194.3	0.92	<LOD	11.00	1.23	2.87
RIK FV2	NL	Vegetable stir-fry mix	<LOD	<LOD	173.9	<LOD	<LOD	1222.2	2.54	<LOD	18.64	1.86	19.90
RIK FV3	NL	Vegetable stir-fry mix	0.62	<LOD	28.41	<LOD	<LOD	54.28	2.70	<LOD	1.22	0.30	0.67
RIK FV4	NL	Vegetable stir-fry mix	<LOD	<LOD	14.87	<LOD	<LOD	139.0	2.24	<LOD	1.61	0.22	1.16
RIK FV5	NL	Vegetable stir-fry mix	<LOD	1.55	34.52	<LOD	<LOD	7.21	4.13	<LOD	0.33	0.26	1.30
RIK FV6	NL	Vegetable stir-fry mix	1.39	2.80	45.19	<LOD	<LOD	2.36	3.79	<LOD	<LOD	0.28	<LOD
RIK FV7	NL	Vegetable stir-fry mix	1.00	1.78	166.1	<LOD	<LOD	1998.0	5.18	0.61	<LOD	2.56	<LOD
RIK FV8	NL	Vegetable stir-fry mix	2.41	3.91	14.80	<LOD	<LOD	63.70	3.42	<LOD	0.91	0.39	0.78
RIK B1	NL	Bell pepper	<LOD	0.51	8.79	<LOD	<LOD	69.65	0.74	<LOD	5.56	0.46	15.83
RIK B2	NL	Bell pepper	<LOD	0.92	7.66	<LOD	<LOD	34.75	0.84	<LOD	0.94	0.81	0.13
RIK B3	NL	Bell pepper	<LOD	0.49	3.89	<LOD	<LOD	43.90	0.46	<LOD	0.39	0.39	0.65
RIK B4	NL	Bell pepper	<LOD	0.56	3.09	<LOD	<LOD	38.27	0.72	0.58	1.86	1.04	4.58
RIK B5	NL	Bell pepper	<LOD	1.01	11.17	<LOD	<LOD	327.0	1.13	0.63	16.34	2.22	2.07
RIK B6	NL	Bell pepper	<LOD	0.93	50.03	<LOD	<LOD	88.34	1.04	0.58	5.17	2.73	10.23
S16-003258	UK	Vegetable stir-fry mix	<LOD	<LOD	43.6	<LOD	<LOD	571.0	1.05	0.70	6.41	<LOD	11.60
S16-003262	UK	Vegetable stir-fry mix	<LOD	<LOD	15.1	<LOD	<LOD	24.1	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003264	UK	Vegetable stir-fry mix	<LOD	<LOD	19.5	<LOD	<LOD	27.9	<LOD	<LOD	0.79	<LOD	0.64
S16-006353	UK	Vegetable stir-fry mix	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-001242	UK	Linseed	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003299	UK	Other seeds	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix X, cont'd. TA concentrations (µg/kg) in positive legumes, stir-fry mixes and oilseeds

Laboratory sample code	Country of sampling	Product	Anisodamine	Apoatropine	Aposcopolamine	Atropine	Homatropine	Littorine	Noratropine	Norscopolamine	Scopolamine
RIK FV1	NL	Vegetable stir-fry mix	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK FV2	NL	Vegetable stir-fry mix	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK FV3	NL	Vegetable stir-fry mix	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK FV4	NL	Vegetable stir-fry mix	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK FV5	NL	Vegetable stir-fry mix	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK FV6	NL	Vegetable stir-fry mix	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK FV7	NL	Vegetable stir-fry mix	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK FV8	NL	Vegetable stir-fry mix	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK B1	NL	Bell pepper	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK B2	NL	Bell pepper	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK B3	NL	Bell pepper	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK B4	NL	Bell pepper	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK B5	NL	Bell pepper	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK B6	NL	Bell pepper	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003258	UK	Vegetable stir-fry mix	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003262	UK	Vegetable stir-fry mix	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-003264	UK	Vegetable stir-fry mix	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-006353	UK	Vegetable stir-fry mix	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
S16-001242	UK	Linseed	<LOD	<LOD	<LOD	0.11	<LOD	<LOD	<LOD	<LOD	0.09
S16-003299	UK	Other seeds	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix Y – Calystegine concentrations (mg/kg) in potato and potato products

Laboratory sample code	Country of sampling	Product	Calystegine					
			A_3	A_5	B_1	B_2	B_3	B_4
RIK P1	NL	New potatoes	62.4	<LOD	<LOD	35.3	<LOD	1.4
RIK P2	NL	Main-crop potatoes	51.0	<LOD	<LOD	27.3	<LOD	0.3
RIK P3	NL	Main-crop potatoes	26.8	<LOD	<LOD	19.7	<LOD	1.2
RIK P5	NL	Main-crop potatoes	127.4	<LOD	<LOD	47.9	<LOD	1.5
RIK P6	NL	Main-crop potatoes	86.6	<LOD	<LOD	32.6	<LOD	1.9
RIK P7	NL	Main-crop potatoes	138.8	<LOD	<LOD	57.2	<LOD	14.5
RIK P8	NL	Main-crop potatoes	109.4	<LOD	<LOD	55.9	<LOD	1.1
RIK P9	NL	Main-crop potatoes	90.5	<LOD	<LOD	34.6	<LOD	5.1
RIK P10	NL	Main-crop potatoes	48.2	<LOD	<LOD	23.3	<LOD	0.3
RIK P12	NL	Main-crop potatoes	113.1	<LOD	<LOD	39.4	<LOD	2.1
RIK P13	NL	Main-crop potatoes	82.0	<LOD	<LOD	38.1	<LOD	1.0
RIK P15	NL	Main-crop potatoes	161.8	<LOD	<LOD	53.3	<LOD	3.2
RIK P16	NL	Main-crop potatoes	163.2	<LOD	<LOD	59.2	<LOD	11.2
RIK P17	NL	Main-crop potatoes	170.4	<LOD	<LOD	64.1	<LOD	2.5
RIK P18	NL	Main-crop potatoes	154.4	<LOD	<LOD	64.3	<LOD	4.8
RIK P19	NL	Main-crop potatoes	141.4	<LOD	<LOD	54.7	<LOD	2.2
RIK P20	NL	Main-crop potatoes	155.1	<LOD	<LOD	65.3	<LOD	13.1
RIK P21	NL	Main-crop potatoes	145.3	<LOD	<LOD	70.8	<LOD	6.9
RIK P22	NL	Main-crop potatoes	69.8	<LOD	<LOD	25.6	<LOD	2.1
RIK P23	NL	Main-crop potatoes	134.3	<LOD	<LOD	56.8	<LOD	1.1
RIK P24	NL	Main-crop potatoes	49.8	<LOD	<LOD	21.1	<LOD	0.3
RIK P25	NL	Main-crop potatoes	90.6	<LOD	<LOD	45.0	<LOD	9.9
RIK P26	NL	Main-crop potatoes	79.8	<LOD	<LOD	31.4	<LOD	2.9
RIK P27	NL	Main-crop potatoes	186.4	<LOD	<LOD	60.0	<LOD	0.6
RIK P28	NL	Main-crop potatoes	109.8	<LOD	<LOD	53.9	<LOD	0.4
RIK P29	NL	Main-crop potatoes	78.1	<LOD	<LOD	38.5	<LOD	5.9
RIK P30	NL	Main-crop potatoes	79.8	<LOD	<LOD	30.1	<LOD	2.4
RIK P31	NL	New potatoes	135.7	<LOD	<LOD	61.4	<LOD	2.2
RIK P32	NL	Main-crop potatoes	126.0	<LOD	<LOD	45.2	<LOD	1.0
RIK P33	NL	Main-crop potatoes	110.7	<LOD	<LOD	44.5	<LOD	0.8
RIK P34	NL	Main-crop potatoes	218.6	<LOD	<LOD	95.9	<LOD	6.3
RIK P36	NL	Main-crop potatoes	120.3	<LOD	<LOD	58.6	<LOD	0.7
RIK P37	NL	Main-crop potatoes	176.6	<LOD	<LOD	92.2	<LOD	2.3
RIK P38	NL	Main-crop potatoes	273.4	<LOD	<LOD	104.6	<LOD	1.4
RIK P39	NL	New potatoes	67.8	<LOD	<LOD	24.6	<LOD	2.8
RIK P40	NL	Main-crop potatoes	178.0	<LOD	<LOD	110.7	<LOD	0.8
RIK P41	NL	Main-crop potatoes	105.2	<LOD	<LOD	43.5	<LOD	5.6
RIK P42	NL	Main-crop potatoes	139.7	<LOD	<LOD	47.2	<LOD	2.2
RIK P43	NL	Main-crop potatoes	180.9	<LOD	<LOD	100.6	<LOD	3.0
RIK P44	NL	Main-crop potatoes	174.4	<LOD	<LOD	72.5	<LOD	5.8
RIK P45	NL	Main-crop potatoes	131.5	<LOD	<LOD	59.0	<LOD	3.4
RIK P46	NL	Main-crop potatoes	165.0	<LOD	<LOD	115.8	0.3	2.7
RIK P47	NL	Main-crop potatoes	81.8	<LOD	<LOD	41.1	<LOD	4.9
RIK P48	NL	Main-crop potatoes	225.9	<LOD	<LOD	72.0	<LOD	0.5
RIK P49	NL	Main-crop potatoes	43.4	<LOD	<LOD	20.6	<LOD	0.4
RIK P50	NL	Main-crop potatoes	128.6	<LOD	<LOD	41.1	<LOD	13.7
RIK P51	NL	Main-crop potatoes	93.9	<LOD	<LOD	45.8	<LOD	5.4
RIK P52	NL	New potatoes	155.5	<LOD	<LOD	91.5	<LOD	1.4

Appendix Y, cont'd. Calystegine concentrations (mg/kg) in potato and potato products

Laboratory sample code	Country of sampling	Product	Calystegine					
			A_3	A_5	B_1	B_2	B_3	B_4
RIK P53	NL	Main-crop potatoes	313.9	<LOD	<LOD	131.3	<LOD	18.1
RIK P54	NL	Main-crop potatoes	129.0	<LOD	<LOD	53.3	<LOD	15.2
RIK P55	NL	Main-crop potatoes	132.3	<LOD	<LOD	39.6	<LOD	0.4
RIK P56	NL	Main-crop potatoes	142.1	<LOD	<LOD	66.3	<LOD	1.4
RIK P57	NL	Main-crop potatoes	62.8	<LOD	<LOD	35.9	<LOD	1.3
RIK P58	NL	Main-crop potatoes	128.1	<LOD	<LOD	41.5	<LOD	1.0
RIK P59	NL	Main-crop potatoes	105.3	<LOD	<LOD	51.7	<LOD	2.9
RIK P60	NL	Main-crop potatoes	97.6	<LOD	<LOD	25.0	<LOD	1.4
RIK P61	NL	Main-crop potatoes	347.4	<LOD	<LOD	121.0	<LOD	12.3
RIK P62	NL	Main-crop potatoes	206.4	<LOD	<LOD	89.2	<LOD	<LOD
RIK P63	NL	Main-crop potatoes	247.4	<LOD	<LOD	131.1	<LOD	4.2
RIK P64	NL	Main-crop potatoes	144.1	<LOD	<LOD	67.0	<LOD	1.4
RIK P65	NL	Main-crop potatoes	161.5	<LOD	<LOD	76.2	<LOD	18.1
RIK P66	NL	Main-crop potatoes	401.5	<LOD	<LOD	94.3	<LOD	11.5
RIK P67	NL	Main-crop potatoes	169.3	6.6	<LOD	74.9	<LOD	9.4
RIK P68	NL	Main-crop potatoes	50.1	<LOD	<LOD	23.8	<LOD	0.4
RIK P69	NL	Main-crop potatoes	95.3	<LOD	<LOD	23.6	<LOD	<LOD
RIK P70	NL	Main-crop potatoes	257.3	7.0	<LOD	155.3	<LOD	0.8
RIK P71	NL	Main-crop potatoes	106.0	<LOD	<LOD	54.9	<LOD	2.0
RIK P72	NL	New potatoes	161.1	<LOD	<LOD	60.7	<LOD	2.0
RIK P73	NL	Main-crop potatoes	197.7	<LOD	<LOD	82.2	<LOD	14.7
RIK P74	NL	Main-crop potatoes	152.1	<LOD	<LOD	89.0	<LOD	23.2
RIK P75	NL	Main-crop potatoes	227.4	<LOD	<LOD	82.3	<LOD	25.5
RIK P76	NL	Main-crop potatoes	159.8	<LOD	<LOD	82.6	<LOD	2.0
RIK P77	NL	Main-crop potatoes	101.5	2.4	<LOD	56.7	<LOD	<LOD
RIK P78	NL	Main-crop potatoes	289.9	<LOD	<LOD	137.5	<LOD	21.7
RIK P79	NL	Main-crop potatoes	98.2	<LOD	<LOD	54.4	<LOD	1.7
RIK P80	NL	Main-crop potatoes	73.5	<LOD	<LOD	32.7	<LOD	4.8
RIK P81	NL	Main-crop potatoes	223.5	3.7	<LOD	166.7	<LOD	5.0
RIK P82	NL	New potatoes	18.8	<LOD	<LOD	8.9	<LOD	0.8
RIK P83	NL	New potatoes	23.7	<LOD	<LOD	12.6	<LOD	1.3
RIK P84	NL	New potatoes	130.2	<LOD	<LOD	79.8	<LOD	6.5
RIK P85	NL	New potatoes	118.3	<LOD	<LOD	45.0	<LOD	1.6
RIK P86	NL	New potatoes	92.4	4.5	<LOD	34.8	<LOD	0.4
RIK P87	NL	New potatoes	138.0	<LOD	<LOD	81.2	<LOD	4.4
RIK P88	NL	Main-crop potatoes	170.0	3.1	<LOD	94.0	<LOD	0.8
RIK P89	NL	Main-crop potatoes	91.8	<LOD	<LOD	67.3	<LOD	0.5
RIK P90	NL	New potatoes	149.6	<LOD	<LOD	68.3	<LOD	10.8
RIK P91	NL	Main-crop potatoes	205.8	<LOD	<LOD	94.1	<LOD	1.0
RIK P92	NL	Main-crop potatoes	91.7	<LOD	<LOD	44.5	<LOD	1.0
RIK P93	NL	Main-crop potatoes	77.2	<LOD	<LOD	13.3	<LOD	15.2
RIK P94	NL	Main-crop potatoes	83.3	<LOD	<LOD	21.9	<LOD	0.3
RIK P95	NL	Main-crop potatoes	104.7	1.8	<LOD	46.3	<LOD	5.6
RIK P96	NL	Main-crop potatoes	207.6	3.9	<LOD	90.6	<LOD	20.5
RIK P97	NL	New potatoes	50.5	<LOD	<LOD	34.0	<LOD	4.4
RIK P98	NL	Main-crop potatoes	287.2	1.4	<LOD	142.5	<LOD	2.6
RIK P99	NL	New potatoes	100.7	<LOD	<LOD	54.5	<LOD	7.5
RIK P100	NL	Main-crop potatoes	88.5	<LOD	<LOD	39.3	<LOD	20.6

Appendix Y, cont'd. Calystegine concentrations (mg/kg) in potato and potato products

Laboratory sample code	Country of sampling	Product	Calystegine					
			A_3	A_5	B_1	B_2	B_3	B_4
RIK P202	NL	New potatoes	107.6	<LOD	<LOD	83.2	<LOD	3.9
RIK P203	NL	Main-crop potatoes	174.6	2.1	<LOD	65.9	<LOD	22.2
RIK P204	NL	Main-crop potatoes	196.7	1.5	<LOD	91.5	<LOD	20.3
RIK P205	NL	Main-crop potatoes	121.7	<LOD	<LOD	63.9	<LOD	6.8
RIK P206	NL	Main-crop potatoes	43.2	<LOD	<LOD	33.5	<LOD	1.7
RIK P207	NL	Main-crop potatoes	56.0	<LOD	<LOD	32.2	<LOD	4.6
RIK P208	NL	Main-crop potatoes	99.9	<LOD	<LOD	60.8	<LOD	5.8
RIK P209	NL	New potatoes	103.6	<LOD	<LOD	40.7	<LOD	4.7
RIK P210	NL	Main-crop potatoes	69.9	<LOD	<LOD	55.5	<LOD	<LOD
RIK P211	NL	Main-crop potatoes	73.9	<LOD	<LOD	38.2	<LOD	6.4
RIK P212	NL	Main-crop potatoes	173.1	<LOD	<LOD	74.3	<LOD	0.4
RIK P213	NL	Main-crop potatoes	169.0	<LOD	<LOD	50.7	<LOD	4.4
RIK P214	NL	Main-crop potatoes	200.4	<LOD	<LOD	94.5	<LOD	5.8
RIK P215	NL	Main-crop potatoes	138.9	<LOD	<LOD	74.1	<LOD	8.2
RIK P216	NL	Main-crop potatoes	100.3	3.0	<LOD	33.5	<LOD	0.7
RIK P217	NL	Main-crop potatoes	272.2	8.2	<LOD	165.5	<LOD	32.4
RIK P218	NL	New potatoes	75.9	<LOD	<LOD	27.0	<LOD	7.4
RIK P219	NL	Main-crop potatoes	71.6	<LOD	<LOD	30.3	<LOD	1.1
RIK P220	NL	Main-crop potatoes	35.2	<LOD	<LOD	19.8	<LOD	8.3
RIK P221	NL	Main-crop potatoes	89.6	<LOD	<LOD	42.2	<LOD	2.2
RIK P222	NL	Main-crop potatoes	122.8	<LOD	<LOD	65.5	<LOD	2.1
RIK P223	NL	Main-crop potatoes	87.7	1.7	<LOD	37.8	<LOD	0.6
RIK P224	NL	Main-crop potatoes	53.5	<LOD	<LOD	20.8	<LOD	4.1
RIK P225	NL	Main-crop potatoes	58.5	<LOD	<LOD	23.1	<LOD	7.1
RIK P226	NL	New potatoes	43.8	<LOD	<LOD	24.3	<LOD	3.6
RIK P227	NL	New potatoes	48.8	<LOD	<LOD	20.3	<LOD	1.1
RIK P228	NL	Main-crop potatoes	74.7	<LOD	<LOD	34.3	<LOD	0.3
RIK P229	NL	Main-crop potatoes	77.0	<LOD	<LOD	34.7	<LOD	<LOD
RIK P230	NL	Main-crop potatoes	163.6	<LOD	<LOD	73.6	<LOD	9.9
RIK P231	NL	Main-crop potatoes	102.9	<LOD	<LOD	42.7	<LOD	1.0
RIK P232	NL	Main-crop potatoes	42.3	<LOD	<LOD	15.6	<LOD	<LOD
RIK P233	NL	Main-crop potatoes	85.4	<LOD	<LOD	38.7	<LOD	2.5
RIK P234	NL	Main-crop potatoes	35.8	<LOD	<LOD	30.7	<LOD	1.2
RIK P235	NL	Main-crop potatoes	73.4	<LOD	<LOD	42.8	<LOD	2.5
RIK P236	NL	Main-crop potatoes	157.2	<LOD	<LOD	87.5	<LOD	<LOD
RIK P237	NL	Main-crop potatoes	221.7	5.5	<LOD	114.2	<LOD	10.6
RIK P238	NL	Main-crop potatoes	210.6	<LOD	<LOD	82.7	<LOD	2.9
RIK P239	NL	Main-crop potatoes	165.7	<LOD	<LOD	76.9	<LOD	2.2
RIK P101	DE	New potatoes	36.9	<LOD	<LOD	25.5	<LOD	0.9
RIK P102	DE	New potatoes	37.6	<LOD	<LOD	26.5	<LOD	1.1
RIK P103	DE	Main-crop potatoes	15.5	<LOD	<LOD	9.1	<LOD	0.6
RIK P104	DE	Main-crop potatoes	12.9	<LOD	<LOD	8.7	<LOD	0.5
RIK P105	DE	Main-crop potatoes	74.0	<LOD	<LOD	19.5	<LOD	0.5
RIK P106	DE	Main-crop potatoes	226.1	<LOD	<LOD	91.6	<LOD	0.4
RIK P107	DE	Main-crop potatoes	56.6	<LOD	<LOD	12.5	<LOD	0.3
RIK P108	DE	Main-crop potatoes	44.8	<LOD	<LOD	25.6	<LOD	2.5
IRTA_TA_0200	ES	Main-crop potatoes	111.3	<LOD	<LOD	30.6	<LOD	3.7
IRTA_TA_0201	ES	Main-crop potatoes	59.0	<LOD	<LOD	33.6	<LOD	1.7

Appendix Y, cont'd. Calystegine concentrations (mg/kg) in potato and potato products

