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Dietary exposure assessment to pyrrolizidine alkaloids in the European population

European Food Safety Authority (EFSA)

Abstract

Chronic and acute dietary exposure to pyrrolizidine alkaloids (PAs) was estimated in the European population via the consumption of plant-derived foods. This resulted in highest estimates of mean chronic dietary exposure of 34.5–48.4 ng/kg body weight (bw) per day in 'Toddlers' (LB–UB) and 154–214 ng/kg bw per day in the highly exposed population (LB–UB, also in 'Toddlers'). Following a rather conservative scenario, the highest estimates of acute mean exposure and 95th percentile exposure were calculated for 'Toddlers', with mean exposure up to 311 ng/kg bw per day and 95th percentile exposure up to 821 ng/kg bw per day. Tea and herbal infusions were by far the main average contributors to the total exposure to PAs. Among consumers only, in the adult population, the mean chronic exposure via the consumption of honey ranged between 0.1 and 7.4 ng/kg bw per day (minimum LB–maximum UB), while for high consumers, it was between 0.4 and 18 ng/kg bw per day (minimum LB–maximum UB). In the young population, for the average consumers of honey, estimates were between 0.3 and 27 ng/kg bw per day (minimum LB–maximum UB), and between 0.7 and 31 ng/kg bw per day (minimum LB–maximum UB) among the high consumers. Ad hoc exposure scenarios for food supplements via consumption of pollen-based supplements showed chronic exposure to PAs that ranged between 0.7 and 12 ng/kg bw per day (minimum LB–maximum UB), while acute exposure was between 2.8 and 44 ng/kg bw per day (minimum LB–maximum UB), in both cases among consumers only. Likewise, the consumption of 150 mL infusion of 2 g of selected plant extracts led to exposures to PAs up to 67,000 ng/kg bw per day (e.g. infusion of Borage).

Keywords: pyrrolizidine alkaloids, dietary exposure, tea, herbal infusions, honey

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Summary

Pyrrolizidine alkaloids (PAs) are plant secondary metabolites against herbivores. More than 6,000 plant species are known to biosynthesise PAs, mainly from the botanical families of the Boraginaceae (e.g. *Heliotropium* spp.), Asteraceae (e.g. *Senecio* spp.) and Fabaceae (e.g. *Crotalaria* spp.). Nowadays, about 600 PAs have been identified. Many PAs can be present both as their free base and as N-oxides, which can be converted to the free base in humans and animals.

The toxicity of PAs depends mainly on the nature of the bond in position 1,2 of the pyrrolizidine ring system. The toxicity of PAs in humans is well known from various poisoning cases following ingestion of PAs containing herbal medicines and teas. Only limited data in experimental animals are available, mostly on 1,2-unsaturated PAs. The available information indicates that the adverse effects of 1,2-unsaturated PAs in experimental animals include hepatotoxicity, developmental toxicity, genotoxicity and carcinogenicity. Based on that the EFSA Panel on Contaminants in the Food Chain (CONTAM) decided, in its scientific opinion in 2011 on PAs in food and feed, to focus on the risk assessment of 1,2-unsaturated PAs and their respective N-oxide forms. A Margin of Exposure (MOE) approach for genotoxic carcinogens to the sum of 1,2-unsaturated PAs was adopted, assuming equal potency. The Panel derived a benchmark dose lower confidence limit for a 10% excess cancer risk ($BMDL_{10}$) of 70 μg/kg body weight (bw) per day for induction of liver haemangiosarcomas by lasiocarpine in male rats, and selected it as the reference point for the assessment of chronic risks. In addition, in this scientific opinion, the EFSA CONTAM Panel also selected a lowest known PA dose associated with acute/short-term toxicity in humans of approximately 2 mg/kg bw per day for the assessment of acute risks.

Based on the outcome of the EFSA 2011 opinion on pyrrolizidine alkaloids (PAs) and two projects aimed at monitoring PAs in different foods, and considering the available analytical standards, the European Commission provisionally selected 28 PAs as relevant in food samples: echimidine, echimidine-*N*-oxide, heliotrine, heliotrine-*N*-oxide, lycopsamine, lycopsamine-*N*-oxide, intermedine, intermedine-*N*-oxide, erucifoline, erucifoline-*N*-oxide, senecionine, senecionine-*N*-oxide, seneci(o)phylline, seneciphylline-*N*-oxide, monocrotaline, monocrotaline-*N*-oxide, jacobine, jacobine-*N*-oxide, senecivernine, senecivernine-*N*-oxide, retrorsine, retrorsine-*N*-oxide, europine, europine-*N*-oxide, lasiocarpine, lasiocarpine-*N*-oxide, senkirkine and trichodesmine.

Considering the relevant 28 PAs provisionally selected, and after applying diverse data cleaning and validation steps, a final data set of 4,581 food samples of plant origin was available for exposure estimations (among them 1,966 on retail honey, and 2,307 on tea and herbal infusions). A total of 825 food samples of animal origin were not considered for the dietary exposure estimations, since for 97% of these samples all analysed PAs were reported as left-censored data and for the rest PAs were present at relatively low levels. The total content of PAs in each food sample was estimated adding up the reported amount for each individual PA analysed. To avoid underestimation on the presence of PAs, only those samples with a minimum number of PAs were included in the final data set; this number was selected after a comprehensive evaluation of the occurrence data in each type of food commodity.

The final data set of retail honey samples was composed of the 1,324 samples already used in the 2011 EFSA opinion (with a common set of 8 PAs) together with those samples recently submitted that contain at least lycopsamine, echimidine and senecionine. The number of PAs per sample varied between 8 and 19. Retail honey unspecified (as usually reported in the consumption database, 94.5% of the eating occasions) contained PA concentrations of 14.5–27.5 μg/kg (lower bound–upper bound (LB–UB)). Among the samples of retail honey, the main average contributors to the total PA concentration were echimidine (44%) and lycopsamine (37%).

The final data set of tea and herbal infusions contained samples of, among others, 'Tea and herbs for infusions, unspecified' (n = 1,002), 'Black tea, infusion' (n = 339), 'Green tea, infusion' (n = 310), 'Camomile flowers' (n = 256), Peppermint (n = 196) and 'Rooibos' (n = 167). The number of PAs analysed per sample in the final data set ranged between 17 and 28. Taking into account the final data set, among the samples of green tea, the main contributors, on average, to the total PA concentration were senecionine-*N*-oxide (19%), retrorsine-*N*-oxide (18%), and intermedine and lycopsamine, both with 16% contribution. In black tea, the main contributors, on average, were intermedine-*N*-oxide (31%), intermedine (20%), lycopsamine (20%) and retrorsine-*N*-oxide (15%), in camomile senecionine-*N*-oxide (28%), intermedine (22%), senecionine and lycopsamine (both 10%), in peppermint seneciphylline-*N*-oxide (28%), senecionine-*N*-oxide (25%), retrorsine-*N*-oxide (13%) and seneci(o)phylline (11%), and in rooibos, senecionine-*N*-oxide (57%), retrorsine-*N*-oxide (19%) and

senecionine (14%). The highest average concentrations of PAs (expressed as consumed) were found in the samples of rooibos (LB = 4.1 μg/L) and peppermint (LB = 3.5 μg/L). Concentrations of PAs in black tea were twice as high as reported for green tea (LB = 1.6 μg/L and LB = 0.8 μg/L, respectively). An apparent downward trend was noted in the levels of PAs in tea and herbal infusions, based on some of the most recently reported levels as compared with the data reported in previous years. This is observed, in particular, at the LB scenario, indicating that this trend may be influenced by the lack of sensitivity of the analytical methods.

Very high levels of PA were reported for certain food supplements. Pollen-based supplements reported average PA concentrations of 235–253 μg/kg (LB–UB); much higher concentrations were reported for some plant extracts consumed as infusions such as Borage (*Borago officinalis*) with levels up to 2,332,558 μg/kg or up to 419,309 μg/kg in Coltsfoot (*Tussilago farfara*). Likewise, other supplements containing plant material sold as capsules/tablets, to be directly ingested, also reported very high levels of PAs (hemp-agrimony (*Eupatorium cannabinum*) up to 2,410,275 μg/kg).

In order to cover the whole range of concentrations of PAs reported for tea and herbal infusions, the estimation of dietary exposure to PAs considered two different scenarios. Together with the other food commodities, a first scenario considered all the samples of tea and herbal infusions submitted by the national authorities and those collected through an EFSA Article 36 grant, while a second scenario assessed exposure based on samples of tea and herbal infusions submitted by Tea & Herbal Infusions Europe (THIE).

Chronic and acute dietary exposure to PAs was estimated via the consumption of foods of plant origin, particularly tea, herbal infusions and honey. Specific scenarios considering only consumers were used to estimate exposure to PAs and to identify possible risks among small subgroups of the population consuming specific commodities (e.g. honey, rooibos, etc.).

Considering all food commodities, and the data on tea and herbal infusions submitted by the EU Member States (MSs) and through an EFSA Article 36 grant, the highest estimates of mean chronic dietary exposure to PAs in the young population ('Infants', 'Toddlers' and 'Other children') were 34.5–48.4 ng/kg bw per day (LB–UB), and 31.1–41.8 ng/kg bw per day (LB–UB) in the adult population ('Adults', 'Elderly', 'Very elderly'). In the highly exposed population, the highest estimates were 153.8–214 ng/kg bw per day and 87.7–127.2 ng/kg bw per day (LB–UB) in the young and the adult population, respectively. When using the data on tea and herbal infusions submitted by THIE, the estimates of chronic exposure were lower as compared with the previous scenario. The highest estimates of mean chronic dietary exposure to PAs in the young population (LB–UB) were 6.1–29.8 ng/kg bw per day and 5.7–33.4 ng/kg bw per day in the adult population. In the highly exposed population, the highest estimates were 23.3–131.6 ng/kg bw per day and 15.9–78.8 ng/kg bw per day (LB–UB) in the young and the adult population, respectively. Overall, in 'Infants' and 'Toddlers' the main average contributors to the chronic dietary exposure to PAs were either 'Tea, unspecified' or 'Tea and herbs for infusions, unspecified'. In the adult population, the main contributor to the chronic exposure to PAs was tea, either reported as 'Tea, unspecified' or as 'Black tea, infusion'.

Among consumers only, in the adult population, the mean chronic exposure via the consumption of honey, ranged between 0.1 and 7.4 ng/kg bw per day (minimum LB–maximum UB), while for high consumers (95th percentile exposure), it was between 0.4 and 17.6 ng/kg bw per day (minimum LB–maximum UB). Higher exposure was estimated among the consumers of honey in the young population. For the average consumers, estimates oscillated between 0.3 and 27 ng/kg bw per day (minimum LB–maximum UB), and between 0.7 and 31.1 ng/kg bw per day (minimum LB–maximum UB) among the high consumers.

Acute dietary exposure to PAs was estimated following a conservative approach considering the presence of high contamination levels in all the different food commodities (occurrence values at the highest reliable percentile, UB estimate), combined with the total daily consumption amount for each corresponding food and adding up all consumed foods (consuming days only). The highest estimates of acute mean exposure and 95th percentile exposure were calculated for 'Toddlers', with mean exposure to PAs up to 311 ng/kg bw per day and 95th percentile exposure up to 821 ng/kg bw per day.

The consumption of 150 mL infusion of 2 g of selected plant extracts (dilution factor 1/75 as used for tea and herbal infusions) can lead to exposures to PAs from 800 ng/kg bw per day for one infusion of mix herbs (among them *Pulmonaria officinalis*) to 67,000 ng/kg bw per day for one infusion of Borage (*B. officinalis*). Chronic exposure to PAs via consumption of pollen-based supplements ranged between 0.7 and 11.5 ng/kg bw per day (minimum LB–maximum UB), while the acute exposure was between 2.8 and 43.9 ng/kg bw per day (minimum LB–maximum UB) in both cases among consumers only.

On estimating dietary exposure to 28 PAs, the UB scenario is highly influenced by the sensitivity of the analytical methods. Based on the current sensitivity of the reported analytical methods, lowest UB concentrations of 53 μg/kg (0.7 μg/L) can be achieved for tea and herbal infusions. This implies mean chronic exposure levels up to 6.1–21 ng/kg bw per day, and up to 14.6–28.3 ng/kg bw per day among the highly exposed consumers (adult–young population), depending on the tea and herbal infusion consumed.

For honey, the lowest UB concentration reported with all eight PAs at levels below the limit of quantification (LOQ) would be 3.6 μg/kg. This would lead to mean chronic exposure estimations up to 3.5 ng/kg bw per day and up to 4.1 ng/kg bw per day among the highly exposed consumers.

Different sources of uncertainty in the estimation of the dietary exposure to PAs were identified. Among others, the most important refer to the large proportion of left-censored data, the fact that not all samples reported analytical data for all 28 PAs, and to the presence of an important number of both eating occasions and occurrence data on unspecified tea and herbs for infusions. Likewise, uncertainty is also associated with how accurately the concentration of PAs reported in the samples of tea and herbal infusions represents the amounts of contaminants the consumers are exposed to. Different methods are used to extract the PAs present in tea and herbal infusions prior to their analysis, and there is uncertainty on how these methods represent the different ways consumers prepare tea and herbal infusions. In addition, many different factors, such as water temperature, water-to-tea ratio, infusion time, stirring and dosage form (loose leaf and tea bag), may have an influence on the extraction of PAs during consumer preparation. Overall, the dietary exposure to PAs calculated in this report is likely to overestimate the exposure levels of the European population.

In order to reduce UB levels, it is recommended to develop more sensitive analytical methods and define performance criteria for the analysis of the most relevant PAs in food. Efforts should continue to collect analytical data on the occurrence of PAs in relevant food commodities, but in particular on tea and herbal infusions to confirm the downward trend in PA levels on the most recently reported samples. Data on the presence of PAs in herbal food supplements other than plant extracts should be also collected. Further investigation should be done on the weeds responsible of the presence of PAs in tea and herbal infusions, as well as to develop adequate measures to control weed infestation.

Table of contents

Abstract 1
Summary 3
1. Introduction 7
1.1. Background and Terms of Reference as provided by the European Commission 9
2. Data and methodologies 9
2.1. Data 9
2.1.1. Occurrence data 9
2.1.1.1. Data collection and validation 9
2.1.1.2. Data analysis 10
2.1.2. Consumption data 10
2.1.2.1. Food consumption data 10
2.1.3. Food classification 11
2.2. Methodologies 11
2.2.1. Dietary exposure assessment 11
2.2.1.1. Dietary exposure assessment in humans 11
3. Assessment 12
3.1. Pyrrolizidine alkaloid occurrence in food 12
3.1.1. Honey 13
3.1.2. Tea and herbal infusions 15
3.1.3. Food supplements 25
3.1.4. Other food commodities 26
3.2. Food consumption data 26
3.2.1. Consumption of honey 26
3.2.2. Consumption of tea and herbal infusions 27
3.2.3. Consumption of food supplements 29
4. Dietary exposure assessment to pyrrolizidine alkaloids 30
4.1. Chronic dietary exposure 30
4.1.1. Chronic dietary exposure using occurrence data submitted by national authorities and occurrence data collected through an EFSA Art 36 grant 30
4.1.2. Chronic dietary exposure using occurrence data submitted by Tea & Herbal Infusions Europe (THIE) 31
4.1.3. Chronic dietary exposure, consumers only 32
4.2. Acute dietary exposure, consumption days only 34
4.3. Dietary exposure through the consumption of plant extracts and pollen-based supplements 35
4.4. Potential dietary exposure at hypothetical PAs concentrations 36
5. Uncertainty 37
6. Conclusions 38
Recommendations 39
References 40
Abbreviations 40
Appendix A – Dietary surveys used for the estimation of dietary exposure to pyrrolizidine alkaloids 42
Appendix B – Levels of pyrrolizidine alkaloids in the dry product (μg/kg) for different types of tea and herbal teas 44
Appendix C – Chronic dietary exposure to pyrrolizidine alkaloids (without using THIE occurrence data). 45
Appendix D – Chronic dietary exposure to pyrrolizidine alkaloids (using occurrence data on tea and herbal infusions only from THIE) 47
Appendix E – Percentage of contribution of eight selected PAs as compared to the total PA concentration in tea and herbal infusions 49
Appendix F – Contribution of different foods to chronic dietary exposure to pyrrolizidine alkaloids 50

1. Introduction

Pyrrolizidine alkaloids (PAs) are plant secondary metabolites against herbivores. More than 6,000 plant species are known to biosynthesise PAs, mainly from the botanical families of the Boraginaceae (e.g. *Heliotropium* spp.), Asteraceae (e.g. *Senecio* spp.) and Fabaceae (e.g. *Crotalaria* spp.) (Smith and Culvenor, 1981).

Figure 1 shows the most important structural features of PAs. From a chemical standpoint, this class of alkaloids is characterised by the presence of a pyrrolizidine ring system (1,2,3,6,7,8-hexahydro-5*H*-pyrrolizine), which is the basic structure of various 1-hydroxymethyl derivatives called necines or necine bases (for a comprehensive overview see Mattocks, 1986; Hartmann and Witte, 1995; Roeder, 1999). The diversity of the various necine bases is attributable to three features:

- the presence of a double bond in position 1,2 resulting in the 1,2-unsaturated PA class of higher toxicological relevance;
- the presence of an additional hydroxy group in position 7, allowing for the formation of open- or cyclic diesters;
- the N-methylation of the pyrrolizidine ring, hindering the conversion to N-oxide (PANO) derivatives.

The rich variety of PAs results from a series of combinations of the various necine bases with a pool of different mono- or dicarboxylic acids (necic acids) to form monoesters, and open- or cyclic diesters. Nowadays, about 600 PAs have been identified. Many PAs can be present both as their free base and as N-oxides, which can be converted to the free base in humans and animals.

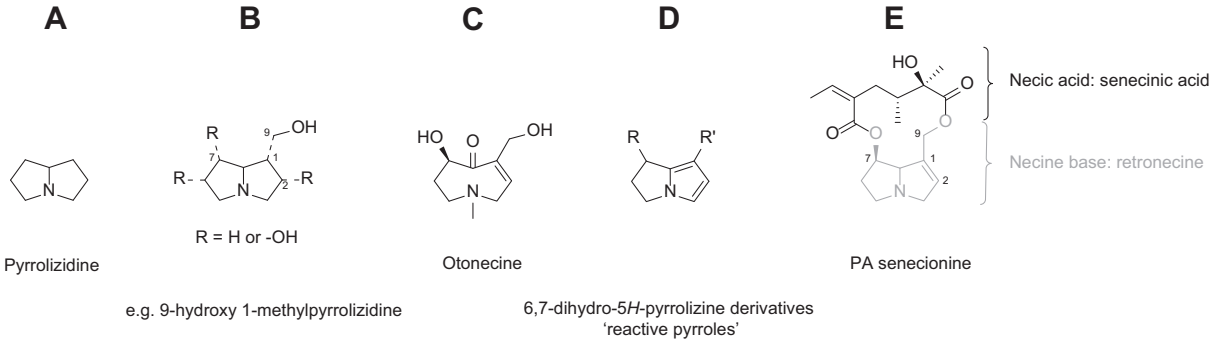

Figure 1: Structural features of PAs. (A) core structural motif pyrrolizidine (1,2,3,6,7,8-hexahydro-5*H*-pyrrolizine); (B) general description of the main necine base parts of naturally occurring PAs including the common necine base numbering; (C) necine base otonecine; a core structural motif of otonecine-type PAs; (D) general pyrrolizine structure motif and (E) structural example of 1,2-unsaturated ester PA senecionine (Figure taken from EFSA CONTAM Panel, 2011)

The toxicity of PAs depends mainly on the nature of the bond in position 1,2 of the pyrrolizidine ring system. Cytochrome P-450 mediated metabolism of 1,2-unsaturated PAs can form pyrroles (dihydropyrrolizine (DHP) and DHP esters), which can readily react with proteins and form DNA adducts (Fu et al., 2004; Wang et al., 2005). Conversely, 1,2-saturated PAs cannot form such reactive metabolites.

