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STATEMENT

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Risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements

EFSA Panel on Contaminants in the Food Chain (CONTAM),
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Abstract

EFSA was asked by the European Commission to deliver a scientific opinion on the risks for human health related to the presence of pyrrolizidine alkaloids (PAs) in honey, tea, herbal infusions and food supplements and to identify the PAs of relevance in the aforementioned food commodities and in other feed and food. PAs are a large group of toxins produced by different plant species. In 2011, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) assessed the risks related to the presence of PAs in food and feed. Based on occurrence data limited to honey, the CONTAM Panel concluded that there was a possible health concern for those toddlers and children who are high consumers of honey. A new exposure assessment including new occurrence data was published by EFSA in 2016 and was used to update the risk characterisation. The CONTAM Panel established a new Reference Point of 237 μg/kg body weight per day to assess the carcinogenic risks of PAs, and concluded that there is a possible concern for human health related to the exposure to PAs, in particular for frequent and high consumers of tea and herbal infusions. The Panel noted that consumption of food supplements based on PA-producing plants could result in exposure levels causing acute/short-term toxicity. From the analysis of the available occurrence data, the CONTAM Panel identified a list of 17 PAs of relevance for monitoring in food and feed. The Panel recommended continuing the efforts to monitor the presence of PAs in food and feed, including the development of more sensitive and specific analytical methods. A recommendation was also issued on the generation of data to identify the toxic and carcinogenic potency of the PAs commonly found in food.

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Keywords: pyrrolizidine alkaloids (PA), origin, chemistry, analysis, exposure, risk assessment, margin of exposure

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Table of contents

1. Introduction

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

1.1.1. Background

The EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted in 2011 a scientific opinion on pyrrolizidine alkaloids in food and feed.[1]

In this scientific opinion, the CONTAM Panel performed estimates of both acute and chronic exposure to pyrrolizidine alkaloids through honey. Due to lack of data on the presence of pyrrolizidine alkaloids (PAs) in foods other than honey, the CONTAM Panel was not able to quantify dietary exposure from food other than honey. A number of PAs of particular importance for food and feed were identified and recommended to be included in future monitoring of the presence of PAs feed and food. The CONTAM Panel concluded that 1,2-unsaturated PAs may act as genotoxic carcinogens in humans. Therefore, the CONTAM Panel decided to apply the Margin of Exposure (MOE) approach. A benchmark dose lower confidence limit for a 10% excess cancer risk ($BMDL_{10}$) of 70 μg/kg body weight (bw) per day was calculated as the reference point (RP) for comparison with the estimated dietary exposure. The CONTAM Panel concluded that there is a possible health concern for those toddlers and children who are high consumers of honey.

It was furthermore concluded that, although no occurrence data were available, exposure to PAs from pollen, tea, herbal infusions and herbal dietary supplements could potentially present a risk of both acute and chronic effects in the consumer.

Following the outcome of this scientific opinion from the CONTAM Panel on PAs in food and feed and the availability of new occurrence data on the presence of PAs in food, the Commission requested EFSA for a dietary exposure assessment to PAs in honey, tea, herbal infusions (herbs) and food supplements.

Following this request, EFSA approved on 13 July 2016 a scientific report on the 'Dietary exposure to PAs in the European population'.[2]

Chronic and acute dietary exposure to 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 bw per day in 'Toddlers' (LB–UB[3]) 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 890 ng/kg bw per day (e.g. infusion of Borage).

Following initial discussions on appropriate risk management measures to ensure a high level of human health protection, it was found appropriate to ask EFSA to assess the health risks related to the estimated exposures to PAs from honey, tea, herbal infusions and food supplements. Furthermore the CONTAM Panel is requested to provide an opinion on the PAs of relevance in honey, tea, herbal infusions and food supplements and other feed and food, based on the availability of new occurrence data.

[1] EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on Pyrrolizidine alkaloids in food and feed. EFSA Journal 2011; 9(11):2406, 134 pp. https://doi.org/10.2903/j.efsa.2011.2406. Available online: www.efsa.europa.eu/efsajournal

[2] EFSA (European Food Safety Authority), 2016. Dietary exposure assessment to pyrrolizidine alkaloids in the European population. EFSA Journal 2016;14(8):4572, 50 pp. https://doi.org/10.2903/j.efsa.2016.4572

[3] LB = Lower bound and UB = Upper bound. 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.

1.1.2. Terms of Reference

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority (EFSA) to assess the human health risks related to the estimated exposures to PAs from honey, tea, herbal infusions and food supplements.

Furthermore the CONTAM Panel is requested to provide an opinion on the PAs of relevance in honey, tea, herbal infusions, food supplements and other feed and food, based on the availability of new occurrence data.

1.2. Interpretation of the Terms of Reference

EFSA received a request from the European Commission to assess the human health risks related to the exposure to PAs from honey, tea, herbal infusions and food supplements estimated in a recent EFSA Technical Report (EFSA, 2016). In addition, an opinion on the PAs of relevance in the aforementioned foods and other feed and food on the basis of the new available occurrence data was requested.