Laboratory sample code	Country of sampling	Product	Calystegine					
			A_3	A_5	B_1	B_2	B_3	B_4
IRTA_TA_0202	ES	Main-crop potatoes	56.5	<LOD	<LOD	21.8	<LOD	<LOD
IRTA_TA_0203	ES	Main-crop potatoes	57.7	<LOD	<LOD	32.9	<LOD	2.1
IRTA_TA_0204	ES	Main-crop potatoes	60.7	<LOD	<LOD	24.3	<LOD	<LOD
IRTA_TA_0205	ES	Main-crop potatoes	124.7	<LOD	<LOD	46.6	<LOD	1.9
IRTA_TA_0206	ES	Main-crop potatoes	52.5	<LOD	<LOD	21.8	<LOD	1.2
IRTA_TA_0207	ES	Main-crop potatoes	81.2	<LOD	<LOD	35.8	<LOD	<LOD
IRTA_TA_0208	ES	Main-crop potatoes	56.8	<LOD	<LOD	38.2	1.7	0.6
IRTA_TA_0209	ES	Main-crop potatoes	42.0	<LOD	<LOD	23.3	<LOD	0.6
IRTA_TA_0210	ES	Main-crop potatoes	73.7	<LOD	<LOD	46.7	<LOD	<LOD
IRTA_TA_0211	ES	Main-crop potatoes	69.0	<LOD	<LOD	32.0	<LOD	4.4
IRTA_TA_0212	ES	Main-crop potatoes	90.8	<LOD	<LOD	69.2	<LOD	0.9
IRTA_TA_0215	ES	Main-crop potatoes	67.5	<LOD	<LOD	31.6	<LOD	1.3
IRTA_TA_0216	ES	Main-crop potatoes	101.5	<LOD	<LOD	43.1	1.4	1.0
IRTA_TA_0217	ES	Main-crop potatoes	88.7	<LOD	<LOD	69.3	<LOD	0.7
IRTA_TA_0218	ES	Main-crop potatoes	212.4	<LOD	<LOD	141.4	1.3	1.5
IRTA_TA_0219	ES	Main-crop potatoes	110.1	1.9	<LOD	99.4	<LOD	<LOD
IRTA_TA_0220	ES	Main-crop potatoes	141.6	<LOD	<LOD	98.6	<LOD	3.2
IRTA_TA_0221	ES	Main-crop potatoes	74.1	<LOD	<LOD	27.6	<LOD	<LOD
IRTA_TA_0222	ES	Main-crop potatoes	100.1	<LOD	<LOD	39.2	<LOD	3.8
IRTA_TA_0223	ES	Main-crop potatoes	85.4	<LOD	<LOD	30.1	<LOD	<LOD
IRTA_TA_0224	ES	Main-crop potatoes	54.7	<LOD	<LOD	44.8	<LOD	0.9
IRTA_TA_0225	ES	Main-crop potatoes	126.2	<LOD	<LOD	48.9	<LOD	3.7
IRTA_TA_0226	ES	Main-crop potatoes	191.2	<LOD	<LOD	86.1	<LOD	1.9
IRTA_TA_0227	ES	Main-crop potatoes	321.8	<LOD	<LOD	165.3	2.9	9.1
IRTA_TA_0800	ES	Main-crop potatoes	75.3	<LOD	<LOD	41.5	<LOD	1.4
IRTA_TA_0801	ES	Main-crop potatoes	136.1	<LOD	<LOD	43.2	<LOD	10.1
IRTA_TA_0802	ES	Main-crop potatoes	95.1	<LOD	<LOD	55.5	<LOD	1.3
IRTA_TA_0803	ES	Main-crop potatoes	134.9	<LOD	<LOD	87.4	<LOD	3.6
IRTA_TA_0804	ES	Main-crop potatoes	53.6	<LOD	<LOD	26.3	<LOD	2.7
IRTA_TA_0805	ES	Main-crop potatoes	83.4	16.8	<LOD	67.7	<LOD	<LOD
IRTA_TA_0806	ES	Main-crop potatoes	169.3	<LOD	<LOD	82.2	<LOD	9.5
IRTA_TA_0807	ES	Main-crop potatoes	59.6	<LOD	<LOD	45.7	<LOD	1.3
IRTA_TA_0808	ES	Main-crop potatoes	50.7	<LOD	<LOD	22.4	<LOD	0.7
IRTA_TA_0809	ES	Main-crop potatoes	66.9	<LOD	<LOD	58.2	<LOD	5.7
IRTA_TA_0810	ES	Main-crop potatoes	93.3	<LOD	<LOD	47.1	<LOD	<LOD
IRTA_TA_0811	ES	Main-crop potatoes	202.1	<LOD	<LOD	111.3	1.5	6.7
IRTA_TA_0812	ES	Main-crop potatoes	149.0	<LOD	<LOD	77.4	1.0	15.6
IRTA_TA_0813	ES	Main-crop potatoes	58.7	<LOD	<LOD	54.8	<LOD	4.2
IRTA_TA_0814	ES	Main-crop potatoes	47.7	<LOD	<LOD	28.8	<LOD	0.6
IRTA_TA_0815	ES	Main-crop potatoes	60.9	<LOD	<LOD	54.2	<LOD	0.8
IRTA_TA_0816	ES	Main-crop potatoes	53.5	<LOD	<LOD	51.9	<LOD	4.4
IRTA_TA_1050	FR	Main-crop potatoes	67.9	<LOD	<LOD	39.8	<LOD	1.6
IRTA_TA_1051	FR	Main-crop potatoes	61.3	<LOD	<LOD	35.0	<LOD	1.4
IRTA_TA_1055	FR	Main-crop potatoes	24.9	<LOD	<LOD	20.8	<LOD	0.8
IRTA_TA_1056	FR	Main-crop potatoes	54.8	<LOD	<LOD	41.7	<LOD	3.1
IRTA_TA_1057	FR	Main-crop potatoes	55.0	<LOD	<LOD	55.0	<LOD	3.6
IRTA_TA_1059	FR	Main-crop potatoes	41.3	<LOD	<LOD	29.3	<LOD	3.2
IRTA_TA_1400	IT	Main-crop potatoes	91.7	<LOD	<LOD	53.4	<LOD	1.0

Appendix Y, cont'd. Calystegine concentrations (mg/kg) in potato and potato products

Laboratory sample code	Country of sampling	Product	Calystegine					
			A_3	A_5	B_1	B_2	B_3	B_4
IRTA_TA_1401	IT	Main-crop potatoes	90.2	1.6	<LOD	70.9	1.1	<LOD
IRTA_TA_1402	IT	Main-crop potatoes	95.4	<LOD	<LOD	66.0	1.1	2.2
IRTA_TA_1403	IT	Main-crop potatoes	105.6	<LOD	<LOD	54.5	<LOD	10.5
IRTA_TA_1404	IT	Main-crop potatoes	103.4	<LOD	<LOD	73.8	1.1	2.1
IRTA_TA_1405	IT	Main-crop potatoes	85.3	<LOD	<LOD	46.6	<LOD	<LOD
IRTA_TA_1406	IT	Main-crop potatoes	52.3	<LOD	<LOD	31.8	<LOD	<LOD
IRTA_TA_1407	IT	Main-crop potatoes	86.8	<LOD	<LOD	54.0	<LOD	0.9
IRTA_TA_1408	IT	Main-crop potatoes	196.8	3.6	<LOD	202.9	2.8	2.4
IRTA_TA_1409	IT	Main-crop potatoes	87.9	<LOD	<LOD	52.6	<LOD	0.8
VV 806/15	CZ	Main-crop potatoes	37.4	<LOD	<LOD	12.4	<LOD	<LOD
VV 807/15	CZ	Main-crop potatoes	60.2	<LOD	<LOD	29.6	<LOD	<LOD
VV 808/15	CZ	Main-crop potatoes	74.7	<LOD	<LOD	41.0	<LOD	<LOD
VV 809/15	CZ	Main-crop potatoes	49.8	<LOD	<LOD	26.4	<LOD	0.3
VV 810/15	CZ	Main-crop potatoes	70.5	<LOD	<LOD	51.5	<LOD	13.0
VV 811/15	CZ	Main-crop potatoes	85.4	<LOD	<LOD	41.3	<LOD	0.4
VV 812/15	CZ	Main-crop potatoes	42.8	<LOD	<LOD	15.7	<LOD	0.3
VV 813/15	CZ	Main-crop potatoes	45.5	<LOD	<LOD	20.1	<LOD	0.7
VV 814/15	CZ	Main-crop potatoes	22.9	<LOD	<LOD	16.9	<LOD	0.3
VV 815/15	CZ	Main-crop potatoes	58.8	<LOD	<LOD	23.6	<LOD	0.3
VV 816/15	CZ	Main-crop potatoes	118.5	<LOD	<LOD	66.1	<LOD	0.4
VV 817/15	CZ	Main-crop potatoes	43.8	<LOD	<LOD	15.3	<LOD	0.3
VV 818/15	CZ	Main-crop potatoes	30.1	<LOD	<LOD	19.3	<LOD	<LOD
VV 819/15	CZ	Main-crop potatoes	81.9	<LOD	<LOD	40.9	<LOD	<LOD
VV 205/16	CZ	Main-crop potatoes	59.8	<LOD	<LOD	32.9	<LOD	<LOD
VV 206/16	CZ	Main-crop potatoes	59.0	<LOD	<LOD	38.8	<LOD	<LOD
VV 207/16	CZ	Main-crop potatoes	51.1	<LOD	<LOD	37.6	<LOD	8.0
VV 208/16	CZ	Main-crop potatoes	59.1	<LOD	<LOD	42.2	<LOD	8.0
VV 209/16	CZ	Main-crop potatoes	47.0	<LOD	<LOD	31.5	<LOD	0.6
VV 210/16	CZ	Main-crop potatoes	48.4	<LOD	<LOD	30.6	<LOD	<LOD
VV 211/16	CZ	Main-crop potatoes	59.8	<LOD	<LOD	45.9	<LOD	0.4
VV 212/16	CZ	Main-crop potatoes	37.6	<LOD	<LOD	22.2	<LOD	0.3
VV 213/16	CZ	Main-crop potatoes	38.9	<LOD	<LOD	27.4	<LOD	<LOD
VV 214/16	CZ	Main-crop potatoes	50.4	<LOD	<LOD	53.0	<LOD	7.0
VV 215/16	CZ	Main-crop potatoes	49.3	<LOD	<LOD	28.5	<LOD	7.0
VV 216/16	CZ	Main-crop potatoes	20.9	<LOD	<LOD	12.2	<LOD	<LOD
VV 217/16	CZ	Main-crop potatoes	34.0	<LOD	<LOD	18.1	<LOD	0.5
VV 218/16	CZ	Main-crop potatoes	77.2	<LOD	<LOD	64.3	<LOD	0.7
VV 219/16	CZ	Main-crop potatoes	32.5	<LOD	<LOD	23.4	<LOD	0.7
VV 220/16	CZ	Main-crop potatoes	25.2	<LOD	<LOD	13.5	<LOD	0.3
VV 1154/15	HU	Main-crop potatoes	67.2	<LOD	<LOD	32.0	<LOD	<LOD
VV 1155/15	HU	Main-crop potatoes	54.8	<LOD	<LOD	41.6	<LOD	<LOD
VV 1156/15	HU	Main-crop potatoes	23.2	<LOD	<LOD	12.5	<LOD	<LOD
VV 1157/15	HU	Main-crop potatoes	131.1	<LOD	<LOD	57.9	<LOD	0.4
VV 1158/15	HU	Main-crop potatoes	78.9	<LOD	<LOD	39.2	<LOD	0.3
VV 1159/15	HU	Main-crop potatoes	43.2	<LOD	<LOD	19.6	<LOD	<LOD
VV 1160/15	HU	Main-crop potatoes	40.0	<LOD	<LOD	18.7	<LOD	0.3
VV 1075/16	HU	Main-crop potatoes	239.9	<LOD	<LOD	87.6	<LOD	11.7
VV 1076/16	HU	Main-crop potatoes	41.8	<LOD	<LOD	29.1	<LOD	0.4

Appendix Y, cont'd. Calystegine concentrations (mg/kg) in potato and potato products

Laboratory sample code	Country of sampling	Product	Calystegine					
			A_3	A_5	B_1	B_2	B_3	B_4
VV 1077/16	HU	Main-crop potatoes	299.0	<LOD	<LOD	164.4	<LOD	0.6
VV 1049/15	PL	Main-crop potatoes	74.8	<LOD	<LOD	27.6	<LOD	0.5
VV 1050/15	PL	Main-crop potatoes	37.0	<LOD	<LOD	22.3	<LOD	<LOD
VV 1051/15	PL	Main-crop potatoes	61.6	<LOD	<LOD	41.8	<LOD	<LOD
VV 1052/15	PL	Main-crop potatoes	64.7	<LOD	<LOD	44.5	<LOD	<LOD
VV 1053/15	PL	Main-crop potatoes	67.0	<LOD	<LOD	43.6	<LOD	<LOD
VV 1054/15	PL	Main-crop potatoes	61.0	<LOD	<LOD	41.8	<LOD	<LOD
VV 1055/15	PL	Main-crop potatoes	101.0	<LOD	<LOD	40.1	<LOD	8.7
VV 1056/15	PL	Main-crop potatoes	61.6	0.7	<LOD	38.3	<LOD	<LOD
VV 1057/15	PL	Main-crop potatoes	64.7	1.1	<LOD	51.6	<LOD	<LOD
VV 1058/15	PL	Main-crop potatoes	45.3	0.7	<LOD	31.2	<LOD	<LOD
S15-100667	UK	Main-crop potatoes	118.3	<LOD	<LOD	51.9	<LOD	4.1
S15-100668	UK	Main-crop potatoes	211.4	1.2	<LOD	127.1	<LOD	6.9
S15-100669	UK	Main-crop potatoes	101.1	<LOD	<LOD	13.1	<LOD	7.6
S15-100670	UK	Main-crop potatoes	167.8	<LOD	<LOD	107.2	<LOD	5.3
S15-100671	UK	Main-crop potatoes	174.0	<LOD	<LOD	72.7	<LOD	4.1
S15-100672	UK	Main-crop potatoes	151.5	<LOD	<LOD	77.0	<LOD	3.7
S15-100673	UK	New potatoes	201.3	<LOD	<LOD	60.9	<LOD	6.7
S15-100674	UK	Main-crop potatoes	96.6	<LOD	<LOD	33.3	<LOD	2.4
S15-100675	UK	Main-crop potatoes	149.7	<LOD	<LOD	40.7	<LOD	1.3
S15-100676	UK	Main-crop potatoes	99.6	<LOD	<LOD	39.3	<LOD	3.1
S15-100677	UK	Main-crop potatoes	193.7	<LOD	<LOD	73.7	<LOD	0.5
S15-100678	UK	New potatoes	69.9	<LOD	<LOD	26.0	<LOD	2.2
S15-100679	UK	New potatoes	205.9	<LOD	<LOD	122.7	<LOD	5.5
S15-100680	UK	Main-crop potatoes	51.8	<LOD	<LOD	15.9	<LOD	8.1
S15-100681	UK	Main-crop potatoes	67.4	<LOD	<LOD	20.2	<LOD	2.2
S15-100682	UK	New potatoes	129.3	<LOD	<LOD	49.2	<LOD	0.7
S15-100683	UK	New potatoes	261.5	<LOD	<LOD	90.2	<LOD	11.2
S15-100684	UK	Main-crop potatoes	142.7	10.6	<LOD	80.2	<LOD	0.6
S15-100685	UK	Main-crop potatoes	262.2	<LOD	<LOD	47.3	<LOD	4.2
S15-100686	UK	New potatoes	131.9	<LOD	<LOD	67.6	<LOD	4.5
S15-100687	UK	Main-crop potatoes	309.9	<LOD	<LOD	56.7	<LOD	4.4
S15-100688	UK	New potatoes	35.1	<LOD	<LOD	10.5	<LOD	1.6
S15-100689	UK	New potatoes	13.6	<LOD	<LOD	16.9	<LOD	1.5
S16-003265	UK	Main-crop potatoes	236.2	4.1	<LOD	126.4	<LOD	13.5
S16-003266	UK	Main-crop potatoes	110.9	5.4	<LOD	105.5	<LOD	4.3
S16-003267	UK	New potatoes	23.7	<LOD	<LOD	14.9	<LOD	2.4
S16-003268	UK	Main-crop potatoes	23.9	<LOD	<LOD	5.9	<LOD	0.7
S16-003269	UK	New potatoes	18.1	<LOD	<LOD	4.1	<LOD	1.2
S16-003270	UK	New potatoes	112.9	6.3	<LOD	109.3	<LOD	4.7
S16-003271	UK	New potatoes	90.9	<LOD	<LOD	24.7	<LOD	0.8
S16-003272	UK	New potatoes	101.3	<LOD	<LOD	23.7	<LOD	2.4
S16-003273	UK	New potatoes	31.5	<LOD	<LOD	17.5	<LOD	0.5
S16-003274	UK	New potatoes	159.5	<LOD	<LOD	51.1	<LOD	16.7
S16-003275	UK	Main-crop potatoes	134.4	<LOD	<LOD	39.5	<LOD	4.0
S16-003276	UK	Main-crop potatoes	132.7	<LOD	<LOD	18.6	<LOD	1.7
S16-003277	UK	Main-crop potatoes	77.1	<LOD	<LOD	45.8	<LOD	2.6
S16-003278	UK	New potatoes	84.2	<LOD	<LOD	27.3	<LOD	0.8

Appendix Y, cont'd. Calystegine concentrations (mg/kg) in potato and potato products

Laboratory sample code	Country of sampling	Product	Calystegine					
			A_3	A_5	B_1	B_2	B_3	B_4
S16-003279	UK	Main-crop potatoes	33.4	<LOD	<LOD	7.4	<LOD	1.6
S16-003280	UK	New potatoes	63.0	2.4	<LOD	21.5	<LOD	4.8
S16-003281	UK	Main-crop potatoes	96.2	<LOD	<LOD	38.2	<LOD	4.0
S16-003282	UK	New potatoes	179.6	<LOD	<LOD	69.0	<LOD	5.6
S16-003283	UK	New potatoes	13.0	<LOD	<LOD	5.4	<LOD	1.1
S16-003284	UK	Main-crop potatoes	221.6	<LOD	<LOD	71.7	<LOD	15.8
S16-003285	UK	New potatoes	75.1	<LOD	<LOD	24.0	<LOD	1.2
RIK P4	NL	Potato boiled	156.6	<LOD	<LOD	44.2	<LOD	6.9
RIK P14	NL	Mashed potato powder	113.4	<LOD	<LOD	56.7	1.9	14.3
RIK P35	NL	Potato boiled	76.0	<LOD	<LOD	26.2	<LOD	1.4
RIK P109	DE	Mashed potato powder	120.1	<LOD	<LOD	38.1	1.3	1.9
RIK P110	DE	Potato boiled	73.2	<LOD	<LOD	21.9	<LOD	2.3
IRTA_TA_0213	ES	Potato boiled	61.6	<LOD	<LOD	17.9	<LOD	2.1
IRTA_TA_0214	ES	Potato boiled	49.6	<LOD	<LOD	12.2	<LOD	<LOD
IRTA_TA_1052	FR	Potato boiled	21.2	<LOD	<LOD	11.3	<LOD	2.5
IRTA_TA_1053	FR	Potato boiled	16.5	<LOD	<LOD	8.6	<LOD	1.2
IRTA_TA_1054	FR	Potato boiled	15.9	<LOD	<LOD	10.4	<LOD	2.1
IRTA_TA_1058	FR	Potato boiled	34.4	<LOD	<LOD	24.8	<LOD	3.5
RIK P11	NL	Sweet potatoes	2.3	<LOD	24.4	22.7	<LOD	2.3
RIK P201	NL	Sweet potatoes	<LOD	5.4	51.3	60.1	<LOD	1.7

Appendix Z – Calystegine concentrations (mg/kg) in aubergine and bell pepper

Laboratory sample code	Country of sampling	Product	Calystegine					
			A_3	A_5	B_1	B_2	B_3	B_4
RIK A1	NL	Aubergine	1.0	<LOD	1.3	2.0	<LOD	<LOD
RIK A2	NL	Aubergine	<LOD	<LOD	0.9	1.9	<LOD	<LOD
RIK A3	NL	Aubergine	1.7	<LOD	4.0	3.0	<LOD	<LOD
RIK A4	NL	Aubergine	<LOD	<LOD	0.5	1.0	<LOD	<LOD
RIK A5	NL	Aubergine	<LOD	<LOD	5.3	3.1	<LOD	<LOD
RIK A6	NL	Aubergine	2.6	<LOD	3.1	5.5	<LOD	<LOD
RIK A7	NL	Aubergine	1.0	<LOD	0.9	2.0	<LOD	<LOD
RIK A8	NL	Aubergine	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK A9	NL	Aubergine	<LOD	<LOD	<LOD	0.3	<LOD	<LOD
RIK A10	NL	Aubergine	0.6	<LOD	3.9	7.0	<LOD	<LOD
RIK A11	NL	Aubergine	4.8	<LOD	6.4	29.4	<LOD	<LOD
RIK A12	NL	Aubergine	6.9	<LOD	10.5	43.1	<LOD	<LOD
RIK A13	NL	Aubergine	2.6	<LOD	5.9	17.3	<LOD	<LOD
RIK A14	NL	Aubergine	0.5	<LOD	3.1	4.8	<LOD	<LOD
RIK A15	NL	Aubergine	0.6	<LOD	1.2	1.4	<LOD	<LOD
RIK A101	DE	Aubergine	<LOD	<LOD	0.3	0.6	<LOD	<LOD
RIK A102	DE	Aubergine	<LOD	<LOD	0.4	0.8	<LOD	<LOD
RIK A103	DE	Aubergine	1.4	<LOD	8.3	6.1	<LOD	<LOD
RIK A104	DE	Aubergine	4.5	<LOD	4.6	22.0	<LOD	<LOD
RIK A105	DE	Aubergine	5.6	<LOD	4.8	23.5	<LOD	<LOD
IRTA_TA_0228	ES	Aubergine	1.4	<LOD	2.0	11.1	<LOD	<LOD
IRTA_TA_0229	ES	Aubergine	5.2	<LOD	3.0	12.5	<LOD	<LOD
IRTA_TA_0230	ES	Aubergine	3.2	<LOD	2.0	9.5	<LOD	<LOD
IRTA_TA_0231	ES	Aubergine	2.7	<LOD	2.0	9.8	<LOD	<LOD
IRTA_TA_0232	ES	Aubergine	1.0	<LOD	1.1	5.7	<LOD	<LOD
IRTA_TA_0233	ES	Aubergine	2.2	<LOD	2.4	8.9	<LOD	<LOD
IRTA_TA_0234	ES	Aubergine	3.0	<LOD	2.7	9.2	<LOD	<LOD
IRTA_TA_0235	ES	Aubergine	3.3	<LOD	2.5	14.4	<LOD	<LOD
IRTA_TA_0236	ES	Aubergine	13.2	<LOD	6.3	45.3	<LOD	<LOD
IRTA_TA_0237	ES	Aubergine	25.1	<LOD	7.2	64.5	<LOD	<LOD
IRTA_TA_0238	ES	Aubergine	10.0	<LOD	6.8	39.1	<LOD	<LOD
IRTA_TA_0239	ES	Aubergine	0.6	<LOD	2.5	2.1	<LOD	<LOD
IRTA_TA_0240	ES	Aubergine	8.8	<LOD	9.5	36.2	<LOD	<LOD
IRTA_TA_0817	ES	Aubergine	0.6	<LOD	6.4	1.1	<LOD	<LOD
IRTA_TA_0818	ES	Aubergine	3.2	<LOD	5.2	14.4	<LOD	<LOD
IRTA_TA_0819	ES	Aubergine	2.4	4.2	11.4	9.9	<LOD	<LOD
IRTA_TA_0820	ES	Aubergine	0.7	<LOD	7.1	4.4	<LOD	<LOD
IRTA_TA_0821	ES	Aubergine	0.9	<LOD	1.8	14.5	<LOD	<LOD
IRTA_TA_0822	ES	Aubergine	1.9	<LOD	2.5	16.3	<LOD	<LOD
IRTA_TA_0823	ES	Aubergine	3.4	<LOD	3.0	16.3	<LOD	<LOD
IRTA_TA_1060	FR	Aubergine	10.9	<LOD	2.5	25.2	<LOD	<LOD
IRTA_TA_1061	FR	Aubergine	5.4	<LOD	2.7	17.7	<LOD	<LOD
IRTA_TA_1062	FR	Aubergine	3.8	<LOD	2.7	14.9	<LOD	<LOD
IRTA_TA_1063	FR	Aubergine	1.9	<LOD	3.1	2.0	<LOD	<LOD
IRTA_TA_1064	FR	Aubergine	1.8	<LOD	1.5	2.1	<LOD	<LOD
IRTA_TA_1410	IT	Aubergine	0.6	<LOD	6.7	4.7	<LOD	<LOD
IRTA_TA_1411	IT	Aubergine	2.1	<LOD	10.5	5.6	<LOD	<LOD
IRTA_TA_1412	IT	Aubergine	1.0	<LOD	7.1	4.9	<LOD	<LOD