The toxicity of PAs in humans is well known from various poisoning cases following ingestion of PA containing herbal medicines and teas, and outbreak cases including deaths associated with the consumption of grain contaminated with PA containing weeds (see e.g. WHO-IPCS, 1988; Prakash et al., 1999; Kakar et al., 2010; Bane et al., 2012; Schneider et al., 2012), with liver and lung as the main target organs. The acute/short-term toxicity of 1,2-unsaturated PAs in humans is characterised mainly by the onset of hepatic veno-occlusive disease (HVOD), associated with high mortality and, or possibly progressing to liver cirrhosis. In its 2011 scientific opinion on the presence of PAs in food and feed, from the evaluation of various human case reports, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) selected a lowest known PA dose associated with acute/short-term toxicity in humans of approximately 2 mg/kg bw per day for the assessment of acute risks (EFSA CONTAM Panel, 2011).

Only limited data in experimental animals are available, mostly on 1,2-unsaturated PAs. The available information indicates that the adverse effects of 1,2-unsaturated PAs in experimental animals include hepatotoxicity, developmental toxicity, genotoxicity and carcinogenicity. The formation of

reactive pyrroles is considered to be the key step related to all the identified adverse effects (Fu et al., 2004). Therefore, the CONTAM Panel decided to focus on the risk assessment of 1,2-unsaturated PAs and their respective N-oxide forms (EFSA CONTAM Panel, 2011). Considering that all 1,2-unsaturated PAs share a common metabolic pathway leading to the formation of genotoxic and carcinogenic reactive pyrroles, the CONTAM Panel concluded that it was not appropriate to establish a health-based guidance value, and decided to adopt the Margin of Exposure (MOE) approach for genotoxic carcinogens to the sum of 1,2-unsaturated PAs, assuming equal potency. The Panel derived a benchmark dose lower confidence limit for a 10% excess cancer risk ($BMDL_{10}$) of 70 μg/kg body weight (bw) per day for induction of liver haemangiosarcomas by lasiocarpine in male rats, and selected it as the reference point for the assessment of chronic risks.

The dietary exposure assessment of the CONTAM Panel 2011 opinion was limited to honey as occurrence data were only available for this food product (EFSA CONTAM Panel, 2011). Two data sets were submitted to EFSA, which included testing of 14 and 17 PAs, respectively, with eight PAs in common between the two data sets. The two data sets included results both from samples of retail honey, that is mostly blended and ready for consumption, and bulk honey which was used by the CONTAM Panel to cover the scenario of honey purchased locally from a single source. For retail honey, chronic exposure levels up to 37.4 ng/kg bw per day and 9.03 ng/kg bw per day were estimated for children and adults (mean consumption in honey consumers only), respectively. Chronic exposure up to 77.8 ng/kg bw per day and 26 ng/kg bw per day were estimated for the two age groups for 95th percentile consumption in children and adults, respectively. Acute exposure levels up to 254 ng/kg bw and 110 ng/kg bw were estimated considering the 95th PAs concentrations and 95th single day consumption for children and adults, respectively. The theoretical exposure calculated for consumption of unblended (bulk) honey was in general about 50–100% higher than the results of the calculations for retail honey. Then CONTAM Panel identified also PAs of particular importance for food and feed, considering the prominent alkaloids present in the main known PA containing plants (EFSA CONTAM Panel, 2011). The list of PAs of particular importance for food and feed identified by the CONTAM Panel (EFSA CONTAM Panel, 2011) was subsequently taken forward by the European Commission in a recommendation for monitoring PAs in food (SCOFCAH, 2014), although it was noted at the time that analytical standards were available only for some of the PAs listed in the European Food Safety Authority (EFSA) opinion. Short after, two projects aimed at monitoring PAs in different foods were performed. One project was carried out by the Federal Institute for Risk Assessment (BfR, 2013), while the second one was a project delivered as a result of an Article 36 grant (GP/EFSA/CONTAM/2013/03) awarded by EFSA to a consortium led by RIKILT and composed also by the Federal Institute for Risk Assessment (BfR) and the Institute for Research and Technology in Food and Agriculture (IRTA). The results of this project were published as an EFSA external Scientific report (Mulder et al., 2015). Based on the outcome of the EFSA 2011 opinion and these two reports, considering the available analytical standards, the European Commission provisionally selected 28 PAs as relevant in food samples (see Table 1).

Regarding the project carried out by BfR, 17 PAs were monitored for which analytical standards were available, in herbal infusions and teas, including two PAs (intermedine and senkirkine) not identified as of particular importance in the EFSA 2011 opinion (BfR, 2013). In the external Scientific report of EFSA (Mulder et al., 2015), analytical standards for 39 PAs were identified. In the project, validated analytical methods were set up for 35 PAs in food of animal origins, and 28 PAs in plant-derived food. The 28 PAs monitored in the plant-derived food included the 17 PAs monitored by BfR in 2013. Out of these 28 PAs, five (intermedine, intermedine-N-oxide, senecivernine, senecivernine-N-oxide and senkirkine) were not identified as of particular importance in honey by the EFSA CONTAM Panel (EFSA CONTAM Panel, 2011). However, two out of the 28 PAs analysed (indicine and intermedine) co-eluted under the chromatographic conditions used by the beneficiaries of the EFSA grant. The list of PAs included in the EFSA external scientific report for the monitoring of plant-derived food (Mulder et al., 2015) is shown in Table 1.

Table 1: List of the 28 PAs provisionally selected by the European Commission as relevant in food samples

	CAS number		CAS number
Echimidine	520-68-3	**Lycopsamine**	10285-07-1
Echimidine-*N*-oxide	41093-89-4	**Lycopsamine-*N*-oxide**	95462-15-0
Erucifoline	40158-95-0	**Monocrotaline**	315-22-0
Erucifoline-*N*-oxide	123864-94-8	**Monocrotaline-*N*-oxide**	35337-98-5
Europine	570-19-4	**Retrorsine**	480-54-6
Europine-*N*-oxide	65582-53-8	**Retrorsine-*N*-oxide**	15503-86-3
Heliotrine	303-33-3	**Seneci(o)phylline**	480-81-9
Heliotrine-*N*-oxide	6209-65-0	**Seneciphylline-*N*-oxide**	38710-26-8
Intermedine	10285-06-0	**Senecionine**	130-01-8
Intermedine-*N*-oxide	95462-14-9	**Senecionine-*N*-oxide**	13268-67-2
Jacobine	6870-67-3	**Senecivernine**	72755-25-0
Jacobine-*N*-oxide	38710-25-7	**Senecivernine-*N*-oxide**	101687-28-9
Lasiocarpine	303-34-4	**Senkirkine**	2318-18-5
Lasiocarpine-*N*-oxide	127-30-0	**Trichodesmine**	548-90-3

CAS: Chemical Abstracts Service.

Maximum levels (ML) for PAs in food are not established in Commission Regulation (EC) No 1881/2006 setting MLs for certain contaminants in foodstuffs.

1.1. Background and Terms of Reference as provided by the European Commission

Following the outcome of EFSA's scientific opinion on pyrrolizidine alkaloids in food and feed in 2011 and the availability of new occurrence data on the presence of pyrrolizidine alkaloids in food, the Commission is considering the possible setting of maximum levels of pyrrolizidine alkaloids in honey, tea, herbal infusions, herbs and food supplements. The exposure assessment in the scientific opinion was only related to the consumption of honey as only occurrence data on pyrrolizidine alkaloids in honey were at that time available. In the meantime data in tea, herbal infusions and food supplements have become available. It would therefore be appropriate to have an updated exposure assessment available (also taking into account the updated comprehensive food consumption database). Given that there are some very high levels of pyrrolizidine alkaloids found in certain samples of honey, tea, herbal infusions and food supplements, it is also appropriate to estimate the changes in exposure by applying specific cut-off levels (not taking into account the data with levels above a certain cut-off value).

In accordance with Art. 31 (1) of Regulation (EC) No 178/2002 the Commission asks EFSA for a dietary exposure assessment to pyrrolizidine alkaloids in honey, tea, herbal infusions (herbs) and food supplements taking into account:

- occurrence data available in the EFSA database;
- updated comprehensive food consumption database;
- changes in estimated dietary exposure by applying specific cut-off values.

2. Data and methodologies

2.1. Data

2.1.1. Occurrence data

2.1.1.1. Data collection and validation

At the moment of the preparation of this scientific report, following the official request from the European Commission (January 2016), a total of 378,752 analytical results on 87 different pyrrolizidine alkaloids (PAs) were available in the EFSA Chemical Occurrence database, among them 345,107

corresponding to food samples and the rest to feed (33,645). As regards food samples, a total of 274,632 analytical results belonged to the 28 PAS provisionally selected by the European Commission for the analysis of PAs in food (listed in Table 1). The concentration of PAs in each food sample was estimated adding up all the individual levels of PAs analysed among the 28 selected by the European Commission.

The data were submitted to EFSA following the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a); occurrence data were managed following the EFSA standard operating procedures (SOPs) on 'Data collection and validation' and on 'Data analysis of food consumption and occurrence data'.

2.1.1.2. Data analysis

Following the EFSA SOP on 'Data analysis and reporting' to guarantee an appropriate quality of the data used in the exposure assessment, the initial data set was carefully evaluated applying several data cleaning and validation steps. Special attention was paid to the number of PAs reported in each sample as well as to different parameters, such as 'Analytical method', 'Reporting unit' and the codification of the different food samples under the FoodEx classification. The outcome of the data analysis is shown in Section 3.

Analytical results were all submitted on a whole weight basis (μg/kg). The left-censored data were treated by the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO/IPCS, 2009). The same method is indicated in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010b) as an option in the treatment of left-censored data. The guidance suggests that the lower-bound (LB) and upper-bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). At the LB, results below the limit of quantification (LOQ) and limit of detection (LOD) were replaced by zero; at the UB, the results below the LOD were replaced by the LOD and those below the LOQ were replaced by the value reported as LOQ. Additionally, as a point estimate between the two extremes, the middle bound (MB) scenario was calculated by assigning a value of LOD/2 or LOQ/2 to the left-censored data.

2.1.2. Consumption data

2.1.2.1. Food consumption data

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) provides a compilation of existing national information on food consumption at individual level. It was first built in 2010 (EFSA, 2011a; Huybrechts et al., 2011; Merten et al., 2011). Details on how the Comprehensive Database is used are published in the Guidance of EFSA (EFSA, 2011a). The latest version of the Comprehensive Database[1] contains results from a total of 51 different dietary surveys carried out in 23 different EU Member States (MSs) covering 94,532 individuals.

Within the dietary studies, subjects are classified in different age classes as described in Table 2; two additional surveys provided information on specific population groups: 'Pregnant women' (Latvia) and 'Lactating women' (Greece).

Table 2: Age classes considered in the EFSA Comprehensive European Food Consumption Database

	Age range
Infants	< 12 months old
Toddlers	≥ 12 months to < 36 months old
Other children	≥ 36 months to < 10 years old
Adolescents	≥ 10 years to < 18 years old
Adults	≥ 18 years to < 65 years old
Elderly	≥ 65 years to < 75 years old
Very elderly	≥ 75 years old

For chronic exposure assessment, food consumption data were available from 44 different dietary surveys carried out in 19 different European countries. Seven additional dietary surveys with only 1 day per subject from seven different countries (covering all age classes except infants) were available for acute exposure assessment. Overall, the food consumption data gathered by EFSA in the

[1] http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb

Comprehensive Database are the most complete and detailed data currently available in the European Union (EU). Consumption data were collected using single or repeated 24- or 48-h dietary recalls, and dietary records covering from 3 to 7 days per subject. Owing to the differences in the methods used for data collection, direct country-to-country comparisons can be misleading.

2.1.3. Food classification

Consumption data were classified according to the FoodEx classification system (EFSA, 2011b). FoodEx is a food classification system developed by EFSA in 2009 with the objective of simplifying the linkage between occurrence and food consumption data when assessing the exposure to hazardous substances. It contains 20 main food groups (first level), which are further divided into subgroups having 140 items at the second level, 1,261 items at the third level and reaching about 1,800 end-points (food names or generic food names) at the fourth level.

For the classification of tea and herbal infusions, the FoodEx classification allows the possibility to codify the samples as solid ('Tea and herbs for infusions', Level 2) or as consumed ('Tea, infusion', Level 2). In order to better describe the reported data and to obtain more accurate dietary exposure estimations, tea and herbal infusions were codified using the category that best matched the food commodity.

2.2. Methodologies

2.2.1. Dietary exposure assessment

2.2.1.1. Dietary exposure assessment in humans

Based on the outcome of the 2011 EFSA Scientific opinion on PAs both acute and chronic exposure were assessed. As suggested by the EFSA Working Group on Food Consumption and Exposure, dietary surveys with only 1 day per subject were considered for acute exposure as they are not adequate to assess repeated exposure (EFSA, 2011a). Similarly, subjects who participated only 1 day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment. Thus, for chronic exposure assessment, food consumption data were used from 35 different and most recent dietary surveys carried out in 19 different European countries present in the latest version of the Comprehensive Database.

For calculating chronic dietary exposure to PAs, food consumption and body weight data at the individual level were accessed in the Comprehensive Database. Occurrence data and consumption data were linked at the lowest FoodEx level possible. In addition, the different food commodities were grouped within each food category to better explain their contribution to the total dietary exposure to PAs. Exposure estimates were calculated for each dietary survey and age class. The mean and the high (95th percentile) chronic dietary exposures were calculated by combining PAs mean occurrence values with the average daily consumption for each food at individual level in each dietary survey.

Acute dietary exposure to PAs in the general population was estimated following a rather conservative approach considering the presence of high contamination levels in the different food commodities (occurrence values at the highest reliable percentile), combined with the total daily consumption amount for each corresponding food and adding up all consumed foods (consuming days only). A total of 41 most recent dietary surveys carried out in 23 different European countries were used.

In Appendix A, the number of available days for each age class used in the acute exposure assessment is described beside the number of subjects available for the chronic exposure assessment.

Specific exposure scenarios (chronic and acute) for selected food commodities were also assessed in order to better understand the exposure to PAs in the European population. Table 3 shows all the different exposure scenarios considered in this scientific report to estimate dietary exposure to PAs.

All analyses were run using the SAS Statistical Software (SAS enterprise guide 5.1).

Table 3: Different scenarios used to estimate chronic and acute dietary exposures to PAs

	Dietary exposure scenarios
A. Chronic exposure assessments	A.1. Chronic dietary exposure in the general population (Section 4.1.1 and 4.1.2)
	A.2. Chronic dietary exposure, honey consumers only (Section 4.1.3)
	A.3. Chronic dietary exposure, tea and herbal infusion consumers only (Section 4.1.3)
	A.4. Chronic dietary exposure at selected PA concentrations (Section 4.3) Honey consumers only Tea and herbal infusion consumers only
	A.5. Chronic dietary exposure to pollen-based supplements (Section 4.4)
B. Acute exposure assessments	B.1. Acute dietary exposure, consumption days only (Section 4.2)
	B.2. Acute dietary exposure to plant extracts[(a)] (Section 4.3)
	B.3. Acute dietary exposure to pollen-based supplements (Section 4.4)

(a): Since only few consumption data on plant extracts were available, this scenario estimates dietary exposure to PAs via the consumption of particular plant extracts using a single consumption of 150 mL infusion of 2 g of plant extract (dilution factor 1/75 as used for tea and herbal teas).

3. Assessment

3.1. Pyrrolizidine alkaloid occurrence in food

As commented in the previous sections, this scientific report focuses on the 28 PAs provisionally selected by the European Commission. This selections is based on the 2011 EFSA scientific opinion where only occurrence data on honey were available (EFSA CONTAM Panel, 2011), and two recent research projects that identified key PAs in tea and herbal infusions (BfR, 2013; Mulder et al., 2015). The list of the 28 selected PAs is shown in Table 1 and covers the following PAs: echimidine, echimidine-*N*-oxide, heliotrine, heliotrine-*N*-oxide, lycopsamine, lycopsamine-*N*-oxide, intermedine, intermedine-*N*-oxide, erucifoline, erucifoline-*N*-oxide, senecionine, senecionine-*N*-oxide, seneci (o)phylline, seneciphylline-*N*-oxide, monocrotaline, monocrotaline-*N*-oxide, jacobine, jacobine-*N*-oxide, senecivernine, senecivernine-*N*-oxide, retrorsine, retrorsine-*N*-oxide, europine, europine-*N*-oxide, lasiocarpine, lasiocarpine-*N*-oxide, senkirkine and trichodesmine.

Considering the relevant 28 PAs, an initial number of 274,632 analytical results on PAs in food samples were available, accounting for a total of 19,332 food samples. Among these samples, the number of PAs analysed per sample ranged between one (n = 29) and 28 (n = 761). As mentioned in Section 2.1.1, the occurrence data were carefully analysed before being used to estimate dietary exposure. The total content of PAs in each food commodity was estimated adding up the reported amount for each individual PA analysed. In order to avoid underestimation on the presence of PA, only those samples with a minimum number of PAs were selected; this number is discussed for the different food categories in the relevant sections.

Special attention was also paid to the presence of two additional PAs that could be relevant due to their toxicity, riddelliine and riddelliine-*N*-oxide. Both of them are found in different *Senecio* plants, with riddelliine defined by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (Group 2B). These two PAs were analysed in 301 samples of tea and herbal infusions, and in all cases were reported below the LOQ. For riddelliine, the LOQs ranged between 5 μg/kg and 50 μg/kg; for riddelliine-*N*-oxide, LOQs ranged between 5 μg/kg and 20 μg/kg.

Food samples mainly belonged to the food group 'Honey' (n = 15,528, FoodEx level 2). A total of 1,722 samples were initially codified as 'Tea and herbs for infusion' (FoodEx level 2), with most of them codified as 'Tea and herbs for infusions, unspecified' that covers a very heterogeneous group of samples that includes mix of herbal infusions, mix of tea and herbal infusions, unspecified herbal infusions, and herbal infusions that could not be classified under FoodEx. A total of 294 samples of 'Food supplements', mainly corresponding to diverse types of 'Plant extract formula' (n = 216, FoodEx level 3), were also available.

In addition to honey samples, other 825 food samples of animal origin were also part of this data set, with 97% of them having all analysed PAs as left-censored data. Previous studies have demonstrated that, in general, the levels of PAs in animal-derived food are much lower than those that can be found in food commodities such as tea and herbal infusions. The recent report published by Mulder et al., 2015, revealed that among 746 samples of animal origin only occasional low levels of

PAs in milk samples (6%) were found, mostly with single PAs (i.e. jacoline, senkirkine, otosenine, lycopsamine, echimidine, retrorsine) in their free base form. Except for two egg samples, PAs were absent in the milk products, eggs, meat and liver samples analysed.

3.1.1. Honey

Honey samples were reported as bulk honey (13,280 samples) and retail honey (2,248 samples). Retail honey is considered as mostly blended, ready for consumption and therefore, representative of what it is habitually consumed. Following the same approach as in the 2011 EFSA opinion, only samples of retail honey were considered to estimate dietary exposure to PAs (EFSA CONTAM Panel, 2011).

Table 4 shows the number of times that the selected 28 PAs were analysed in the different samples of retail honey. Most of the retail honey samples had in common eight different PAs (echimidine, echimidine-*N*-oxide, heliotrine, lycopsamine, retrorsine, senecionine, seneci(o)phylline and senkirkine). These eight PAs seems to represent around 75–90% of the total PA levels measured in honey, with echimidine-*N*-oxide having minor contribution (EFSA CONTAM Panel, 2011). Among the samples with these eight PAs, there are 1,324 samples (codified as 'Honey, unspecified') that were already available in the 2011 EFSA opinion (EFSA CONTAM Panel, 2011); they correspond to two different data sets reported separately, one of 1,116 samples (with eight PAs) and another one of 208 (with 14 PAs).