The CONTAM Panel concluded that the EC request can be addressed by a Panel statement including:

- An update of the risk characterisation for human health, considering the new exposure levels estimated by EFSA
- An analysis of the available data sets on the occurrence of PAs in food and feed to recommend a list of PAs of relevance for monitoring in food and feed

The CONTAM Panel concluded that a systematic update of the hazard identification and characterisation performed in the previous opinion (EFSA CONTAM Panel, 2011) was not necessary, also considering the ongoing systematic review under finalisation by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), cfr. Summary Report of the Eightieth JECFA meeting (FAO/WHO, 2015). However, the CONTAM Panel noted that an update of the benchmark dose (BMD) modelling approach applied in the previous opinion is warranted, in view of the new guidance of the EFSA Scientific Committee on the use of BMD in risk assessment (EFSA Scientific Committee, 2017).

1.3. Additional information

Pyrrolizidine alkaloids are a large group of natural toxins synthesised as secondary metabolites by different plant species. Several PAs are known to be highly toxic to humans and animals as a result of their presence in the food chain. In 2011, the CONTAM Panel evaluated the risks to human and animal health related to the presence of PAs in food and feed (EFSA CONTAM Panel, 2011).

PAs can be described as a combination of pyrrolizidine-derived moieties (defined as necine bases) with a pool of different mono- or dicarboxylic acids (defined as necic acids). In particular, the PAs with a double bond in position 1,2 of the pyrrolizidine ring system (1,2-unsaturated PAs) are considered of higher toxicity due to their potential to undergo metabolic activation and form reactive pyrrole species, which can readily react with proteins and form DNA adducts. An in-depth description of the chemistry and biochemistry of PAs is present in the previous CONTAM opinion (EFSA CONTAM Panel, 2011).

The toxicity of PAs in humans is documented in a series of case reports of intoxication 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. Short-term toxicity of PAs includes liver and lung as the main target organs, and in particular it is associated with the onset of hepatic veno-occlusive disease (HVOD). Although most PAs have not been extensively tested in experimental animals or in vitro systems, information on the tested PAs includes hepatotoxicity, developmental toxicity, genotoxicity and carcinogenicity. In particular, 1,2-unsaturated PAs are considered as genotoxic and carcinogenic substances due to their potential to undergo metabolic activation into reactive pyrroles. Based on available data, the International Agency for Research on Cancer (IARC) classified lasiocarpine, monocrotaline and riddelliine as being possibly carcinogenic to humans (category 2B), while other PAs assessed were not classifiable (category 3) due to the limited information available (IARC 1983, 1987, 2002).

1.3.1. Conclusions of the previous opinion of the CONTAM Panel

In 2011, the CONTAM Panel performed a comprehensive risk assessment for the presence of PAs in food and feed considering the information available at the time.

The CONTAM Panel assessed both chronic and acute risks related to the human dietary exposure to PAs. For the chronic effects, the Panel concluded that all 1,2-unsaturated PAs share a common metabolic pathway leading to the formation of genotoxic and carcinogenic reactive pyrroles. The Panel carried out a dose–response analysis for the incidence of liver tumours observed in rodents for two PAs tested in carcinogenicity studies by the National Toxicology Programme (NTP), lasiocarpine and riddelliine. A $BMDL_{10}$ for excess cancer risk of 70 μg/kg bw per day for induction of liver haemangiosarcomas by lasiocarpine in male rats was selected as the RP for the assessment of chronic risks and applied in a MOE approach. In view of the lack of long-term studies for other 1,2-unsaturated PAs, the CONTAM Panel assumed a carcinogenic potency equal to lasiocarpine. This was considered as a conservative approach since lasiocarpine was among the more toxic PAs when comparing intraperitoneal (i.p.) and intravenous (i.v.) acute LD_{50}s, and toxicity of PAs is considered to influence their carcinogenic potency.

The risks related to the possible adverse effect of acute exposure to PAs were assessed considering the available human data. While the CONTAM Panel could not set an acute reference dose (ARfD), the limited information available from human poisoning cases allowed identifying a lowest known dose of approximately 2 mg/kg bw per day associated with acute/short-term effects. This was based on a case of a 6-month-old girl who received a daily dose of approximately 0.8–1.7 mg PA/kg bw for 2 weeks and was diagnosed for HVOD, and a 2-month-old boy who was administered an approximate dose of 3 mg/kg bw for 4 days, with a fatal outcome.

The dietary exposure assessment of the CONTAM Panel 2011 opinion was limited to honey as occurrence data were only available for this food product. Using occurrence data on 14 and 17 PAs from two independent data sets submitted to EFSA, with eight PAs in common between the two data sets, the CONTAM Panel estimated dietary exposure for the consumption of retail honey and for 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 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 exposure estimates calculated in the scenario of honey produced locally from a single source were in general about 50–100% higher than the results of the calculations for retail honey.