Appendix Z, cont'd. Calystegine concentrations (mg/kg) in aubergine and bell pepper

Laboratory sample code	Country of sampling	Product	Calystegine					
			A_3	A_5	B_1	B_2	B_3	B_4
IRTA_TA_1413	IT	Aubergine	1.0	<LOD	8.3	6.2	<LOD	<LOD
IRTA_TA_1414	IT	Aubergine	<LOD	<LOD	3.2	1.8	<LOD	<LOD
VV 820/15	CZ	Aubergine	<LOD	<LOD	0.3	7.7	<LOD	<LOD
VV 821/15	CZ	Aubergine	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 822/15	CZ	Aubergine	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
VV 823/15	CZ	Aubergine	<LOD	<LOD	0.3	<LOD	<LOD	<LOD
VV 824/15	CZ	Aubergine	<LOD	<LOD	0.3	<LOD	<LOD	<LOD
VV 825/15	CZ	Aubergine	<LOD	<LOD	0.4	11.1	<LOD	<LOD
VV 826/15	CZ	Aubergine	<LOD	<LOD	0.7	16.2	<LOD	<LOD
VV 221/16	CZ	Aubergine	0.6	<LOD	0.4	13.7	<LOD	<LOD
VV 222/16	CZ	Aubergine	0.8	<LOD	6.0	19.6	<LOD	<LOD
VV 223/16	CZ	Aubergine	<LOD	<LOD	9.0	6.0	<LOD	<LOD
VV 224/16	CZ	Aubergine	1.4	<LOD	13.0	61.7	<LOD	<LOD
VV 225/16	CZ	Aubergine	<LOD	<LOD	0.5	14.5	<LOD	<LOD
VV 226/16	CZ	Aubergine	<LOD	<LOD	0.4	11.9	<LOD	<LOD
VV 227/16	CZ	Aubergine	<LOD	<LOD	0.5	14.5	<LOD	<LOD
VV 228/16	CZ	Aubergine	<LOD	<LOD	0.4	9.4	<LOD	<LOD
VV 1161/15	HU	Aubergine	1.2	<LOD	0.5	14.5	<LOD	<LOD
VV 1162/15	HU	Aubergine	<LOD	<LOD	0.4	12.8	<LOD	<LOD
VV 1163/15	HU	Aubergine	<LOD	<LOD	0.4	12.8	<LOD	<LOD
VV 1078/16	HU	Aubergine	<LOD	<LOD	0.3	8.9	<LOD	<LOD
VV 1079/16	HU	Aubergine	<LOD	<LOD	5.1	17.6	<LOD	<LOD
VV 1080/16	HU	Aubergine	<LOD	<LOD	8.3	17.9	<LOD	<LOD
VV 1081/16	HU	Aubergine	1.2	<LOD	8.6	24.7	<LOD	<LOD
VV 1082/16	HU	Aubergine	<LOD	<LOD	0.5	16.8	<LOD	<LOD
VV 1083/16	HU	Aubergine	<LOD	<LOD	0.5	16.3	<LOD	<LOD
VV 1059/15	PL	Aubergine	0.6	<LOD	0.4	11.9	<LOD	<LOD
S15-100690	UK	Aubergine	5.8	<LOD	7.1	22.4	<LOD	<LOD
S15-100691	UK	Aubergine	6.1	<LOD	6.6	18.8	<LOD	<LOD
S15-100692	UK	Aubergine	3.6	<LOD	4.8	20.5	<LOD	<LOD
S15-100693	UK	Aubergine	5.9	<LOD	6.2	30.5	<LOD	<LOD
S15-100694	UK	Aubergine	8.3	<LOD	8.1	40.6	<LOD	<LOD
S15-100695	UK	Aubergine	6.1	<LOD	6.8	33.2	<LOD	<LOD
S15-100696	UK	Aubergine	7.5	<LOD	5.8	29.6	<LOD	<LOD
S15-100697	UK	Aubergine	0.7	<LOD	7.4	4.7	<LOD	<LOD
S16-003232	UK	Aubergine	1.1	<LOD	0.9	1.5	<LOD	<LOD
S16-003286	UK	Aubergine	<LOD	<LOD	0.5	0.7	<LOD	<LOD
S16-003287	UK	Aubergine	1.1	<LOD	2.8	11.6	<LOD	<LOD
S16-003288	UK	Aubergine	3.8	<LOD	1.9	3.5	<LOD	<LOD
S16-003289	UK	Aubergine	1.6	<LOD	3.4	14.4	<LOD	<LOD
S16-003290	UK	Aubergine	2.1	<LOD	1.2	1.9	<LOD	<LOD
S16-003315	UK	Aubergine	28.1	<LOD	28.6	124.8	<LOD	<LOD
RIK B-1	NL	Bell pepper	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK B-2	NL	Bell pepper	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK B-3	NL	Bell pepper	<LOD	<LOD	0.4	<LOD	<LOD	<LOD
RIK B-4	NL	Bell pepper	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK B-5	NL	Bell pepper	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
RIK B-6	NL	Bell pepper	<LOD	<LOD	0.5	<LOD	<LOD	<LOD

Annex A. Literature Search - Occurrence of tropane alkaloids in food in the EU

A1. Introduction

The European Food Safety Authority (EFSA, 2013) has delivered a scientific opinion on the risks to human and animal health related to the presence of tropane alkaloids (TAs) in food and feed. Although more than 200 different TAs have been identified in various plants, data on their toxicity and occurrence in food are limited and the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) could only perform a risk assessment on (-)-hyoscyamine and (-)-scopolamine.

EFSA reported on the levels of TAs in 124 food samples collected in the Netherlands and Germany (Mulder et al., 2015). Most of the food samples (83%) did not contain detectable TAs but almost all of the food samples that did contain TAs were in the infants and young children food category and comprised simple cereals (wheat, maize, rye, oats, rice and mixtures thereof). The EFSA CONTAM Panel recommended that the occurrence of TAs in food either naturally or as contaminants be better characterised.

The presence of calystegines in foods such as potatoes, eggplants, and sweet potatoes poses the question as to the effect that these compounds might have on humans.

The objective of this literature study is to provide a recommendation of the relevant food products in the EU to be analysed for TAs and the relevant TAs to be analysed in those food products within the EFSA project, with the aim to obtain statistically representative data for use in future risk assessment studies by EFSA.

A1.1. Production of TAs

TA producing plants in general produce a profile of TAs. This is mainly correlated to the genetic make-up of the plant family (Asano et al., 1997a; Griffin and Lin, 2000; Schimming et al., 2005). However, growth state, cultivar and environmental factors such as climate or handling of the product can influence the occurrence and the concentration of the produced TAs (Ashtiania and Sefidkonb, 2011; Dhar and Bhat, 1982; Petersson et al., 2013). It is believed that plants may produce the TAs as a defence mechanism as TA occurrence may prevent grazing or insect damage.

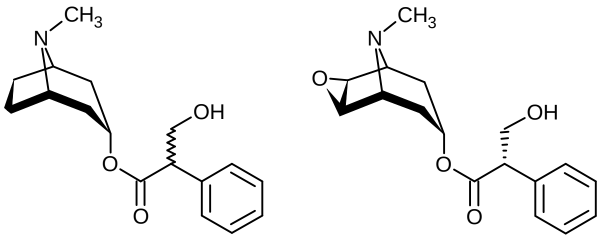
A1.2. Tropane alkaloids

TAs are esters of hydroxytropanes (α-tropanol, α-tropane-diol or α-tropane-triol) with short-chain acids such as acetic acid, propanoic acid, isobutyric acid, isovaleric acid, 2-methylbutyric acid, tiglic acid, (+)-α-hydroxy-β-phenylpropionic acid, tropic acid and atropic acid (EFSA, 2013). The alkaloid part of TAs is a two-ringed structure characterised by a pyrrolidine and a piperidine ring sharing a single nitrogen atom and two carbon atoms. The asymmetric α-carbon of tropic acid esters allows the formation of two stereoisomers.

Atropine is the racemic mixture of (–)-L-hyoscyamine and (+)-D-hyoscyamine (Mulder et al., 2014).

Hyoscyamine is the ester of tropane-3α-ol (3α-hydroxytropane) and S-(-)-tropic acid.

Atropine and scopolamine are esters of tropane-3α-ol (and the 6-7 epoxide of tropane-3α-ol) and tropic acid (Figure A1).



Atropine Scopolamine

Figure A1: Structures of atropine and scopolamine

Tropinone reductases are key enzymes of TA formation. Two different tropinone reductases are involved, one forming tropine, leading to esterified alkaloids, and the other forming pseudotropine that is converted to the related TAs known as calystegines (Dräger et al., 1994; Keiner and Dräger, 2000).

Hyoscyamine is synthesised in the plant as the optically active S-(-)- form and undergoes racemisation over time, so that both the R-(+)- and the S-(-)- forms are found in plants in varying ratio (Eich, 2008; Zhang et al., 2007). Analytical extraction procedures may affect the isomerisation and usually do not distinguish between the products and so in this review the name atropine will refer to the racemic mixture or an unspecified isomer.

TAs are synthesised in the young root cells and translocated to the aerial parts of the plant (Hashimoto et al., 1991). The synthesis proceeds from ornithine and produces tropinone and then tropine (Figure A2) (Zhang et al., 2007) which is esterified to form littorine. Littorine is transformed to (-)-hyoscyamine by at least two routes and scopolamine is formed from (-)-hyoscyamine (Li et al., 2006).

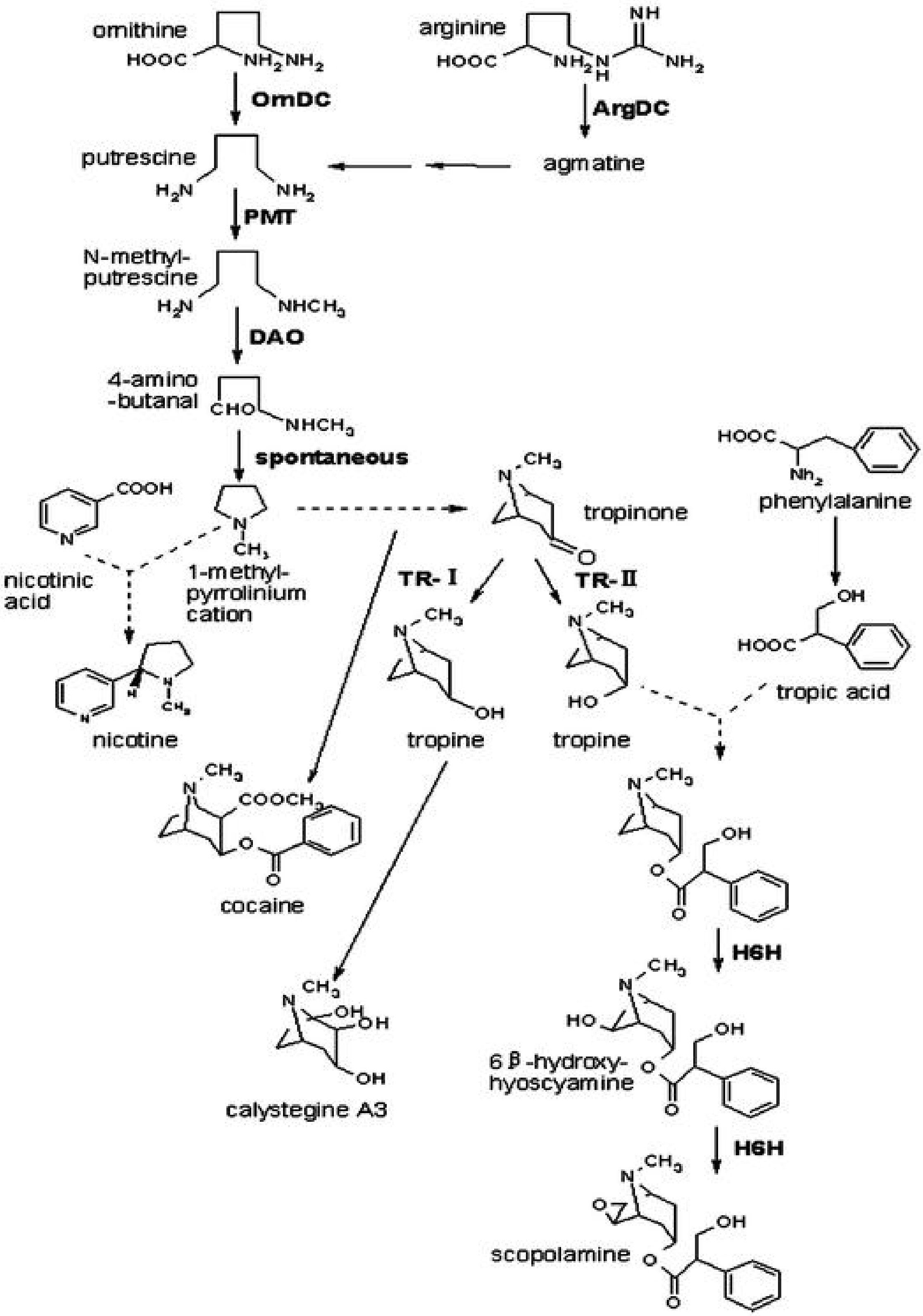


Figure A2: Biosynthesis pathways of pyridine and tropane alkaloids in transgenic *Hyoscyamus niger* hairy root cultures (Figure taken from: Zhang et al., 2007)

A1.3. Calystegines

Calystegines are polyhydroxy alkaloids with a nortropane skeleton (Dräger et al., 1994; EFSA, 2013; Schimming et al., 2005). About 15 calystegines are known, and those most commonly reported include calystegine A_3; A_5; B_1; B_2; B_3; B_4; B_5; N-methyl-calystegine B_2; C_1; C_2; and N-methyl-calystegine C_1. Not all of these are available commercially as reference standards (Figure A3).

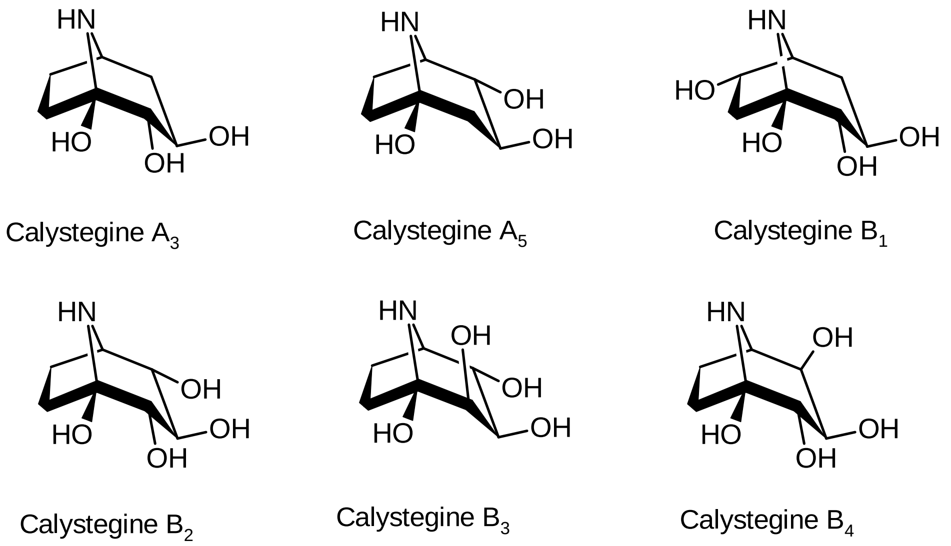


Figure A3: Structures of the most common calystegines

A1.4. Literature survey

The literature study presented here reviews the occurrence of TAs in food plants and in co-occurring weed plants that both contain TAs and have a strong potential to contaminate foods. The information was obtained by means of a literature search performed at the start of the project in March 2015, using the terms of an agreed strategy provided in Table A1, and through studies in the Fera Information Centre and its available on-line sources. The search did not include literature that was not in the English language. TAs for which no standard were believed to be available and cocaine and other TAs used for recreational drugs were excluded. The search provided about 3,500 results with the majority associated with atropine and scopolamine. In view of the considerable quantity of data concerning the occurrence and toxicity of atropine and scopolamine and the EFSA interest in the remaining TAs the focus of the review is on the less familiar TAs including calystegines. During the course of the project (until May 2016), new available information was added, where appropriate. No new extended literature search was performed after March 2015.

The results of the literature survey were evaluated on the following criteria: the likelihood of relevant TA-containing plants contaminating food products in the EU, TA composition of these plants and, if available, toxicity data of individual TAs. The latter also applied as selection criteria for the inherent TAs in foods.

A2. Plants and TAs associated with intoxications and RASFF notifications

To identify the TAs containing plants that might contaminate food, cases of intoxication by TAs reported in the EU were studied and associated plants and TAs characterised when known. In addition the European Union's Rapid Alert System for Food and Feed (EU) was searched for alerts and notifications on TAs or the presence of TA-producing plants.

A2.1. Reported cases of human intoxication in the EU

Many reports of the acute adverse effects of TAs in humans have resulted from deliberate ingestion in order to experience hallucinogenic effects (Boumba et al., 2004). Accidental ingestion of tropane-containing plants is also comparatively common. The cases are described below and the results are summarised in Table A2.

A2.1.1. Intoxications related to TAs from *Atropa belladonna*

Serious poisoning of a nine year old boy resulted from the ingestion of 20-25 *Atropa belladonna* berries (Lange and Toft, 1990), the symptoms being confused with a psychosis.

A similar confusion surrounded the accidental poisoning of an elderly but healthy man with *A. belladonna* berries (Trabattoni et al., 1984).

Three of four adults who ingested cooked ripened *A. belladonna* berries became delirious with visual hallucinations, one becoming comatose. One adult and four children exhibited mild peripheral anticholinergic symptoms (Schneider et al., 1996).

Eight adults were poisoned in the Netherlands in 2013 in two or more incidents involving consumption of herbal tea prepared from dried marshmallow root (*Althaea officinalis*) (approximately 20 g), some of the cases were linked, and in all cases the tea came from the same supplier (van Riel et al., 2014). A similar case linked to the same product was reported in France with the herbal tea being purchased in the Netherlands. The atropine content of the tea was high (1–10 mg/g) and the plant source believed to be *A. belladonna* (deadly nightshade). The *Althaea officinalis* was harvested in Bulgaria and sold only in the Netherlands. The patients had anticholinergic symptoms (dry mucous membranes, nausea, blurred vision, hallucinations, tachycardia, and urinary retention) typical of tropane poisoning.

A single case of poisoning by a tea (described as a 'biodrug') in Germany, causing 'respiratory insufficiency' was reported by a physician in 2001 (BfR, 2006). The toxicological relevant substance was reported to be *A. belladonna*.

Tropane alkaloid caused poisoning in Austrian consumers after drinking nettle tea (*Urtica*) contaminated with leaves of 'belladonna' (Adamse et al., 2014; Scholz et al., 1980).

In the UK, A 30-year old male suffered from atropine like symptoms after drinking comfrey tea (*Symphytum*), to relieve flatulence, as well as an elderly couple who drank comfrey tea (Galizia, 1983; Routledge and Spriggs, 1989). The teas were accidentally contamined with atropine, the leaves of one of the teas contained 4 mg atropine per 28 g (Routledge and Spriggs, 1989)

A2.1.2. Intoxications related to *Datura* in EU

Datura is an invasive wild plant that appears to readily contaminate buckwheat (*Fagopyrum sagittatum*) and has serious health effects after ingestion, even at low doses. Buckwheat products are attractive to people who are intolerant of the gluten found in wheat.

A stock of Breton buckwheat that contained seeds of *Datura* caused poisoning in a large area of south-eastern France in September 2012 after it was purchased by a mill in the Alpes de Haute Provence (Glaizal et al., 2013). All of the victims had consumed bread, or in two cases homemade foods, prepared from organic buckwheat flour. The flour was sold in various bakeries and speciality food stores but was supplied by the same source, which was a grain company operating throughout the European Union. Tens of tons of this stock were distributed to four specialised organic mills in Brittany and to a lesser extent in Provence. Flour produced from the seeds was sold in over one hundred bakeries and was passed to retail outlets, organic shops, and restaurants. Contaminated flour or finished products such as black bread, and pancakes, etc. were distributed from the only mill located in Provence to not only the six departments of the Provence-Alpes-Côte d'Azur region but also to Corsica, Rhone-Alpes and Languedoc-Roussillon. Within less than 2 months, 24 people from the Provence-Alpes-Cotes d'Azur and Languedoc-Roussillon regions went to accident and emergency departments with symptoms of intoxication. Poisoning by buckwheat contaminated with *Datura* was confirmed in most (19) of these patients who were from 12 different households. In Rhone-Alpes, 14 people from 9 different households were intoxicated. The distribution chain and chemical analysis linked the contamination of buckwheat to seeds of *Datura* sp. in the Breton area. Levels of atropine and scopolamine in the flour sent to the Provençal region were much higher (16,467 and 7,042 mg/kg respectively) than in other mills (no data were provided for Brittany). Cases of poisoning were recorded from some places over a month after the withdrawal and in Rhône-Alpes a case was reported after five months.

Seven people were poisoned by consumption of cooked wild collected spinach (blites) that contained leaves of *Datura innoxia* (Papoutsis et al., 2010). The cooked vegetables contained atropine and scopolamine at 0.8 and 1.2 mg/kg, respectively. The patients recovered with hospital treatment.

A 10 year old girl in Germany suffered from *D. stramonium* intoxication after making tea from *D. stramonium* leaves. The leaves were not intended for tea making but for the production of fumes to be inhaled for asthma treatment (van_der_Heide, 1988).

In Slovenia in 2003, 73 cases of domestic food poisoning with mild to moderate effect were associated with ingestion of a traditional dish containing buckwheat flour, which is commonly used in Slovenia (Perharič, 2005). Four samples of the flour were shown to contain atropine and scopolamine but it is not clear if the scope of analysis included other TAs. The whole buckwheat grain contained up to 190 seeds of *Datura stramonium* per kg of grain. Atropine and/or scopolamine were detected (> 3 µg/kg) in 20 of 43 buckwheat samples with the highest levels (26 mg/kg and 12 mg/kg of atropine and scopolamine respectively) found in the flour consumed by a family of eight people. The level of consumption was between 53 and 140 µg/kg body weight for atropine and between 25 and 64 µg/kg body weight for scopolamine. In Slovenia all buckwheat flour and buckwheat flour products were recalled and legislation on grain purity and buckwheat flour amended.