Table 4: Number of times (N) the selected 28 PAs were analysed in the 2,248 retail honey samples, and the percentage being left-censored data (LC %)

	N	LC %		N	LC %		N	LC %
Senecionine	2,245	80.8	**Senecionine-*N*-oxide**	989	95.4	**Trichodesmine**	224	98.2
Seneci (o)phylline	2,245	86.4	**Seneciphylline-*N*-oxide**	989	96.3	**Jacobine**	170	89.4
Senkirkine	2,243	96.8	**Retrorsine-*N*-oxide**	966	98.6	**Erucifoline**	170	94.7
Retrorsine	2,222	87.9	**Lasiocarpine**	977	98.1	**Erucifoline-*N*-oxide**	169	89.3
Heliotrine	2,198	98.2	**Monocrotaline**	758	98.8	**Europine**	169	88.2
Lycopsamine	2,073	57.2	**Heliotrine-*N*-oxide**	519	99.6	**Europine-*N*-oxide**	169	92.3
Echimidine	2,114	48.2	**Monocrotaline-*N*-oxide**	400	93.5	**Senecivernine**	116	82.8
Echimidine-*N*-oxide	1,324	99.3	**Lycopsamine-*N*-oxide**	316	97.5	**Jacobine-*N*-oxide**	116	100
			Lasiocarpine-*N*-oxide	311	99.7	**Senecivernine-*N*-oxide**	116	99.1
			Intermedine	310	83.5	**Intermedine-*N*-oxide**	116	96.6

Among the 924 honey samples received after the 2011 EFSA opinion, the number of PAs reported per sample varied between 1 and 25. In 634 of these samples, the eight PAs mentioned above were all analysed, except echimidine-*N*-oxide. In the samples where at least one PA was quantified, the most important PAs were lycopsamine (average contribution of 37%), echimidine (average contribution of 33%) and senecionine (average contribution of 10%). Other PAs that seem to be important in the total concentration of PAs in honey are intermedine (average contribution of 9.5%) and europine (average contribution of 8%).

Considering all samples of retail honey as shown in Table 5, the number of PAs analysed went from a minimum of one PA (one sample) to a maximum of 25 PAs (116 samples), with eight PAs being the most habitual number of PAs analysed (1,117 samples).

The final data set of retail honey samples used for exposure estimations was composed of the 1,324 samples already used in the 2011 EFSA opinion, together with samples recently submitted that were analysed for at least lycopsamine, echimidine and senecionine. Based on this, a total number of 1,966 samples of honey were used to estimate dietary exposure to PAs. The final data set for honey contains analytical data on 24 different PAs, with a number of PAs per sample varying between a minimum of eight (n = 1,116) and a maximum of 19 (n = 105).

Table 5: Number of PAs analysed in samples of bulk honey and different types of retail honey. Bulk honey and samples of retail honey not analysed for lycopsamine, echimidine and senecionine (the three PAs together) were excluded from the final data set

	Number of pyrrolizidine alkaloids analysed in each sample															Total
	1	**2**	**5**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**16**	**17**	**18**	**19**	**25**	
Bulk honey	0	0	0	4,897	0	0	0	0	0	8,383	0	0	0	0	0	**13,280**
Retail honey																
Honey, unspecified	0	0	38	1,116	3	17	4	75	3	208	6	18	0	0	16	**1,504**
Honey, monofloral	1	1	7	1	46	41	7	134	34	0	13	11	2	48	35	**381**
Honey, polyfloral	0	0	2	0	72	14	1	31	14	0	13	23	0	31	5	**206**
Honey, blended	0	0	0	0	0	0	0	0	0	0	0	0	0	24	57	**81**
Honeydew honey	0	0	0	0	1	9	2	38	10	0	2	2	0	2	1	**67**
Comb honey	0	0	1	0	0	3	0	2	1	0	0	0	0	0	2	**9**

PA: pyrrolizidine alkaloids.

Looking at Table 6, it can be seen the average contribution of each of the PAs analysed in the total PA concentration for the 1,966 samples of honey. Echimidine (44%) and lycopsamine (37%) were, by far, the most important PAs in terms of contribution to the levels of PAs in honey.

Table 6: Average contribution of different PAs to the total PA concentration in the samples of honey included in the final data set

	Average contribution (%)		Average contribution (%)
Echimidine	43.6	Lycopsamine	37.3
Echimidine-*N*-oxide	0.1	Lycopsamine-*N*-oxide	0.0
Erucifoline	0.1	Monocrotaline	0.1
Erucifoline-*N*-oxide	0.0	Monocrotaline-*N*-oxide	0.0
Europine	6.7	Retrorsine	3.9
Europine-*N*-oxide	0.0	Retrorsine-*N*-oxide	0.4
Heliotrine	0.7	seneci(o)phylline	4.4
Heliotrine-*N*-oxide	0.0	Senecionine	7.4
Intermedine	8.9	Senecionine-*N*-oxide	0.7
Jacobine	1.1	Seneciphylline-*N*-oxide	0.3
Lasiocarpine	0.7	Senkirkine	0.9
Lasiocarpine-*N*-oxide	0.0	Trichodesmine	0.0

Table 7 shows the PAs levels estimated in diverse types of retail honey in the final data set. Only for comparison purposes the samples of bulk honey are also included in this table although they were not used to estimate dietary exposure to PAs. It can be seen that, overall, the average levels of PAs in bulk honey were more than twofold the levels in retail honey.

Most of the samples of honey were analysed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) and high-performance liquid chromatography–hydride generation–atomic fluorescence spectrometry detection (LC–HG–AFS). For LC–MS/MS, the minimum LOQ was reported for echimidine (0.18 μg/kg), while a maximum LOQ of 7.5 μg/kg was reported for several PAs (retrorsine, retrorsine-*N*-oxide and senecionine-*N*-oxide). The use of LC–HG–AFS allowed higher sensitivity than LC–MS/MS; a minimum LOQ of 0.05 μg/kg was submitted for all PAS except for intermedine and lycopsamine. For most of the PAs, the maximum LOQ was 0.3 μg/kg although for particular ones, such as retrorsine and seneci(o)phylline, the maximum LOQ was 10 μg/kg.

Table 7: Levels of PAs (μg/kg) in different types of retail honey. Bulk honey is only included for comparison purposes

	N[(a)]	%LC[(b)]	PAs analysed	Variable[(c)]	Mean	Percentiles[(d),(e)]				
						P5	P25	Median	P75	P95
Retail honey										
Honey, unspecified	1,429	24	8–17	LB	14.5	0.0	2.0	8.0	20.0	55.0
				MB	21.0	6.5	8.5	14.5	25.5	59.0
				UB	27.5	13.0	17.0	22.0	31.0	64.0
Honey, monofloral	275	63	9–19	LB	5.0	0.0	0.0	0.0	2.5	17.6
				MB	11.3	4.0	6.5	7.0	9.6	22.5
				UB	17.6	6.4	13.0	14.0	17.4	26.8
Honey, polyfloral	183	45	9–19	LB	7.3	0.0	0.0	1.3	6.0	39.9
				MB	14.1	6.5	8.5	9.6	11.5	44.4
				UB	20.9	10.5	14.8	17.4	19.3	47.5
Honey, blended	24	46	9–19	LB	14.7	0.0	0.0	0.3	14.0	
				MB	17.2	2.8	2.8	3.0	16.0	
				UB	19.8	5.7	5.7	5.7	17.9	
Honeydew honey	52	60	11–19	LB	8.7	0.0	0.0	0.0	15.0	–
				MB	14.4	6.4	6.5	7.0	19.5	–
				UB	20.1	10.9	13.0	14.0	24.0	–
Comb honey	3	67	12–13	LB	1.0	–	–	–	–	–
				MB	7.5	–	–	–	–	–
				UB	14.0	–	–	–	–	–
Bulk honey	13,280	33	8–14	LB	33.3	0.0	0.0	7.0	32.0	144.0
				MB	39.7	7.0	8.5	13.0	37.5	149.0
				UB	46.1	14.0	17.0	20.0	43.0	154.0

PA: pyrrolizidine alkaloids.
(a): Number of samples.
(b): LC = samples with all PA analysed reported as left-censored data.
(c): LB: lower bound; MB: middle bound; UB: upper bound.
(d): P5/P25/75/95: 5th/25th/75th/95th percentiles.
(e): The estimation of high percentiles is not reliable when too few observations are available (less than 11 for the P75, 29 for the P90, 60 for the P95 and 298 for the P99).

3.1.2. Tea and herbal infusions

After the evaluation of the samples, a total of 2,374 samples of tea and different herbal infusions were initially available for dietary exposure estimations. Among them, 1,722 were codified at FoodEx level 2 as 'Tea and herbs for infusions'. Within this food category, the most important group was the one containing unspecified herbal infusions and fruit teas (n = 1,040), accompanied by camomile (n = 269), peppermint (n = 205) and rooibos (n = 168).

Regarding the samples of tea, a total of 14 samples were codified as 'Tea, unspecified', that corresponded to samples reported without specifying whether they referred to green or black tea, and 12 were codified as 'Tea, decaffeinated'. Together with them, a total of 649 samples corresponded to either black or green tea, and were classified as 'Black tea, infusion' or 'Green tea, infusion', respectively. Samples of white tea, a non-fermented or lightly fermented tea were classified as 'Green tea, infusion', while few samples of oolong tea, often described as semi-fermented tea, were classified as 'Black tea, infusion'.

For most of the samples, the concentration of PAs was submitted in μg/kg, referring to the dry product; in these cases the concentration of PAs was divided by a factor of 75 to obtain the concentration in the product as consumed (μg/L). This factor was selected based on the protocol used in the EFSA external scientific report (Mulder et al., 2015) where the samples of tea and herbal infusions were prepared according to the standard protocol specified in the DIN standard 10809 (2 g of dried plant material in 150 mL boiling water).

Table 8 shows the number of PAs submitted for each of the 2,374 samples of tea and different herbal infusions initially available. It can be seen that the number of PAs per sample varied between one (for 28 samples) and 28 PAs (for 386 samples). It is important to mention that for some samples diverse PAs that could not be chromatographically separated were provided as one analytical result. Further details for these specific cases are given later in the report.

Table 8: Number of individual PAs reported as analysed in samples of tea and herbal infusions

	Number of pyrrolizidine alkaloids analysed in each sample																	Total
	1	2	6	11	12	16	17	18	19	21	22	23	24	25	26	27	28	
Tea and herbs for infusions, unspecified	28	0	1	5	2	6	141	12	43	2	11	83	77	15	423	106	85	**1,040**
Tea, unspecified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	**14**
Tea, decaffeinated	0	0	0	0	0	0	0	0	5	0	2	0	3	0	1	1	0	**12**
Black tea	0	0	0	0	0	0	27	3	40	0	0	1	129	3	7	40	89	**339**
Green tea	0	0	0	0	0	0	14	23	7	2	0	2	162	2	20	12	68	**310**
Instant tea, powder	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	**3**
Camomile	0	0	2	1	0	10	65	0	5	0	41	11	22	1	48	4	59	**269**
Peppermint	0	1	0	0	0	8	39	2	5	0	4	6	22	13	60	6	39	**205**
Rooibos	0	0	0	1	0	0	36	1	6	0	0	11	43	4	25	11	30	**168**
Ginseng	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	**3**
Yerba mate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	**3**
Hibiscus	0	0	0	0	0	0	0	1	2	0	0	0	0	0	1	2	0	**6**
Rose petals	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	**1**
Lime	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	**1**

Based on two recent research projects that identified key PAs in tea and herbal infusions (BfR, 2013; Mulder et al., 2015), all the samples for which less than 17 individual PAs were reported were excluded from the final data set, except four samples that reported 16 PAs + two co-eluting PAs. Likewise, three samples codified as 'Ginseng root (*Panax ginseng*)' and three as 'Instant tea, powder' with all PAs as left-censored data were also excluded.

At the end, a total of 2,307 samples of tea and herbal infusions were available to estimate dietary exposure to PAs with the number of PAs in each sample ranging from 17 to 28. Some samples of 'Tea for infants and young children' (n = 39) were also available; either 17 or 19 PAs were submitted for these samples (Table 9). The highest average concentrations of PAs were found in the samples of rooibos (LB = 4.1 μg/L) and peppermint (LB = 3.5 μg/L). Concentrations of PAs in black tea were twice as high as reported for green tea (LB = 1.6 μg/L and LB = 0.8 μg/L, respectively). An additional table with the levels of PAs in the 2,307 samples of tea and herbal infusions expressed as μg/kg in the dry product is shown in Appendix B.

The data on tea and herbal infusions were submitted by seven different data providers. Five data sets were submitted by national authorities from the diverse MSs (MS1-DS–MS4-DS), while one data set (Art36-DS) was the result of Article 36 grant (GP/EFSA/CONTAM/2013/03) awarded by EFSA to a consortium led by RIKILT and composed also by the Federal Institute for Risk Assessment (BfR) and the Institute for Research and Technology in Food and Agriculture (IRTA) (Mulder et al., 2015). A seventh data set (DS-THIE) was provided by Tea & Herbal Infusions Europe (THIE), the European association representing the interests of producers and traders of tea (*Camellia sinensis*) and herbal infusions.

For the data set MS2A-DS, the samples of tea and herbal infusions were all collected between 2010 and 2013, in the data sets MS2B-DS and MS3-DS in 2014, in the data sets MS1-DS and MS4-DS in 2015 and in the data set Art36-DS between 2014 and 2015. For the samples submitted by THIE, they were collected in two different years, 2015 and 2016.

Table 10 shows the concentration of PAs reported for the different samples of tea and herbal infusions grouped by data providers. The number of PAs per sample in each data set was different. Two data sets reported the 28 PAs for each sample (MS4-DS and Art36-DS), one reported 19 PAs per sample (MS3-DS) and two reported 17 PAs (MS2A-DS and MS2B-DS), while for the samples provided by THIE, the number of individual PAs varied between 16 and 28. For the data set Art36-DS, baseline

Table 9: Levels of PAs in the product as consumed (μg/L) in different types of tea and herbal infusions, together with samples of 'Tea for infants and young children'

	N[(a)]	%LC[(b)]	PAs analysed	Variable[(c)]	Mean	Percentiles[(d),(e)]				
						P5	P25	Median	P75	P95
Tea and herbal infusions										
Tea and herbs for infusions, unspecified	1,002	56	16–28	LB	1.8	0.0	0.0	0.0	0.8	5.4
				MB	3.4	0.9	1.7	1.9	3.1	6.4
				UB	5.1	1.8	3.5	3.6	5.1	7.7
Tea, unspecified	14	50	28	LB	0.9	–	0.0	0.1	0.6	–
				MB	3.9	–	3.1	3.2	3.1	–
				UB	6.9	–	6.1	6.2	6.1	–
Tea, decaffeinated	12	58	19–27	LB	0.2	–	0.0	0.0	0.2	–
				MB	1.2	–	0.7	1.0	1.7	–
				UB	2.3	–	1.3	1.5	3.5	–
Black tea, infusion	339	55	17–28	LB	1.6	0.0	0.0	0.0	0.5	7.6
				MB	2.9	0.7	1.2	1.7	2.4	8.9
				UB	4.2	1.3	2.0	3.5	3.8	10.3
Green tea, infusion	310	73	17–28	LB	0.8	0.0	0.0	0.0	0.2	3.7
				MB	2.3	0.6	1.7	1.7	2.3	4.2
				UB	3.8	0.8	3.3	3.5	3.8	6.1
Camomile flowers	256	38	17–28	LB	2.3	0.0	0.0	0.4	2.2	9.8
				MB	3.5	0.3	1.5	2.0	3.9	10.4
				UB	4.8	0.5	2.8	3.6	5.8	11.2
Peppermint	196	36	17–28	LB	3.5	0.0	0.0	0.4	1.6	10.2
				MB	4.9	0.7	1.8	2.0	3.1	11.0
				UB	6.2	1.3	2.9	3.6	5.0	11.8
Hibiscus flowers	6	83	18–27	LB	0.0	–	–	0.0	–	–
				MB	2.0	–	–	2.4	–	–
				UB	4.1	–	–	4.8	–	–
Rose petals	1	100	22	LB	0.0	–	–	–	–	–
				MB	1.5	–	–	–	–	–
				UB	2.9	–	–	–	–	–
Lime (linden)	1	100	18	LB	0.0	–	–	–	–	–
				MB	3.0	–	–	–	–	–
				UB	5.9	–	–	–	–	–
Rooibos leaves	167	9	17–28	LB	4.1	0.0	1.1	2.3	4.4	10.3
				MB	5.2	0.7	2.2	3.7	5.4	10.6
				UB	6.3	1.2	3.6	5.1	6.6	11.3
Yerba mate	3	33	26–28	LB	0.4	–	–	–	–	–
				MB	2.2	–	–	–	–	–
				UB	3.9	–	–	–	–	–
Tea for infants and young children	39	26	17–19	LB	0.6	–	0.0	0.2	0.9	–
				MB	1.0	–	0.1	0.8	1.6	–
				UB	1.4	–	0.2	1.2	2.2	–

(a): Number of samples.
(b): LC = samples with all PA analysed reported as left-censored data.
(c): LB: lower bound; MB: middle bound; UB: upper bound.
(d): P5/P25/75/95: 5th/25th/75th/95th percentiles.
(e): The estimation of high percentiles is not reliable when too few observations are available (less than 11 for the P75, 29 for the P90, 60 for the P95 and 298 for the P99).

separation between indicine and intermedine as well as their respective N-oxides was not possible, although analytical results were submitted either as intermedine or intermedine-*N*-oxide. This means that in case of a positive finding of intermedine, it could be that indicine or a mixture of indicine and intermedine is present in the samples; the same applies to indicine-*N*-oxide and intermedine-*N*-oxide. The data set DS-THIE also contained diverse co-elutions of two-three PAs when all 28 PAs were not reported individually (intermedine/lycopsamine, intermedine/lycopsamine-indicine, intermedine-*N*-oxide/lycopsamine-*N*-oxide, intermedine-*N*-oxide/lycopsamine-*N*-oxide/indicine-*N*-oxide, senecionine/senecivernine, senecionine-*N*-oxide/senecivernine-*N*-oxide, indicine-*N*-oxide/lycopsamine-*N*-oxide). MS1-DS contains samples analysed for a total of 18 individual PAs together with six additional PAs that co-eluted in pairs (jacobine-*N*-oxide/retrorsine-N-oxide, senecionine-*N*-oxide/senecivernine-*N*-oxide, senecionine/senecivernine). Analytical results reported as co-elutions were also used to estimate the total concentration of PAs per sample. Overall, the highest number of individual PAs reported was 26 (n = 586 samples) followed by 24 (n = 458 samples).

The average contribution of each individual PA to the total PA concentration for each sample of tea and herbal infusions in the final data set was also assessed (Table 11). The average contribution of each PA was estimated in those samples where the PA was analysed and at least one PA was quantified. Although some uncertainty may be associated to these estimations as not all the samples were analysed for the 28 PAs, similar results in terms of main contributors to the total PA concentration were obtained when assessing only the samples analysed for the 28 PAs. Looking at this table, it can be seen that the identity of the PAs with the highest contributions was slightly different depending on the food commodity, although some of the PAs were main average contributors in several teas and herbal infusions. Taking into account the final data set, among the samples of green tea, the main average contributors to the total PA concentration were senecionine-*N*-oxide (19%), retrorsine-*N*-oxide (18%), and intermedine and lycopsamine, both with 16% contribution. A similar profile was observed for black tea, although intermedine-*N*-oxide gains importance reaching an average contribution of 31%. Among the samples of camomile, senecionine-*N*-oxide and intermedine were the main average contributors, with 28% and 22%, respectively, followed by senecionine and lycopsamine, both with 10%. In the herbal infusion peppermint, the main average contributors to the total PA concentration were seneciphylline-*N*-oxide (28%) followed by senecionine-*N*-oxide (25%), retrorsine-*N*-oxide (13%) and seneci(o)phylline (11%). In the samples of rooibos, the concentration of PAs seems to be clearly dominated by senecionine-*N*-oxide that represents, as an average, 57% of the total concentration, followed by retrorsine-*N*-oxide (19%) and senecionine (14%). It should be noted that for as many as 15 PAs their average contributions to the total concentration of PAs were below 5%, in many cases even below 1%: senecivernine, senecivernine-*N*-oxide, monocrotaline, monocrotaline-*N*-oxide, jacobine, jacobine-*N*-oxide, lasiocarpine, lasiocarpine-*N*-oxide, erucifoline, erucifoline-*N*-oxide, trichodesmine, europine, senkirkine, heliotrine, and retrorsine. The three last PAs are included among the typical 8 PAs analysed in honey, although apart from retrorsine the contribution of senkirkine and heliotrine to the total concentration of PAs is almost negligible (EFSA CONTAM Panel, 2011). When adding up the average contribution of these 15 PAs in different teas and herbal infusions, they contributed on average 8% (in samples of peppermint) to 14% (in samples of green tea) of the total PA concentration.