In relation to PAs in retail honey, the calculated MOEs for adults (all consumers) ranged from 3,500,000 to 57,000, and from > 7,000,000 to 7,400, at the mean and high (95th percentile) long-term consumption, respectively. For 'toddlers' (all consumers), the MOEs ranged between 7,000,000 and 14,000, and between 7,000,000 and 1,200 for mean and high (95th percentile) long-term consumption, respectively. In the scenarios for consumers only, MOEs for adults were in the range 700,000–7,800 and 230,000–2,700 for mean and 95th percentile consumption, respectively. For 'toddlers', MOEs ranged from 175,000 to 1,900 and from 66,000 to 900 for mean and 95th percentile consumption, respectively. Estimated exposure levels for 'other children' were intermediate between those of 'toddlers' and adults, with corresponding MOEs for all consumers in the ranges of 1,800,000–25,000 and > 7,000,000–3,900 at mean and 95th percentile consumption, respectively. Overall, the Panel concluded that there was a possible health concern for those toddlers and children who are high consumers of honey. Estimates of acute dietary exposure to PAs in honey were four orders of magnitude lower than the lowest known PA dose associated with acute/short-term toxicity in humans, indicating no risk of PA acute toxicity related to consumption of honey. The Panel noted that much higher exposure levels to PAs could result from pollen and herbal dietary supplements than dietary exposure from honey, but data were not available for the CONTAM Panel to perform exposure assessments or risk characterisation for these sources.

For the risk to animal health related to the presence of PAs in feed, no quantitative risk assessment was possible in view of the limited data on occurrence and toxicity of PAs in livestock and domestic animals. Exposure to PAs may occur via the consumption of forage and roughage, or herbs and herbal mixtures contaminated with PA producing plants (e.g. Senecioneae and Boraginaceae spp.). All animal species were considered sensitive to the toxic effects of PAs, with small ruminants and rabbits being more resistant than other species. Overall, the CONTAM Panel concluded that the risk of PA poisoning in the European Union (EU) appears to be low and most poisoning cases reported have been due to accidental exposure.

Finally, the CONTAM Panel identified also PAs of particular importance for food and feed, considering the prominent alkaloids present in the main known PA containing plants. This list 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 EFSA opinion.

1.3.2. Conclusions of the EFSA scientific report on exposure assessment to PAs in food

Following a Commission request, EFSA published in August 2016 a scientific report on the dietary exposure to PAs through the consumption of honey, tea, herbal infusions (herbs) and food supplements (EFSA, 2016).

The scientific report considered the 28 PAs provisionally selected by the European Commission, based on the EFSA opinion (EFSA CONTAM Panel, 2011) and two reports, one EFSA external Scientific report (Mulder et al., 2015) and the other produced by the German Federal Institute for Risk Assessment (BfR, 2013). Initially, 274,632 analytical results were available for the exposure estimations; the concentration of PAs in each food sample was estimated adding up all the individual levels of PAs analysed. For tea and herbal infusions, samples with a minimum of 17 and a maximum of 28 analysed PAs were selected to estimate dietary exposure, while for honey, the number of PAs per sample in the final data set varied between 8 and 19.

Retail honey contained PA concentrations of 14.5–27.5 μg/kg (lower bound–upper bound (LB–UB)). 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 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 g/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). Certain food supplements contained very high levels of PAs. Average PA concentrations of 235–253 μg/kg (LB–UB) were reported for pollen-based supplements. Much higher concentrations were reported for some plant extracts consumed as infusions such as borage (*Borago officinalis*) with levels up to 31,101 μg/kg or for comfrey (*Symphytum officinale*) (up to 29,694 μg/kg), both concentrations expressed in the dry product. Some supplements containing plant material and sold as capsules/tablets to be directly ingested possessed the highest levels of PAs (hemp-agrimony (*Eupatorium cannabinum*) up to 2,400 mg/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 the samples of tea and herbal infusions submitted by national authorities and those collected through an EFSA Article 36 grant (Scenario A), while a second scenario assessed exposure based on samples of tea and herbal infusions submitted by Tea & Herbal Infusions Europe (THIE) (Scenario B).

In the Scenario A, the highest estimates of mean chronic dietary exposure were rather similar in both the youngest age classes ('Infants' and 'Toddlers') and the oldest age classes ('Elderly', 'Very elderly'). In 'Toddlers' the maximum exposure estimate was 34.5–48.4 ng/kg bw per day (LB–UB) while for 'Very elderly' was 31.1–41.8 ng/kg bw per day (LB–UB). In the highly exposed population (95th percentile), 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 'Toddlers' and 'Elderly'–'Very Elderly', respectively.

In Scenario B, the estimates of chronic exposure were lower as compared to the previous scenario. 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'.

Considering the relatively high levels of PAs in honey and its possible regular consumption by particular subgroups of the population, an ad hoc exposure scenario was applied to estimate the exposure amongst consumers only. 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. Although based on very limited number of eating occasions (n = 32), chronic exposure to PAs via the

consumption of pollen-based supplements was also estimated and ranged between 0.7 and 11.5 ng/kg bw per day among consumers only (minimum LB–maximum UB).