D. stramonium seeds present in millet-carrot balls caused illness in eight persons, of which one had to hospitalised, in Austria (Fretz et al., 2007).

In France, canned beans contaminated with seeds of *D. stramonium* were reported to cause three poisonings (Le_Parisien, 2010).

Frozen mixed vegetables contaminated with *D. stramonium* were sold via the largest market chain in Finland in May of 2013 (Finland_Times, 2013; Termala et al., 2014). A total of 28 poisonings were suspected and 10 patients had anticholinergic symptoms. The products were recalled and 30,000 customers were warned to avoid the vegetables. It was pointed out that the outbreak could have lasted much longer on account of the potential long storage time of the frozen product.

A2.1.3. Intoxications related to other TAs containing plants symptoms in the EU

A mistaken identity caused intoxication of two consumers of what they thought was *Borago officinalis* but turned out to be *Mandragora officinarum* leaves (Tsiligianni, 2009).

A2.1.4. Poisoning centre reports

Plant exposures are the fourth most common category of poisoning in Germany, accounting for 22% of paediatric exposures. 58,641 cases involving 248 different plant genera were reported from 1998 to 2004 of which about 10% of cases had noticeable symptoms. In a separate German analysis of 111,313 calls to a regional poison control centre over a twenty year period from 1974, 56% referred to children and 44% to adults with plant ingestion being the cause of 9.7% of cases (Pietsch et al., 2008; Plenert et al., 2012). Intoxications with the plant genera *Taxus*, *Ligustrum* and *Ficus* continued over the 10 year period under investigation but exposures to the plant genera *Brugmansia* and *Datura* showed time-dependent changes (Plenert et al., 2012).

Analysis of severe poisonings by plants reported to the Swiss Toxicological Information Center from 1966 to 1994 showed that of 24,950 cases the clinical course was either mild or unknown (Jaspersen-Schib et al., 1996). Poisoning was severe in 152 cases which could be associated with 24 plants which included *A. belladonna* (42 cases), *Aconitum napellus* (4 cases) and *Hyoscyamus niger* (3 cases).

From 174 exposures of children to toxic plants recorded in the Czech Republic over a 6-year period from 1996 to 2001 the most frequent cases (15%) resulted from ingestion of *Datura* seeds (Vichova and Jahodar, 2003).

A2.2. Search of the EU RASFF system for alerts and notifications related to TAs in food products

A search of the RASFF Rapid Alert system (EU), updated on November 18, 2016, produced relevant results from the search terms: atropine, *Belladonna*, *Datura*, *Hyocyamus* and *Solanum nigrum*. No results were obtained for the names of other TAs or other TA-producing plants. All results for scopolamine were also returned under the atropine search.

The RASFF system at that date contained a total of 46 notifications and/or alerts for atropine, *Belladonna*, *Datura*, *Hyoscyamus* and *Solanum nigrum*, as shown in Table A3.1 and A3.2 and summarised in Table A3.3.

Atropine/scopolamine

A total of 25 notifications and/or alerts were reported for atropine/scopolamine in the period 1994 to November 18, 2016. Six reports to RASFF concerned the occurrence of atropine and scopolamine in buckwheat (*Fagopyron esculentum*) flour. All were reported by Slovenia. In addition, there were ten notifications and/or alerts on atropine contamination of millet and millet-based products, usually in combination with scopolamine, four on corn-based products and two on sorghum-based products. Two notifications and/or alerts concerned porridge and one on tea.

Belladonna

Four alerts were reported for the presence of *A. belladonna*. Three alerts were on tea and one alert on Burdoch root not further specified.

Datura

In the period 2006-2013 there were nine notifications and/or alerts for *Datura*. Five notifications and/or alerts of *D. stramonium* seeds in food were related to millet. *Datura* seeds were found in vegetable / bacon stir-fry mix from Spain in 2007 and in frozen vegetable-bean-seed mix from Belgium/Spain. *D. stramonium* fruits (presumably unripe seed cases) were found in canned green beans from Hungary in 2006 and in 2007.

Hyoscyamus

Seeds of *Hyoscyamus niger* (henbane) were found as contaminants in poppy seed from the Czech Republic in 2007 and 2008. The contamination level was between 0.13% and 0.42%. Two of the samples were sold in the Slovak Republic and the third in the Czech Republic.

Solanum nigrum

In 2015 plant material of *Solanum nigrum* was rejected at the border of Portugal after the attempted import of food supplements from India. *Solanum nigrum* fruits were, furthermore, reported in young and canned beans (3 alerts) and in frozen green beans (one alert).

A3. Food plants with inherent TAs in the EU

Several TA-containing plants are consumed in Europe. They are either grown in Europe or can be imported. They belong to the families of Solanaceae, Convolvulaceae and Brassicaceae. Very little is known on the incidence and concentration of the TAs in the food plants as consumed. The TAs containing food plants consumed in the EU are described below and listed in Table A4.

A3.1. Food plants of the Brassicaceae family

Many Brassicaceae plants are part of the staple European diet, particularly those from the species *B. oleracea*, including broccoli, cauliflower, kale, kohlrabi and Brussels sprouts. Other commonly consumed brasssicas are *B. rapa* (turnip, white turnip), *B. napobrassica* (swede, rutabaga, Chinese cabbage), the colloquial names of which vary across Europe (Table A4.1). Other Brassicas are consumed to a lesser but growing extent in Europe, including *Raphanus sativus* (radishi), *Eruca sativa* (rocket) and *Nasturtium officinale* (watercress). In addition many are consumed at lower level as ingredients or spices, such as *B. juncea* (brown mustard), *B. nigra* (black mustard), *Sinapis alba* (white mustard), or as sauce components such as *Armoracia rusticana* (horseradish) and *Wasabi japonica* (wasabi).

A3.2. Food plants of the Convolvulaceae family

The most relevant food plants of the Convolvulaceae family are *Ipomoea*, of which about 700 species are found in most tropical and subtropical regions. The most important plant is *I. batatas*, the sweet potato or yam (Table A4.2). Sweet potatoes are grown in many countries and consumed throughout most of the world (Bovell-Benjamin, 2007; Zhao et al., 2005).

Another *Ipomoea*, *I. aquática* is a food crop in much of Asia, where the leaves are consumed as a rich source of nutrients and essential amino acids. Some related ipomoea species eaten to a lesser degree are *I. alba* L., *I. albivenia*, *I. involucrata* and *I. leptophylla*.

A3.3. Food plants of the Moraceae family

The leaves and fruits of the white mulberry tree *Morus alba* have been shown to contain calystegines (Asano et al., 1994; Bajpai et al., 2012; Singh et al., 2013; Singhal et al., 2010) (Table A4.3). The white mulberry is a tree of economic importance in Asia as the leaves are the sole food source for silkworms and they are sometimes eaten as vegetable. *M. alba* is considered a weed in the USA and is found in warmer European countries. The fruit is consumed in Asia and the USA and is becoming popular in Europe, although the *M. rubra* (red) and *M. nigra* (black) species are preferred. Calystegines have not been reported in these two species.

A3.4. Food plants belonging to the Solanaceae family

Most food plants that naturally contain TAs belong to the Solanaceae family. This is one of the most diverse and varied groups of plants with 90 genera and just over 4000 species. It is found worldwide with the majority of species in Central and South America. Food plants of the Solanaceae family relevant for food consumption in the EU were identified in this study (Table A4.4).

Capsicum

Capsicum spp. peppers comprise very many species of which five - the sweet bell pepper (*C. annuum*) and four 'chilli' types: *C. frutescens, C. chinense, C. baccatum* and *C. pubescens* have been domesticated.

Lycium

Lycium barbarum and *L. chinense*. The fruits of the *Lycium barbarum,* goji berry plant, have recently been marketed as foods beneficial to health. Plant parts of *Lycium* have a history of use for medicinal purposes in China but in the West usage is restricted to the berries of *L. barbarum* (Potterat, 2010). The berries are generally produced in and exported from China but the plant is believed to originate from the Mediterranean Basin (Genaust, 1996) and might be cultivated in Europe in future.

Physalis

Physalis peruviana. The Cape Gooseberry (*P. peruviana*) is a popular fruit in much of Europe but not eaten in quantity. This plant and the related *P. alkekengi* (Chinese lantern, bladder berry) contain several tropane and secotropane alkaloids in the roots and/or leaves (Griffin and Lin, 2000) but there are no reports of the TAs in the edible parts (fruits). The roots of *P. alkekengi* contain the minor TAs tigloidine, 3α-tigloyloxytropane, cuscohygrine and phygrine over the range 0.02 to 0.025% fresh weight or 0.084-0.104% dry weight (Basey et al., 1992).

Physalis philadelphica or Physalis ixocarpa . The tomatillo is becoming popular in Europe as a result of interest in Mexican foods. Its cultured root has been shown to contain calystegine A_3 but not A_5, B_1 or B_2 which were present in related species (Azemi et al., 2006).

Solanum

Plants of the genus *Solanum* do only contain calystegines and do not contain atropine or scopolamine (Griffin and Lin, 2000).

Solanum betaceum (syn. Cyphomandra betacea). The tamarillo or tree tomato is popular in New Zealand, Mexico and South America where it is eaten raw as a fruit or vegetable dish and also cooked, but is receiving attention in Europe. Some related species also have edible fruits. It has not been reported to contain TAs.

Solanum lycopersicum (Syn. *Lycopersicum esculentum).* Tomatoes were imported from South America to Europe in the 15th century and have since been grown and hybridised extensively in Europe. The cultivated S. lycopersicum can be hybridised with *S. lycopersicoides*, *S. ochrantum* and *S. sitiens*.

Solanum melongena. Aubergines or eggplant are believed to originate in Asia or Africa but it has been grown in the Mediterranean basin since the 7th century. Several minor species physically similar to aubergine are cultivated on a small scale for food, including scarlet and gboma eggplants (*S. aethiopicum* and *S. macrocarpon*).

Solanum muricatum (pepino), *S. betaceum* (tree tomato), and *S. quitoense* (naranjilla) are popular in Mexico and South America and have potential to become popular in Europe, as does *S. melanocerasum* (garden huckleberry).

Solanum tuberosum. Potatoes originated in South America and have become a staple item in the European diet since the 18th century.

A3.5. Summary

Table A5 gives an indication of the relative consumption of food with inherent plant toxins in Europe, based on the data obtained in section A7.2. Table A6 is the summary of the information presented in Tables A4 and A5 for foods that contain inherent TAs and are consumed in moderate or high quantities.

In summary the most important food plants for the European situation, based on the amount consumed, that naturally contain TAs have been identified as the Solanums (potato, aubergine and pepper), the Brasssica's (cabbage, broccoli) and sweet potatoes.

A4. Literature search for TAs associated plant species that could potentially contaminate field crops in the EU

Plant species that could potentially contaminate field crops in the EU were identified from the search results and from searches of databases of weed science organisations. Plants identified in these searches that were designated problematic as agricultural weeds and also known to contain TAs are described below and presented in Table A7.

In addition to the searches carried out using the Fera Information Centre the databases of and reports issued by the following weed science organisations were searched:

- The European Network on Invasive Alien Species (NOBANIS) (http://www.nobanis.org/)
- The Global Biodiversity Information Facility (http://www.gbif.org/)
- The International Weed Science Society (http://www.iwss.info/)
- The European Weed Research Society (EWRS) (http://www.ewrs.org/)
- The EWRS Invasive Plants Working Group.
- The EWRS Weed Mapping Working Group.
- Association Française de Protection des Plantes (conference proceedings)
- Sociedad Española de Malherbología (http://www.semh.net/)
- Italian Weed Research Society
- The Plant List maintained by the Royal Botanic Gardens, Kew and Missouri Botanical Garden (http://www.theplantlist.org/)

The major plant species known to contain TAs that contaminate field crops in the EU or had a strong potential to do so were identified as *Solanum* and *Convolvulus* species with a lesser risk from *Brassica, Atropa* and *Datura* species.

A4.1. Associated plants of the Brassicaceae family

Cochlearia species (scurvy grass) are annual Brassicaceae plants that do not have an impact on crops but were the first brasssicas to be shown to contain tropanes (Brock et al., 2006).

Calystegines were found in two species, *Berteroa incana* and *Bunias orientalis*, that can be invasive weeds. *Berteroa incana* is an invasive and toxic weed, especially of alfalfa and clover forages, although the identity of the toxin has not been confirmed (Pieper et al., 2010). *Bunias orientalis* is a brassica native to the Caucasus and southern Russia. It has spread rapidly throughout Europe through the human use as animal feed and is now naturalised in many countries in Europe and is still spreading intensively (Birnbaum, 2006), especially in Estonia where it is listed as particularly invasive and fast-spreading.

Calystegines were also detected in *Barbarea vulgaris* and *Bunias erucago* (Brock et al., 2006), two minor weeds that, however, have been proposed as human salad or vegetable foods (PFAF, 2015)

Brassica tournefortii (Sahara mustard, African mustard, Asian mustard, Mediterranean turnip, Sahara mustard, Tournefort's birdrape, wild turnip) is a native annual herb in Europe, common in disturbed sites such as roadsides and abandoned fields, but is not considered invasive in Europe. *B. tournefortii* is also found in the Middle East and in the European and neighbouring countries Azerbaijan, Cyprus, Egypt, Greece, Italy, Morocco, Spain, Tunisia, Turkey, Turkmenistan and Uzbekistan. It is widely used as food in some North African countries (DAISIE).

Brassica elongata (elongated mustard, long-stalked rape) is a biennial or perennial herb that is native to Eastern Europe, Russia and Central Asia. In some areas it competes with native flora and has the potential to spread. *B. elongata* is cultivated in Estonia (USDA_ARS, 2006).

Brassica campestris L. (wild turnip). This plant is generally distributed as a weed in temperate zones of Europe and is cultivated plant in parts of the Middle East and Asia. It is found throughout the Western part of Russia, in the Caucasus, Western and in Eastern Siberia. It has been described as a pernicious weed infesting all spring crops, both grain and vegetative types (AgroAtlas_Project, 2009).

A number of other brassicas are mentioned for containing calystegines but can probably not be considered a health threat: *Arabidopsis thaliana, Coringia orientalis* (toxic to livestock, noxious weed), *Cronopus squamatus, Diplotaxis murales, Isatis tinctoria, Lunaria annua* (not onsidered to have weed potential), *Lunaria rediviva* (not onsidered to have weed potential), *Matthiola incana* (not onsidered to have weed potential), *Neslia paniculata* (commonly affect wheat and millet), *Peltaria alliacea, Sisymbrium strictissimum* (AgroAtlas_Project, 2009).

A4.2. Associated plants of the Convolvulaceae family

Convolvulus arvensis (field bindweed) is considered one of the worst weeds in the world by agriculturists and horticulturists (Austin, 2000). It grows very successfully in temperate and Mediterranean climates where it is a weed of cereals, beans and potatoes. It is problematic in France, Germany, Greece, the former Yugoslavia, and many countries outside Europe. *C. arvensis* ranked joint 7th in a comparison of the overall importance of European weeds, 15th in the rankings for its importance in spring cereals crops and in winter rapeseed, and 6th in maize and sorghum (Schimming et al., 2005; Schroeder et al., 1993; Shukla et al., 2006).

Kaçan *et al.* described these weeds as a major obstacle to sunflower seed production in Turkey (Kaçan et al., 2013). *C. arvensis* was among the major weeds found in 2012.

A4.3. Associated plants of the Solanaceae family

Genus *Datura*

Datura is a frequent contaminant of food crops as is evidenced by the reports under the RASFF system. However there were no further descriptions of the scale of the associated weed problem in the results from the search. *Datura stramonium* (Jimson weed, devil's snare) was among the major weeds described as a major obstacle to sunflower seed production in Turkey in 2012 (Kaçan et al., 2013).

Genus *Hyoscyamus*

Hyoscyamus albus is the henbane plant which has been described as a natural weed in much of Europe (Hanf, 1983; Williams and Hunyadi, 1987; Williams, 1982).

Hyoscyamus niger (Black Henbane) is a weed of poppy, wheat, and millet crops. This plant produces very many seeds, up to 400,000 per plant (AgroAtlas_Project, 2009). The literature search did not provide information on the scale of the associated weed problem but again the RASFF reports indicate a significant degree of food contamination.

Genus *Solanum*

Solanum nigrum, known as the Common nightshade or Black nightshade was the most frequently reported *Solanum* weed although confusion with related similar species is very likely. It is an annual weed growing to 0.6 x 0.3 m. which flowers in late summer. It produces fruits that are at first green and might be unseen in vegetables. These turn black on maturity and might then be mistaken as edible fruits. *S. nigrum* ranked joint 13th in a comparison of the overall importance of European weeds (Schroeder et al., 1993). It ranked 6th in importance in relation to infestation of maize and sorghum.

Solanum elaeagnifolium known as yellow tomatito or silverleaf nightshade is a highly infective toxic weed of vegetable crops, cotton and pastures in the eastern Mediterranean countries (Boyd et al., 1984; Mekki, 2007). It is considered one of the worst weeds in the world. *S. elaeagnifolium* became invasive in many countries after it was unintentionally introduced to Europe and is now widespread in or considered a risk to Albania, Algeria, Bosnia and Herzegovina Bulgaria, Croatia, Cyprus, France, Georgia, Greece, Hungary, Israel, Italy, Jordan, Kazakhstan, Moldova, Malta, Morocco Portugal, Spain, Romania, Russia, Serbia and Montenegro, Slovenia, Tunisia, Turkey, Ukraine and Uzbekistan. It colonises roadsides, pastures and grasslands and infests crops. Maize, sorghum and wheat are considered to be very affected by *S. elaeagnifolium* which can be abundant in fields. In Morocco it has caused serious damage to maize crops (Gmira et al., 1998; Taleb and Bouhache, 2006). According to a report from the European and Mediterranean Plant Protection Organisation (EMPPO, 2006) the plant is present in the five continents and in several countries where it is invasive.

Solanum plants that have weed status in Russia with the potential to spread westwards are: *Solanum carolinense* L. (horse-nettle) and *Solanum cornutum* (buffalobur). *S. carolinense* is a native of the USA but spreading in Russia. It is an extremely competitive weed that infests corn and other grain crops, potato, soybean, and tomato (AgroAtlas_Project, 2009). *S. cornutum* is a native of Mexico and the southwest USA now found in the European part of the Former Soviet Union, and described as a pernicious quarantine weed of spring grain crops, poisonous to animals (AgroAtlas_Project, 2009).

A4.4. Minor associated plants of the Solanaceae family of the genera: *Atropa, Physalis, Mandragora* and *Duboisia*

Atropa belladonna and *Physalis* species contain TAs but are not reported as troublesome weeds in Europe.

Mandragora autumnalis, the main Mediterranean species of the mandrake plant contains many toxic tropanes (Hanus et al., 2005) but is not particularly effective as a weed.

The Australian native trees *Duboisia leichhardtii* and *D. myrporoıdes* that grow well in the warmer parts of Europe contain high concentrations of atropine-like alkaloids especially atropine and scopolamine but the trees are too few in number to cause a weed problem (Gaillard and Pepin, 1999).

A5. Occurrence of TAs in food

A5.1. Food plants containing inherent TAs

Overviews of the TAs occurring in food plants are published (Dräger, 1995; Griffin and Lin, 2000; Pomilio et al., 2008). Calystegines A_3, B_1, B_2, and C_1 were detected in samples of the Solanaceae and Convolvulaceae tested (Asano, 2000; Asano et al., 1997a; Asano et al., 1997b; Dräger et al., 1994). The occurrence of calystegines were reported in over 70 varieties of potatoes and other edible species such as aubergine and sweet potato (Watson et al., 2000).

Occurrence data of several calystegines inherent to food are described below and are collected in Table A4.

Brassicaceae family

Brassicaceae species used as food do not contain atropine or scopolamine (Griffin and Lin, 2000), however the leaves of the majority of Brassicaceae contain calystegines. Calystegines are accumulated throughout the life cycle of brassica plants and are found in all parts.

Convolvulaceae family

The Convolvulacea contain convolvine (3α-veratroyloxynortropane), and convolidine (3α-vanilloyloxynortropane) (Griffin and Lin, 2000). In a survey using chromatography/mass spectrometry (GC-MS) the composition of 129 convolvulaceous species belonging to 29 genera (all 12 tribes) were determined. From one to six calystegines in 62 species belonging to 22 genera of all tribes except *C. uscuteae* were detected (Schimming et al., 2005). *Ipomoea* had the highest number of calystegine-positive species. Calystegines B_1, B_2, B_3 and C_1 have all been reported in the sweet potato *I. batatas*, as well as in the related *I. alba, I. aquatica, I. carnea, I. eremnobrocha, I. obscura, I. pes-caprae, I. setifera, I. violacea* and *I. hederifolia*.

The levels of calystegines reported in the edible *I. batatas* tuber by Asano *et al.* (1997a) were A_3 (0.11), B_1 (2.4-16 mg/kg) and B_2 (1.1-19 mg/kg fresh weight), C_1 (0.61-9) (Asano et al., 1997a).

Moraceae family

The leaves and fruits of the white mulberry tree, *Morus alba,* have been shown to contain calystegines but they have not been found in the red or black varieties, *M. rubra* and black *M. nigra* (Bajpai et al., 2012; Singh et al., 2013). Asano *et al.* (2001a) found 17 polyhydroxylated alkaloids in the plant including calystegine B_2 in the leaves, 2-hydroxymethyl-3,4-dihydroxy-pyrrolidine-*N*-propionamide in the root bark and 4-O-α-D-galactopyranosyl-calystegine (calystegine B_2) and 3β,6β-dihydroxynortropane in the fruit (Asano et al., 2001a).

Solanaceae family

Capsicum

Capsicum annuum has been found to contain high levels of calystegines. The calystegines B_1, B_2 and C_1 were detected at levels of respectively 12, 37, and 4 mg/kg fresh weight, but no A_3 was detected. Chili pepper (*C. frutescens*) contained 0.24 mg/kg A_3 and 0.27 mg/kg B_2 but no A_3 or C_1 (Asano et al., 1997a).

Lycium

A variety of 14 N-methylcalystegines have been reported to occur in root of goji berry (*Lichium chinense*) (Asano et al., 1997b).

The fruits from an Indian goji berry plant have been reported to contain atropine at a level 9.5 g/kg and hyoscyamine at 2.9 g/kg dry fruit but this degree of contamination would have led to poisoning incidents (Harsh, 1989).