Supplementary electronic information in Appendix E is provided showing the contribution of the main eight PAs in the different samples of tea and herbal infusions as compared to the total concentration in each of the samples where all the 28 PAs were analysed. Overall, these eight PAs were the main responsible of the PA levels reported for tea and herbal infusions, with contributions below 60% of the total in only 25% of the samples of peppermint, in 17% of the samples of camomile, in 14% of the samples of green tea, in 8% of the samples of black tea and in only 4% of the samples of rooibos.

Bringing the attention to lasiocarpine, one of the most toxic PAs that have been tested (EFSA CONTAM Panel, 2011), it was analysed in 2,250 of the samples of tea and herbal infusions included in the final data set (96% of the samples). However, this PA was only quantified in 103 samples (< 5% of the samples), mostly in samples codified as 'Tea and herbs for infusions, unspecified' (n = 51) although also in 'Tea for infants and young children' (n = 21). It was in the latter group where lasiocarpine showed the highest contributions to the total PA concentration, 42% on average, among the 29 samples where this PA was analysed. In the rest of the samples of tea and herbal infusions, the average contribution of lasiocarpine was almost negligible, from not being quantified in any of the samples of black tea to a maximum average contribution of 1.7% in 'Tea and herbs for infusions, unspecified' (Table 11).

Table 10: Total concentration (μg/L) of PAs in different samples of tea and herbal infusions as reported in different data sets

		MS1-DS			MS2A-DS			MS2B-DS			MS3-DS			MS4-DS			Art36-DS			THIE-DS			ALL MS-DS + Art 36-DS		
Sampling year		**2015**			**2010–2013**			**2014**			**2014**			**2015**			**2014–2015**			**2015–2016**			**2010–2015**		
PAs analysed		**18[a]**			**17**			**17**			**19**			**28**			**28**			**16–28[b]**			**17–28**		
		N	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**
Tea and herbs for infusions, unsp.	**LB**	**12**	2.2	–	**137**	2.3	12.0	**3**	0.0	–	**41**	20.5		**24**	0.0		**20**	5.9	–	**765**	0.6	2.8	**237**	5.5	15.5
	MB		4.1	–		3.2	12.9		0.6	–		21.1			3.1			6.0	–		2.5	4.3		6.5	16.1
	UB		6.0	–		4.1	13.7		1.2	–		21.7			6.2			6.1	–		4.3	6.4		7.6	16.3
Tea, unsp.	**LB**		–	–		–	–	–	–	–	**1**	2.0	–	**14**	0.9	–		–	–	**476[d]**	0.5	2.0	**187[e]**	3.0	14.3
	MB		–	–		–	–	–	–	–		2.6	–		3.9	–		–	–		2.1	3.2		4.0	14.5
	UB		–	–		–	–	–	–	–		3.1	–		6.9	–		–	–		3.7	5.8		5.0	15.4
Tea, decaff.	**LB**		–	–		–	–	–	–	–	**5**	0.3	–		–	–		–	–	**7**	0.0		**5**	0.3	–
	MB		–	–		–	–	–	–	–		0.9	–		–	–		–	–		1.4			0.9	–
	UB		–	–		–	–	–	–	–		1.5	–		–	–		–	–		2.9			1.5	–
Black tea, infusion	**LB**	**3**	0.0	–	**27**	3.6	–	–	–	–	**40**	0.7	–		–	–	**33**	7.6	–	**236**	0.6	2.1	**103**	3.7	17.6
	MB		3.0	–		4.4	–	–	–	–		1.4	–		–	–		7.8	–		2.3	3.2		4.3	17.8
	UB		5.9	–		5.3	–	–	–	–		2.0	–		–	–		8.0	–		3.9	6.1		4.9	18.0
Green tea, infusion	**LB**	**23**	0.0	–	**14**	1.3	–	–	–	–	**6**	0.0	–		–	–	**26**	5.6	–	**240**	0.4	2.0	**70**	2.4	14.1
	MB		2.7	–		2.2	–	–	–	–		0.7	–		–	–		5.8	–		2.2	3.2		3.6	14.2
	UB		5.5	–		3.1	–	–	–	–		1.3	–		–	–		6.0	–		3.9	5.4		4.8	14.4
Peppermint	**LB**	**2**	0.0	–	**30**	1.5	–	**9**	2.9	–	**5**	1.4	–	**6**	46.6		**30**	6.6	–	**114**	1.2	3.2	**82**	6.8	13.0
	MB		3.0	–		2.4	–		3.6	–		2.0	–		49.4			6.8	–		2.9	5.2		7.6	13.1
	UB		5.9	–		3.4	–		4.2	–		2.5	–		52.3			6.9	–		4.7	7.2		8.4	13.2
Hibiscus flowers	**LB**	**1**	0.0	–		–	–	–	–	–	**2**	0.0	–		–	–		–	–	**3**	0.0	–	**3**	0.0	–
	MB		3.0	–		–	–	–	–	–		0.7	–		–	–		–	–		2.6	–		1.5	–
	UB		5.9	–		–	–	–	–	–		1.3	–		–	–		–	–		5.3	–		2.9	–
Camomile flowers	**LB**	**0**	–	–	**39**	5.4		**26**	2.4	–	**5**	6.5	–	**11**	0.9	–	**35**	3.7	–	**140**	1.0	3.5	**116**	3.8	16.0
	MB		–	–		6.4			2.6	–		7.0	–		3.9	–		3.8	–		2.7	5.6		4.6	16.1
	UB		–	–		7.4			2.9	–		7.4	–		6.8	–		4.0	–		4.3	7.8		5.3	16.2

		MS1-DS			MS2A-DS			MS2B-DS			MS3-DS			MS4-DS			Art36-DS			THIE-DS			ALL MS-DS + Art 36-DS		
Sampling year		**2015**			**2010–2013**			**2014**			**2014**			**2015**			**2014–2015**			**2015–2016**			**2010–2015**		
PAs analysed		**18[a]**			**17**			**17**			**19**			**28**			**28**			**16–28[b]**			**17–28**		
		N	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**	**N**	**Mean**	**P95[c]**
Lime	**LB**	**1**	0.0	–		–	–		–	–		–	–		–	–		–	–		–	–	**1**	0.0	–
	MB		3.0	–		–	–		–	–		–	–		–	–		–	–		–	–		3.0	–
	UB		5.9	–		–	–		–	–		–	–		–	–		–	–		–	–		5.9	–
Rooibos leaves	**LB**	**1**	2.6	–	**21**	7.9	–	**15**	1.7	–	**6**	4.9	–		–	–	**22**	8.0	–	**102**	2.7	7.2	**65**	6.1	15.1
	MB		5.7	–		8.8	–		1.7	–		5.4	–		–	–		8.1	–		4.3	8.2		6.6	15.2
	UB		8.9	–		9.7	–		1.8	–		5.9	–		–	–		8.3	–		5.8	10.0		7.0	15.4
Rose petals	**LB**		–	–		–	–		–	–		–	–		–	–		–	–	**1**	0.0	–		–	–
	MB		–	–		–	–		–	–		–	–		–	–		–	–		1.5	–		–	–
	UB		–	–		–	–		–	–		–	–		–	–		–	–		2.9	–		–	–
Yerba mate	**LB**		–	–		–	–		–	–		–	–		–	–		–	–	**3**	0.4	–		–	–
	MB		–	–		–	–		–	–		–	–		–	–		–	–		2.2	–		–	–
	UB		–	–		–	–		–	–		–	–		–	–		–	–		3.9	–		–	–

PA: pyrrolizidine alkaloids; MS: Member State; N: number of samples; THIE: Tea & Herbal Infusions Europe.
(a): A total of 18 individual PAs were reported individually, together with six additional PAs that co-eluted in pairs (jacobine-*N*-oxide/retrorsine-*N*-oxide, senecionine-*N*-oxide/senecivernine-*N*-oxide, senecionine/senecivernine).
(b): A variable number between 16 and 28 PAs were reported individually, together with diverse co-elutions of two-three PAs (intermedine/lycopsamine, intermedine/lycopsamine-indicine, intermedine-*N*-oxide/lycopsamine-*N*-oxide, intermedine-*N*-oxide/lycopsamine-*N*-oxide/indicine-*N*-oxide, senecionine/senecivernine, senecionine-*N*-oxide/senecivernine-*N*-oxide, indicine-*N*-oxide/lycopsamine-*N*-oxide).
(c): P95 = 95th percentile; 95th percentile is not shown when the number of samples was lower than 60 as this percentile is not statistically significant.
(d): Concentration of PAs for 'Tea, unspecified' is derived using the samples of 'Black tea, infusion' and 'Green tea, infusion'.
(e): Concentration of PAs for 'Tea, unspecified' is derived using the samples of 'Black tea, infusion' and 'Green tea, infusion' and 14 samples that were reported as 'Tea, unspecified'.

Having in mind the average contribution of each individual PA to the total PA concentration, it is important to mention that in four of the data sets, namely MS1-DS, MS2A-DS, MS2B-DS and MS3-DS, intermedine-*N*-oxide and lycopsamine-*N*-oxide are among the PAs that were not analysed. These PAs have a relatively high contribution to the total concentration of PAs in samples of black tea, on average 30.9% in the case of intermedine-*N*-oxide (Table 11). As a result, considerable underestimation of the total concentration of PAs could be expected for the samples of black tea contained in these data sets (70 samples, see Table 10). It is also worth mentioning that in the data set DS-THIE some PAs, with an important contribution to the total concentration of PAs, were not analysed in a number of samples. As an example, several herbal infusion samples were not analysed for intermedine-*N*-oxide (42 samples of camomile and 37 of unspecified herbal infusions, among others). Similarly, in 36 samples of camomile, seneciphylline-*N*-oxide and lycopsamine (average contribution of ~ 10% and ~ 7%, respectively) were not analysed, or senecionine-*N*-oxide that represents in average between 9% and 57% of the total content of PAs was not analysed in 24 samples of diverse types of tea and herbal infusions. Therefore, some underestimation when reporting the total concentration of PAs in these samples cannot be discarded.

Table 11: Average contribution of different PAs to the total PA concentration in the samples of tea and herbal infusions included in the final data set

	Unspecified herbal infusions, fruit teas and mixes	Green tea	Black tea	Camomile flowers	Peppermint	Rooibos leaves
Mean contribution (%)[a]						
Echimidine	5.5	1.5	0.0	6.0	0.1	0.2
Echimidine-*N*-oxide	1.7	0.2	0.0	4.1	1.0	0.5
Erucifoline	0.0	0.0	0.4	0.0	0.4	0.0
Erucifoline-*N*-oxide	0.3	0.4	0.1	0.0	0.0	0.0
Europine	1.5	0.7	1.4	0.1	0.3	0.0
Europine-*N*-oxide	19.0	0.2	0.0	0.9	6.1	0.1
Heliotrine	1.4	1.0	0.0	1.4	1.0	0.1
Heliotrine-*N*-oxide	10.3	0.4	0.4	0.2	5.5	0.2
Intermedine	14.5	16.2	19.6	22.2	0.3	0.4
Intermedine-*N*-oxide	6.8	4.2	30.9	7.2	0.0	0.7
Jacobine	0.0	2.3	0.9	0.0	0.0	0.0
Jacobine-*N*-oxide	0.1	3.8	1.8	0.0	0.0	0.0
Lasiocarpine	1.7	1.1	0.0	0.6	0.4	0.0
Lasiocarpine-*N*-oxide	3.4	0.0	0.2	0.3	1.7	0.2
Lycopsamine	7.5	16.4	19.5	9.5	0.8	0.0
Lycopsamine-*N*-oxide	5.3	1.6	7.0	5.3	0.8	0.2
Monocrotaline	0.3	0.0	0.0	0.0	0.0	0.1
Monocrotaline-*N*-oxide	0.2	0.0	0.3	0.0	0.0	0.0
Retrorsine	1.1	1.9	2.3	0.2	1.3	3.2
Retrorsine-*N*-oxide	6.3	18.2	14.5	4.1	13.1	18.6
seneci(o)phylline	0.9	0.2	0.0	2.7	11.0	0.3
Senecionine	3.9	3.2	1.6	9.9	5.1	14.2
Senecionine-*N*-oxide	18.7	18.8	9.3	27.5	24.5	56.6
Seneciphylline-*N*-oxide	2.8	0.4	0.0	7.1	27.8	1.3
Senecivernine	0.4	0.1	0.0	0.9	1.7	1.8
Senecivernine-*N*-oxide	1.8	0.8	0.5	2.4	0.6	4.4
Senkirkine	1.1	1.7	0.0	1.3	0.1	0.4
Trichodesimine	0.2	0.2	0.0	0.0	0.0	0.0

	Unspecified herbal infusions, fruit teas and mixes	Green tea	Black tea	Camomile flowers	Peppermint	Rooibos leaves
PAs reported as co-eluting						
Intermedine/ lycopsamine or Intermedine/ lycopsamine/indicine	7.5	7.1	12.4	27.6	1.8	0.0
Senecionine/ senecivernine	1.4	0.2	5.0	0.0	0.0	3.1
Senecionine-*N*-oxide/ senecivernine-*N*-oxide	43.7	34.0	3.7	0.2	0.0	68.4
Intermedine-*N*-Oxide/ lycopsamine-*N*-oxide or Intermedine-*N*-oxide/ lycopsamine-*N*-oxide/ Indicine-*N*-oxide	11.7	56.9	67.4	2.2	6.7	0.0
Jacobine-*N*-oxide/ retrorsine-*N*-oxide	6.9	–	–	–	–	0.0

PA: pyrrolizidine alkaloids.
(a): The average contribution of each PA was estimated in those samples where the PA was analysed and at least one PA was quantified.

Two main analytical methods were used to analyse the samples of tea and herbal infusions submitted to EFSA: LC–HG–AFS and LC–MS/MS. Table 12 shows the different LOQs reported for each of the analytical methods used for different data sets. Both methods seem to be able to reach similar sensitivity in the analysis of PAs; using LC–HG–AFS, an LOQ as low as 0.5 μg/kg was reported for the analysis of heliotrine. The same LOQ was reported for LC–MS/MS in the analysis of several PAs such as intermedine, monocrotaline, and senecionine among others.

Despite the high sensitivity that could be reached with both methods, relatively high LOQs were also reported. In addition, it is also noticed that specific data sets provided relatively high LOQs for several or even for all PAs analysed, as observed for instance in the data sets MS1-DS and MS2A-DS. In other occasions very wide ranges of LOQs were reported for the same PAs using the same analytical method (e.g. 1.0–45.9 μg/kg for lasiocarpine). A detailed evaluation of the LOQs indicates that in some specific data sets, the lowest LOQs for each PA analysed are only reported for very few samples, while the highest LOQ of the range is reported for most of the analytical results. As an example, for senecionine, with a LOQ range of 1–20 μg/kg, LOQ = 1 was reported for two analytical results and LOQ = 10 or 20 reported for 90% of the analytical results. These relatively high LOQs can lead to scenarios where for different teas and herbal infusions the LB is zero or close to zero and the UB estimates are rather high, as observed in some of the data sets (Table 10).

Considering the maximum LOQs submitted for the different data sets, in a scenario with all the PAs submitted as below LOQ, the UB estimates could be as high as 1,340 μg/kg (17.9 μg/L), or 445 μg/kg (5.9 μg/L) if all PAs were reported as below LOD, in both cases also considering the analytical results reported as co-eluting PAs. At the same time, considering the two data sets with the minimum LOQs and reporting all 28 PAs, if all of them were below the LOQ, the minimum UB scenarios would range between 53 μg/kg (0.7 μg/L) and 98.5 μg/kg (1.3 μg/L).

When comparing the PA concentrations in the different data sets, it can be seen that in general the concentrations are lower in the data submitted by THIE as compared to the rest of data sets (Table 10). This is particularly evident when comparing THIE results to the data collected through the EFSA Article 36 grant (Art36-DS). Although the data set with the oldest samples (MS2A-DS, sampling years = 2010–2013) showed the highest PA concentrations among the data submitted by the national authorities, the data submitted through the Art 36 grant contains samples collected between 2014 and 2015 and also showed relatively high concentrations of PAs. In the data sets that contained samples collected in 2015 (MS1-DS and MS4-DS), the LB estimations for certain food commodities were close to those estimated for THIE. However, the relatively high UB estimates indicate that the analytical methods may not be sensitive enough to quantify all the PAs potentially present in the samples.

Table 12: Limits of quantification (LOQs), expressed as dry product (μg/kg), of the different analytical methods used to analyse PAs in tea and herbal infusions

	MS1-DS		MS2A-DS		MS2B-DS		MS3-DS		MS4-DS		Art36-DS		THIE-DS			
	N = 43		N = 268		N = 53		N = 111		N = 55		N = 166		N = 1,621			
	LC–MS/MS		LC–MS/MS		LC–MS/MS		LC–HG–AFS		LC–HG–AFS		LC–MS/MS		LC–MS/MS		LC–HG–AFS	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Echimidine	30	30	2.9	2.9	0.5	11.9	5.0	5.0	10	10	2.6	2.6	1.0	10.0	1.0	10.0
Echimidine-*N*-oxide	–	–	–	–	–	–	–	–	10	10	6.1	6.1	1.0	10.0	1.0	10.0
Erucifoline	30	30	–	–	–	–	5.0	5.0	50	50	1.9	1.9	5.0	50.0	1.0	10.0
Erucifoline-*N*-oxide	30	150	–	–	–	–	5.0	5.0	20	20	3.8	3.8	2.5	20.0	1.0	10.0
Europine	30	30	–	–	–	–	–	–	10	10	2.1	2.1	2.0	10.0	1.0	10.0
Europine-*N*-oxide	50	60	–	–	–	–	–	–	10	10	2.3	2.3	2.0	14.0	1.0	10.0
Heliotrine	15	20	2.9	2.9	1.0	43.8	5.0	5.0	10	10	1.7	1.7	1.0	10.0	0.5	10.0
Heliotrine-*N*-oxide	60	60	13.3	13.3	0.5	17.4	5.0	5.0	10	10	2.0	2.0	1.0	10.0	2.0	20.0
Intermedine	15	15	4.7	4.7	0.5	14.1	5.0	5.0	10	10	3.1	3.1	1.0	20.0	2.0	10.0
Intermedine-*N*-oxide	–	–	–	–	–	–	–	–	10	10	3.8	3.8	2.0	20.0	1.0	10.0
Jacobine	–	–	–	–	–	–	5.0	5.0	50	50	4.0	4.0	5.0	50.0	4.0	10.0
Jacobine-*N*-oxide	–	–	–	–	–	–	–	–	20	20	4.2	4.2	2.5	20.0	2.0	10.0
Lasiocarpine	30	30	3.4	3.4	1.0	45.9	5.0	5.0	10	10	2.4	2.4	1.0	10.0	1.0	10.0
Lasiocarpine-*N*-oxide	30	30	12.2	12.2	0.5	39.3	5.0	5.0	10	10	2.8	2.8	1.0	10.0	1.0	20.0
Lycopsamine	15	15	3.9	3.9	5.0	10.2	5.0	5.0	10	10	6.4	6.4	1.0	20.0	2.0	10.0
Lycopsamine-*N*-oxide	–	–	–	–	–	–	–	–	10	10	4.9	4.9	1.0	20.0	2.0	10.0
Monocrotaline	60	60	3.2	3.2	0.5	20.3	5.0	5.0	20	20	2.8	2.8	2.5	20.0	1.0	20.0
Monocrotaline-*N*-oxide	60	60	6.2	6.2	1.0	17.4	5.0	5.0	20	20	5.4	5.4	2.5	20.0	1.0	10.0
Retrorsine	150	150	151.8	151.8	5.0	15.1	5.0	5.0	20	20	2.7	2.7	1.0	20.0	3.0	10.0
Retrorsine-*N*-oxide	–	–	48.7	48.7	1.0	29.4	5.0	5.0	20	20	4.6	4.6	1.0	20.0	5.0	20.0
Senecionine	–	–	64.1	64.1	0.5	25.2	5.0	5.0	20	20	5.9	5.9	1.0	20.0	2.0	20.0
Senecionine-*N*-oxide	–	–	46.8	46.8	1.0	30.9	5.0	5.0	10	10	2.9	2.9	1.0	20.0	2.0	20.0
Seneci(o)phylline	60	60	7.8	7.8	2.5	51.7	10.0	10.0	20	20	4.0	4.0	1.0	20.0	2.0	20.0

	MS1-DS		MS2A-DS		MS2B-DS		MS3-DS		MS4-DS		Art36-DS		THIE-DS			
	N = 43		N = 268		N = 53		N = 111		N = 55		N = 166		N = 1,621			
	LC–MS/MS		LC–MS/MS		LC–MS/MS		LC–HG–AFS		LC–HG–AFS		LC–MS/MS		LC–MS/MS		LC–HG–AFS	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Seneciphylline-*N*-oxide	60	60	14.3	14.3	0.5	16.3	5.0	5.0	10	10	2.7	2.7	1.0	20.0	2.0	10.0
Senecivernine	–	–	–	–	–	–	–	–	20	20	5.3	5.3	5.0	20.0	2.0	10.0
Senecivernine-*N*-oxide	–	–	–	–	–	–	–	–	20	20	2.6	2.6	5.0	20.0	2.0	10.0
Senkirkine	60	60	5.3	5.3	1.0	17.5	5.0	5.0	10	10	2.4	2.4	1.0	20.0	1.0	10.0
Trichodesmine	60	60	3.5	3.5	0.5	20.7	–	–	10	10	3.1	3.1	1.0	20.0	1.0	20.0

N: Number of samples; THIE: Tea & Herbal Infusions Europe; LC–MS/MS: liquid chromatography–tandem mass spectrometry; MS: Member State; DS: data set; LC–HG–AFS: liquid chromatography–hydride generation–atomic fluorescence spectrometry detection.