Acute dietary exposure to PAs was estimated following a conservative approach considering the presence of high contamination levels in all the different food commodities combined with the total daily consumption for each corresponding food (consuming days only). 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. Likewise, the consumption of 150 mL infusion of 2 g of certain plant extracts with relatively high PA levels can lead to exposure to PAs up to 890 ng/kg bw per day as estimated for one infusion of borage (*B. officinalis*). For pollen-based supplements, the acute exposure was between 2.8 and 43.9 ng/kg bw per day (minimum LB–maximum UB), among consumers only.

On estimating dietary exposure to PAs, the UB levels were highly influenced by the sensitivity of the analytical methods and the large proportion of left-censored data. This was particular evident in the Scenario B, where 93% of the analytical data were left-censored, with almost 60% of the samples of tea and herbal infusions with not a single PA quantified. Based on the current sensitivity of the reported analytical methods for the 28 PAs, the lowest UB concentration that can be achieved for tea and herbal infusions is 33.5 μg/kg (0.45 μg/L). This would correspond to mean chronic exposure UB levels (across age groups) up to 3.9–13.5 ng/kg bw per day, and up to 9.5–18.2 ng/kg bw per day among the highly exposed consumers, depending on the tea and herbal infusion consumed. For honey, the lowest UB concentration that could be reported with the eight selected PAs all at levels below the limit of quantification (LOQ) would be 3 μg/kg. This would lead to mean chronic exposure estimations up to 2.9 ng/kg bw per day, and up to 3.4 ng/kg bw per day among the highly exposed consumers.

In addition to continue ongoing efforts to collect analytical data on the occurrence of PAs in relevant foods, there is a need 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.

2. Assessment

2.1. Updated dose–response analysis

The CONTAM Panel agreed that an update of the dose response analysis performed for the chronic effects of PAs in the previous opinion is warranted in view of the updated guidance of the EFSA Scientific Committee on the use of benchmark modelling in risk assessment (EFSA Scientific Committee, 2017).

The CONTAM Panel reviewed the dose–response analysis carried out in 2011, briefly described in Section 1.3.1 of this statement and applied the BMD model averaging approach on the data sets on the incidence of liver haemangiosarcoma in male and female rats exposed to lasiocarpine (NTP, 1978) and riddelliine (NTP, 2003). When analysing the data sets, the CONTAM Panel noted that weaknesses are present in both studies in relation to the application of the BMD approach.

The NTP (1978) study on lasiocarpine reports that 24 rats/sex were tested in each treatment group, a relatively low number of animals considering the population size currently recommended for long-term studies in widely accepted test guideline documents. In addition, high mortality was observed at an early stage of the exposure period in both males and females exposed to the highest tested dose (1.5 mg/kg bw per day), and to a lesser extent the mid tested dose (0.75 mg/kg bw per day). In particular, in males an increased mortality started after week 60 and no rats in the high-dose group survived beyond week 88. Mortality affected more severely the study in female rats, with all animals in the high dose group dying approximately between week 30 and week 68. The impact of early mortality on the incidence of liver haemangiosarcoma was evident in female rats and hindered the possibility to perform BMD analysis on that data set. The Panel noted that early mortality could have also affected the likelihood of observing tumours in males exposed to 1.5 mg lasiocarpine/kg bw per day. Finally, the CONTAM Panel noted that the data set has limitations for the performance of BMD modelling, since all the three tested doses were associated with an increased incidence in liver haemangiosarcoma higher than the default benchmark response (BMR) of 10%.

The study on riddelliine was conducted with an adequate number of animals per dose group, following a tailored study design with six female groups (control and five riddelliine doses) and only two male groups (control and high dose). Also, in this case, early mortality was observed at the top dose (0.714 mg/kg bw per day), however, compared to lasiocarpine, a higher incidence of liver

haemangiosarcoma was observed in both sexes exposed to this high dose (76% and 86% for female and male rats, respectively), suggesting a low impact of the early mortality in the observed dose–response relationship. Even though the study design was particularly suitable for the performance of BMD modelling, the data set on the incidence of liver haemangiosarcoma in female rats was considered by the CONTAM Panel to have limitations as only the highest tested dose induced a statistically significant increase in tumour incidence. No tumour incidence was observed in the control group and in the lower three doses ranging from 0.007 to 0.071 mg/kg bw per day. The increased incidence in liver haemangiosarcoma observed at 0.236 mg/kg bw per day (3 female rats out of 50), although not achieving statistical significance, was considered of biological significance in view of the low spontaneous incidence of this type of tumour in rats (Zwicker et al., 1995).

The BMD modelling of the incidence of liver haemangiosarcoma in male rats exposed to lasiocarpine and in female rats exposed to riddelliine led to BMD_{10} confidence intervals (CIs) ($BMDL_{10}$–$BMDU_{10}$) of 8–343 and 237–548 μg/kg bw per day, respectively, based on model averaging.