A later investigation of the atropine content of eight samples of goji berries from China and Thailand based on liquid chromatography-mass spectrometry (LC-MS) found atropine in one sample of dried berries at 0.019 mg/kg whereas for seven others the level was <0.01 mg/kg (Adams et al., 2006).

A further evaluation by the German Federal Institute for Risk Assessment (BfR) published by Klenow *et al.* (2012) concluded that the original test was not specific for tropanes and the plant was probably misidentified *Lycium europaeum*. Analysis of the two species by liquid chromatography-tandem mass spectrometry (LC-MS/MS) showed an atropine content of 0.59 mg/kg for fruits of *L. europaeum* and atropine at the detection limit (0.01 mg/kg) in the fruits of *L. barbarum* from China (Klenow et al., 2012).

The roots of *L. chinense* contain at least 14 calystegines and N-methylcalystegines and other compounds with β-glucosidase and/or α-galactosidase inhibitory activity (Asano et al., 1997b).

Physalis

Cape Gooseberry contains no scopolamine, hyoscyamine, littorine or ditigloyl esters. Tigloidine (3-β-tigloyloxytropane) and 3-α-tigloyloxytropane were isolated form the root (Beresford and Woolley, 1974; Griffin and Lin, 2000). The roots also contain the secotropane alkaloids (+)-physoperuvine, racemic physoperuvine and (+)-N,N-dimethyl-physoperuvinium, which are metabolites of TAs (Sahai and Ray, 1980). Both leaves and roots contain tropine, 3α-tigloyloxytropane, 3-β-acetoxytropane, hygrine, two isomers of N-methylpyrrolidinylhygrine, physoperuvine, and cuscohygrine (Kubwabo et al., 1993). The related *P. alkekengi* contains calsytegine A5 and B3 (Asano et al., 1995). In analyses by Asano *et al* (Asano et al., 1997a) *Physalis peruviana* fruit contained calystegines A_3 at 0.003 mg/kg, B_1 at 0.038 mg/kg and B_2 at 0.048 mg/kg fresh weight.

A study of the potential of the root of *P. peruviana* root for use as a functional food found that it contained cuscohygrine, with anolides and flavonoids. Five compounds were isolated and their structures elucidated by different spectral analysis techniques. Animal experiments showed that root extracts improved oxidative stress, liver function and kidney disorders, protecting the liver and kidney against fibrosis (El-Gengaihi et al., 2013).

Solanum

Solanum tuberosum. Calystegines have been found in all edible potatoes (Asano et al., 1997a), and potato plants contain calystegines in all parts. They have been measured in over 70 edible varieties of potatoes (Friedman et al., 2003; Nash et al., 1998; Watson et al., 2000).

Potatoes can contain up to 2,300 mg/kg fresh weight calystegines in their sprouts and up to 450 mg/kg in the peel of the tuber (Keiner and Dräger, 2000).

A total calystegine level of 10 mg/kg (fresh weight in the tuber flesh) was reported for potatoes obtained from supermarkets in the UK (Nash et al., 1993).

Calystegine A_3 and B_2 were reported in potatoes obtained in the UK and calystegines B_2 in potatoes obtained from Japan, in the range of 1.17 to 7 mg/kg total tuber fresh weight (Asano et al., 1997a).

Calystegine A_3 and B_2 were measured in potatoes at a total alkaloid content of 1.17 to 7 mg/kg fresh weight (Asano et al., 1997a; Watson et al., 2000). The ratio of B_2:A_3 was typically 2:1 (Watson et al., 2000).

Details of the variation of calystegine levels in various parts of a single cultivar (Liu) of *S. tuberosum* during the life cycle of the plant were investigated. In the mature plant total calystegines reached 25 to 75 mg/kg in the leaves and about 100 to 250 mg/kg in the growing tuber. Directly after harvest the tubers contained a maximum of about 400 mg/kg calystegines in the eyes and skin, which rose to maxima at 5 months after harvest to 1000 mg/kg in the skin and 3000 mg/kg in the young sprouts emerging from the tuber when stored in the dark at 4°C. Calystegines A_3, B_2 and B_4 were the most prominent and could be quantified in all tissues (roots, tubers, leaves, sprouts and eyes), and calystegines A_5, B_1 and B_3 were believed to be present in very small quantities (<10 mg/g fresh weight). N-methyl calystegine B_2 was not detected in any part (Keiner and Dräger, 2000). Contrary to potato glycoalkaloids, calystegine levels in the skin do not increase on exposure to light but actually decrease. The major changes in calystegine levels during tuber production and post-harvest probably account for the variations in the levels reported in the literature.

Calystegines in potato (and probably other vegetables) are relatively unstable through cooking and other food processing operations (Watson et al., 2000). Boiling and oven roasting decreased total calystegine content to 15% of the starting value, micro wave preparation and deep fat frying reduced the calystegines content to 20% (Watson et al., 2000). Storage of potatoes at 5°C increased the proportions of the 4-*O*-alpha-D-galactoside of calystegine B_2 and the trihydroxylated calystegine A_3. Watson et al (2000) reported the calystegine content of seven prepared potato products cooked following the recommended domestic procedures. All products contained calystegines A_3 and B_2. The highest levels were in French fries for microwave cooking (A_3 and B_2 at 15 and 20 mg/kg product respectively), instant mashed potato (9 and 23 mg/kg respectively), and potato crisps (5 and 21 mg/kg respectively) (Watson et al., 2000).

Solanum melongena. TAs have been reported in the fruit of the aubergine or eggplant (Das and Barua, 2013; Nash et al., 1993). Tubers from UK contained calystegine A_3 at 0.3 and B_2 at 0.5 mg/kg fresh weight, while tubers from Japan contained no calystegines A_3 but contained calystegine B_1 at 7 and B_2 at 73 mg /kg fresh weight (Asano et al., 1997a). There has been some interest in the grafting of food plants on to the root stock of related species for food production. There is a single report of poisoning associated with consumption of aubergine that that been grafted on to the root of *Datura metel* (Oshiro et al., 2008).

Other plants

The berries of *A. belladonna have* been mistaken for edible berries such as bilberries, blueberries, cranberries, and huckleberries, and the leaf has been mistaken for *Malva sylvestris* (mallow), used occasionally as a vegetable (Adamse et al., 2014). Several parts (leaves, seeds and roots) of other plants such as *Datura* have been mistaken for common vegetables and herbs.

A5.2. Non-food plants containing inherent TAs

Overviews of the TAs occurring in associated plants are collected in Table A8 and described below.

Brassicaceae

Camelina sativa

Camelina sativa, (camelina, a weed of flax seed, also contains calstegines A_3, A_5, B_2 and B_3, at levels of 1 to 5 mg/kg (Brock et al., 2006). The plant is increasingly used for production of biodiesel in USA and Europe (Russo and Reggiani, 2012).

Convolvulaceae

Convolvulus arvensis

The TAs of *Convolvulus arvensis* have been studied by Asano *et al.* (1997a) and Asano (2000) and others (Asano, 2000; Asano et al., 1997a).

Early studies (Evans and Somanabandhu, 1974; Jenett-Siems and Eich, 1994) reported tropinone and feruloyltropanol.

Fresh aerial parts of *C. arvensis* plants which had poisoned horses were not shown to contain calystegines (Todd et al., 1995) but pseudotropine (a biosynthetic precursor of the calystegines) meso-cuscohygrine, a cuscohygrine stereoisomer and traces of tropine and tropinone were found.

Todd *et al.* (1995) found no calystegines in the aerial parts but Schimming *et al.* (2005) reported calystegines A_3, A_5, B_1, B_2, B_3 and B_4, with the flowers containing only A_5 and B_2 (Schimming et al., 2005; Todd et al., 1995).

De Simone *et al.* (2008) found that the roots of *C. arvensis* contain calystegines B_1, B_2 and A_3 (De Simone et al., 2008).

Other *Convolvulus* spp. Related compounds convolidine (3α-vanilloyloxynortropane), convolamine (*N*-methyltropan-3α-yl-veratrate), confoline (3α-veratroyl-*N*-formylnortropine), confolidine ((±)3α-vanillyl-*N*-formylnortropane) and convolamine-*N*-oxide were detected in various other *Convolvulus* spp. (De Simone et al., 2008).

Solanaceae

Atropa belladonna

Atropa belladonna contains mainly atropine and scopolamine both in leaves and seeds (Ashtiania and Sefidkonb, 2011; Dhar and Bhat, 1982)

Leaves of *A. belladonnna* L. contained apoatropine, (Ashtiania and Sefidkonb, 2011) tropine, belladonnine, norhyoscyamine, hyoscyamine, 6-β-hydroxyhyoscyamine and scoplamine (Arraez-Roman et al., 2008),

Atropine represent 90-95% of the TAs and scopolamine 5-10% in leaves and berries. Several minor metabolites do occur such as apoatropine, tigloytropeine, aposcopolamine, hydroxyhyoscyamine and tigloyloxytropane (Gaillard and Pepin, 1999).

The presence of littorine was reported occasionally (Nakanishi et al., 1998).

Tropanes are present in all parts of the plant but the total TA levels vary considerably between different varieties and harvesting stages (Dhar and Bhat, 1982).

Atropine and scopolamine are always the major TAs with atropine always the most abundant, up to 10 mg/g dry weight in the root and 3 mg/g in the leaves (Simola et al., 1988).

Fresh plants from Iran *A. belladonna* L. contained about 3% total TAs in the leaves 8% in the roots, 1% in the stem and 5% in the seeds (Ashtiania and Sefidkonb, 2011).

An overview of quantitative data available in the literature is given in the EFSA Opinion (EFSA, 2013). The range of total atropine + scopolamine was 20 to 5,000 mg/kg in leaves and 960 to 7,300 in seeds.

A. belladonna also contains calystegines A_3, B_1 and B_3 (Dräger, 1995; Dräger et al., 1994). Calystegines were located in all parts but mainly in the young upper leaves (Dräger, 1995) where the levels vary but can reach about 400 mg/kg dry weight (Bekkouche et al., 2001). The presence of calystegine B4 is unconfirmed.

Datura

The TA profiles of *Datura* species have been much studied and over 65 TAs have been reported. Most of the information relates to atropine and scopolamine which predominate in all species investigated (Berkov et al., 2006; Doncheva et al., 2006; Philipov et al., 2007).

A total of 19 TAs were reported to occur in three varieties of *D. stramonium*, of which 10 occurred in all three cultivars: 3-(hydroxyacetoxy)-tropane, 3α-phenylacetoxytropane, apoatropine, hyoscyamine, 6–hydroxyhyoscyamine, 3-(3′-acetoxytropoyloxy)-tropane, 3α-tigloiloxy-6β-hydroxytropane, 3-tigloyloxy-6-(2′-methylbutyryloxy)-tropane, 3α,6β-ditigloyloxytropane and scopolamine (Doncheva et al., 2006).

TA patterns in *Datura* are influenced more strongly by environmental factors than by genetic ones, but the proportion of the less common TAs in the seed of European *Datura* is low compared to those of atropine and scopolamine.

Ionkova *et al.* (1994) reported quite high levels of apoatropine (3α-apotropoyloxytropane) in the seeds of a cultured plant (calculated as 27% of the gas chromatography-mass spectrometry response) (Ionkova et al., 1994).

Very few other TAs were reported in seeds in these studies, with the greatest variety of TAs being in the leaf tissue, It is likely therefore that atropine and scopolamine are the metabolic end products for storage in the seed.

Duboisia

Duboisia leichhardtii is a tree cultured in Australia the leaves of which contain calystegines B_1, B_2, B_4, C_1 and C_2 (Kato et al., 1997), but the plant is not considered invasive in Europe.

Hyoscyamus niger

Atropine and scopolamine are also the major TAs in *Hyoscyamus niger* which unusually has a higher scopolamine level than that of atropine (Gaillard and Pepin, 1999). The species contains calystegines in addition: A_3, A_5, A_6, B_1, B_2, B_3 and N_1 (Dräger, 2004). *H. muticus* (Egyptian henbane) is particularly rich in TAs (Mandal et al., 1991; Oksman_Caldentey et al., 1987). *Hyoscyamus albus* contains calystegines A_3, B_1, B_2 and B_3 in its cultured roots (Bekkouche et al., 2001).

Mandragora

A number of poisonings by the mandrake *Mandragora autumnalis* have been reported in Italy, the native habitat for this plant (Piccillo et al., 2002). However it is not a seriously invasive weed. *M. autumnalis* and *M. vernalis* contained hyoscyamine, hyoscine, cuscohygrine, apoatropine, 3 α-tigloyloxytropane and 3,6-ditigloyloxytropane. Belladonnine was present in the dried roots, but could not be detected in fresh roots (Jackson and Berry, 1973).

Scopolia

Contains several TAs but is not seriously invasive (Asano et al., 1996). Besides hyoscyamine and scopolamine, it contains calystegines A_3, A_5, B_1, B_2, B_3, B_4, C_1.

Solanum dulcamara

Solanum dulcamara was reported to contain calystegines A_3, B_1 and B_2 by Asano *et al.* (2001b) (Asano et al., 2001b).

The most frequently encountered calystegines were A_3, B_1, B_2 and B_3, while distribution of N_1 and C_1 was more limited (Bekkouche et al., 2001). Bekkouche *et al.* (2001) did not detect A_5 and B_4 (Bekkouche et al., 2001).

A5.3. Occurrence of TAs in non-TA containing foods

Data on occurrence of TAs in foods are scarce. Some references are available on the occurrence of atropine and/or scopolamine in food. The identity of the plant delivering the TAs is often unknown since it is assumed that the contamination occurs via co-harvested weeds.

Atropine and scopolamine were found in buckwheat in France in 2007, and examination of the data in 2008 led to the setting a provisional threshold level of 0.02 mg/kg in flour intended for human consumption in France. The sum of atropine and scopolamine in the samples measured in 2007 was above 1000 µg/kg in ten samples with a maximum of 7.4 mg/kg. Five samples of buckwheat grain and 29 samples of buckwheat flour were measured in 2008 of which the sum of atropine and scopolamine exceeded 1 mg/kg in two samples (AFSSA, 2008).

An episode of TA contamination in buckwheat was reported in Serbia (Rancic and Spasic, 2009). A large consignment (about 3000 kg) of buckwheat flour imported from a neighbouring country into Serbia in 2001 was analysed after a case of poisoning was reported. Seeds resembling those of *D. stramonium* were separated and analysed by automated multicolumn high pressure liquid chromatography (HPLC) with UV detection which confirmed the presence of atropine and scopolamine.

In 2011 atropine and scopolamine were measured in buckwheat fruits, flours and retail foods sold in Italy (Caligiani et al., 2011). No TAs were found above an LOQ of 1 µg/g in buckwheat fruits (2), flours (7) or food products made from buckwheat (3 pasta, 2 porridge, 1 cracker, and 1 flakes).

A food poisoning incident in 2005 involving *Datura* in buckwheat in Slovenia was followed up by a survey of 75 samples of buckwheat grain and buckwheat food products collected in Slovenia (Perharič et al., 2013). The survey samples comprised 12 wholegrain buckwheat, 13 samples of groats, 34 flour, 8 pasta, 4 bread and 4 žganci (semi-prepared fermented buckwheat product) products. Fifty of the products were analysed by gas chromatography with mass spectrometry (GC-MS). 18 samples contained atropine and scopolamine at levels above the limit of quantification (LOQ) of 30 µg/kg. Of these eleven were flours, four were pasta, and three were 'žganci'. The maximum contamination level of 26 mg/kg atropine and 12 mg/kg scopolamine was in buckwheat flour from Hungary. Of the 18 positive samples 11 were from Hungary, 4 from the Czech Republic, 2 from China and 1 from Slovenia. The TA content of the positive samples was 1.9 mg/kg atropine and 1 mg/kg scopolamine with a median content of 0.25 and 1.0 mg/kg, respectively.

In the survey data reported in the EFSA Opinion of 2013 of food samples from the Netherlands (112 samples) and Germany (12 samples), 93 were within the food group 'Food for infants and small children'. Of those, 19 samples in the 'Simple cereals that are or have to be reconstituted with milk or other appropriate nutritious liquids' had mean atropine + scopolamine concentrations of 4.5 µg/kg at the Lower Bound (LB) limit and 4.9 µg/kg at the Upper Bound (UB) limit. The major contamination frequency (50%) being in the cereal products that were indicated specifically for toddlers (a total of 28) with food for both toddlers and infants, having lower levels in five products (EFSA, 2013; Mulder et al., 2015).

The EFSA Opinion on TAs in food also indicated that other food could be contaminated such as oilseeds, especially sunflower seeds, and in millet (EFSA, 2013).

In the RASFF system, atropine levels in the food concerned range from 3.7 to 1500 µg/kg product and scopolamine levels range from 2.0 to 460 µg/kg product are reported in the EU.

A6. Toxicity data of individual TAs

Many experiments have been performed to study the toxicological and pharmacological effects of atropine and scopolamine. For other individual TAs far less data on their toxicity and pharmacological properties are available. Data from plant poisoning cases are difficult to link to individual TAs. In addition to the TAs, other plant toxins may have been present.

A6.1. Datura-type TAs

Ingestion of TAs causes a variety of symptoms in humans, including: a dry mouth and dryness of the upper digestive and respiratory tracts, hot red skin, pupil dilation (mydriasis) and blurred vision, changes in heart rate (-tachycardia), and respiratory depression, urinary retention, ataxia, speech disturbance, disorientation and visual hallucinations (Brunton et al., 2011).

The Datura-type TAs are anticholinergic compounds and the poisoning symptoms are due entirely to inhibition of muscarinic acetylcholine receptors (AChE) in the central nervous system (CNS) and the autonomic nervous system (ANS). There are five different subtypes of the muscarinic receptor (M1-M5). All subtypes are expressed in the CNS, and M2 – M5 are also expressed in the PNS. Due to their location within different parts of the brain and body, different functions can be assigned to the subtypes of the receptor (Wess et al. 2007; Abrams et al. 2006).

The muscarinic receptors have allosteric and orthosteric binding sites. The orthosteric binding site do not differ between receptor subtypes, at this site the enzymatic activity of the receptor is directly regulated. Atropine binds competitively to the orthosteric binding site of the M-receptors, so it does not have more affinity for a specific subtype of the receptor (Schmitz et al. 2014). Scopolamine is an antagonist of the muscarinic AChE receptors. The effects of atropine and scopolamine on these receptors are similar. However, in animal studies is found that the effect of scopolamine on the CNS is more potent compared to atropine. The brain is affected by scopolamine blocking the muscarinic receptors in multiple regions of the brain, predominantly the M1-receptor (Klinkenberg & Blokland

2010; Falsafi et al. 2012). Scopolamine does not specifically block the M1-receptor, other M-receptors in the CNS and PNS are also affected, causing multiple other symptoms (reviewed by (Klinkenberg & Blokland 2010)). Next to the muscarinic pathway, the nicotinic AChE receptors in the hippocampus are also affected by exposure to scopolamine. The receptors become upregulated after scopolamine exposure, indicating an inhibition of these receptors by scopolamine (Falsafi et al. 2012). Another study confirmed these findings, and found a decreasing of expression of the N-methyl-D-aspartate receptors (NMDR) after chronic exposure to scopolamine. This could indicate a role for both the cholinergic and glutamatergic systems in the brain in the memory impairment caused by scopolamine (Doguc et al. 2012).

Studies in mice have shown no clear evidence of teratogenicity (Arcuri and Gautieri, 1973). Atropine and scopolamine are not mutagenic in bacterial assays (EMEA, 1998).

Ingestion of TAs causes a variety of symptoms in humans, including: a dry mouth and dryness of the upper digestive and respiratory tracts, hot red skin, pupil dilation (mydriasis) and blurred vision, changes in heart rate, tachycardia, and respiratory depression, urinary retention, ataxia, speech disturbance, disorientation and visual hallucinations (Brunton et al., 2011). Studies in mice have shown no clear evidence of teratogenicity (Arcuri and Gautieri, 1973). Atropine and scopolamine are not mutagenic in bacterial assays (EMEA, 1998).

The metabolic pathways of atropine and scopolamine in humans differ. Atropine has a high oral bioavailability (Lewis and Elvin-Lewis, 1977) whereas scopolamine does not (Ali-Melkkilä et al., 1993). Following absorption, both alkaloids have a high volume of distribution (EMEA, 1997, 1998). The toxic effects are derived from cleavage of hyoscyamine into an alkamine and tropic acid by hepatic esterases (Glick and Glaubach, 1941; Wada et al., 1994). Most atropine (60%) is excreted unchanged in the urine but for scopolamine urinary excretion is lower than 5% (Ali-Melkkilä et al., 1993; Chen et al., 2006c; Putcha et al., 1991; Renner et al., 2005).

Studies in mouse and rat found primarily glucuronide conjugates of polar metabolites in rodent urine, whereas in human studies these conjugates are not found or in much lower concentrations ((Kalser 1971); reviewed by (EFSA, 2013)). This indicates that rodent studies concerning the metabolism and toxic dose of atropine are difficult to extrapolate to the human situation.

Scopolamine undergoes oxidative demethylation and first-pass metabolism mediated by cytochrome P450 CYP3A, so that only a small fraction (<5%) of the dose is excreted as the parent compound, however, the oxidation products have not yet been identified (Renner et al., 2005). When scopolamine is administered orally, an absolute maximum of 13% of the dose becomes bioavailable. Scopolamine has a half-life of approximately 1 h in humans and can have an effect for up to 6 h. The relative low bioavailability cannot be caused by the chemical properties of scopolamine, as these should allow quick absorption from the intestine. Thus, the limited bioavailability indicates first-pass kinetics of scopolamine in the human body via metabolism. Another cause of the low bioavailability of scopolamine is most likely the distribution of scopolamine across several tissues in the body (Renner et al. 2005). The metabolism of scopolamine in humans is not well known. The chemical, and its metabolites, are thought to be excreted via the urine. However, neither the compound nor metabolites could be retrieved from the faeces in animal and human studies (reviewed (Renner et al. 2005)). It is thought that glucuronidation is one of the pathways involved in the metabolism, as the glucuronidate metabolites were found in the urine of human volunteers (Renner et al. 2005; Kentala et al. 1990).

In 2013 the European Food Safety Authority derived a health-based guidance value for the sum of (-)-hyoscyamine and (-)-scopolamine based on the similar acute pharmacological effects of these compounds. The ARfD (acute reference dose) established was 0.016 µg/kg body weight (expressed as the sum of (-)-hyoscyamine and (-)-scopolamine) (EFSA, 2013).

A6.2. Calystegines

Calystegines are potent inhibitors of β-glucosidase (Asano, 2000; Asano et al., 1997a; Asano et al., 1995; Molyneux et al., 1993). Calystegines of the roots of *C. arvensis* and of the hedge bindweed (*C. sepium*) are potent inhibitors of β-glucosidase. They cause neurological dysfunction associated with glycosidase inhibition and lysosomal storage disorders (Watson et al., 2001).