In order to cover the whole range of concentrations of PAs reported for tea and herbal infusions, the estimation of dietary exposure to PAs considered two different scenarios. Together with the other food commodities, a first scenario considered all the samples of tea and herbal infusions submitted by the national authorities and those collected through the Art 36 grant, while a second scenario took into account the samples of tea and herbal infusions submitted by THIE.

3.1.3. Food supplements

A final number of 278 samples of different food supplements were available in the final data set used to estimate dietary exposure to PAs. The most common number of PAs reported per sample was 28 (n = 193), followed by 19 (n = 78), with the minimum number being 9 for just one sample.

Most of the food supplements were codified as 'Plant extract formula' (n = 200), followed by 'Pollen-based supplements' (n = 41). Some samples of 'Supplements containing special fatty acids' (n = 34), 'Algae formula' (n = 2) and 'Enzyme-based supplements' (n = 1) were also analysed, with all PAs below the LOQs (Table 13). These three food supplements were not used for exposure estimations.

Table 13: Levels of PAs for different types of food supplements (μg/kg)

	N[(a)]	Variable[(b)]	Mean	90th percentile[(c)]	95th percentile[(c)]
Supplements containing special fatty acids	34	LB	0	–	–
		MB	45	–	–
		UB	90	–	–
Plant extract formula	200	LB	47,110	–	55,459
		MB	47,138	–	55,471
		UB	47,166	–	55,484
Enzyme-based supplements	1	LB	0	–	–
		MB	50	–	–
		UB	100	–	–
Algae formula (e.g. *Spirulina*, *Chlorella*)	2	LB	0	–	–
		MB	101	–	–
		UB	203	–	–
Pollen-based supplements	41	LB	235	967	–
		MB	244	970	–
		UB	253	974	–

PA: pyrrolizidine alkaloids.
(a): Number of samples.
(b): LB = lower bound; MB = middle bound; UB = upper bound.
(c): 90th and 95th percentile are not shown when the number of samples were lower than 29 and 60, respectively, as they are not statistically significant.

The concentration of PAs reported for the plant extracts varied enormously. In some of the supplements codified as 'Plant extract formula' no presence of PAs was reported (e.g. garlic extracts, ginseng extracts); however, some other supplements possessed relatively high concentrations of PAs. Among the latter is important to mention diverse supplements containing plant material from *Eupatorium* sp., *Tussilago farfara* (coltsfoot), *Borago officinalis* (borage) and *Pulmonaria officinalis* (lungwort). Table 14 shows the plant extracts with the highest concentrations (above 25,000 μg/kg), most of them intended to be consumed as tea infusions. In these samples, the analyses were carried out in the plant extracts prepared as standard infusions (dilution factor of 1/75, i.e. 2 g in 150 mL), and the concentrations are also shown in μg/L. The highest average contributions to the total concentration of PAs came from lycopsamine, intermedine and their N-oxides. An exception was the samples of coltsfoot, where 80–90% of the total concentration of PAs came from senkirkine.

When both occurrence and consumption data were available for particular plant extracts, they were included in the general scenarios developed to estimate dietary exposure to PAs; these plant extract are further detailed in Section 3.2.3. An additional scenario considering those samples with the highest levels of PAs (Table 13) for which no consumption data are available is also presented in this scientific report (Section 4.3).

Table 14: Levels of PAs for individual samples of plant extracts (μg/kg)

Type of plant extract[(a)]	Levels of PAs	
	Dry product (μg/kg)	Infusion (μg/L)[(b)]
Hemp-agrimony (*Eupatorium cannabinum*)	2,410,275	–
Boneset (*Eupatorium perfoliatum*)	1,077,547	–
Borage (*Borago officinalis*)	2,332,558	31,101
Borage (*Borago officinalis*)	2,151,891	28,692
Coltsfoot (*Tussilago farfara*)	419,309	5,591
Common gromwell (*Lithospermum officinale*)	312,271	4,164
Lungwort (*Pulmonaria officinalis*)	141,401	1,885
Lungwort (*Pulmonaria officinalis*)	129,001	1,720
Coltsfoot (*Tussilago farfara*)	114,907	1,532
Lungwort (*Pulmonaria officinalis*)	60,295	804
Lungwort (*Pulmonaria officinalis*)	50,624	675
Eupatorium odoratum Linn. Herbal Tea for Health	40,877	545
Comfrey (*Symphytum officinale* L.)	29,694	396
Mix of different herbs (including *Pulmonaria officinalis*)	27,943	373

PA: pyrrolizidine alkaloids.
(a): All plant extracts except the two-first (Hemp-agrimony and Boneset) refers to supplements intended to be prepared as tea infusion. Hemp-agrimony and Boneset are intended to be directly ingested.
(b): Levels of PAs in μg/L were reported for those plant extracts which are intended to be prepared as tea infusion before consumption; the reported approach was the same as used for tea and herbal infusions (2 g in 150 mL).

3.1.4. Other food commodities

Together with the samples described above, a small number of other plant-origin food samples with measured levels of PAs were available.

There were 13 samples of 'Processed spinaches' that reported average values of 0.2 μg/kg at the LB and 0.9 μg/kg at the UB, with the number of PAs analysed between 8 and 11. Likewise, one sample of fresh spinach was reported with no levels of PAs detected among the 11 PAs analysed. However, the average value for the frozen spinaches was imputed to account for the consumption of fresh spinaches.

For 10 samples of unspecified mixed herbs, either 11 or 16 PAs were analysed. The average levels were 1.7 μg/kg (LB) and 10 μg/kg (UB), with lycopsamine being the PA more frequently reported, and at the highest concentration.

Additionally, 39 samples of tea and herbal infusions for children were also submitted with average values of 0.6 μg/kg (LB) and 1.4 μg/kg (UB). These values were, in general, lower than those for tea and herbal infusions. Samples were submitted in two different data sets, one with 17 PAs and the other one with 19 PAs. Among the different PAs, the highest average contribution to the total concentration came from lasiocarpine (42%) and its N-oxide (14%).

Finally, four samples of 'Composite food', vegetable-based meals (spinaches with cream), were available with 9 and 10 PAs analysed, and average values of 0.05 μg/kg (LB) and 0.8 μg/kg (UB).

3.2. Food consumption data

3.2.1. Consumption of honey

Among the 17,046 eating occasions for honey available in the EFSA consumption database (all dietary surveys considered, see Appendix A), the vast majority were codified at FoodEx level 2 as 'Honey' without further information (~ 94.5%), with few eating occasions codified as 'Honey, monofloral' (4%) and 'Honey polyfloral' (1.5%).

When considering chronic consumption (only dietary surveys with at least 2 days reported), in the adult population ('Adults', 'Elderly' and 'Very elderly'), the percentage of honey consumers varied from 5% to 50% of the total population, with average chronic consumption ranging from 0.002 to 0.06 g/kg bw per day and a high chronic consumption (95th percentile) between 0 and 0.32 g/kg bw per day.[2] In the

[2] For the 95th percentile consumption, those dietary surveys with less than 60 honey consumers were not considered as the number of subjects is not statistically significant.

young population ('Infants', 'Toddlers' and 'Other children'), the percentage of honey consumers in the total population ranged between 1% and 40%. The average chronic consumption varied between 0 and 0.14 g/kg bw per day, and between 0 and 0.49 g/kg bw per day in the high consumers (95th percentile).[2]

Since honey is a food commodity that is not regularly consumed in the population, the average consumption values from all participants in the dietary survey are much lower than considering consumers only. When considering consumers only, in the adult population the average chronic consumption ranges from 0.01 to 0.27 g/kg bw per day, while in the high consumers (95th percentile),[2] it varied between 0.03 and 0.64 g/kg bw per day.[2] In the young population, consumers only, the average chronic consumption of honey across dietary surveys is between 0.02 and 0.98 g/kg bw per day, and between 0.05 and 1.13 g/kg bw per day in the high consumers (95th percentile).[2]

The acute consumption of honey on each individual day was also estimated (consuming days only). The percentage of days with consumption of honey varied from 2% to 27% in the adult population, and between 0.3% and 14% in the young population. Across the different dietary surveys, in the adult population, the average amount of honey consumed considering only the days with honey consumption was as high as 0.52 g/kg bw per day with a minimum average acute consumption of 0.01 g/kg bw per day. In the adult populations, high consumption (95th percentile)[2] varied between 0.05 and 1.22 g/kg bw per day of honey consumption. For the youngest populations, the corresponding average acute consumption across dietary surveys, considering only consuming days, ranged between 0.06 and 1.35 g/kg bw per day, while among the high consumers (95th percentile),[2] the amounts varied between 0.21 and 1.88 g/kg bw per day.

3.2.2. Consumption of tea and herbal infusions

Among the approximately 118,000 eating occasions for tea and herbal infusions reported in the EFSA consumption database (all dietary surveys considered), almost 96% were reported as consumed (liquid). The remaining 4% reported as solid (~ 8,000 eating occasions) were converted into liquid by multiplying the reported amount by a factor of 75 (~ 2 g in 150 mL of water) before dietary exposure was estimated. As commented in Section 3.2.1, this factor was selected based on the protocol used in the EFSA external scientific report on the occurrence of PAs in food (Mulder et al., 2015).

The different eating occasions were codified following the same strategy described for the occurrence data. Around 91,000 eating occasions referred to tea; in about half of the cases (n = 44,400), it was not specified whether the consumption referred to green or black tea ('Tea, unspecified'). Since no occurrence data were codified for 'Instant tea, liquid', these eating occasions and those reported as ice tea-type drinks (ready-to-drink tea) were also recodified as 'Tea, unspecified' assuming a similar composition in PAs as the standard tea infusions. A great number of eating occasions were codified as 'Black tea, infusion' (n = 41,000), with around 5,000 reporting the consumption of 'Green tea, infusion'. Under the food group 'Tea and herbs for infusions, unspecified' (n = 23,000) were the eating occasions reported as 'Herbal tea, infusion' without further information, those herbal infusions for which occurrence data were not available, and the eating occasions reported as 'Fruit tea, infusion'. Among the herbal infusions for which detailed consumption data were reported were 'Rooibos' (n = 2,454), 'Camomile' (n = 612) and Peppermint (n = 553).

Chronic consumption of tea and herbal infusions

Table 15 shows the chronic consumption of tea and different herbal infusions across dietary surveys in Europe for the adult population ('Adults', 'Elderly' and 'Very elderly') and the young population ('Infants', 'Toddlers' and 'Other children'). It can be seen that while in the adult population the highest percentage of consumers was for the consumption of black tea (up to 94%), in the young population was for 'Tea and herbs for infusions, unspecified' (up to 83.8%).

The percentage of consumers varied considerably across the different dietary surveys and this had obviously a clear impact in the range of chronic consumption in the whole population. When looking at consumers only, the highest mean consumption expressed as mL/kg bw per day was for black tea (8.7 mL/kg bw per day); among the high consumers, the highest value was for 'Tea and herbs for infusions, unspecified', with consumption up to 20.8 mL/kg bw per day.

In the young population, for the whole population the mean consumption went up to 5.3 mL/kg bw per day for 'Tea, unspecified', and up to 23 mL/kg bw per day in the high consumers (for 'Tea and herbs for infusions, unspecified'). Among consumers only, the highest mean consumption reached 30 mL/kg bw per day for 'Tea and herbs for infusions, unspecified' while high consumers reported consumption up to 40.4 mL/kg bw per day also for the same food category.

Table 15: Range of chronic consumption of tea and different herbal infusions across dietary surveys in Europe for the adult and young population

	Consumers (%)	Whole population (mL/kg bw per day)		Consumers only (mL/kg bw per day)	
		Mean consumption	95th consumption[a]	Mean consumption	95th consumption[a]
Adult population[b]					
Tea and herbs for infusions, unspecified	2.6–51.9	0.02–3.4	0.0–14.3	0.03–7.2	0.1–20.8
Tea unspecified	0.4–69.9	0.0–2.5	0.0–11.3	0.3–7.4	2.0–17.9
Tea unspecified, decaffeinated	0.3–10.8	0.0–0.9	0.7	1.5–8.4	21.0
Black tea, infusion	0.7–94.8	0.0–8.3	2.1–18.9	0.5–8.7	4.3–19.0
Green tea, infusion	0.9–12.0	0.02–0.6	0.4–4.3	1.0–6.4	6.4–17.4
Camomile flowers	0.2–21.8	0.0–0.7	3.4	0.5–3.7	10.5
Peppermint	0.03–4.0	0.0–0.1	–	0.1–5.0	–
Rooibos leaves	1.4–13.5	0.03–0.6	0.0–2.8	1.8–5.9	5.4–15.8
Young population[c]					
Tea and herbs for infusions, unspecified	0.3–83.8	0.0–5.0	0.1–23.0	0.1–30.0	0.1–40.4
Tea unspecified	0.2–77.5	0.0–5.3	1.9–19.3	0.2–11.3	4.9–31.0
Tea unspecified, decaffeinated	0.2–1.1	0.0–0.4	–	1.4–6.6	–
Black tea, infusion	1.6–19.5	0.02–1.2	0.3–6.0	0.4–11.2	12.0–17.4
Green tea, infusion	0.1–1.6	0.0–0.1	–	0.5–4.8	–
Camomile flowers	0.2–4.1	0.0–0.3	–	2.2–8.5	–
Peppermint	0.5–13.3	0.0–0.4	2.9	0.1–4.4	9.1
Rooibos leaves	2.5–6.0	0.1–0.3	–	2.2–11.5	–
Tea for infants and young children	0.8–29.6	0.0–2.4	–	0.3–17.7	–

bw: body weight.
(a): 95th percentile is not shown when the number of consumers within one specific survey was lower than 60 as this percentile is not statistically significant.
(b): Adult population includes 'Adults', 'Elderly' and 'Very elderly'.
(c): Young population includes 'Infants', 'Toddlers' and 'Other children'.

Acute consumption of tea and herbal infusions (consumption days only)

Table 16 shows the acute consumption of tea and herbal infusions in the European population, divided into adult population ('Adults', 'Elderly' and 'Very elderly') and young population ('Infants', 'Toddlers' and 'Other children'). Acute consumption refers to the total consumption of one specific commodity in one single day, and it is represented as consumption day.

Among the adult population, the average acute consumption for tea and herbal infusions ranged between 0.3 mL/kg bw per day for peppermint and 14.5 mL/kg bw per day for 'Tea and herbs for infusions, unspecified'. For the high consumption days (95th percentile), the values varied between 0.1 mL/kg bw per day for 'Tea and herbs for infusions, unspecified' and 32.3 mL/kg bw per day for 'Tea, unspecified'

In the young population, the acute mean consumptions ranged from 0.1 mL/kg bw per day for 'Tea and herbs for infusions, unspecified' to 33.7 mL/kg bw per day for camomile. Among the high consumption days (95th percentile), the highest value was reported for 'Tea and herbs for infusions, unspecified' with 64.7 mL/kg bw per day with the lowest value also reported for the same food group (0.2 mL/kg bw per day).

Table 16: Range of acute consumption (consumption days only) of tea and different herbal infusions across dietary surveys in Europe for the adult and young population

	Consumption days (%)	Consumption days only (mL/kg bw day)	
		Mean consumption	95th consumption[a]
Adult population[b]			
Tea and herbs for infusions, unspecified	1.6–40.7	0.4–14.5	0.1–26.5
Tea unspecified	0.1–41.3	0.4–10.6	3.9–32.3
Tea unspecified, decaffeinated	0.1–8.3	3.0–10.5	23.3
Black tea, infusion	0.3–89.9	3.1–9.5	5.2–19.9
Green tea, infusion	0.5–8.8	2.1–9.3	9.4–22.8
Camomile flowers	0.03–14.2	3.3–5.7	8.3–14.7
Peppermint	0.01–2.0	0.3–10.0	–
Rooibos leaves	0.7–9.7	3.6–9.3	7.9–23.7
Young population[c]			
Tea and herbs for infusions, unspecified	0.1–64.9	0.1–30.0	0.2–64.7
Tea unspecified	0.1–58.3	0.4–18.1	9.7–38.2
Tea unspecified, decaffeinated	0.1–0.5	3.8–11.7	–
Black tea, infusion	0.5–12.5	0.6–16.8	4.8–43.9
Green tea, infusion	0.04–0.8	2.5–11.3	–
Camomile flowers	0.1–1.0	5.5–33.7	–
Peppermint	0.2–4.5	0.4–13.9	18.2–25.7
Rooibos leaves	1.1–2.3	7.2–25.1	–
Tea for infants and young children	0.4–15.7	0.1–20.3	42.5–56.6

bw: body weight.
(a): 95th percentile is not shown when the number of consumers within one specific survey was lower than 60 as this percentile is not statistically significant.
(b): Adult population includes 'Adults', 'Elderly' and 'Very elderly'.
(c): Young population includes 'Infants', 'Toddlers' and 'Other children'.

3.2.3. Consumption of food supplements

A very limited number of eating occasions (n = 1,818) was available for the food supplements for which analytical results on the presence of PAs was reported (see Table 13), mostly on plant extract formula (n = 1,786) and few on pollen-based supplements (32 eating occasions). A detailed evaluation of the consumption data allowed the identification of specific food supplements for which occurrence data were available. As a result, diverse eating occasions for plant extracts were identified, some of them for which all reported data were left-censored (garlic extracts or ginseng extract, among others). However, several eating occasions of plant extracts with relatively high concentrations of PAs were also identified, such as those of St John's wort (*Hypericum perforatum*) for which an average value of 1,543 μg/kg was reported (n = 7). Other samples for which both occurrence and consumption data were available were extracts of the *Echinacea* sp., sage leaf extracts and artichoke leaf extracts, among others.

Those plant extracts that were not represented in both the consumption and the occurrence databases were not considered in the general scenarios developed to estimate dietary exposure to PAs. However, as mentioned in Section 3.1.3, an additional scenario was carried out to estimate potential exposure to PAs through the consumption of plant extracts with the highest levels of PAs (Section 4.3).