Applying model averaging, the BMD_{10} CI for lasiocarpine was affected by a high degree of uncertainty, with a $BMDU_{10}$ to $BMDL_{10}$ ratio of about 40-folds and $BMDL_{10}$–$BMDU_{10}$ intervals below the tested dose range for all the accepted individual models. On the other hand, the BMD modelling for riddelliine using model averaging resulted in a narrower $BMDL_{10}$–$BMDU_{10}$ interval, fully included within the two higher tested doses (equivalent to 237–714 μg/kg bw per day), despite the relatively high uncertainty related to the poor information on the dose response relationship of the study.

Despite the marked difference between the $BMDL_{10}$ for lasiocarpine and riddelliine, mainly due to the aforementioned limitations of the two data sets, a partial overlap of the $BMDL_{10}$–$BMDU_{10}$ CIs calculated using model averaging was observed, suggesting that the two substances could have similar carcinogenic potency. This was more evident when a BMR falling within the tested dose ranges for both substances, such as 30%, was selected. BMD_{30} of 491 and 435 μg/kg bw per day were calculated for lasiocarpine and riddelliine, respectively, using model averaging. The respective $BMDL_{30}$–$BMDU_{30}$ intervals were 211–811 μg/kg bw per day for lasiocarpine and 373–622 μg/kg bw per day for riddelliine. Overall, this additional modelling supported the assumption that the two PAs can be considered of similar carcinogenic potency.

In conclusion, the CONTAM Panel selected the $BMDL_{10}$ of 237 μg/kg bw per day, derived for the incidence of liver haemangiosarcoma in female rats exposed to riddelliine as RP for the chronic risk assessment of PAs. Considering the general degree of uncertainty related to the available studies used for the dose response analysis and the fact that both riddelliine and lasiocarpine are classified among the most potent PAs, the CONTAM Panel concluded that the change in the RP maintains the conservative nature of the previous risk assessment.

The full details of the BMD modelling are given in Appendices A and B.

2.2. Updated risk characterisation

The CONTAM Panel considered that the recent report on dietary exposure assessment to PAs in the European population (EFSA, 2016), and the updated RP for the assessment of carcinogenicity warranted the update of the conclusions on the risks to human health of the previous scientific opinion.

Chronic risks

With regard to the chronic exposure, the CONTAM Panel applied an MOE approach considering the different chronic exposure scenarios presented in the latest exposure assessment, using the chronic RP of 237 μg/kg bw per day for the sum of 1,2-unsaturated PAs assuming equal potency. The EFSA Scientific Committee concluded that, for substances that are both genotoxic and carcinogenic, an MOE of 10,000 or higher, based on a $BMDL_{10}$ from an animal study, and taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view (EFSA, 2005).

Considering the all consumers scenario using the MS and Art 36 occurrence data sets (Scenario A described in Section 1.3.2), the Panel calculated MOEs ranging from > 10,000,000 to about 4,900 (min LB–max UB across dietary surveys and age classes) for the mean exposure in the younger age classes (infants–adolescents) and from > 1,000,000 to 5,700 (min LB–max UB across dietary surveys and age classes) for adults, as shown in Table 1.

The CONTAM Panel noted that MOEs calculated for all age groups when considering the maximum LB exposure levels are similar to respective MOEs at the maximum UB, indicating that the differences

in consumption data present in the various surveys, rather than the analytical uncertainties in the occurrence data, are mainly responsible of the high variability observed in the minimum LB–maximum UB MOEs.

When considering the 95th percentile exposure levels calculated in Scenario A, MOEs below 10,000 were calculated for all age groups both at the maximum LB and maximum UB. For the younger age classes MOEs ranged from > 10,000,000 to about 1,100 (min LB–max UB across dietary surveys and age classes), and for adults from > 200,000 to about 1,900 (min LB–max UB across dietary surveys and age classes). The median LB–UB 95th percentile ranged from about 16,200 (median LB in 'adolescents') to about 4,200 (median UB in 'toddlers') (see Table 1).

Table 1: Exposure levels calculated in the EFSA report on dietary exposure assessment to pyrrolizidine alkaloids (PAs) (EFSA, 2016), considering data submitted by EU Member States and from an Article 36 Grant project (Mulder et al., 2015) and related Margin of Exposure (MOEs) using the Reference Point of 237 μg/kg bw per day for the sum of all 1,2-unsaturated PAs

Age class		Mean dietary exposure (ng/kg bw per day)						MOEs Mean dietary exposure					
		Lower bound[a]			Upper bound[a]			Lower bound			Upper bound		
	N	Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
Infants	6	0	4.1	30.2	0	5.9	42.8	> 1,000,000	57,805	7,848	> 1,000,000	40,169	5,537
Toddlers	10	0	3.2	34.5	0	5.2	48.4	> 1,000,000	74,063	6,870	> 1,000,000	45,577	4,897
Other children	18	0.7	4.2	24.1	1.2	6.4	34.3	338,571	56,429	9,834	197,500	37,031	6,910
Adolescents	17	0.3	3.7	18.4	0.6	5.7	26.1	790,000	64,054	12,880	395,000	41,579	9,080
Adults	17	0.2	6.7	21.3	0.4	10.6	28.8	1,185,000	35,373	11,127	592,500	22,358	8,229
Elderly	14	3.0	8.1	29.5	4.3	12.4	39.9	79,000	29,259	8,034	55,116	19,113	5,940
Very elderly	12	3.9	9.2	31.1	5.7	13.9	41.8	60,769	25,761	7,621	41,579	17,050	5,670