The calystegines B1 and C1 competitively inhibit the β-glucosidase in both human and rodent liver cells (Asano, Kato, Matsui, et al. 1997). The enzyme β-xylosidase is inhibited by calystegines A_3, B_1, B_2, and C_1 in human liver cells. And α-galactosidase is moderately inhibited by the calystegine A_3 (Asano, Kato, Matsui, et al. 1997).

In studies using mammalian tissues Asano et al. (1997a) found that calystegines B_1 and C_1 were potent competitive inhibitors of the bovine, human, and rat β-glucosidase activity and calystegine B_2 was a strong competitive inhibitor of the α-galactosidase activity in liver (Asano et al., 1997a). Calystegine B_1 is also a potent competitive inhibitor of almond β-glucosidase and bovine liver β-galactosidase but it does not inhibit α-galactosidases. Calystegine B_2 is also a potent competitive inhibitor of almond β-glucosidase and coffee bean α-galactosidase (Asano et al., 1995).

Mice fed *Convolvulus* alone ate it readily but soon showed excitement and then depression, anorexia, hunched posture and death. Lesions were found in their stomachs and livers, with acute gastritis erosions and ulcerations, and hepatic necrosis. Mice offered bindweed as a choice ate it but had far milder effects. Human β-xylosidase was inhibited by all calystegines tested. Calystegines A_3 and B_2 selectively inhibited the rat liver β-glucosidase activity. Calystegine A_3 is weaker than calystegine B_2. Calystegine A_5, had no activity against any glycosidases. Calystegine B_3 (a 2-epimer of calystegine B_2) has weak inhibitory activity, suggesting that the equatorially oriented -OH group at C_2 is necessary for strong binding at the active site of glycosidases. Convolvine blocks M2 and M4 cholinergic muscarinic receptors (Mirzaev and Aripova, 1998).

Asano et al. (1997a) proposed that the potent inhibition of mammalian glucosidases in vitro raised concerns that calystegines were toxic to humans consuming large amounts of plants that contain calystegines (Asano et al., 1997a).

Knowledge on the bioavailability and metabolites of calystegines in the human body is scarce. One study mentions hardly any transfer of calystegines from the apical side to the basolateral side in a transwell system using Caco2-cells (Jocković et al. 2013).

A6.3. Minor TAs

There is very little information on the toxicity of individual TAs other than atropine and scopolamine. Pseudotropine causes intestinal contraction in the dog, followed by an inhibition of spontaneous contractions but tropine augments intestinal contractions (Hamlte 1931 quoted in (Todd et al., 1995)). Cuscohygrine suppresses an immune response in mice (Watson et al., 1983).

Anisodine produces four known metabolites (norscopine, scopine, hydroxyanisodine, anisodine *N*-oxide) as determined in rat plasma following ingestion (Chen et al., 2006b).

A7. Identification of relevant food products

A7.1. Identification of foods associated with TAs

The information gathered in the literature review revealed that relevant food for TA-contamination originates from either a) food commodities that do contain inherent TAs or b) food commodities that do not contain inherent TAs but are contaminated with TAs by co-harvest of TA-containing weeds.

A7.1.1. Foods containing inherent TAs

Human intoxications resulting from the consumption of foods with inherent TAs are only reported when berries of *Atropa belladonna* were consumed by accident (3) and when *Atropa belladonna* and *Datura* stramonium were used as medicinal plant (2). However, *Atropa belladonna* and *Datura stramonium*, the plant involved in these cases, are not considered as food in this study.

The foods containing inherent TAs are presented in Table A4 and Table A5 and summarised in Table A6. The consumption is rated low – moderate – high according to the consumption levels in Europe as explained in section A7.2.

Foods most relevant in this respect are potatoes and aubergine. Potatoes are the food within this category that is consumed in largest quantities in the EU.

It should be taken into consideration that diets are changing as more 'superfoods' are consumed and more gluten free food is available. Several of those 'superfoods' are plants containing more inherent TAs, such as *B. oleracea* and goji berries.

A7.1.2. Foods associated with the TA-containing weeds

Food containing co-occurring TAs associated with intoxications in humans in the EU is presented in Table A2 and concerns: tea contaminated with *Atropa belladonna* (3); buckwheat/millet bread/flour contaminated with *Datura* (3); leafy vegetables collected from the wild containing/composed of *Datura* or *Mandragora officinarium* (2) and vegetables canned /frozen contaminated with *Datura* (2).

Table A3 shows that six food product categories are involved in TA occurrence in food in the EU as reported in the EU RASFF system: cereals and bakery products containing atropine or *Datura stramonium* (24 results); fruits and vegetables contaminated with *Datura stramonium* or *Solanum nigrum* (8 results); dietetic foods, food supplements, fortified foods contaminated with atropine and *Solanum nigrum* (5 results); cocoa, coffee and tea contaminated with atropine and *Atropa belladonna* (3 results); nuts, nut products and seeds contaminated with *Hyoscyamus niger* (3 results); and herbs and spices contaminated with *Atropa belladonna* (2 results). Tea is in two categories: cocoa, coffee and tea and herbs and spices. On product level, most results are retrieved for millet and millet-based products (12 results), followed by buckwheat (6 results) and vegetable mix (5 results).

The occurrence of TAs from co-occurring weeds in food in the EU is described in section A5.3. It concerns buckwheat (4) and foods for infants and small children (1).

TAs in foods can originate from co-occurring weeds as is discussed before in this report. Table A7 shows a summary of the result of TAs and TA-containing co-occurring weeds in foods, as reported also presented in Table A2, Table A3 and section A5.3.

Foods most relevant in this respect are cereal-based foods, particularly those intended for infants and young children and foods based on particular seeds, principally buckwheat, millet, corn and poppy.

A second important category in this category is vegetables, particularly green beans, both frozen and canned, which are occasionally contaminated with *Datura* fruit and with toxic species of *Solanum*.

The third important group is tea.

EFSA also indicated oilseeds, especially sunflower seeds as food possible contaminated with TAs by co-occurring weeds.

A7.2. Consumption levels

A summary of the consumption levels of the tropane-containing plants in European countries for which data was available via the FAO/WHO Chronic Individual Food Consumption Database – summary statistics (CIFOCOss) was obtained as published in 2015 (FAO_WHO, 2015) and a summary of this is are provided in Table A11. Data are provided in grams of plant per kg body weight per day (g/kg bw/day), although data have not been reported for some important countries. The data show that the highest levels of potato consumption to occur in children in Bulgaria (about 3.5 g/kg bw/day), in adolescents and children in the Czech Republic (2-3 g/kg bw/day), in adults and elderly people in Hungary and adults in Spain. There are no data for potato consumption in Belgium, France and the UK.

The highest levels of fresh tomato consumption are in Italy and Bulgaria (about 3 g/kg bw/day). The highest levels of brassica consumption are in the North Western European countries, and the highest consumption of green beans (included on account of more regular tropane contamination) are in France, Belgium, and Bulgaria.

Summaries of the consumption levels of the TA-containing plant foods and of foods that have been contaminated with TAs in 19 European countries (Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, the Netherlands, Romania, Spain, Sweden, United Kingdom) as reported by the EFSA Food Consumption Database (EFSA) are provided in Tables A11-A19.

The tables generally show the number of subjects of the survey, the number of consumers of the products, and the average consumption in grams per day for all consumers. The EFSA consumer descriptions are for pregnant women, lactating mothers, infants, toddlers, other children, adolescents, adults, elderly, and very elderly. In most tables the consumer descriptions have a number prefix to enable sorting by age. Where there is more than one entry for a consumer description this is because data for more than one consumption survey is reported.

Table A12 shows the average consumption in grams per day for all subjects and all consumers of a range of potatoes and potato products. Boiled potatoes are the major product in this group.

Table A13 shows a breakdown by country of the data for potato supplied in Table A12 filtered to show only the higher consumers, i.e. those consuming more than the average quantity. The heaviest consumers are in northern, western and central Europe and tend to be adults and elderly.

Table A14 shows the average consumption in grams per day for infants, toddlers and other children of cereal-based foods for infants and young children. High consumers are shown in bold. In this table (only) data are reported for all subjects including the non-consumers, in order to show the range of consumption in these vulnerable groups. There appears to be evidence of some targeting of subject groups in the data for Finland and Germany where in some cases several hundred subjects provide no consumers and in other cases there are a number of consumers. Consumption levels appear much higher in the data for toddlers in Spain.

Table A15 shows the average consumption in grams per day for high consumers of aubergines. There were approximately 4,000 consumers from 80,000 subjects, with an average intake of 1.5 grams/day. The highest consumers by some margin were in Romania and Italy.

Table A16 shows the average consumption in grams per day for high consumers of beans with and without pods. These data are provided on account of the occasional contamination of green beans and vegetable mixes with *Datura*. From 80,000 subjects there were approximately 7,000 consumers of beans without pods and 10,000 consumers of beans without pods with an average intake of 2.5 and 4.0 grams/day respectively. The highest consumers of beans without pods were across a range of ages in the UK where subjects typically consumed 7 x the European average. However these are likely to be beans cooked and canned in tomato sauce, in which contaminating vegetable material from *Datura* would be more easily identified. For beans with pods the high consumers were in the Netherlands, Romania and Spain (3 to 5 x the average).

Table A17 shows the average consumption in grams per day for all consumers of buckwheat products. In the case of Latvia, the presence of a high proportion of consumers is associated with a particularly high consumption level. These factors indicate that the product is popular in that country. In other countries elevated consumption levels are also related to a higher proportion of consumers.

Table A18 shows the average consumption in grams per day for all consumers of millet products. The proportion of consumers is low but notably higher in toddlers and other children in Finland. In most countries the consumption level does not greatly exceed the European average.

Table A19 shows the average consumption in grams per day for all consumers of poppy seeds. Consumption levels are highest in the Czech Republic, Hungary and Romania.

A7.3. Availability of these foods on the EU market

All of the foods described above are available on the EU market to degrees that vary with location. The foods that have been contaminated with TA-containing weeds are easily available across the EU. Many food plants with endogenous TAs (potatoes, aubergines, peppers, sweet potatoes) are widely available while others such as Cape gooseberry and goji berries have limited availability or market demand is current low.

A8. Identification of the relevant TAs in food in the EU

A8.1. Identification of the inherent TAs in food in the EU

The data in Table A7 show that calystegines A_3; A_5; B_1; B_2; B_4; C_1 are reported in one or more species from the genus *Solanum* that are reported as most relevant for consumption in the EU. These calystegines do occur inherent in these foods.

Buckwheat is an example of a gluten free food product, replacing wheat products. Buckwheat is more often contaminated with TAs derived from co-occurring weeds. If the consumption of these foods indeed increases, the TA exposure could increase significantly for EU citizens.

A8.2. Identification of the relevant co-occurring TAs in food in the EU

The data in Table A10 show that atropine and scopolamine are reported to occur in foods in the EU. The reports on weeds associated with food in the EU are *Datura stramonium*; *Atropa belladonna*; *Solanum nigrum*; *Hyoscyamus niger* and *Mandragora.*

A8.3. Identification of emerging co-occurring TAs in food in the EU

Table A10 summarises Table A8 and Table A9 for the weeds that are considered invasive in Europe.

Some assessment was made of toxic weed plants from outside the EU likely to contaminate foods imported into the EU. The demand for soya beans in the EU exceeds its cultivation and over 30 million tons are imported each year of which about 5% is used directly as an ingredient in human foods. Soya is frequently contaminated by seeds of toxic *Solanum* species often described as black nightshade (*Solanum nigrum* L.) but which is more likely eastern black nightshade (*Solanum ptycanthum*) (Binning, 1993; Crotser and Witt, 2000). The plant is common in cultivated fields and its fruits have been harvested with peas, snap beans and soybeans (Crotser and Witt, 2000). Other solanums invasive and common in the USA and Asia are *Solanum viarum* and *Solanum torvum* (turkeyberry).

A paper from Adamse et al. (2014) clearly points to contamination with seeds from *D. stramonium* (Jimson weed or thorn apple) as the most common problem, however the authors indicate that seeds from other *Datura* spp. as well as berries of *A. belladonna* and seeds of *Hyoscyamus niger* have been reported as impurities in food (Adamse et al., 2014).

TAs are found in the genera *Atropa*, *Brugmansia*, *Datura*, *Hyoscyamus*, *Mandragora* and *Scopolia* among the plants that are herbaceous and therefore more likely to act as weeds, and in many plants less likely to be found in Europe or in the vicinity of food crops, such as *Iochroma*, *Juanulloa*, and *Solandra*.

TAs (tropine, tropinone, cuscohygrine, hygrine) have been reported in *Convolvulus arvensis* and *Convolvulus sepium*, now known as *Calystegia sepium* (Goldmann et al., 1990).

Specialised Nutrition Europe (SNE) is a trade association representing the specialised nutrition industry across the European Union. SNE members are the national associations of 17 EU Member States and their companies produce foods for specific groups, including the foods specifically intended for infants and young children. An unreported survey by SNE carried out in 2014 confirmed data from the Dutch authorities that showed contamination risks in sorghum and millet. SNE has expressed its favour in setting up an Indicative Value of 250 µg/kg for the sum of hyoscyamine and scopolamine) in sorghum, millet and buckwheat used to manufacture cereal-based foods for infants and young children (SNE, 2014).

A9. Conclusion and recommendations Literature review

It can be concluded that information on the occurrence of TAs in food in the EU is scarce. Moreover, the available information is not always relevant to food, e.g. many reports concern occurrence of TAs in non-edible plant parts such as roots and flowers.

Most information is available on atropine and scopolamine and little information, which is often also very dated, is available on the other TAs occurring in foods. Likewise, knowledge on toxic effects of TAs, other than atropine and scopolamine, are scarce. This means that at this moment all TAs are considered toxic and it is advised to measure all TAs for which an analytical standard is available.

It is very difficult to predict which co-occurring weeds, and thus which TAs, will be present in the food in the EU. Areas where food is sourced can change quickly and are not only limited to the EU. Regarding contamination of cereal crops with weeds, the major weeds currently contaminating food crops in the EU are *Datura stramonium* and *Convolvulus arvensis*. The TAs tropine, tropinone, cuscohygrine, hygrine have been reported in *Convolvulus arvensis* and *C. sepium* (*Calystegia sepium*). Toxic weed plants from outside the EU likely to contaminate foods imported into the EU are *Solanum* species *S. ptycanthum*, *S. nigrum*, *S. viarum* and *S. torvum*. These plants are common in cultivated fields and have been harvested with peas, snap beans and soya beans (Binning, 1993; Crotser and Witt, 2000). Other solanums invasive and common in the USA and Asia are *S. viarum* and *S. torvum* (turkeyberry). It is therefore advised to analyse all foods for all TAs for which an analytical standard is available.

The EFSA Opinion on tropane alkaloids in 2013 indicates contamination of a high proportion of cereal-based food for infants and young children, and a lesser contamination of a high proportion of cereal-based food intended for both toddlers and infants. Other contamination has been revealed in food and feed based on oilseeds, especially sunflower seeds, and in millet and related plants such as sorghum.

From reports under RASFF the greatest incidence of contamination has concerned *Datura* seeds in millet (mainly organic and mainly originating in Austria) and *Datura* fruit (berries) in processed bean-based vegetables. Frozen green beans have also been contaminated with *Solanum* and poppy seeds with *Hyoscyamus* (henbane).

Considering the widespread contamination of herbal products with pyrrolizidine alkaloids and a single incidence of contamination of tea prepared from marshmallow root with *A. belladonna* root there might be a requirement to survey herbal teas from areas with potential tropane-containing plant growth.

Whilst there are many reports of contamination by *Datura* tropanes there are no reports of calystegine contamination of foods in which they do not occur naturally. There appears to be widespread and sometimes heavy growth of *Convolvulus* in cereal fields, but consumer exposure to calystegines will be greater and more sustained through consumption of potato products.

The primary recommendations are for a focus on:

- The review has highlighted the emerging potential for food contamination from non-food weed plants that are encroaching on field crops in Europe. *Convolvulus* species (bindweeds) present a particular problem that should be addressed in future projects.
- Analysis of all TAs for which a reliable analytical standard is available.

Foods to be analysed for inherent TAs

- Potato products of all types and cultivars should be analysed for calystegines as priority crop.
- Include aubergines in the survey to be analysed for calystegines based on consumption pattern in the EU.
- Fututure work should be extented to the occurrence of calystegines in *Capsicum annuum*, *Solanum lycopersicum*, *Brassica napa* and *B. oleracea.*
- It is recommended to also include emerging superfoods that do contain inherent TAs when appropriate, e.g. *B. oleracea* and goji berries.

Foods to be analysed for co-occurring TAs

Analyse for low molecular weight TAs, Convolvulaceae-type TAs, Datura-type TAs

- Processed cereal foods intended for babies, infants and toddlers, including a high proportion of organic products.
- Processed and unprocessed cereal foods for adults, including a high proportion of organic products, including some samples with poppy seeds and where identified some products containing millet.
- Processed foods and flours based on buckwheat, including a high proportion of organic products, including some samples with poppy seeds and where identified some products containing millet.
- Canned and frozen vegetables with a high proportion of green beans.
- Tea, specifically herbal teas.

Analyse for calystegines

- It is recommended to extend the calystegines survey to the field cereal products since some co-occurring weeds do also contain calystegines. However, concentration in the final product will be extremely low and may not be very relevant for exposure.

Considerations for sampling

- It is advised to include more than one harvest year.
- The sampling should include food from as many different sources as possible, including food sourced from outside the EU.

The table below lists the suggested search topics and combinations for searching.

Provisional indications are that so few results are obtained from including 'calystegine' as a search term that an independent single search for this topic will be the best approach.

Searching in multiple databases on 'Web of Science' did not include use of the Chemical Abstracts Registry (CAS) Number. The searches can be repeated using the 'CAB Abstracts® and Global Health®' databases, using the CAS numbers to replace the chemical names in # 2.

Table A1: Strategy for identification and characterisation of relevant tropane alkaloids and food products

Search 1 returns records on toxic effects of tropane alkaloids	
# 16	# 15 AND # 2
# 15	# 12 OR # 13 OR # 14
# 14	TOPIC: (intox*)
# 13	TOPIC: (food* SAME poison*)
# 12	TOPIC: (tox* OR harmful* OR adverse*)
Search 2 returns records on occurrence of tropane alkaloids in food, feed, and plants	
# 11	# 10 AND # 2
# 10	# 9 OR # 8 OR # 7 OR # 6 OR # 5 OR # 4 OR # 3
# 9	TOPIC: (weed OR weeds OR cereal* OR pea* solanac OR brassica*)
# 8	TOPIC: (plant* SAME deriv* SAME contam*)
# 7	TOPIC: ((plant or plants) NEAR/2 assess*)
# 6	TOPIC: (risk* NEAR/2 assess*)
# 5	TOPIC: (provenance OR source*)
# 4	TOPIC: (natural* NEAR/2 occur*)
# 3	TOPIC: (food* OR feed*)
# 2	TOPIC: (Tropane OR Tropinone OR Tropine OR Pseudotropine OR Scopine OR Scopoline OR Tigloytropine OR Tigloidine OR Cusohygrine OR *Tropanyl phenylacetate* OR Apoatropine OR Homatropine OR Convolidine OR Phygrine OR Apohyoscine OR Aposcopolamine OR Littorine OR Convoline OR Atroscine OR Convolamine OR *Beta-hydroxyhyoscyamine OR Hydroxyhyoscyamine OR Anisodine OR Belladonine OR Atropa OR Brugmansia OR *Datura* OR Duboisia OR Erycibe OR Hyoscyamus OR Latua OR Lycium OR MandragORa OR Nicotiana OR Physalis OR Scopolia OR Brassica OR Calystegia OR Convolvulus)
# 1	TOPIC: (Calystegine)

Table A2: Reported cases of human intoxication in the EU

Plant involved	Product involved (Reference)
Atropa belladonna	Berries - accidental - Denmark (Lange and Toft, 1990) Berries - accidental - Italy (Trabattoni et al., 1984) Cooked Berries - accidental - France (Schneider et al., 1996) Tea - co-contamination – the Netherlands and France (van Riel et al., 2014) Tea - Medicinal herb - Germany (BfR, 2006)
'atropine' symptoms	Nettle tea - co-contamination – Austria (Adamse et al., 2014) Comfrey tea - co-contamination – United Kingdom (Galizia, 1983; Routledge and Spriggs, 1989)
Datura	Buckwheat flour bread - co-contamiantion - France (Glaizal et al., 2013)
Datura innoxia	Wild collected spinach - co-contamination - (Papoutsis et al., 2010)
Datura stramonium	Tea from *Datura* leaves - Medicinal herb - Germany (van_der_Heide, 1988) Buckwheat flour - co-contamination - Slovenia (Perharič, 2005) Seeds in millet-carrot balls - co-contamination - Austria (Fretz et al., 2007) Seeds in canned beans - co-contamination - France (Le_Parisien, 2010) Frozen vegetables - co-contamination - Finland (Termala et al., 2014)
Mandragora officinarum	Leaves - co-contamination - Greece (Tsiligianni, 2009)

Table A3.1: RASFF notifications and/or alerts for tropane alkaloids atropine and scopolamine in food (Nov. 18, 2016)

Type	Date of case	Reference	Country	Product Category	Food	Origin	Concentration (µg/kg)	
							Atropine	Scopolamine
Alert	20/9/2016	2016.1298	Austria	Cereals and bakery products	Millet flour	Hungary	23.5	9.5
Alert	22/07/2016	2016.0975	Austria	Cereals and bakery products	Corn	Germany	10.5; 12.2; 12.3	2.0; 3.8; 3.1
Alert	13/04/2016	2016.0144	Czech Republic	Dietetic foods, food supplements, fortified foods	Baby porridge without milk	Spain	7.9	NM
Alert	01/02/2016	2016.0106	Czech Republic	Cereals and bakery products	Gluten-free baking mix based on sorghum	Czech Republic	180; 130	36; 27
Alert	26/11/2015	2015.1487	Czech Republic	Cereals and bakery products	Sorghum flour	Czech Republic/ Slovakia	1200; 1500	360; 460
Alert	18/09/2015	2015.1190	Germany	Cereals and bakery products	Microwave popcorn	Spain	29	6
Alert	04/06/2015	2015.0684	Germany	Cereals and bakery products	Organic polenta cornmeal	Germany	198.5	45
Alert	31/03/2015	2015.0399	Germany	Cereals and bakery products	Millet balls	Hungary	384	388
Alert	30/03/2015	2015.0388	Austria	Cereals and bakery products	Gluten free organic millet	Austria	30	24
Alert	30/03/2015	2015.0387	Austria	Cereals and bakery products	Millet honey poppies	Germany	26	11
Alert	20/03/2015	2015.0339	Austria	Cereals and bakery products	Millet dumplings	Hungary	304	358
Alert	20/03/2015	2015.0338	Austria	Cereals and bakery products	Organic millet dumplings	Hungary	481	533
Alert	20/02/2015	2015.0210	Germany	Cereals and bakery products	Organic polenta cornmeal	Germany	156.2; 207.5	27.2; 31.3
Alert	20/02/2015	2015.0203	Austria	Cereals and bakery products	Brown millet	Germany	62	33
Alert	18/12/2014	2014.1724	Germany	Dietetic foods, food supplements, fortified foods	Millet/cereal porridge with rice	Hungary	36.6	NM
Alert	11/12/2014	2014.1694	Germany	Dietetic foods, food supplements, fortified foods	Organic baby food apple pear millet	Germany	3.7; 6.7; 31.9	NM
Alert	04/12/2014	2014.1652	Germany	Cereals and bakery products	Brown millet flour	Austria/Hungary/ the Netherlands	46	25
Alert	21/11/2014	2014.1596	Germany	Dietetic foods, food supplements, fortified foods	Baby food porridge	Germany/Austria	12.1	NM
Alert	13/06/2013	2013.0829	Slovenia	Cereals and bakery products	Buckwheat flour	Austria/Slovakia	18	5.5
Alert	22/05/2013	2013.0706	Slovenia	Cereals and bakery products	Buckwheat flour	Czech Republic	14	11
Information	08/06/2012	2012.0794	Slovenia	Cereals and bakery products	Buckwheat flour	Slovenia	20	6.1
Information	30/04/2009	2009.0558	Slovenia	Cereals and bakery products	Buckwheat flour	Hungary	110	47
Information	03/07/2006	2006.BMT	Slovenia	Cereals and bakery products	Buckwheat flour	Ukraine	37	48
Alert	03/07/2006	2006.0424	Slovenia	Cereals and bakery products	Buckwheat flour	Czech Republic	35	65
Alert	28/10/1994	1994.18	United Kingdom	Cocoa, coffee and tea	Burdock root tea	United Kingdom	NM	NM

NM = not mentioned.