4. Dietary exposure assessment to pyrrolizidine alkaloids

Different dietary exposure scenarios to PAs were considered. Based on the outcome of the 2011 EFSA Scientific opinion on PAs (EFSA CONTAM Panel, 2011), both acute and chronic exposure were assessed.

4.1. Chronic dietary exposure

Two main general scenarios were carried out to estimate chronic dietary exposure to PAs. In both cases, the occurrence data were linked, at the lowest FoodEx level possible, to consumption data from 35 different dietary surveys with at least 2 days assessment (19 different European countries).

In the first general scenario, only the occurrence data submitted by national authorities and those collected through an EFSA Art 36 grant on tea and herbal infusions were considered; these data are described in Table 10. In the second scenario, data on tea and herbal infusions submitted by THIE were used. In both scenarios, occurrence data on other food commodities (e.g. honey, food supplements) as described in Section 3.1 were also considering when estimating dietary exposure to PAs.

4.1.1. Chronic dietary exposure using occurrence data submitted by national authorities and occurrence data collected through an EFSA Art 36 grant

Table 17 shows the summary statistics of the dietary exposure estimates to PAs when using the occurrence data on tea and herbal infusions from national authorities and the EFSA Art 36 grant, together with the other food commodities (honey, food supplements, etc.). Detailed information for each individual dietary survey and each age class is shown in Appendix C.

The median of the mean estimates across the different dietary surveys were, in general, higher in the oldest age classes. However, the highest mean dietary exposure in 'Toddlers' was 48.4 ng/kg bw per day (UB), for the age class 'Very elderly' went up to 41.8 ng/kg bw per day (UB). The highest estimates of mean dietary exposure were rather similar in both the youngest age classes ('Infants' and 'Toddlers') and the oldest age classes ('Elderly', 'Very elderly').

In the highly exposed population, referring to the 95th percentile of the distribution of the exposure for each dietary survey and age class, the highest estimates were in 'Toddlers' for both LB and UB, with values of 153.8 ng/kg bw per day and 214.0 ng/kg bw per day, respectively. The highest estimate of the highly exposed population was also rather high in the age class 'Infants'. The maximum exposure estimates for 'Infants' and 'Toddlers' corresponded to one dietary survey where the main contributor was 'Tea and herbs for infusions, unspecified', with a contribution at the MB exposure scenario of 78–85%, ('Toddlers'-'Infants').

Dietary exposure to PAs in specific groups of the population, namely 'Pregnant women' and 'Lactating women', were within the range of exposure estimates in the adult population.

Detailed information of the main contributors to the dietary exposure to PA among the different age classes across dietary surveys is provided as Supplementary electronic information in Appendix F.

Overall, using the MB estimations in 'Infants' and 'Toddlers', the main average contributors were either 'Tea, unspecified' or 'Tea and herbs for infusions, unspecified'. In 'Infants', the contribution of 'Tea and herbs for infusions, unspecified' showed an average contribution up to 98% (median = 43%), and in 'Toddlers', a maximum of 81%, although in more than half of the dietary surveys did not have any contribution. Regarding 'Tea, unspecified' in 'Infants', the maximum contribution was 95%, with no contribution in four out of six surveys, while in 'Toddlers' went up to 96% but with rather low median contribution (3%). For 'Toddlers', honey was, overall, the main contributor (up to 86%) in those dietary surveys where the consumption of tea and herbal infusion seemed to be secondary. In general, these dietary surveys were those with the lowest estimates of exposure among 'Toddlers'.

In the adult population ('Adults', 'Elderly', 'Very elderly'), the main contributor to the exposure to PAs was tea; the consumption of tea in the different dietary surveys was either reported as 'Tea, unspecified' or as 'Black tea, infusion'. The maximum average contribution reached up to 99% for 'Tea, unspecified' while for 'Black tea, infusion' went up to 90%. As observed in the young population, the lowest estimates of exposure were for those dietary surveys where others foods, particularly honey but also spinaches, became main contributors. Honey was especially important in one country, where its consumption contributed up to 53% to the total exposure to PAs.

Table 17: Summary statistics of chronic dietary exposure assessments to PAs across European dietary surveys, considering the data on tea and herbal infusions submitted by national authorities and those collected through an EFSA Art 36 grant[a]

Age class[b]	N	Lower bound[d]			Upper bound[d]		
		Min	Median	Max	Min	Median	Max
Mean dietary exposure (ng/kg bw per day)							
Infants	6	0.0	4.1	30.2	0.0	5.9	42.8
Toddlers	10	0.0	3.2	34.5	0.0	5.2	48.4
Other children	18	0.7	4.2	24.1	1.2	6.4	34.3
Adolescents	17	0.3	3.7	18.4	0.6	5.7	26.1
Adults	17	0.2	6.7	21.3	0.4	10.6	28.8
Elderly	14	3.0	8.1	29.5	4.3	12.4	39.9
Very elderly	12	3.9	9.2	31.1	5.7	13.9	41.8
95th percentile dietary exposure[c] (ng/kg bw per day)							
Infants	5	0.0	_[e]	133.6	0.0	_[e]	185.2
Toddlers	7	0.0	42.8	153.8	0.0	57.1	214.0
Other children	18	3.3	21.2	90.5	6.3	32.5	125.6
Adolescents	17	0.8	14.6	68.4	2.4	24.6	95.1
Adults	17	1.1	30.1	85.7	2.0	42.9	120.0
Elderly	14	15.3	33.8	87.7	21.4	52.7	123.3
Very elderly	9	15.9	30.8	86.7	22.9	42.8	127.2

bw: body weight; Max: maximum; Min: minimum; N: number of surveys.
(a): Occurrence data on other food commodities (e.g. honey, food supplements) as described in Section 3.1 were also considered when estimating dietary exposure to PAs.
(b): Section 2.1.2.1 describes the age range within each age class.
(c): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.
(d): Estimates were rounded to one decimal place.
(e): A minimum number of six dietary surveys is required to estimate a statistically robust median (EFSA, 2011b).

4.1.2. Chronic dietary exposure using occurrence data submitted by Tea & Herbal Infusions Europe (THIE)

Table 18 shows the summary statistics of the dietary exposure estimates to PAs by using the occurrence data on tea and herbal infusions submitted by THIE (Table 9), and the occurrence data available for other food commodities (honey, food supplements, etc.). Detailed information for each individual dietary survey and each age class is shown in Appendix D

Overall, the estimates of dietary exposure to PAs using the data from THIE were lower than those obtained by using the data submitted by national authorities and those collected through the EFSA Art 36 grant. These differences are expected as relatively high differences are observed in the occurrence data for food commodities that are key contributors to the exposure to PAs, 'Tea and herbs for infusions, unspecified', 'Tea, infusion' and 'Black tea, infusion'. It is important to note that the differences in the estimates using the two occurrence data sets decreased for the UB exposure estimates.

Detailed information of the main contributors to the dietary exposure to PA among the different age classes across dietary surveys is provided as Supplementary electronic information in Appendix F.

Table 18: Summary statistics of chronic dietary exposure assessments to PAs across European dietary surveys, considering the occurrence data on tea and herbal infusions submitted by Tea & Herbal Infusions Europe (THIE)[a]

Age class[b]	N	Lower bound[d]			Upper bound[d]		
		Min	Median	Max	Min	Median	Max
Mean dietary exposure (ng/kg bw per day)							
Infants	6	0.0	0.6	5..5	0.0	3.6	26.6
Toddlers	10	0.0	1.0	6.1	0.0	4.6	29.8
Other children	18	0.2	1.2	4.4	1.0	5.2	23.7
Adolescents	17	0.2	0.7	3.4	0.5	4.4	18.1
Adults	17	0.1	1.2	3.7	0.4	8.1	22.6
Elderly	14	0.7	1.8	5.4	3.4	9.8	31.6
Very elderly	12	0.9	1.8	5.7	4.3	10.9	33.4
95th percentile dietary exposure[c] (ng/kg bw per day)							
Infants	5	0.0	_[e]	19.0	0.0	_[e]	106.2
Toddlers	7	0.0	7.6	23.3	0.0	45.6	131.3
Other children	18	1.3	7.0	14.3	6.3	26.7	77.0
Adolescents	17	0.8	3.7	13.1	2.4	18.5	64.9
Adults	17	0.9	5.4	14.7	1.9	33.7	78.1
Elderly	14	3.0	6.7	14.7	15.9	37.2	78.8
Very elderly	9	4.0	8.2	15.9	18.2	33.9	76.9

bw: body weight; Max: maximum; Min: minimum; N: number of surveys.
(a): Occurrence data on other food commodities (e.g. honey, food supplements) as described in Section 3.1 were also considered when estimating dietary exposure to PAs.
(b): Section 2.1.2.1 describes the age range within each age class.
(c): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.
(d): Estimates were rounded to one decimal place.
(e): A minimum number of six dietary surveys is required to estimate a statistically robust median (EFSA, 2011b).

4.1.3. Chronic dietary exposure, consumers only

The standard scenario considering the whole population and the consumption of all commodities is the most appropriate to reflect chronic exposure to hazardous chemicals. However, this scenario could not be sufficient to protect small subgroups of the population consuming certain foods (e.g. honey) in a regular basis when these foods present relatively high levels of the chemical under investigation. In these circumstances, a scenario considering the exposure among consumers only can be useful to identify possible risks in these subgroups of the population.

When assessing the overall exposure to PAs, tea and herbal infusions are the main sources of exposure due to their high number of consumers, whereas honey is never appearing as an important contributor despite the relatively high levels identified. Considering the relatively high levels of PAs in honey and its possible regular consumption among consumers only, an ad hoc exposure scenario has been considered. As result, Tables 19 and 20 shows dietary exposure estimates considering consumers only, using the two different occurrence data sets that were also used in the general scenarios. Occurrence data used to estimate exposure are those described in Table 10, while the consumption data are depicted in Section 3.2. The Tables 19 and 20 show not only the exposure via the consumption of honey but also for tea and different herbal infusions for which occurrence data are available in order to show the potential risk associated to their consumption.

Looking at Table 19, in the adult population ('Adults', 'Elderly', 'Very elderly'), the mean chronic exposure via the consumption of honey, among consumers, ranged between 0.1 and 7.4 ng/kg bw per day (minimum LB–maximum UB), while for high consumers (95th percentile exposure) was between 9.3 and 17.6 ng/kg bw per day (minimum LB–maximum UB). Higher exposure was estimated among the consumers of honey in the young population ('Infants', 'Toddlers' and 'Other children'). For the average consumers, estimates ranged between 0.3 and 27.0 ng/kg bw per day (minimum LB–maximum UB), and between 0.7 and 31.1 ng/kg bw per day (minimum LB–maximum UB) among the high consumers.

The highest estimates of chronic exposure among consumers only were for 'Tea and herbs for infusions, unspecified'. This is a food category grouping different herbal infusions unspecified, in few cases because no occurrence data were available for specific consumed commodities, but mostly to the fact that the consumption data were reported without specifying the type of herbal infusion consumed. Mean exposure estimates up to 228 ng/kg bw per day (UB) in average consumers, and up to 307 ng/kg bw per day (UB) in high consumers were calculated among the young population.

With the focus on individual types of tea and herbal infusions, the consumption of rooibos seems to lead, somewhat, to the highest estimates of chronic exposure among consumers only. In the chronic scenario, the exposure to PAs for the mean consumption of rooibos in the adult population was up to 36.0–41.3 ng/kg bw per day (LB–UB), estimates that were also similar for black tea and peppermint. Among the high consumers of rooibos, it was up to 96.4–110 ng/kg bw per day (LB–UB). In the young population, the highest mean exposure also came via the consumption of rooibos (70.2–80.5 ng/kg bw per day, LB–UB), while in the highest exposure among high consumers was for black tea (64.4–85.3 ng/kg bw per day, LB–UB). Since the number of consumers of rooibos per dietary survey in the young population was less than 60, their 95th percentile estimates (high consumers) are not shown as they are not statistically robust (EFSA, 2011b). Similar conclusions can be extracted looking at Table 20 (using THIE data) for the food commodities leading to the highest estimates of exposure among consumers only. For tea and herbal infusions, the estimates were relatively lower based on the occurrence data used, with lower levels of PAs. This was particularly evident on the exposure estimation at the LB scenario. The consumption of rooibos led, overall, to the highest estimates of exposure among consumers only in both the young and the adult population. In the young population, the highest exposure went up to 66.7 ng/kg bw per day (UB) in the average consumers, while in the adult population, the highest mean exposure went up to 34.6 ng/kg bw per day (UB) and the highest 95th percentile exposure up to 91.6 ng/kg bw per day.

Table 19: Exposure estimates to PAs across different dietary surveys considering consumers only (Section 3.2), and the data on tea and herbal infusions submitted by national authorities and those collected through an EFSA Art 36 grant (Table 10)

	Range of chronic dietary exposure (ng/kg bw per day)[d], consumers only							
	Adult population[a]				Young population[b]			
	Mean exposure		95th exposure[c]		Mean exposure		95th exposure[c]	
	LB	UB	LB	UB	LB	UB	LB	UB
Tea and herbs for infusions, unspecified	0.2–39.6	0.2–54.7	0.6–114.4	0.8–158.1	0.6–165.0	0.8–228.0	5.5–222.2	7.6–307.0
Tea, unspecified	0.9–22.2	1.5–37.0	6.0–53.7	10.0–89.5	0.6–33.9	1.0–56.5	14.7–93.0	24.5–155.0
Tea unspecified, decaffeinated	0.5–2.5	2.3–12.6	6.3	31.5	0.4–2.0	2.1–9.9	–	–
Black tea, infusion	1.9–32.2	2.5–42.6	15.9–70.3	21.1–93.1	1.5–41.4	2.0–54.9	44.4–64.4	58.8–85.3
Green tea, infusion	2.4–15.4	4.8–30.7	15.4–41.8	30.7–83.5	1.2–11.5	2.4–23.0	–	–
Camomile flowers	1.9–14.1	2.7–19.6	39.9	55.7	8.4–32.3	11.7–45.1	–	–
Peppermint	0.7–34.0	0.8–42.0	–	–	0.7–29.9	0.8–37.0	61.9	76.4
Rooibos leaves	11.0–36.0	12.6–41.3	32.9–96.4	37.8–110.6	13.4–70.2	15.4–80.5	–	–
Tea for infants and young children	–	–	–	–	0.2–10.6	0.4–24.8	–	–
Honey	0.1–3.9	0.3–7.4	0.4–9.3	0.8–17.6	0.3–14.2	0.6–27.0	0.7–16.4	1.4–31.1

bw: body weight; LB: lower bound; UB: upper bound.
(a): Adult population comprises the age classes 'Adults', 'Elderly' and 'Very elderly' across the different dietary surveys.
(b): Young population comprises the age classes 'Infants', 'Toddlers' and 'Other children' across the different dietary surveys.
(c): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.
(d): Estimates were rounded to one decimal place.

Table 20: Exposure estimates to PAs across different dietary surveys considering consumers only (Section 3.2) and the occurrence data on tea and herbal infusions submitted by Tea & Herbal Infusions Europe (THIE) (Table 10)

	Range of chronic dietary exposure (ng/kg bw per day)[d], consumers only							
	Adult population[a]				Young population[b]			
	Mean exposure		95th exposure[c]		Mean exposure		95th exposure[c]	
	LB	UB	LB	UB	LB	UB	LB	UB
Tea and herbs for infusions, unspecified	0.0–4.3	0.1–31.0	0.1–12.5	0.4–89.4	0.1–18.0	0.4–129.0	0.6–24.2	4.3–173.7
Tea, unspecified	0.2–3.7	1.1–28.1	1.0–9.0	7.6–68.0	0.1–5.7	0.8–42.9	2.5–15.5	18.6–117.8
Tea unspecified, decaffeinated	–	4.4–24.4	–	60.9	0.0–0.0	4.1–19.1	–	–
Black tea, infusion	0.3–5.2	2.0–33.9	2.6–11.4	16.8–74.1	0.2–6.7	1.6–43.7	7.2–10.4	46.8–67.9
Green tea, infusion	0.3–1.9	3.6–23.0	1.9–5.2	23.0–62.6	0.2–1.4	1.8–17.3	–	–
Camomile flowers	0.5–3.7	2.2–15.9	10.5	45.2	2.2–8.5	9.5–36.6	–	–
Peppermint	0.6–6.0	2.4–23.5	–	–	0.1–5.3	0.5–20.7	10.9	42.8
Rooibos leaves	4.9–15.9	10.4–34.2	14.6–42.7	31.3–91.6	5.9–31.1	12.8–66.7	–	–

PA: pyrrolizidine alkaloid; bw: body weight; LB: lower bound; UB: upper bound.
(a): Adult population comprises the age classes 'Adults', 'Elderly' and 'Very elderly' across the different dietary surveys.
(b): Young population comprises the age classes 'Infants', 'Toddlers' and 'Other children' across the different dietary surveys.
(c): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.
(d): Estimates were rounded to one decimal place.

4.2. Acute dietary exposure, consumption days only

Acute dietary exposure to PAs was estimated following a conservative approach considering the presence of high contamination levels in all the different food commodities (occurrence values at the highest reliable percentile,[3] UB estimate), combined with the total daily consumption amount for each corresponding food (consuming days only). A total of 41 different and most recent dietary surveys carried out in 23 different European countries were used.

In comparison to the previous Section 4.1, where chronic consumption in consumers only was estimated separately for different foods to identify possible risks in small subgroups of the population that consume these foods on a regular basis (e.g. honey), this scenario estimates the exposure adding up all consumed foods during one single day. Estimations of acute dietary exposure were carried out using the occurrence data set generated with the data submitted by national authorities and those collected through an EFSA Art 36 grant. The highest reliable percentile (UB estimate) for the most important food commodities were as follows: 'Black tea infusion' (95th percentile = 18 μg/L), 'Green tea, infusion' (95th percentile = 14.4 μg/L), 'Camomile flowers' (95th percentile = 16.2 μg/L), 'Peppermint' (95th percentile = 13.2 μg/L), 'Rooibos leaves' (95th percentile = 15.4 μg/L), 'Tea and herbs for infusions, unspecified' (95th percentile = 16.3 μg/L) and 'Tea, unspecified' (95th percentile = 15.4 μg/L).

Table 21 shows the summary statistics of the acute exposure to PAs among consuming days only (UB estimates). Highest estimates of acute mean exposure and 95th percentile exposure were calculated for 'Toddlers', with mean acute exposure to PAs up to 310.8 ng/kg bw per day and 95th percentile exposure up to 820.5 ng/kg bw per day.

[3] The selection of the highest reliable percentiles was based on the number of samples available, 60 samples for the 5th and 95th percentile, 11 samples for 25th and 75th percentile, and six samples for the median. Otherwise, the percentiles may not be statistically robust.

Table 21: Summary statistics of acute dietary exposure to PAs (consumption days only) considering the data on tea and herbal infusions submitted by national authorities and those collected through an EFSA Art 36 grant (occurrence values at the highest reliable percentile, UB estimate)

Age class[d]	Acute dietary exposure (ng/kg bw per day, consumption days only)[a],[b]							
	Upper bound estimations							
	Mean exposure				95th percentile dietary exposure[c]			
	N	Min	Median	Max	N	Min	Median	Max
Infants	6	1.2	98.7	212.8	5	154.1	–[e]	719.7
Toddlers	10	1.7	109.6	310.8	7	261.8	419.4	820.5
Other children	18	19.6	88.2	248.1	18	125.5	276.6	505.1
Adolescents	17	8.9	71.5	151.5	17	90.4	194.5	415.0
Adults	17	5.8	86.8	159.9	17	22.4	226.4	495.5
Elderly	14	29.2	76.7	161.9	14	81.5	234.2	388.9
Very elderly	12	33.1	84.1	168.1	9	110.4	218.1	382.8

PA: pyrrolizidine alkaloid; bw: body weight; Max: maximum; Min: minimum; N: number of surveys.
(a): Occurrence data on other food commodities (e.g. honey, food supplements) as described in Section 3.1 were also considered when estimating acute dietary exposure to PAs.
(b): Estimates were rounded to one decimal place.
(c): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.
(d): Section 2.1.2.1 describes the age range within each age class.
(e): A minimum number of six dietary surveys is required to estimate a statistically robust median (EFSA, 2011b).