Age class		95th percentile dietary exposure[b] (ng/kg bw per day)						MOEs 95th percentile dietary exposure					
		Lower bound[a]			Upper bound[a]			Lower bound			Upper bound		
	N	Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
Infants	5	0	_[c]	133.6	0	_[c]	185.2	> 10,000,000		1,774	> 10,000,000		1,280
Toddlers	7	0	42.8	153.8	0	57.1	214	> 10,000,000	5,537	1,541	> 10,000,000	4,151	1,107
Other children	18	3.3	21.2	90.5	6.3	32.5	125.6	71,818	11,179	2,619	37,619	7,292	1,887
Adolescents	17	0.8	14.6	68.4	2.4	24.6	95.1	296,250	16,233	3,465	98,750	9,634	2,492
Adults	17	1.1	30.1	85.7	2.0	42.9	120.0	215,455	7,874	2,765	118,500	5,524	1,975
Elderly	14	15.3	33.8	87.7	21.4	52.7	123.3	15,490	7,012	2,702	11,075	4,497	1,922
Very elderly	9	15.9	30.8	86.7	22.9	42.8	127.2	14,906	7,695	2,734	10,349	5,537	1,863

bw: body weight.
(a): Estimates were rounded to one decimal figure.
(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011). Those estimates were not included in this table.
(c): A minimum number of six dietary surveys is required to estimate a statistically robust median (EFSA, 2011).

The second all consumers chronic scenario was run using the occurrence data set for tea and herbal infusion submitted by THIE (Scenario B in Section 1.3.2). This led to consistently lower exposure estimates compared to the previous scenario, and consequently to higher MOEs. This is reflected in particular in the mean exposure scenario, in which MOEs calculated using maximum UB exposure levels were slightly below 10,000 for 'infants' and 'toddlers' (approximately around 8,000–9,000), higher than 10,000 for 'adolescents' and 'adults' (13,100 and 10,500, respectively) and below 10,000 for 'elderly' and 'very elderly' (7,500 and 7,100, respectively). Maximum UB MOEs calculated for the 95th percentile consumption were consistently below 10,000 for all age groups (ranging approximately from 1,800 to 3,700). A greater difference was observed for MOEs calculated using LB exposure estimates, reflecting the possible limitations of this data set identified in the dietary exposure assessment report, including a lower number of analysed PAs in some samples and a lower analytical sensitivity (EFSA, 2016) (see Appendix C, Table C.1).

Finally the chronic exposure was estimated for consumers only (see Appendix C, Table C.2), considering different types of teas, herbal infusions and honey. In particular, for Scenario A, minimum LB–maximum UB MOEs calculated for the means for only consumers of unspecified herbs and infusions ranged from > 1,000,000 to 4,300 and from > 1,000,000 to 1,000 for the adult and young population, respectively. The 95th percentile MOEs ranged from 395,000 to 1,500 and from 43,000 to 770 for the adult and young population, respectively. When looking at specific types of infusions, lower MOEs were calculated for the consumption of camomile flowers in particular in the young population (minimum LB–maximum UB approximately at 28,200–5,300 for mean consumption, 95th percentile not calculated), and for rooibos leaves both for adults (ranges of 21,500–5700 and 7,200–2,100 for mean and 95th percentile consumption, respectively) and the young population (range of 17,700–2,900, mean consumption, 95th percentile not calculated). A similar trend to the one reported in the scenarios on all consumers was observed when calculating MOEs for only consumers in Scenario B (data not shown).

MOEs calculated for adult consumers only of retail honey ranged between > 1,000,000 and 32,000 (minimum LB–maximum UB) and between 593,000 and 13,500 for mean and 95th percentile consumption, respectively. For the young population, MOEs ranged from 790,000 to 8,800, and from 339,000 to 7,600 for mean and 95th percentile consumption, respectively.