Table A3.2: RASFF notifications and/or alerts for Belladonna, Datura, Hyoscyamus, and Solanum niger in food (Nov. 18, 2016)

Type	Date of case	Reference	Country	Product Category	Food	Origin	Contaminant	Content
Alert	23/01/2013	2013.0079	the Netherlands	Herbs and spices	Marshmallow tea	Germany/ Bulgaria	*Atropa belladonna* root	NM
Alert	29/06/1989	1989.15	France	Herbs and spices	Burdoch root	NM	Belladonna	NM
Alert	23/01/1984	1984.03	France	Cocoa, coffee and tea	Bardane herbal tea	NM	Belladonna	NM
Alert	04/02/1983	1983.03	United Kingdom	Cocoa, coffee and tea	Tea	NM	Belladonna	NM
Alert	17/05/2013	2013.0696	Finland	Fruits and vegetables	Frozen vegetable-bean-seed mix	Belgium/Spain	*Datura stramonium* seeds	NM
Information	02/10/2007	2007.CGO	Spain	Fruits and vegetables	Vegetable bacon stir-fry	Spain	*Datura stramonium* seeds	NM
Alert	05/09/2007	2007.0613	Austria	Fruits and vegetables	Canned green beans	Hungary	*Datura stramonium* fruit	NM
Alert	27/11/2006	2006.0835	Austria	Fruits and vegetables	Canned green beans	Hungary	*Datura stramonium* fruit	NM
Alert	27/11/2006	2006.0833	Austria	Cereals and bakery products	Organic millet	Austria/Hungary	*Datura stramonium* seeds	130 seeds/kg
Information	20/11/2006	2006.CRE	Austria	Cereals and bakery products	Brown millet	Austria	*Datura stramonium* seeds	NM
Information	08/11/2006	2006.COH	Austria	Cereals and bakery products	Organic millet	Austria	*Datura stramonium* seeds	NM
Alert	24/10/2006	2006.0737	Austria	Cereals and bakery products	Organic millet	Austria	*Datura stramonium* seeds	NM
Information	10/10/2006	2006.CFX	Austria	Cereals and bakery products	Peeled bio gold millet	Austria	*Datura stramonium* seeds	NM
Alert	06/05/2008	2008.0520	Czech Republic	Nuts, nut products and seeds	Poppy seeds	Czech Republic	*Hyoscyamus niger* seeds	0.13%
Alert	13/04/2007	2007.0267	Slovakia	Nuts, nut products and seeds	Poppy seeds	Czech Republic	*Hyoscyamus niger* seeds	0.24%
Alert	10/04/2007	2007.0256	Slovakia	Nuts, nut products and seeds	Poppy seeds	Czech Republic	*Hyoscyamus niger* seeds	0.42%
Border rejection	06/03/2015	2015.AKE	Portugal	Dietetic foods, food supplements, fortified foods	Food supplement	India	*Solanum nigrum*	NM
Alert	07/06/2013	2013.0804	Germany	Fruits and vegetables	Young green beans	the Netherlands	*Solanum nigrum* berries	NM
Alert	18/02/2004	2004.086	France	Fruits and vegetables	Frozen green beans	NM	*Solanum nigrum* berries	NM
Alert	06/11/1985	1985.15	Germany	Fruits and vegetables	Canned green beans	NM	*Solanum nigrum* berries	NM
Alert	13/10/1982	1982.07	Italy	Fruits and vegetables	Frozen green beans	NM	*Solanum nigrum* berries	NM

NM = not mentioned.

Table A3.3: Foods involved in RASFF notifications and/or alerts for atropine and scopolamine; Belladonna, Datura, Hyoscyamus and Solanum niger in food (August 2, 2016)

Product Category	Sub-product category	RASFF Reference	Contaminant
Cereals and bakery products	Buckwheat	2013.0829; 2013.0706; 2012.0794; 2009.0558; 2006.BMT; 2006.0424	Atropine, scopolamine
	Corn-based food	2016.0975; 2015.1190; 2015.0684; 2015.0210	Atropine, scopolamine
	Millet and millet-based foods	2016.1298; 2015.0399; 2015.0388; 2015.0387; 2015.0339; 2015.0338; 2015.0203; 2014.1652; 2006.0833; 2006.CRE; 2006.COH; 2006.0737; 2006.CFX	Atropine, scopolamine, *Datura stramonium* seeds
	Sorghum-based food	2016.0106; 2015.1487	Atropine, scopolamine
Cocoa, coffee and tea	Tea	1994.18; 1984.03; 1983.03	Atropine; *Atropa belladonna*
Dietetic foods, food supplements, fortified foods	Porridge (baby)	2016.0144; 2014.1596	Atropine
	Food supplement	2015.AKE	*Solanum nigrum*
	Millet and millet-based food	2014.1724; 2014.1694	Atropine
Fruits and vegetables	Vegetable mix	2013.0696; 2007.CGO; 2013.0804; 2004.086; 1982.07	*Datura stramonium* seeds; *Solanum nigrum* berries
	Canned beans	2007.0613; 2006.0835; 1985.15	*Datura stramonium* fruit; *Solanum nigrum* berries
Herbs and spices	Tea	2013.0079; 1989.15	*Atropa belladonna*
Nuts, nut products and seeds	Poppy seeds	2008.0520; 2007.0267; 2007.0256	*Hyoscyamus niger* seeds

Table A4.1: Food plants from the Brassicaceae family associated with TAs in the EU

Brassicaceae					
Genus	**Species**	**Name**	**Tropane alkaloids (mg/kg dry matter)**		**Reference**
			Atropine	**Calystegine**	
Amorica	*A. rusticana*	Horseradish	NR	NR	
Brassica	*B. juncea*	Brown mustard	NR	NR	
	B. napobrassica	Swede, rutabaga, Chinese cabbage	NR	NR	
	B. nigra	Black mustard	NR	A_3 (15); A_5 (2); B_2 (1)	(Brock et al. 2006)
	B. oleracea	Broccoli, cauliflower, kale, Brussels sprouts	NR	A_3 (4-28); A_5 (5); B_2 (5)	(Brock et al. 2006)
	B. rapa	Turnip	NR	NR	
	/ pekinensis	Chinese cabbage	NR	NR	
Eruca	*E. sativa*	Rocket	NR	NR	
Nasturtium	*N. officinale*	Water cress	NR	NR	
Raphanus	*R. sativus*	Radish	NR	NR	
Sinapis	*S. alba*	White mustard	NR	NR	
Wasabi	*W. japonica*	Wasabi	NR	NR	

NR = no reports found.

Table A4.2: Food plants form the Convolvulaceae family associated with TAs in the EU

Convolvulaceae					
Genus	**Species**	**Name**	**Tropane alkaloids (mg/kg fresh weight)**		**Reference**
			Atropine	**Calystegine**	
Ipomoea	*I. alba* L	Tropical white morning-glory, moonflower	NR	Ecuador: A_3; A_5; B_1; B_2; B_3; C_1	(Schimming et al., 2005)
	I. albivenia	Wild cotton	NR	NR	
	I. aquatica	Water spinach	NR	Thailand: A_3; B_1; B_2; B_4	(Schimming et al. 2005)
	I. batatas	Sweet potato	NR	Japan: B_1 (16); B_2 (19); C_1 (9) UK: A_3 (0.11); B_1 (2.37); B_2 (1.12); C_1 (0.61) Mexico, Panama, Japan: A_3; A_5; B_1; B_2; B_3	(Asano et al., 1997a; Schimming et al., 2005)
	I. involucrata	UNK	NR	Tanzania: B_1; B_3	(Schimming et al., 2005)
	I. leptophylla	Bush morning-glory	NR	NR	

NR = no reports found.
UNK= no common name known.

Table A4.3: Food plants form the Moraceae family associated with TAs in the EU

Moraceae					
Genus	**Species**	**Name**	**Tropane alkaloids (mg/kg dry weight)**		**Reference**
			Atropine	**Calystegine**	
Morus	*M. alba*	White mulberry	NR	B_2 (7)	(Asano et al., 1997a)
	M. nigra	Black mulberry	NR	NR	
	M. rubra	Red mulberry	NR	NR	

NR = no reports found.

Table A4.4: Food plants form the Solanaceae family associated with TAs in the EU

Solanaceae					
Genus	**Species**	**Name**	**Tropane alkaloids (µg/g fresh weight)**		**Reference**
			Other TA	**Calystegine**	
Capsicum	*C. annuum var. angulosum*	Sweet peppers	NR	Japan: B_1 (12); B_2 (37); C_1 (4)	(Asano et al., 1997a)
	C. baccatum	Ají Amarillo, peppadew lemon drop	NR	NR	
	C. chinense	Various hot peppers	NR	NR	
	C. frutenscens	Cayenne pepper	NR	UK: A_3 (0.24); B_2 (0.27)	(Asano et al., 1997a)
	C. pubescens	Rukutu, ruqutu, luqutu, Manzano pepper	NR	NR	
Lychium	*L. barbarum (syn. L. chinense)*	Goji berry	Atropine: 0.02 µg/g DW	NR	(Klenow et al., 2012)
	L. europaeum	Goji berry	Atropine: 0.59 µg/g DW	NR	(Klenow et al., 2012)
Physalis	*P. alkekengi*	Chinese lantern	NR	NR	
	P. ixocarpa (Syn. P. philadelphica)	Tomatillo	NR	UK: B_2 (0.002)	(Asano et al., 1997a)
	P. peruviana	Cape gooseberry	Tigloidine	UK: A_3 (0.003); B_1 (0.038); B_2 (0.048); C_1 (0.005)	(Asano et al., 1997a; Beresford and Woolley, 1974)
Solanum	*S. aethiopicum*	Garden egg	NR	NR	
	S. betaceum (syn. Cyphomandra betaceae)	Tamarillo or tree tomato	NR	UK: B_2 (0.002)	(Asano et al., 1997a)
	S. esculentum (syn. Lycopersicum esculentum)	Tomato	NR	UK: A_3 (1.1); B_2 (4.5)	(Asano et al., 1997a)
	S. macrocarpon	African eggplant or gboma	NR	NR	
	S. melongena	Aubergine or eggplant	NR	Japan: B_1 (7); B_2 (73) UK: A_3 (0.3); B_2 (0.5)	(Asano et al., 1997a)
	S. muricatum	Pepino	NR	NR	
	S. quitoense	Naranjilla	NR	NR	
	S. melanocerasum (syn. *S. scabrum)*	Garden huckleberry	NR	B_2 (0.007)	(Asano et al., 1997a)

Solanaceae					
Genus	Species	Name	Tropane alkaloids (µg/g fresh weight)		Reference
			Other TA	Calystegine	
	S. tuberosum	Potato (fresh tuber flesh)	NR	Japan: B_2 (7); UK: A_3 (1.17); B_2 (2.22); Sweden: A_3 (9-84); B_2 (10-56); B_4 (2-30) Japan, USA: A_3 (0.2-11.1); B_2 (0.8-56.5) UK: Total calystegines (10) French fries for microwave cooking: A_3 (15); B_2 (20) Oven ready chips: A_3 (1); B_2 (1) Instant mashed potato: A_3 (9); B_2 (23) Potato waffles: A_3 (3); B_2 (4) Potato crisps: A_3 (5); B_2 (21) Crisps from feeze dried potato granules: A_3 (0.2); B_2 (0.3) Hula hoops: A_3 (1); B_2 (5)	(Asano et al., 1997a; Friedman et al., 2003; Nash et al., 1993; Petersson et al., 2013; Watson et al., 2000)

NR = no reports found.
DW = Dry weigth.

Table A5: Food plants that contain inherent calystegines

Species	Name	Consumption in Europe
Brassica family		
Armoracia rusticana	Horseradish	Low
Brassica juncea	Brown mustard	Low
B. napa	Turnip, white turnip	High
B. nigra	Black mustard	Low
B. oleracea	Broccoli, cauliflower, kale, Brussels sprouts	High
B. rapa	Swede, rutabaga, Chinese cabbage	Moderate
Eruca sativa	Rocket	Low
Nasturtium officinale	Watercress	Low
Raphanus sativus	Kohlrabi	Moderate
Sinapis alba	White mustard	Low
Wasabi japonica	Wasabi	Low
Convolvulaceae family		
Ipomoea alba	Moonflower, moon vine	Low
I. albivenis		Low
I. aquática	Water spinach	Low
I. batatas	Sweet potato	Moderate
I. involucrate		Low
I. leptophylla		Low
Morus family		
Morus alba	White mulberry	Low
M. nigra	Black mulberry	Low
M. rubra	Red mulberry	Low
Solanaceae family		
Capsicum annuum	Sweet pepper, chili pepper, paprika	High
C. baccatum	Chilli pepper	
C. chinensis	Various hot peppers	Low
C. frutenscens	Cayenne pepper	Low
C. pubescens	Manzano pepper	Low
Lycium barbarum and *L. chinense*	Goji berry	Low
P. ixocarpa or *P. philadelphica*	Tomatillo	Low
Physalis peruviana	Cape Gooseberry	Low
P. alkekengi		
Solanum betaceum	Tamarillo or tree tomato	Low
S. esculentum	Tomato	High
hybrid with S. lycopersicoides	UNK	
hybrid with S. ochrantum	UNK	
hybrid with S. sitiens	UNK	
S. melongena	Aubergine or eggplant	High
S. aethiopicum	Scarlet aubergine (limited area of production)	
S. macrocarpon	Gboma aubergine (limited area of production)	
S. muricatum	Pepino	Low
S. quitoense	Naranjilla	Low
S. tuberosum	Potato	High

UNK= no common name known

Table A6: Summary of Table A4 and Table A5: Food plants that contain calystegines and are consumed in moderate or high amounts

Species	Name	Calystegines	Consumption in Europe
Brassica family			
Brassica napa	Turnip, white turnip	NR	High
B. oleracea	Broccoli, cauliflower, kale, Brussels sprouts	A_3; A_5; B_2	High
B. rapa	Swede, rutabaga, Chinese cabbage	NR	Moderate
Raphanus sativus	Kohlrabi	NR	Moderate
Convolvulaceae family			
Ipomoea batatas	Sweet potato	A_3; A_5; B_1; B_2; B_3; C_1	Moderate
Solanaceae family			
Capsicum annuum	Sweet pepper, chili pepper, paprika	B_1; B_2; C_1	High
S. esculentum	Tomato	A_3 ; B_2	High
hybrid with S. lycopersicoides	UNK	NR	
hybrid with S. ochrantum	UNK	NR	
hybrid with S. sitiens	UNK	NR	
S. melongena	Aubergine or eggplant	A_3; B_1; B_2	High
S. aethiopicum	Scarlet aubergine (limited area of production)	NR	
S. macrocarpon	Gboma aubergine (limited area of production)	NR	
Solanum tuberosum	Potato	A_3; B_2; B_4	High

NR = no reports found.
UNK = no common name known.

Table A7: Summary of TAs and TA-containing co-occurring weeds in foods. TAs as reported in Table A2, Table A3 and section A5.3.

Food	Contaminant	Reference to table or section (number of results)
Food available in retail		
Cereals and bakery products; buckwheat flour	*Datura;* atropine, scopolamine	Table A2 (2); A3 (6); section A5.3 (4)
Cereals and bakery products: corn	Atropine, scopolamine	Table A3 (4)
Cereals and bakery products; millet flour	*Datura stramonium*; atropine, scopolamine	Table A2 (1); A3 (12)
Cereals and bakery products: sorghum-based food	Atropine, scopolamine	Table A3 (2)
Cocoa, coffee and tea; herbs and spices; tea	*Atropa belladonna*; atropine	Table A2 (3); A3 (5)
Dietetic foods, food supplements, fortified foods; foods for infants and small children	Atropine; *Solanum nigrum*	Table A3 (5); section A5.3 (1)
Fruit and vegetables; vegetables canned/frozen	*Datura stramonium; Solanum nigrum*	Table A2 (2); A3 (8)
Nuts, nut products and seeds: poppy seeds	*Hyoscyamus niger*	Table A3 (3)
Collected in the wild by consumer		
Leafy vegetables	*Datura / Mandragora*	Table A2 (2)

Table A8.1: Co-occurring plant species from the Brassicacea family associated with TAs in the EU

Brassicaceae					
Genus	**Species**	**Common name**	**Calystegines (µg/g DM)**	**Other TA (µg/g fresh weight)**	**Reference**
Barberea	*Barbarea vulgaris*[(a)]	Yellow rocket, Garden yellow rocket	NR	NR	(PFAF, 2015)
Bertoroa	*Berteroa incana*	Hoary alyssum, false hoary madwort, hoary berteroa, and hoary alison	NR	NR	(Pieper et al., 2010)
Brassica	*Brassica campestris*	Wild turnip	A_3 (18); A_5 (3)	NR	(AgroAtlas_Project, 2009; Brock et al., 2006)
	Brassica elongata	Elongated mustard, long-stalked rape	NR	NR	(USDA_ARS, 2006)
	Brassica tournefortii[(a)]	Sahara mustard, African mustard, Asian mustard, Medditeranean turnip, Tournefort's birdrape, wild turnip	NR	NR	(DAISIE)
Bunias	*Bunias erucago*[(a)]	Corn rocket, crested warty-cabbage	NR	NR	(PFAF, 2015)
	Bunias orientalis	Turkish warty-cabbage, warty-cabbage, or Turkish rocket	NR	NR	(Birnbaum, 2006)
Camelina	*Camelina sativa*	False flax	A_3 (1); A_5 (5); B_2 (3); B_3 (4)	NR	(Brock et al., 2006)
Cochleria	*Cochlearia* spp.	Scurvy grass	A_3 (25-250); A_5 (40-4722); B_2 (10-25); B_3 (5-250)	Tropine (1-52); pseudotropine (1-62)	(Birnbaum, 2006; Brock et al., 2006)

NR = no reports found.
(a): Also used as food (*B. tournefortii*) or recommended as salad (*B. vulgaris* and *B. erucago*).

Table A8.2: Co-occurring plant species from the Convolvulaceae family associated with TAs in the EU

Convolvulaceae					
Genus	**Species**	**Common name**	**Calystegines**	**Other TA**	**Reference**
Convolvulus	*Convolvulus arvensis*	Field bindweed	A_3, A_5, B_1, B_2, B_3, B_4, B_5, C_1 Flowers: A_5 and B_2	tropine, pseudotropine, tropinone, cuscohygrine	(Austin, 2000; De Simone et al., 2008; Kaçan et al., 2013; Schimming et al., 2005; Schroeder et al., 1993; Shukla et al., 2006; Todd et al., 1995)

Table A8.3: Co-occurring plant species from the Solanaceae family associated with TAs in the EU

Solanaceae					
Genus	**Species**	**Common name**	**Calystegines (µg/g fresh)**	**Other TA (µg/kg fresh weight)**	**Reference**
Atropa	*Atropa belladonna*	Deadly nightshade	A_3; B_1; B_2; B_3 Leaves: A_3 (40-60) Root: A_3 (14); B_1 (4); B_2 (13) Leaf: A_3 (62); B_1 (16); B_2 (70) Young leaf: A_3 (280); B_1 (80); B_2 (380) Flower: A_3 (146); B_1 (57); B_2 (263) Fruit: A_3 (5); B_2 (16) Root culture: A_3 (1200 DW); A_5 (400 DW); B_2 (320 DW)	Atropine, scopolamine, apoatropine, tropine, belladonnine, norhyoscyamine, hyoscyamine, 6-β-hydroxyhyoscyamine, tigloytropeine, aposcopolamine, hydroxyhyoscyamine, tigloyloxytropane, littorine	(Arraez-Roman et al., 2008; Ashtiania and Sefidkonb, 2011; Bekkouche et al., 2001; Dräger et al., 1994; Dräger et al., 1995; EFSA, 2013; Gaillard and Pepin, 1999; Nakanishi et al., 1998; Simola et al., 1988)
Datura	*Datura stramonium*	Jimson weed, Devil's snare	NR	Scopolamine, hyoscyamine, 3-(hydroxyacetoxy)-tropane, 3α-phenylacetoxytropane, apoatropine, 3-(3′-acetoxytropoyloxy)-tropane, 3α-tigloyloxy-6β-hydroxytropane, 3-tigloyloxy-6-(2′-methylbutyryloxy)-tropane, 3α,6β-Ditigloyloxytropane, 6–hydroxyhyoscyamine	(Doncheva et al., 2006; Kaçan et al., 2013; Philipov et al., 2007)
Duboisia	*Duboisia leichhardti*	Not known	NR	L-hyoscyamine, scopolamine	(Gaillard and Pepin, 1999; Zhang et al., 2007)

Solanaceae					
Genus	**Species**	**Common name**	**Calystegines (µg/g fresh)**	**Other TA (µg/kg fresh weight)**	**Reference**
	Duboisia myrporoides	Not known	NR	L-hyoscyamine, scopolamine	(Gaillard and Pepin, 1999)
Hyoscyamus	*Hyoscyamus albus*	Henbane	A_3; B_1; B_2; B_3	L-hyoscyamine, scopolamine	(Bekkouche et al., 2001; Gaillard and Pepin, 1999; Hanf, 1983; Williams and Hunyadi, 1987; Williams, 1982)
	Hyoscyamus niger	Black henbane	Root: A_3 (19–23); B_1 (22); B_2 (45) Leaf: A_3 (2–14); B_1 (12); B_2 (15) Young leaf: A_3 (28); B_1 (35); B_2 (57) Flower: A_3 (7); B_2 (3) Root culture A_3 (100–120)	Atropine, scopolamine	(AgroAtlas_Project, 2009; Dräger, 2004)
Mandragora	*Mandragora autumnalis*	Mandrake	A_3 (30 DW); B_2 (20 DW) Leaves: B_2, B_3 Roots: A_3; B_1; B_2; B_3	Scopolamine, hyoscyamine, hyoscine, cuscohygrine, apoatropine, 3α-tigloyloxytropane, 3,6-ditigloyloxytropane, belladonnine	(Bekkouche et al., 2001; Dräger, 2004; Hanus et al., 2005; Jackson and Berry, 1973)
Physalis	*Physalis spp.*	Ground berry / golden berry	A_3 (6.99); A_5 (4.41); B_1 (8.52); B_2 (14.7) A_3 (0.003); B_1 (0.038); B_2 (0.048)	NR	(Azemi et al., 2006; Dräger, 2004)
Scopolia	*Scopolia*		A_3; A_5; B_1; B_2; B_3; B_4; C_1	Atropine, scoplamine	(Asano et al., 1996)
Solanum	*Solanum carolinense*	Horse-nettle	NR	NR	(AgroAtlas_Project, 2009)
	Solanum cornutum	Buffalobur	NR	NR	(AgroAtlas_Project, 2009)
	Solanum elaeagnifolium	Yellow tomatillo or silverleaf nightshade	NR	NR	(Boyd et al., 1984; EMPPO, 2006; Gmira et al., 1998; Mekki, 2007; Taleb and Bouhache, 2006)
	Solanum nigrum	Common nightshade or black nightshade	NR	NR	(Schroeder et al., 1993)

DW = Dry weigth.
NR = no reports found.