As observed for the estimates of chronic dietary exposure, using the occurrence data set from THIE results in lower acute exposure estimates as compared to those described in Table 21. Highest estimates of acute mean exposure and 95th percentile exposure were also calculated for 'Toddlers', with mean acute exposure to PAs up to 147.8 ng/kg bw per day and 95th percentile exposure up to 413.8 ng/kg bw per day.

4.3. Dietary exposure through the consumption of plant extracts and pollen-based supplements

The occurrence data reported for 'Plant extracts' were diverse and with a wide range of concentrations of PAs. When there were available consumption data, exact linking was made with the occurrence data, and the plant extracts were included in the general scenarios of exposure. However, for several of the plant extracts with PAs levels, no specific consumption data were available so they were excluded to avoid introducing uncertainty in the exposure estimation. Some of these plant extracts featured very high levels of PAs as compared to the levels reported for tea and herbal infusions (see Table 14), and their consumption, therefore, could lead to high exposure to PAs.

In the absence of consumption data, the potential exposure from the plant extracts intended to be consumed as infusions was estimated using a single consumption of 150 mL infusion of 2 g of plant extract (dilution factor 1/75 as used for tea and herbal infusions). With this consumption, the estimated exposure to PAs via this particular plant extracts would range between 800 ng/kg bw per day for one sample of mix herbs, among them *Pulmonaria officinalis,* and 67,000 ng/kg bw per day for one sample of Borage (*Borago officinalis*). Likewise, for two samples of plant extracts reported to be ingested directly (as capsules/tablets), the consumption of 1 unit (standard weight 0.75 g) would imply exposure to PAs of 808,160 ng/kg bw per day for Boneset (*Eupatorium perfoliatum*) and 1,807,706 ng/kg bw per day for Hemp-agrimony (*Eupatorium cannabinum*).

Regarding pollen-based supplements, as commented in the consumption section, only 32 eating occasions are available in the consumption database. Based on these data and the reported occurrence data, some rough estimations of the exposure to PAs via the consumption of pollen-based supplements were done. For chronic consumption, using the occurrence value, the reported mean concentration (LB = 235 μg/kg, UB = 253 μg/kg), the chronic exposure would range between 0.7 and 11.5 ng/kg bw per day (minimum LB–maximum UB) among consumers only. The acute exposure, using as occurrence value the highest reliable percentile (LB-P90 = 966 μg/kg, UB-P90 = 974 μg/kg), would be between 2.8 and 43.9 ng/kg bw per day (minimum LB–maximum UB) among consumers only.

4.4. Potential dietary exposure at hypothetical PAs concentrations

The influence on the dietary exposure to PAs when consuming particular food commodities with selected concentrations was assessed. These concentrations were based on the performance of the reported analytical methods and, when possible, on the distribution of the reported occurrence data. The targeted food commodities were tea, herbal infusions and honey, all of them key contributors to the exposure to PAs.

As commented above, the most recent data seems to indicate somehow lower levels of PAs in different teas and herbal infusions as compared to the samples collected in the previous years (Table 10). However, this is more evident at the LB scenario which opens the possibility that this trend is not due to a decrease in the levels but rather to differences in the sensitivity of the applied methods. Sensitive analytical methods are required to avoid situations where LB scenarios are zero or close to zero, while the UB scenarios show relatively high concentrations of PAs. Considering the two studies that analysed the 28 PAs and reported the lowest LOQs (see Table 12), if all PAs were at levels below the LOQ, the UB scenario for the total concentration of PAs would be between 53 and 98.5 μg/kg in the dry product (0.7 μg/L and 1.3 μg/L in the prepared infusions). Regarding honey, and with the focus on the samples that reported the data set of 8 PAs described in Section 3.1.1, the lowest UB concentration that could be reported for a sample of honey with the eight PAs below the LOQ would be 3.6 μg/kg.

Table 22 shows the estimations of exposure among consumers only of tea and herbal infusion, and among consumers of honey only when chronic consumption data are combined with minimum UB concentrations that could be reported for these food commodities based on the sensitivity of the analytical methods. Additional chronic exposure estimations, also for honey consumers only, were carried out assuming a concentration of 5 μg/kg based on the median concentration (LB) among all reported samples of honey.

For tea and herbal infusions, based on the current sensitivity of the reported analytical methods for 28 PAs, UB concentrations below 53 μg/kg (0.7 μg/L) cannot be excluded, what implies mean exposure levels in the young population up to 21.0 ng/kg bw per day and up to 28.3 ng/kg bw per day among the highly exposed consumers, in both cases for consumers only of 'Tea and herbs for infusion, unspecified' (for more details see Table 22).

The chronic consumption of honey, at hypothetical PA concentrations of 3.6–5 μg/kg, led to maximum mean exposure estimations among consumers that ranged between 3.5 and 4.9 ng/kg bw per day, and between 1.0 and 1.4 ng/kg bw per day, in the adult and young population, respectively. For the same PA concentrations, the highest exposures among high consumers of honey ranged between 2.3 and 3.2 ng/kg bw per day, and between 4.1 and 5.7 ng/kg bw per day in the adult and young population, respectively.

Table 22: Chronic exposure estimates to PAs across different dietary surveys considering consumers only and selected concentrations of PAs in different food commodities as described in the text

	Concentration of PAs (μg/L)	Young population[a]		Adult population[b]	
		Mean exposure	95th exposure[c]	Mean exposure	95th exposure[c]
		ng/kg bw per day[d]		ng/kg bw per day[d]	
Tea and herbs for infusions, unspecified	0.7	0.1–21.0	0.7–28.3	0.0–5.0	0.1–14.6
Tea unspecified	0.7	0.1–7.9	3.4–21.7	0.2–5.2	1.4–12.5
Tea unspecified, decaffeinated	0.7	1.0–4.6	–	1.1–5.9	14.7
Black tea, infusion	0.7	0.3–7.8	8.4–12.2	0.4–6.1	3.0–13.3
Green tea, infusion	0.7	0.4–3.4	–	0.7–4.5	4.5–12.2
Camomile flowers	0.7	1.5–6.0	–	0.4–2.6	7.4–7.4
Peppermint	0.7	0.1–3.1	6.4–6.4	0.1–3.5	–
Rooibos leaves	0.7	1.5–8.1	–	1.3–4.1	3.8–11.1
Tea for infants and young children	0.7	0.2–12.4	–	–	–
Honey	3.6[e]	0.1–3.5	0.2–4.1	0.0–1.0	0.1–2.3

	Concentration of PAs (μg/L)	Young population[a]		Adult population[b]	
		Mean exposure	95th exposure[c]	Mean exposure	95th exposure[c]
		ng/kg bw per day[d]		ng/kg bw per day[d]	
Tea and herbs for infusions, unspecified	1.3	0.1–39.0	1.3–52.5	0.0–9.4	0.1–27.0
Tea unspecified,	1.3	0.3–14.7	6.4–40.3	0.4–9.6	2.6–23.3
Tea unspecified, decaffeinated	1.3	1.8–8.6	–	2.0–10.9	27.3
Black tea, infusion	1.3	0.5–14.6	15.6–22.6	0.7–11.3	5.6–24.7
Green tea, infusion	1.3	0.7–6.2	–	1.3–8.3	8.3–22.6
Camomile flowers	1.3	2.9–11.1	–	0.7–4.8	13.7
Peppermint	1.3	0.1–5.7	11.8–11.8	0.1–6.5	–
Rooibos leaves	1.3	2.9–15.0	–	2.3–7.7	7.0–20.5
Tea for infants and young children	1.3	0.4–23.0	–	–	–
Honey	5[e]	0.1–4.9	0.3–5.7	0.1–1.4	0.2–3.2

PA: pyrrolizidine alkaloid; bw: body weight.
(a): Young population comprises the age classes 'Infants', 'Toddlers' and 'Other children' across the different dietary surveys.
(b): Adult population comprises the age classes 'Adults', 'Elderly' and 'Very elderly' across the different dietary surveys.
(c): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.
(d): Estimates were rounded to one decimal place.
(e): The concentration of PAs in honey refers to μg/kg.

5. Uncertainty

A qualitative evaluation of the inherent uncertainties in the assessment of the dietary exposure to PAs was performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006).

Considering the relevant 28 PAs provisionally selected by the European Commission, a final data set of 4,581 samples of plant origin was available for exposure estimations. The total content of PAs in each food sample was estimated adding up the reported amount for each individual PA analysed. To avoid underestimation on the presence of PAs, only those samples with a minimum number of PAs were included in the final data set; this number was selected after a comprehensive evaluation of the occurrence data in each type of food commodity. As there were samples in the final data set that did not contain analytical data for all 28 PAs, this may cause some underestimation in the dietary exposure to PAs.

A total of 1,002 samples were codified as 'Tea and herbs for infusions, unspecified'. This food category covers a very heterogeneous group of samples that includes mix of herbal infusions, mixes of tea and herbal infusions, unspecified herbal infusions, and herbal infusions that could not be classified under FoodEx. This adds uncertainty to the estimations of exposure via the consumption of 'Tea and herbs for infusions, unspecified'.

The large proportion of left-censored data introduces uncertainty to the overall dietary exposure estimates. In total, 90% of the analytical results were left-censored, and for 40% of the samples, the levels for all measured PAs were left-censored. This is particular important in this case as the concentration of PAs in the samples is the result of adding up the individual levels of up to 28 PAs. In this situation, the reported LOQ has a large influence on the calculation of the UB levels, and large variances in LOQs were seen for the different analytical methods. While the LB values tend to underestimate the estimations of dietary exposure to PAs, UB values tend to overestimate it.

Uncertainty is also associated to how accurately the concentration of PAs reported in the samples of tea and herbal infusions represents the amounts of contaminants the consumers are exposed to. Different methods are used to extract the PAs present in tea and herbal infusions previous to their analysis, and there is uncertainty on how these methods represent the different ways the consumers use to prepare tea and herbal infusions. In addition, many different factors, such as water temperature, water-to-tea ratio, infusion time, stirring, and dosage form (loose leaf and tea bag), may have influence on the extraction of PAs during consumer preparation.

Uncertainties and limitations related to the use of the EFSA Comprehensive Food Consumption Database have already been described in EFSA (EFSA, 2011b) and are not further detailed; only those with a particular repercussion on the dietary exposure to PAs are mentioned here. Among them is the consumption data on tea and herbal infusions reported as unspecified (~ 55% considering all dietary surveys), either as 'Tea and herbs for infusions, unspecified' or 'Tea, unspecified'. The latter food category includes, among others, many eating occasions of ready-to-drink tea (ice tea-type drinks) that may possess variable amount of tea in its composition (Section 3.2.2). This adds uncertainty also to the estimations of exposure via the consumption of 'Tea, unspecified' together with the above mentioned uncertainty coming via the consumption of 'Tea and herbs for infusions, unspecified' (Table 23).

Table 23: Summary of the qualitative evaluation of the impact of uncertainties on the dietary exposure to PAs

Sources of uncertainty	Direction[(a)]
Measurement uncertainty of analytical results associated to the methods of analysis	±
Using the substitution method at the lower bound (LB) scenario	–
Using the substitution method at the upper bound (UB) scenario	+
Samples in the final data set that did not contain analytical data for all 28 PAs	–
Representativity of occurrence data to the whole of Europe	±
High number of samples reported as 'Tea and herbs for infusions, unspecified'	±
Eating occasions of ready-to-drink tea (ice tea-type drinks) all classified as 'Tea, unspecified'	+
High number of eating occasions on tea and herbal infusions reported as unspecified ('Tea and herbs for infusions, unspecified' or 'Tea, unspecified')	±
Extrapolation of analytical methods to the domestic preparation of tea and herbal infusions	±
Variability on the domestic preparation of tea and herbal infusions	±
Estimation of acute dietary exposure using occurrence values at the highest reliable percentile (UB estimate) for all foods	+

(a): + = uncertainty with potential to cause over-estimation of exposure; – = uncertainty with potential to cause underestimation of exposure.

Overall, the dietary exposure to PAs calculated in this report is likely to overestimate the exposure levels of the European population.

6. Conclusions

- Based on the different data sets reported, for tea and herbal infusions, samples with a minimum of 17 and a maximum of 28 analysed PAs were selected to estimate dietary exposure to PAs.
- For honey, the number of PAs per sample in the final data set varied between 8 and 19, based on the common PAs reported in all the samples used in the EFSA 2011 opinion, and the recently reported samples analysed for at least echimidine, lycopsamine, and senecionine.
- Among the samples of retail honey, the main contributors to the total PA concentration in each sample were, on average, echimidine (44%) and lycopsamine (37%).
- Among the samples of tea and herbal infusions, the main contributors to the total PA concentration were, on average: lycopsamine, intermedine, intermedine-*N*-oxide, senecionine, senecionine-*N*-oxide, seneci(o)phylline, seneciphylline-*N*-oxide, and retrorsine-*N*-oxide.
- Taking into account the final data set, in black tea these eight PAs represented, on average, 95% of the total PA concentration, 92% in samples of rooibos, 90% in samples of camomile, 83% in samples of peppermint and 78% in green tea.
- Looking at the contribution of these eight PAs as compared to the 28 PAs in those samples where all 28 PAs were analysed, similar average contributions were observed, with contributions below 60% of the total, in only 25% of the samples of peppermint, in 17% of the samples of camomile, in 14% of the samples of green tea, in 8% of the samples of black tea and in only 4% of the samples of rooibos.
- An apparent downwards trend was noted in the levels of PAs in tea and herbal infusions, based on some of the most recently reported levels. This is observed, in particular, at the LB scenario, indicating that this trend may be influenced by the lack of sensitivity of the analytical methods.

- Considering all food commodities, and the data on tea and herbal infusions submitted by MS and through an EFSA Article 36 grant, the highest estimates of mean chronic dietary exposure to PAs in the young population ('Infants', 'Toddlers' and 'Other children') were 34.5–48.4 ng/kg bw per day (LB–UB) and 31.1–41.8 ng/kg bw per day (LB–UB) in the adult population ('Adults', 'Elderly', 'Very elderly'). In the highly exposed population, the highest estimates were 153.8–214.0 ng/kg bw per day and 87.7–127.2 ng/kg bw per day (LB–UB) in the young and the adult population, respectively.
- When using the data on tea and herbal infusions submitted by Tea & Herbal Infusions Europe (THIE), the estimates of chronic exposure were lower as compared to the previous scenario. The highest estimates of mean chronic dietary exposure to PAs in the young population (LB–UB) were 6.1–29.8 ng/kg bw per day and 5.7–33.4 ng/kg bw per day in the adult population. In the highly exposed population, the highest estimates were 23.3–131.6 ng/kg bw per day and 15.9–78.8 ng/kg bw per day (LB–UB) in the young and the adult population, respectively.
- Overall, in 'Infants' and 'Toddlers', the main average contributors were either 'Tea, unspecified' or 'Tea and herbs for infusions, unspecified'. In the adult population, the main contributor to the exposure to PAs was tea; either reported as 'Tea, unspecified' or as 'Black tea, infusion'.
- In the adult population, the mean chronic exposure via the consumption of honey, among consumers only, ranged between 0.1 and 7.4 ng/kg bw per day (minimum LB–maximum UB), while for high consumers (95th percentile exposure), it was between 9.3 and 17.6 ng/kg bw per day (minimum LB–maximum UB). In the young population, for the average consumers estimates ranged between 0.3 and 27.0 ng/kg bw per day (minimum LB–maximum UB), and between 0.7 and 31.1 ng/kg bw per day (minimum LB–maximum UB) among the high consumers.
- The highest estimates of acute mean and high (95th percentile) exposure were calculated for 'Toddlers', being up to 311 ng/kg bw per day and up to 821 ng/kg bw per day, respectively.
- The consumption of 150 mL infusion of 2 g of certain plant extracts with relatively high PA levels can lead to exposure to PAs from 800 ng/kg bw per day for one infusion of mixed herbs to 67,000 ng/kg bw per day for one infusion of Borage.
- Chronic exposure to PAs via the consumption of pollen-based supplements ranged between 0.7 and 11.5 ng/kg bw per day (minimum LB–maximum UB), while the acute exposure was between 2.8 and 43.9 ng/kg bw per day (minimum LB–maximum UB) in both cases among consumers only.
- On estimating dietary exposure to 28 PAs, the UB scenario is highly influenced by the sensitivity of the analytical methods. Based on the current sensitivity of the reported analytical methods, lowest UB concentrations of 53 μg/kg (0.7 μg/L) can be achieved for tea and herbal infusions. This implies mean chronic exposure levels up to 6.1–21.0 ng/kg bw per day, and up to 14.6–28.3 ng/kg bw per day among the highly exposed consumers (adult–young population), depending on the tea and herbal infusion consumed.
- For honey, the lowest UB concentration that could be reported with all eight PAs at levels below the LOQ would be 3.6 μg/kg. This would lead to mean chronic exposure estimations up to 3.5 ng/kg bw per day and up to 4.1 ng/kg bw per day among the highly exposed consumers.

Recommendations

- To develop more sensitive analytical methods allowing the reduction in UB levels, and define performance criteria for the analysis of the most relevant PAs in food. Alternatively, to identify the most relevant PAs in the different food commodities to be included in the UB scenario.
- To continue ongoing efforts to collect analytical data on the occurrence of PAs in relevant food commodities such as honey, but in particular on tea and herbal infusions (including 'Tea for infants and young children') to confirm the downwards trend in PA levels on the most recently reported samples.
- Data on the occurrence of PAs in herbal food supplements other than plant extracts should be collected.
- To obtain information on the sources of PAs in tea, i.e. the weeds responsible for the contamination and develop adequate measures to control weed infestation.