Regarding the interpretation of the calculated MOEs, the CONTAM Panel noted that a substantial degree of uncertainty remains in relation to the assumption that all 1,2-unsaturated PAs share the same mode of action and have carcinogenic potencies equal to the one selected for the establishment of the RP for neoplastic effects, riddelliine. While it is plausible to assume that following systemic absorption all 1,2-unsaturated PAs will generate the reactive pyrrole species likely responsible of the adverse effects, a large variability in toxicokinetic and toxicodynamic can be also expected in view of the large structural diversity in this group of substances, which could result in a marked variability in the carcinogenic potency of the individual PAs. In a recent work, Merz and Schrenk (2016) proposed provisional potency factors for a series of 1,2-unsaturated PAs, based on available data on i.p. and i.v. acute LD_{50}s in rat and mouse, genotoxic potency in *Drosophila melanogaster*, and *in vitro* cytotoxicity data in a model of chicken hepatocytes. From the analysis of this composite data set, the authors proposed a rationale to differentiate carcinogenic potency of 1,2-unsaturated PAs, based on the structure and stereochemistry of their necic acid moieties. In particular, cyclic diesters and open-chained diesters with 7*S* configuration (e.g. lasiocarpine, riddelliine or senecionine) were assigned a relative potency factor (RPF) of 1; monoesters with 7*S* configuration (e.g. heliotrine) were assigned RPF of 0.3; and finally open-chained diesters with 7*R* configuration (e.g. echimidine) and 7*R*-monoesters (e.g. intermedine or lycopsamine) were assigned RPF values of 0.1 and 0.01, respectively. In a more recent approach, Chen et al. (2017) proposed to derive RPFs for a series of PAs for which information on tumour incidence following exposure in rats is available. Beside the two PAs with available oral carcinogenicity studies (lasiocarpine and riddelliine), this series includes monocrotaline, clivorine, senkirkine and symphytine, for which limited information is available on their carcinogenic potency. Namely, these substances were studied in tests with design limitations (only one dose group and a control group, limited number of animals and non-standard exposure regime, including shorter durations and treatment frequencies). In addition, only the study on clivorine was carried out using the oral route of exposure, whereas i.p. injection was used in the studies on senkirkine and symphytine, and s.c. injection for monocrotaline. Chen et al. (2017) derived RPFs by estimating the doses associated with an increase of 10% in tumour incidence (T10) for monocrotaline, clivorine, senkirkine and symphytine and comparing them with the RP derived by EFSA in 2011 for lasiocarpine. In the case of riddelliine, the lowest $BMDL_{10}$ calculated by EFSA in 2011 was selected for

the comparison. This resulted in RPFs of 1, 0.39, 0.05, 0.23, 0.03 and 0.02 for lasiocarpine, riddelliine, monocrotaline, clivorine, senkirkine and symphytine, respectively. Finally, in a comparative 28-day oral toxicity study recently performed by Dalefield et al. (2016) on echimidine and lasiocarpine, Wistar rats (10/sex per dose) were exposed to either one of these two PAs at doses of 0.6, 1.2 or 2.5 mg/kg bw, including a common negative control group. A significant decrease in body weight gain was observed in male and female rats treated with lasiocarpine at $\geq$ 1.2 and 2.5 mg/kg bw per day, respectively, while no effects on body weight gain were observed in the groups treated with echimidine. No other adverse effects were observed for the two substances. The CONTAM Panel concluded that, due to the limitations in the analysed data set and the provisional nature of the semi-quantitative approach proposed by Merz and Schrenk (2016), it is not adequate to use the derived RPFs for the cumulative risk assessment of PAs in food. Similarly, the approach proposed by Chen et al. (2017) has also important limitations and its use is not considered adequate for the risk assessment of PAs. However, altogether these publications suggest that several of the PAs mainly contributing to the dietary exposure levels calculated in the EFSA report (2016) could be of substantially lower potency than riddelliine or lasiocarpine. As already discussed in the 2011 opinion, The CONTAM Panel therefore confirmed the conservative nature of the RP based on potent PAs such as riddelliine or lasiocarpine for the cumulative risk assessment of PAs in food.

The CONTAM Panel concluded that the MOEs calculated for all consumers in the mean and high (95th percentile) consumption scenarios indicate a possible concern for human health. In particular a concern was expressed for frequent and high consumers of teas or herbal infusions.

Acute risks

As described in Section 1.3.1, an approximate lowest known dose of 2 mg PA/kg bw per day associated with acute/short-term toxicity in humans was used by the CONTAM Panel for the assessment of acute risks, based on information from human cases indicating short-term toxicity following exposure in the range 1–3 mg PA/kg bw per day for periods ranging from 4 days up to 2 weeks.

In the 2016 EFSA report, acute dietary exposure to PAs was estimated considering the presence of high contamination levels in all the different food commodities, combined with the total daily consumption for each corresponding food. This conservative approach resulted in acute exposure levels ranging from approximately 1 to 300 ng/kg bw per day and from 6 to 170 ng/kg bw per day for mean consumers in the younger age classes (infants–adolescents) and adults, respectively. Exposure for the 95th percentile consumption levels was well below 1 μg/kg bw per day in all age classes. In view of the margin of more than three orders of magnitude between the estimated exposure levels and the lowest known dose range of 1–3 mg PA/kg bw per day at which human acute/short-term toxicity has been reported, the CONTAM Panel concluded that there is a low risk related to acute dietary exposure to PAs through the consumption of teas, herbal infusions and honey.