Table A9: Occurrence of associated weeds that contain TAs

Family	Species	Common name	Invasive weed in Europe
Brassicaceae	*Barbarea vulgaris*[(a)]	Yellow rocket, garden yellowrocket	Low
	Berteroa incana	Hoary alyssum, false hoary madwort, hoary berteroa, and hoary alison	High
	Brassica campestris	Wild turnip	Moderate
	Brassica elongata	Elongated mustard, long-stalked rape	Low
	Brassica tournefortii[(a)]	Sahara mustard, African mustard, Asian mustard, Medditeranean turnip, Tournefort's birdrape, wild turnip	Low
	Bunias erucago[(a)]	Corn rocket, crested warty-cabbage	Low
	Bunias orientalis	Turkish wartycabbage, warty-cabbage, or Turkish rocket	High
	Cochlearia spp.	Scurvy grass	Low
Convolvulaceae	*Convolvulus arvensis*	Field bindweed	High
Solananceae	*Atropa belladonna*	Deadly nightshade	Low
	Datura stramonium	Jimson weed, Devil's snare	High
	Duboisia leichhardti	Not known	Low
	Duboisia myrporoides	Not known	Low
	Hyoscyamus albus	Henbane	High
	Hyoscyamus niger	Black henbane	High
	Mandragora autumnalis	Mandrake	Low
	Physalis spp.	Ground berry / golden berry	Low
	Scopolia		Low
	Solanum carolinense	Horse-nettle	Moderate*
	Solanum cornutum	Buffalobur	Moderate*
	Solanum elaeagnifolium	Yellow tomatillo or silverleaf nightshade	High
	Solanum nigrum	Common nightshade or black nightshade	High

(a): Noxious weed in European part of Russia spreading to rest of Europe.

Table A10: Summary of Table A8 and Table A9: Co-occurring weeds that contain TAs

Species	Common name	Calystegines	Other TA	Invasive weed in Europe
Brassicaceae				
Berteroa incana	Hoary alyssum, false hoary madwort, hoary berteroa, and hoary alison	NR	NR	High
Brassica campestris	Wild turnip	A_3; A_5	NR	Moderate
Bunias orientalis	Turkish wartycabbage, warty-cabbage, or Turkish rocket	NR	NR	High
Convolvulaceae				
Convolvulus arvensis	Field bindweed	A_3, A_5, B_1, B_2, B_3, B_4, B_5, C_1	tropine, pseudotropine, tropinone, cuscohygrine	High
Solanaceae				
Atropa belladonna	Deadly nightshade	A_3, A_5, B_1, B_2, B_3	Atropine, scopolamine, apoatropine, tropine, belladonnine, norhyoscyamine, hyoscyamine, tigloytropeine, 6-β-hydroxyhyoscyamine, aposcopolamine, hydroxyhyoscyamine, tigloyloxytropane, littorine	Low
Datura stramonium	Jimson weed, Devil's snare	NR	3-(hydroxyacetoxy)-tropane, 3α-phenylacetoxytropane, apoatropine, hyoscyamine, 6-hydroxyhyoscyamine, 3-(3′-Acetoxytropoyloxy)-tropane, 3α-tigloyloxy-6β-hydroxytropane, 3-tigloyloxy-6-(2′-methylbutyryloxy)-tropane, 3α,6β-Ditigloyloxytropane, scopolamine	High
Hyoscyamus albus	Henbane	A_3; B_1; B_2; B_3	Atropine, scopolamine	High
Hyoscyamus niger	Black henbane	A_3; B_1; B_2	Atropine, scopolamine	High
Solanum carolinense	Horse-nettle	NR	NR	Moderate
Solanum cornutum	Buffalobur	NR	NR	Moderate*
Solanum elaeagnifolium	Yellow tomatillo or silverleaf nightshade	NR	NR	High
Solanum nigrum	Common nightshade or black nightshade	NR	NR	High

NR = No reports found.

Table A11: Consumption levels of calystegine-containing plants in European countries for which data was available from the FAO/WHO Chronic Individual Food Consumption Database

Country	Age class	Potato	Tomato	Sweet potato	Brassica	Green beans
Belgium	Adolescents	0.00	0.53	-	0.011	0.054
Belgium	Adults	0.01	0.50	-	0.024	0.071
Belgium	Elderly	0.00	0.41	-	0.018	0.150
Belgium	Other children	-	0.66	-	0.006	-
Belgium	Toddlers	-	0.65	-	0.043	-
Belgium	Very elderly	-	-	-	0.027	0.116
Bulgaria	Other children	3.54	3.20	-	-	0.124
Bulgaria	Toddlers	3.87	3.07	-	-	0.144
Bulgaria	Infants	1.64	0.80	-	-	0.030
Czech Republic	Adolescents	2.08	0.37	-	0.009	-
Czech Republic	Adults	1.17	0.25	-	0.010	-
Czech Republic	Other children	2.87	0.35	-	0.018	-
Denmark	Adolescents	-	0.71	-	0.016	-
Denmark	Adults	-	0.54	-	0.021	-
Denmark	Elderly	-	0.45	-	0.020	-
Denmark	Other children	-	0.94	-	0.018	-
Denmark	Very elderly	-	0.44	-	0.023	-
Finland	Adults	0.24	0.43	0.004	0.020	-
Finland	Elderly	0.34	0.37	0.003	0.015	-
Finland	Other children	0.12	0.50	-	0.027	-
Finland	Other children	-	-	-	0.029	-
Finland	Toddlers	0.04	0.37	-	0.001	-
France	Adolescents	-	0.37	-	-	0.247
France	Adolescents	-	-	0.004	-	-
France	Adults	-	0.38	-	-	0.247
France	Adults	-	-	0.002	-	-
France	Elderly	-	0.39	-	-	0.281
France	Other children	-	0.67	-	-	-
France	Other children	-	-	0.002	-	0.546
France	Very elderly	-	0.38	-	-	0.324
Greece	Other children	-	0.44	-	-	-
Hungary	Adults	1.53	0.14	-	0.000	-
Hungary	Elderly	1.43	0.14	-	-	-
Hungary	Very elderly	1.68	0.13	-	-	-
Ireland	Adults	0.07	0.52	0.005	0.001	-
Italy	Adolescents	-	1.55	-	-	0.057
Italy	Adults	0.00	1.16	0.001	-	0.044
Italy	Elderly	0.00	1.04	0.001	-	0.056
Italy	Other children	-	2.65	-	-	0.076
Italy	Toddlers	-	2.60	-	-	0.119
Italy	Infants	-	0.08	-	-	-
Italy	Very elderly	-	0.98	-	-	0.040
Latvia	Adolescents	-	-	-	0.001	0.020
Latvia	Adults	-	-	-	0.001	0.005
the Netherlands	Adults	-	-	-	0.076	-
the Netherlands	Other children	-	-	-	0.043	-
the Netherlands	Toddlers	-	-	-	0.089	-
Spain	Adolescents	1.34	0.63	0.002	-	-
Spain	Adolescents	-	0.45	-	-	-
Spain	Adolescents	-	0.32	-	-	-

Country	Age class	Potato	Tomato	Sweet potato	Brassica	Green beans
Spain	Adults	0.81	0.91	0.001	-	0.004
Spain	Adults	1.14	0.94	-	-	0.002
Spain	Other children	-	0.76	0.026	-	-
Spain	Other children	-	0.30	-	-	-
Spain	Toddlers	-	0.27	0.108	-	-
Sweden	Adolescents	0.00	0.15	-	0.000	0.004
Sweden	Adults	0.00	0.37	-	0.003	0.005
Sweden	Other children	0.01	-	-	0.002	0.009
United Kingdom	Adults	0.23	0.39	0.004	0.010	-

- = no data.

Table A12: EFSA Average potato and potato product consumption for all countries (grams/day)

Item	No. Subjects	No. Consumers	All Subjects	All Consumers
Potato boiled	80697	34578	30.7	45.8
Main-crop potatoes	725965	92210	8.1	32.0
Potatoes and potato products	80697	19890	20.4	28.2
Potato fried	80697	6813	4.5	9.8
New potatoes	80697	2617	2.4	8.1
Potato baked	80697	4995	2.6	4.8
Potato crisps	80697	11306	2.1	2.4
Mashed potato powder	80697	4232	0.8	2.2
Potato croquettes	80697	755	0.3	0.8

Table A13: EFSA Consumption data for potatoes and potato products

Product	Consumer	No. Subjects	No. Consumers	g/day
Potato boiled	**High consumers**			
Belgium	Elderly, Very elderly	1215	1002	113
Ireland	Adults, Elderly, Very elderly	2458	2140	114
Latvia	Pregnant women, Adolescents, Adults	2726	1991	83
the Netherlands	Elderly, Very elderly	912	660	77
Sweden	Other children, Adolescents	2491	2077	66
Sweden	Adults, Elderly, Very elderly	3007	2386	100
Main-crop potatoes	**High consumers**			
Czech Republic	4. Adolescents	298	223	92
Czech Republic	3. Other children	389	304	72
Czech Republic	5. Adults	1666	1222	87
Hungary	7. Very elderly	80	73	114
Hungary	6. Elderly	206	179	104
Hungary	5. Adults	1074	912	108
Romania	7. Very elderly	45	43	75
Romania	6. Elderly	83	80	88
Romania	5. Adults	1254	1151	75
Potatoes and potato products	**High consumers**			
Belgium	3. Other children	625	535	50
Belgium	2. Toddlers	36	27	45
Denmark	4. Adolescents	377	351	70
Denmark	4. Adolescents	479	453	84
Denmark	5. Adults	1739	1683	89
Denmark	5. Adults	2822	2750	105
Denmark	6. Elderly	274	270	128
Denmark	6. Elderly	309	306	143
Denmark	1. Infants	826	764	39
Denmark	3. Other children	298	284	53
Denmark	3. Other children	490	470	58
Denmark	2. Toddlers	917	842	24
Denmark	7. Very elderly	12	12	89
Denmark	7. Very elderly	20	20	175

Table A14: EFSA Consumption data for cereal-based food for infants and young children

Country	Consumer	No. Subjects	No. Consumers	g/day
Austria	3. Other children	128	0	0.0
Belgium	2. Toddlers	36	0	0.0
Belgium	3. Other children	625	1	0.0
Bulgaria	1. Infants	859	9	0.2
Bulgaria	2. Toddlers	428	2	0.2
Bulgaria	3. Other children	433	0	0.0
Czech Republic	3. Other children	389	4	0.2
Denmark	1. Infants	826	0	0.0
Denmark	2. Toddlers	917	0	0.0
Denmark	3. Other children	490	0	0.0
Denmark	3. Other children	298	0	0.0
Finland	1. Infants	500	0	0.0
Finland	2. Toddlers	500	0	0.0
Finland	2. Toddlers	497	72	7.3
Finland	3. Other children	750	0	0.0
Finland	3. Other children	250	0	0.0
Finland	3. Other children	933	7	0.4
France	3. Other children	482	0	0.0
Germany	1. Infants	159	0	0.0
Germany	2. Toddlers	348	0	0.0
Germany	2. Toddlers	261	15	2.9
Germany	3. Other children	293	0	0.0
Germany	3. Other children	835	0	0.0
Germany	3. Other children	660	3	0.7
Greece	3. Other children	838	0	0.0
Italy	1. Infants	16	0	0.0
Italy	2. Toddlers	36	0	0.0
Italy	3. Other children	193	0	0.0
Latvia	3. Other children	187	0	0.0
the Netherlands	2. Toddlers	322	52	2.7
the Netherlands	3. Other children	447	0	0.0
the Netherlands	3. Other children	957	42	0.8
Spain	2. Toddlers	17	8	41.6
Spain	3. Other children	399	0	0.0
Spain	3. Other children	156	7	2.4
Sweden	3. Other children	1473	33	4.7
UK	1. Infants	1369	45	0.3
UK	2. Toddlers	185	4	0.2
UK	2. Toddlers	1314	47	0.4
UK	3. Other children	651	2	0.0
	Sum	80697	357	0.6
	Average for all consumers			3.2

Table A15. EFSA Consumption data for aubergines

Country	Consumer	No. Subjects	No. Consumers	g/day
Bulgaria	2. Toddlers	428	27	2.8
Bulgaria	3. Other children	433	37	6.2
France	5. Adults	2276	588	2.4
France	6. Elderly	264	61	2.8
Greece	0. Lactating women	65	10	4.1
Italy	2. Toddlers	36	4	2.2
Italy	3. Other children	193	26	6.5
Italy	4. Adolescents	247	34	9.2
Italy	5. Adults	2313	427	9.9
Italy	6. Elderly	290	43	8.9
Italy	7. Very elderly	228	33	7.6
Romania	5. Adults	1254	542	11.8
Romania	6. Elderly	83	45	15.1
Romania	7. Very elderly	45	24	18.6
Spain	5. Adults	410	38	3.4
Spain	5. Adults	981	69	2.2
	Sum	80697	3971	
	Average for consumers			1.5

Table A16.1: EFSA Consumption data for high consumers of beans (*Phaseolus vulgaris*)

Country	Consumer	No. Subjects	No. Consumers	g/day
Romania	6. Elderly	83	40	8.1
Romania	7. Very elderly	45	22	9.0
Spain	3. Other children	399	54	9.1
Spain	4. Adolescents	651	94	12.5
United Kingdom	2. Toddlers	185	94	15.7
United Kingdom	2. Toddlers	1314	483	8.9
United Kingdom	3. Other children	651	284	16.1
United Kingdom	4. Adolescents	666	234	17.6
United Kingdom	5. Adults	1266	417	17.6
United Kingdom	5. Adults	1724	865	16.7
United Kingdom	6. Elderly	166	47	14.5
United Kingdom	7. Very elderly	139	31	8.6
	Average for all consumers			2.5

Table A16.2: EFSA Consumption data for high consumers high consumers of beans (*Phaseolus vulgaris*) with pods

Country	Consumer	No. Subjects	No. Consumers	g/day
Hungary	6. Elderly	206	29	8.8
the Netherlands	4. Adolescents	1142	211	9.6
the Netherlands	5. Adults	2057	385	11.6
the Netherlands	6. Elderly	173	42	18.8
the Netherlands	6. Elderly	289	75	20.1
the Netherlands	7. Very elderly	450	134	23.4
Romania	5. Adults	1254	893	14.9
Romania	6. Elderly	83	61	17.1
Romania	7. Very elderly	45	31	16.9
Spain	2. Toddlers	17	4	17.1
Spain	4. Adolescents	209	39	10.5
Spain	4. Adolescents	86	33	14.9
Spain	5. Adults	410	94	11.1
Spain	5. Adults	981	393	16.0
	Average for all consumers			4.0

Table A17: EFSA Consumption data for all consumers of buckwheat

Country	Consumer	Product	No. Subjects	No. Consumers	g/day
Germany	2. Toddlers	Bread	261	2	0.596
Denmark	5. Adults	Flour	1739	7	0.007
Finland	5. Adults	Flour	1575	9	0.181
Finland	6. Elderly	Flour	463	10	0.970
Germany	2. Toddlers	Flour	261	1	0.024
Germany	3. Other children	Flour	835	1	0.003
Germany	3. Other children	Flour	660	3	0.012
Germany	5. Adults	Flour	10419	1	0.000
Belgium	5. Adults	Grain	1292	1	0.024
Belgium	7. Very elderly	Grain	704	1	0.122
Finland	1. Infants	Grain	500	2	0.016
Finland	2. Toddlers	Grain	500	16	0.571
Finland	3. Other children	Grain	750	35	0.227
Finland	4. Adolescents	Grain	306	1	0.032
Germany	2. Toddlers	Grain	348	1	0.000
Germany	3. Other children	Grain	293	1	0.005
Germany	3. Other children	Grain	660	1	0.002
Germany	3. Other children	Grain	835	1	0.024
Germany	5. Adults	Grain	10419	2	0.007
Germany	6. Elderly	Grain	2006	1	0.043
Italy	4. Adolescents	Grain	247	4	0.270
Italy	5. Adults	Grain	2313	14	0.100
Italy	6. Elderly	Grain	290	4	0.224
Italy	7. Very elderly	Grain	228	1	0.073
the Netherlands	6. Elderly	Grain	289	2	0.007
Sweden	3. Other children	Grain	1473	1	0.014
UK	1. Infants	Grain	1369	1	0.001
UK	2. Toddlers	Grain	185	1	0.095
UK	2. Toddlers	Grain	1314	7	0.068
UK	3. Other children	Grain	651	2	0.031
Austria	5. Adults	Groats	308	3	0.386
Ireland	5. Adults	Groats	1274	1	0.005
Latvia	3. Other children	Groats	187	31	10.896
Latvia	4. Adolescents	Groats	453	52	9.575
Latvia	5. Adults	Groats	1271	154	10.250
Latvia	Pregnant women	Groats	1002	178	16.205
Ireland	5. Adults	Milling products	958	1	0.008
Finland	2. Toddlers	Milling products	497	11	0.405
Finland	3. Other children	Milling products	933	31	0.204

Table A18: EFSA Consumption data for all consumers of millet

Country	Consumer	Product	No. Subjects	No. Consumers	g/day
Belgium	4. Adolescents	Grain	576	1	0.069
Belgium	6. Elderly	Grain	511	1	0.093
Finland	2. Toddlers	Grain	500	18	0.179
Finland	3. Other children	Grain	750	61	0.201
Finland	6. Elderly	Grain	413	1	0.097
Germany	1. Infants	Grain	159	5	0.254
Germany	2. Toddlers	Grain	261	3	0.074
Germany	2. Toddlers	Grain	348	14	0.168
Germany	3. Other children	Grain	293	10	0.057
Germany	3. Other children	Grain	660	7	0.069
Germany	3. Other children	Grain	835	3	0.166
Germany	4. Adolescents	Grain	393	1	0.068
Germany	6. Elderly	Grain	2006	2	0.066
Hungary	7. Very elderly	Grain	80	1	0.063
Italy	6. Elderly	Grain	290	1	0.092
Sum			80697	154	
Austria	5. Adults	Groats	308	2	0.263
Latvia	3. Other children	Groats	187	1	0.535
Latvia	0. Pregnant women	Groats	1002	9	0.773
Sweden	5. Adults	Groats	1210	1	0.017
Sum			80697	13	
Austria	5. Adults	Flakes	308	4	0.195
Austria	7. Very elderly	Flakes	25	2	0.600
Denmark	2. Toddlers	Flakes	917	7	0.028
Germany	1. Infants	Flakes	159	7	0.329
Germany	2. Toddlers	Flakes	261	2	0.026
Germany	5. Adults	Flakes	10419	6	0.015
Germany	6. Elderly	Flakes	2006	3	0.007
Sum			79871	31	
Finland	2. Toddlers	Flour	497	20	0.170
Finland	3. Other children	Flour	933	31	0.040
Finland	5. Adults	Flour	1575	1	0.018
Finland	6. Elderly	Flour	463	3	0.100
Germany	2. Toddlers	Flour	348	1	0.003
Germany	3. Other children	Flour	835	1	0.002
Germany	3. Other children	Flour	660	1	0.008
Germany	3. Other children	Flour	293	1	0.014
		Sum	80697	59	
		Average for all consumers			0.045

Table A19: EFSA Consumption data for all consumers of poppy seed

Country	Consumer	No. Subjects	No. Consumers	g/day
Austria	3. Other children	128	3	0.30
Austria	4. Adolescents	237	5	0.19
Austria	5. Adults	308	1	0.02
Czech Republic	3. Other children	389	22	0.39
Czech Republic	4. Adolescents	298	11	0.41
Czech Republic	5. Adults	1666	40	0.25
Germany	2. Toddlers	261	1	0.03
Germany	3. Other children	293	1	0.01
Germany	3. Other children	660	2	0.02
Germany	3. Other children	835	6	0.03
Germany	5. Adults	10419	3	0.00
Hungary	5. Adults	1074	57	0.46
Hungary	6. Elderly	206	18	0.60
Hungary	7. Very elderly	80	3	0.28
Ireland	5. Adults	1274	1	0.00
Ireland	6. Elderly	149	1	0.00
Romania	5. Adults	1254	37	0.12
Romania	6. Elderly	83	1	0.03
United Kingdom	3. Other children	651	4	0.00
United Kingdom	5. Adults	1266	6	0.01
United Kingdom	6. Elderly	166	1	0.02
	Sum	80697	224	
	Average for all consumers			0.15