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Abbreviations

BfR	Federal Institute for Risk Assessment
$BMDL_{10}$	benchmark dose lower confidence limit for a 10 % excess cancer risk

bw	body weight
CAS	Chemical Abstracts Service
CONTAM	EFSA Panel on Contaminants in the Food Chain
DHP	dihydropyrrolizine
FAO/WHO	Food and Agriculture Organization/World Health Organization
HVOD	hepatic veno-occlusive disease
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
IRTA	Institute for Research and Technology in Food and Agriculture
LB	lower bound
LC–HG–AFS	liquid chromatography–hydride generation–atomic fluorescence spectrometry detection
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MB	middle bound
ML	maximum level
MOE	Margin of Exposure
MSs	EU Member State
PA	pyrrolizidine alkaloids
SOP	standard operating procedure
THIE	Tea & Herbal Infusions Europe
UB	upper bound

Appendix A – Dietary surveys used for the estimation of dietary exposure to pyrrolizidine alkaloids

Country	Survey acronym	Survey period	No of days per subject	No of subjects/No of days						
				Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
Austria	ASNS – Adults	2010–2012	2	–	–	–	–	308/726	67/181	25/85
	ASNS – Children	2010–2012	3	–	–	128/384	237/706	–	–	–
Belgium	Regional Flanders	2002–2002	3	–	36/108	625/1,875	–	–	–	–
	Diet National 2004	2004	2	–	–	–	576/1,187	1,292/2,648	511/1,045	704/1,408
Bulgaria	NSFIN	2004	1	–	–	–	–/162	–/691	–/151	–/200
	NUTRICHILD	2007	2	861/1,720	428/856	433/867	–	–	–	–
Cyprus	Childhealth	2003	3	–	–	–	303/909	–	–	–
Czech Republic	SISP04	2003–2004	2	–	–	389/778	298/596	1,666/3,332	–	–
Denmark	DANSDA 2005-08	2005–2008	7	–	–	298/2,085	377/2,622	1,739/12,127	274/1,916	12/84
	IAT 2006 07	2006–2007	7	826/5,771	917/6,388	–	–	–	–	–
Estonia	NDS 1997	1997	1	–	–	–	–	–/1,866	–	–
Finland	DIPP 2001 2009	2001–2009	3	500/1,500	500/1,500	750/2,250	–	–	–	–
	NWSSP07 08	2007–2008	4	–	–	–	306/1,186	–	–	–
	FINDIET2012	2012	2	–	–	–	–	1,295/2,590	413/826	–
France	INCA2	2007	7	–	–	482/3,315	973/6,728	2,276/15,727	264/1,824	84/571
Germany	VELS	2001–2002	6	159/927	348/1,947	293/1,610	–	–	–	–
	EsKiMo	2006	3	–	–	835/2,498	393/1,179	–	–	–
	National Nutrition Survey II	2007	2	–	–	–	1011/2,022	10,419/20,838	2,006/4,012	490/980
Greece	Regional Crete	2004–2005	3	–	–	838/2,508	–	–	–	–
	DIET LACTATION GR	2005–2007	3	–	–	–	–	65/350	–	–
Hungary	National Repr Surv	2003	3	–	–	–	–	1,074/3,222	206/618	80/240
Ireland	NANS 2012	2008–2010	4	–	–	–	–	1,274/5,096	149/596	77/308
Italy	INRAN SCAI 2005 06	2005–2006	3	16/48	36/108	193/579	247/741	2,313/6,939	290/870	228/684
Latvia	EFSA TEST	2008	2	–	–	187/377	453/979	1,271/2,655	–	–
	FC PREGNANTWOMEN 2011	2011	2	–	–	–	–	1,002/2,005	–	–

Country	Survey acronym	Survey period	No of days per subject	No of subjects/No of days						
				Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
Netherlands	VCP kids	2006–2007	3	–	322/644	957/1,914	–	–	–	–
	VCPBasis AVL2007 2010	2007–2010	2	–	–	447/894	1,142/2,284	2,057/4,114	173/346	
	VCP-Elderly	2010–2012	2	–	–	–	–	–	289/578	450/900
Poland	IZZ FAO 2000	2000	1	–	–/79	–/409	–/666	–/2,527	–/329	–/124
Romania	Dieta Pilot Children	2012	1	–	–	–/205	–/567	–	–	–
	Dieta Pilot Adults	2012	7	–	–	–	–	1,254/8,770	83/581	45/315
Slovakia	SK MON 2008	2008	1	–	–	–	–	2,761	–	–
Slovenia	CRP 2008	2007–2008	1	–	–	–	–	407	–	–
Spain	enKid	1998–2000	2	–	17/34	156/312	209/418	–	–	–
	AESAN	1999–2001	3	–	–	–	–	410/828	–	–
	NUT INK05	2004–2005	2	–	–	399/798	651/1,302	–	–	–
	AESAN FIAB	2009	3	–	–	–	86/226	981/2,748	–	–
Sweden	NFA	2003	4	–	–	1,473/5,875	1,018/4,047	–	–	–
	Riksmaten 2010	2010–2011	4	–	–	–	–	1,430/5,680	295/1,167	72/288
United Kingdom	NDNS-RollingProgrammeYears1-3	2008–2011	4	–	185/737	651/2,595	666/2,653	1,266/5,040	166/662	139/552
	DNSIYC 2011	2011	4	1,369/5,446	1,314/5,217	–	–	–	–	–

Appendix B – Levels of pyrrolizidine alkaloids in the dry product (μg/kg) for different types of tea and herbal teas

Table B.1: Levels of pyrrolizidine alkaloids in the dry product (μg/kg) of different types of tea and herbal teas, and samples of 'Tea for infants and young children'

	N[(a)]	Variable	Mean	Percentiles[(b),(c)]				
				P5	P25	Median	P75	P95
Tea and herbal teas								
Tea and herbs for infusions, unspecified	1,002	Lower bound	133	0	0	0	61	408
		Middle bound	257	67	130	140	230	478
		Upper bound	381	134	260	270	380	580
Tea, unspecified	14	Lower bound	70	–	0	10	108	–
		Middle bound	293	–	230	237	328	–
		Upper bound	515	–	460	465	548	–
Tea, decaffeinated	12	Lower bound	11	–	0	0	14	–
		Middle bound	93	–	55	90	123	–
		Upper bound	174	–	102	132	241	–
Black tea, infusion	339	Lower bound	116	0	0	0	39	568
		Middle bound	216	50	88	130	181	669
		Upper bound	315	95	148	260	287	770
Green tea, infusion	310	Lower bound	57	0	0	0	12	276
		Middle bound	172	47	130	130	173	317
		Upper bound	287	61	250	260	282	460
Camomile flowers	256	Lower bound	173	0	0	29	165	735
		Middle bound	265	20	110	147	293	778
		Upper bound	358	35	207	270	432	836
Peppermint	196	Lower bound	263	0	0	31	119	766
		Middle bound	365	54	135	152	230	827
		Upper bound	467	100	218	270	372	887
Hibiscus flowers	6	Lower bound	1	–	–	0	–	–
		Middle bound	154	–	–	179	–	–
		Upper bound	306	–	–	358	–	–
Rose petals	1	Lower bound	0	–	–	–	–	–
		Middle bound	110	–	–	–	–	–
		Upper bound	220	–	–	–	–	–
Lime (linden)	1	Lower bound	0	–	–	–	–	–
		Middle bound	223	–	–	–	–	–
		Upper bound	445	–	–	–	–	–
Rooibos leaves	167	Lower bound	305	0	84	169	330	774
		Middle bound	388	50	166	274	403	799
		Upper bound	471	89	270	384	499	845
Yerba mate	3	Lower bound	32	–	–	–	–	–
		Middle bound	162	–	–	–	–	–
		Upper bound	292	–	–	–	–	–
Fruit juice and herbal tea for infants and young children								
Tea for infants and young children	39	Lower bound	47	–	0	16	71	–
		Middle bound	75	–	9	61	120	–
		Upper bound	103	–	13	87	163	–

(a): Number of samples.
(b): P5/P25/75/95: 5th/25th/50th/75th/95th percentiles.
(c): The estimation of high percentiles is not reliable when too few observations are available (less than 11 for the P75, 29 for the P90, 60 for the P95 and 298 for the P99).

Appendix C – Chronic dietary exposure to pyrrolizidine alkaloids (without using THIE occurrence data)

Table C.1: Mean and 95th percentile (P95) chronic dietary exposure[(a)] to pyrrolizidine alkaloids (ng/kg bw per day) for total population in lower bound (LB) and upper bound (UB) scenarios, considering the data on tea and herbal infusions submitted by national authorities and those collected through an EFSA Art 36 grant

Dietary surveys[(c)]	Range of dietary exposure (LB–UB) (ng/kg bw per day)[(a)]													
	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
ASNS – Adults	–	–	–	–	–	–	–	–	0.2–0.4	1.1–2.0	11.4–16.9	40.0–55.6	19.2–28.3	–[(b)]
ASNS – Children	–	–	–	–	13.2–20.2	49.1–74.1	8.0–12.6	37.5–64.1	–	–	–	–	–	–
Regional Flanders	–	–	0.1–0.5	–[(b)]	1.9–3.3	10.7–18.0	–	–	–	–	–	–	–	–
Diet National 2004	–	–	–	–	–		4.4–7.2	23.4–38.5	0.4–0.8	2.0–4.2	5.8–8.7	35.9–50.2	5.0–7.2	30.8–42.8
NUTRICHILD	13.6–20.0	65.1–90.9	16.8–24.8	62.1–89.3	13.4–19.6	50.9–73.1	–	–	–	–	–	–		–
Childhealth	–	–	–	–	–	–	1.7–2.9	9.1–16.1	–	–	–	–	–	
SISP04	–	–	–	–	6.1–10.5	18.6–31.7	4.4–7.5	13.2–23.0	2.6–3.9	14.6–20.9	–	–		–
DANSDA 2005-08	–	–	–	–	1.8–3.0	10.4–17.6	2.8–4.7	14.6–24.6	3.4–5.5	12.7–20.9	6.4–10.9	31.8–53.4	7.4–12.5	–[(b)]
IAT 2006 07	1.0–1.6	3.0–5.0	1.8–3.1	9.8–16.4	–	–	–	–	–	–	–	–	–	–
DIPP 2001 2009	–	–	–	–	1.7–1.3	5.2–10.4	–	–	–	–	–	–	–	–
NWSSP07 08	–	–	–	–	–	–	1.9–3.3	11.7–20.5	–	–	–	–	–	–
FINDIET2012	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	–	–	–	–	6.2–10.6	32.1–53.9	4.5–7.8	19.5–32.9	–	–
INCA2	–	–	–	–	1.5–2.8	8.7–14.6	1.9–3.2	10.4–17.9	6.7–11.3	33.6–56.4	7.1–12.2	30.9–51.9	3.9–6.9	19.4–32.5
VELS	30.2–42.8	133.6–185.2	34.5–48.4	153.8–214.0	24.1–34.3	90.5–125.6	–	–	–	–	–	–	–	–
EsKiMo	–	–	–	–	21.4–30.7	80.6–115.2	18.4–26.1	68.4–95.1	–	–	–	–	–	–
National Nutrition Survey II	–	–	–	–			14.6–21.4	66.2–91.7	3.0–5.1	10.1–17.3	24.0–34.2	87.7–123.3	26.5–37.6	86.7–127.2
Regional Crete	–	–	–	–	1.4–2.5	7.3–13.9	–	–	–	–	–	–	–	–
DIET LACTATION GR	–	–	–	–	–	–	2.6–4.7	9.9–17.3	2.6–4.7	9.9–17.3	–	–	–	–
National Repr Surv	–	–	–	–	–	–	–	–	8.6–13.9	23.7–39.1	9.0–12.5	23.1–34.9	11.0–15.2	28.9–41.9
NANS 2012	–	–	–	–	–	–	–	–	9.6–13.3	31.0–41.7	29.5–39.9	64.5–86.0	31.1–41.8	69.3–92.3
INRAN SCAI 2005 06	7.3–10.2	–[(b)]	4.5–7.3	–[(b)]	4.6–7.4	27.5–38.6	3.7–5.7	19.5–28.8	13.5–19.9	56.1–80.7	3.0–4.3	15.3–21.4	3.9–5.7	15.9–22.9
EFSA TEST	–	–	–	–	19.1–31.2	44.7–75.1	14.6–23.7	38.6–62.3	18.6–26.4	70.2–96.6	–	–	–	–
FC PREGNANTWOMEN 2011	–	–	–	–	–	–	14.9–25.3	34.6–58.0	14.9–25.3	34.6–58.0	–	–	–	–
VCP kids	–	–	9.3–15.8	57.5–96.6	9.0–15.3	44.5–73.8	–	–	–	–	–	–	–	–

Dietary surveys[c]	Range of dietary exposure (LB–UB) (ng/kg bw per day)[a]													
	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
VCPBasis AVL2007 2010	–	–	–	–	11.8–17.7	53.1–78.7	12.0–18.0	47.4–68.6	19.6–26.9	59.5–81.7	17.3–24.1	56.8–80.2	–	–
VCP-Elderly	–	–	–	–	–	–	–	–	–	–	21.5–29.7	65.4–90.0	22.7–31.2	54.4–71.3
Dieta Pilot Adults	–	–	–	–	–	–	–	–	20.2–28.6	85.7–120.0	6.7–10.8	19.2–29.0	6.8–11.3	–[b]
enKid	–	–	0.3–0.8	–[b]	0.7–1.2	3.3–6.3	0.4–0.7	0.8–2.4	–	–	–	–	–	–
AESAN	–	–	–	–	–	–	–	–	5.5–8.4	29.5–42.9	–	–	–	–
NUT INK05	–	–	–	–	0.7–1.2	3.5–6.6	0.6–0.9	4.0–7.7	–	–	–	–	–	–
AESAN FIAB	–	–	–	–			0.3–0.6	2.2–4.1	6.1–10.5	30.1–54.3	–	–	–	–
NFA	–	–	–	–	1.1–2.3	7.1–12.6	1.9–3.5	10.6–18.1	–	–	–	–	–	–
Riksmaten 2010	–	–	–	–	–	–	–	–	21.3–28.8	59.6–79.8	6.5–9.3	25.2–39.6	5.5–8.1	24.6–40.9
NDNS-RollingProgrammeYears1-3	–	–	7.7–10.7	42.8–57.1	3.7–5.3	23.8–33.4	4.6–6.3	21.5–30.3	6.7–9.5	29.6–40.9	24.3–33.8	67.7–93.0	26.0–35.7	64.0–85.8
DNSIYC 2011	0.5–0.8	0.3–1.9	1.6–2.6	7.9–13.1	–	–	–	–	–	–	–	–	–	–

bw: body weight; P95: 95th percentile.
(a): Occurrence data on other food commodities (e.g. honey, food supplements) as described in Section 3.1 were also considered when estimating dietary exposure to PAs.
(b): 95th percentile calculated over a number of observations lower than 60 requires cautious interpretation as the results may not be statistically robust (EFSA, 2011a,b).
(c): Details on the dietary surveys and the number of subjects are given in Appendix A.

Appendix D – Chronic dietary exposure to pyrrolizidine alkaloids (using occurrence data on tea and herbal infusions only from THIE)

Table D.1: Mean and 95th percentile (P95) chronic dietary exposure[a] to pyrrolizidine alkaloids (ng/kg bw per day) for total population in lower bound (LB) and upper bound (UB) scenarios, considering the occurrence data on tea and herbal infusions submitted by Tea & Herbal Infusions Europe (THIE)

Dietary surveys[c]	Range of dietary exposure (LB–UB) (ng/kg bw per day)[a]													
	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
ASNS – Adults	–	–	–	–	–	–	–	–	2.1–13.1	8.4–58.0	2.2–12.0	7.9–35.4	3.0–19.1	–[b]
ASNS – Children	–	–	–	–	2.4–13.7	7.4–45.7	1.4–8.9	6.5–45.7	–	–	–	–	–	–
Regional Flanders	–	–		–[b]	0.5–2.5	3.8–14.3	–	–	–	–	–	–	–	–
Diet National 2004	–	–	–	–	–		0.8–5.3	3.7–27.7	1.0–5.9	5.0–31.4	1.1–6.0	6.2–34.7	0.9–5.1	5.6–30.9
NUTRICHILD	1.8–12.5	7.6–55.9	2.4–15.3	8.4–53.6	2.0–11.9	7.8–44.1	–	–	–	–	–	–		–
Childhealth	–	–	–	–	–	–	0.5–2.3	3.4–12.2	–	–	–	–	–	
SISP04	–	–	–	–	2.0–8.4	9.0–25.4	1.2–5.9	4.8–18.3	0.7–3.9	3.0–13.7	–	–		–
DANSDA 2005-08	–	–	–	–	0.4–2.4	1.9–13.3	0.5–3.5	2.3–18.5	1.1–8.5	5.4–42.4	1.1–8.2	5.0–40.1	1.2–9.4	–[b]
IAT 2006 07	0.2–1.2	0.7–3.8	0.5–2.5	2.1–12.8	–	–	–	–	–	–	–	–	–	–
DIPP 2001 2009	–	–	–	–	0.2–1.1	1.3–8.2	–	–	–	–	–	–	–	–
NWSSP07 08	–	–	–	–	–	–	0.3–2.5	1.9–15.5	–	–	–	–	–	–
FINDIET2012	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0					0.9–7.7	4.6–39.5	0.7–5.7	3.0–24.7	–	–
INCA2	–	–	–	–	0.5–2.3	2.7–13.1	0.4–2.5	2.4–14.1	1.2–8.1	6.0–40.9	1.5–9.4	6.6–38.9	1.1–5.5	4.0–24.4
VELS	5.5–26.6	19.0–106.2	6.1–29.8	23.3–131.3	4.4–21.4	13.9–77.0	–	–	–	–	–	–	–	–
EsKiMo	–	–	–	–	3.9–19.6	14.3–70.6	3.4–16.8	13.1–64.9	–	–	–	–	–	–
National Nutrition Survey II	–	–	–	–			2.3–14.2	10.3–61.7	3.6–18.8	14.5–78.1	4.3–22.5	14.7–78.8	4.5–24.3	13.6–76.9
Regional Crete	–	–	–	–	1.1–2.4	6.6–13.4	–	–	–	–	–	–	–	–
DIET LACTATION GR	–	–	–	–	–	–	2.1–4.5	7.7–16.6	2.1–4.5	7.7–16.6	–	–	–	–
National Repr Surv	–	–	–	–	–	–	–	–	2.2–10.8	7.0–33.7	2.2–10.2	6.7–28.5	2.4–12.3	8.2–33.9
NANS 2012	–	–	–	–	–	–	–	–	3.7–22.6	10.3–62.9	5.4–31.6	11.7–67.3	5.7–33.4	12.3–73.7
INRAN SCAI 2005 06	0.9–5.9	–[b]	2.6–6.7	–[b]	1.4–6.1	7.6–34.0	0.7–4.4	3.9–23.3	0.6–3.0	3.2–15.9	0.7–3.4	3.7–15.9	0.9–4.3	4.1–18.2
EFSA TEST	–	–	–	–	3.2–23.7	9.3–56.3	2.5–18.1	6.6–47.6	1.6–10.7	4.8–30.3	–	–	–	–
FC PREGNANTWOMEN 2011	–	–	–	–	–	–	2.6–19.2	6.5–43.5	2.6–19.2	6.5–43.5	–	–	–	–
VCP kids	–	–	1.7–12.0	9.9–72.4	1.6–11.6	8.1–56.0	–	–	–	–	–	–	–	–

Dietary surveys[c]	Range of dietary exposure (LB–UB) (ng/kg bw per day)[a]													
	Infants		Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
VCPBasis AVL2007 2010	–	–	–	–	2.4–13.4	10.0–58.9	2.2–13.6	8.9–51.5	3.7–19.7	14.7–74.3	3.6–18.3	14.3–57.8	–	–
VCP-Elderly	–	–	–	–	–	–	–	–	–	–	4.6–22.6	14.5–67.1	5.0–24.4	15.9–56.9
Dieta Pilot Adults	–	–	–	–	–	–	–	–	0.6–4.1	2.5–15.6	1.2–7.8	3.2–19.1	1.2–8.3	–[b]
enKid	–	–	0.3–0.8	–[b]	0.4–1.0	3.3–6.3	0.3–0.7	0.8–2.4	–	–	–	–	–	–
AESAN	–	–	–	–	–	–	–	–	0.1–0.4	0.9–1.9	–	–	–	–
NUT INK05	–	–	–	–	0.6–1.2	3.3–6.6	0.3–0.7	1.9–6.5	–	–	–	–	–	–
AESAN FIAB	–	–	–	–			0.2–0.5	1.1–4.1	0.3–0.7	1.8–4.2	–	–	–	–
NFA	–	–	–	–	0.3–1.9	1.8–10.0	0.4–2.7	2.2–13.6	–	–	–	–	–	–
Riksmaten 2010	–	–	–	–	–	–	–	–	1.5–7.4	7.0–33.1	1.5–7.3	5.9–29.4	1.2–6.3	6.1–28.8
NDNS-RollingProgrammeYears1-3	–	–	1.4–7.8	7.6–45.6	0.9–4.4	6.0–28.1	0.9–5.1	2.2–13.6	3.4–21.5	10.6–63.5	4.4–28.2	12.3–76.6	4.6–29.5	11.1–68.5
DNSIYC 2011	0.2–0.7	0.3–1.8	0.6–2.2	3.4–13.0	–	–	–	–	–	–	–	–	–	–

bw: body weight; P95: 95th percentile.
(a): Occurrence data on other food commodities (e.g. honey, food supplements) as described in Section 3.1 were also considering when estimating dietary exposure to PAs.
(b): 95th percentile calculated over a number of observations lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011a,b).
(c): Details on the dietary surveys and the number of subjects are given in Appendix A.

Appendix E – Percentage of contribution of eight selected PAs as compared to the total PA concentration in tea and herbal infusions

Appendix E can be found in the online version of this output ('Supporting information' section): http://dx.doi.org/10.2903/j.efsa.2016.4572

Appendix F – Contribution of different foods to chronic dietary exposure to pyrrolizidine alkaloids

Appendix F can be found in the online version of this output ('Supporting information' section): http://dx.doi.org/10.2903/j.efsa.2016.4572