In specific scenarios, the acute (or short-term, assuming daily consumption of the same food supplement batch for few days/weeks) exposure to PAs related to the consumption of food supplements was estimated. In the 2016 dietary exposure report of EFSA, a wide range of PA concentrations was reported for herbal food supplements, reaching total PA levels of more than 2 g/kg in some samples. Acute/short-term exposure was estimated for plant extracts intended to be consumed following infusion (by assuming the same dilution factor used for teas and herbal infusions) or to be ingested as capsules/tablets. A single consumption occasion of a *B. officinalis* infusion led to an estimated acute/short-term exposure of 890 ng/kg bw per day. In another scenario, ingestion of one tablet/capsule of boneset (*Eupatorium perfoliatum*) or hemp-agrimony (*E. cannabinum*) corresponded to estimated acute/short-term exposure levels of about 800–1,800 μg/kg bw per day, respectively. Acute/short-term exposure through the consumption of pollen-based supplements showed much lower exposure estimates in the range of 3–44 ng/kg bw per day.

The CONTAM Panel concluded that the consumption of herbal food supplements based on PA-producing plants could reach acute/short-term exposure levels in the range of doses associated with severe acute/short-term effects in humans. This is supported by a series of human cases of intoxication following the consumption of herbal remedies derived from PA-producing plants (EFSA CONTAM Panel, 2011). In view of the uncertainty on the possible toxicity levels of PAs in humans and of the severity of the effects, the CONTAM Panel concluded that exposure levels less than 100 times lower than the aforementioned dose range of 1–3 mg PA/kg bw per day may be associated with the risk of acute/short-term effects.

Consumption of pollen-based supplements is not considered to pose acute risks to human health.

2.3. Recommended PAs for monitoring in food and feed

2.3.1. Food

Together with the estimation of the dietary exposure to PAs in the European population, the 2016 EFSA scientific report carried out an exhaustive evaluation of the available occurrence data in diverse food commodities, including the contribution of each PAs to the total contamination levels in the samples (EFSA, 2016).

Considering the final data set of tea and herbal infusions, the main average contributors to the total PA concentration in green tea 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 seneciphylline (11%); and in rooibos, senecionine-*N*-oxide (57%), retrorsine-*N*-oxide (19%) and senecionine (16%).

Overall, 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, seneciphylline, seneciphylline-*N*-oxide and retrorsine-*N*-oxide. 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.

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%). Similar main contributors had been already described for the 1,324 samples available in the 2011 CONTAM opinion that were also part of the 1,966 samples used in the 2016 EFSA scientific report.

For food supplements (plant extracts and pollen-based supplements) overall, the highest average contributions to the total PA levels 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.

Together with their occurrence in the different food commodities, other criteria such as the toxicology and chromatographic separation were also considered when selecting a set of PAs to be monitored.

From an analytical point of view, the analysis of certain PA isomers such as intermedine/lycopsamine or senecionine/senecivernine as well as their *N*-oxide derivatives present certain difficulties. It is reported that by using high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS), the most habitual analytical method for the analysis of PAs, a baseline chromatographic separation is not always achieved for these PAs (Crews, 2013). In addition, they have the same molecular weight and cannot be distinguished by mass spectrometry. Due to this fact, it seems that an accurate quantification of the individual PAs is not always possible. For the pair intermedine/lycopsamine also co-elution of indicine could happen (Mulder et al., 2015). An identical situation is observed for indicine-*N*-oxide and the *N*-oxide derivatives of intermedine/lycopsamine.

Another issue to be considered is that the ratio between the two forms usually present, the PA-*N*-oxide (PANO) and the free tertiary base, strongly depends of many factors among them the sample preparation and extraction conditions (EFSA CONTAM Panel, 2011). Therefore, a general recommendation is to analyse both forms regardless of the PAs selected.

Other PAs that should be monitored, although not relevant in terms to their contribution to the total occurrence in the current data set, are lasiocarpine and senkirkine. Lasiocarpine is among the most toxic of the PAs that have been tested, and the $BMDL_{10}$ for induction of liver haemangiosarcomas in male rats was used as RP in the previous EFSA opinion (EFSA CONTAM Panel, 2011). In addition, and although lasiocarpine was only quantified in less than 5% of the samples analysed, in certain food categories in particular in 'Tea for infants and young children', this PA represented on average 42% of the total concentration among the samples where it was analysed. Regarding, senkirkine, its average contribution to the occurrence levels in the food commodities (honey and tea/herbal infusions) was negligible (e.g. 0.9% in honey and 1.7% in green tea). However, senkirkine can be of particular importance in certain plant extracts, such as *Tussilago farfara* (coltsfoot), samples with reported PA levels above 400 μg/L and with this PA contributing to 80–90% of the total concentration.

Based on this information, the CONTAM Panel proposed a set of 17 PAs to be monitored in food: intermedine/lycopsamine, intermedine-*N*-oxide/lycopsamine-*N*-oxide, senecionine/senecivernine, senecionine-*N*-oxide/senecivernine-*N*-oxide, seneciphylline, seneciphylline-*N*-oxide, retrorsine, retrorsine-*N*-oxide, echimidine, echimidine-*N*-oxide, lasiocarpine, lasiocarpine-*N*-oxide and senkirkine. When considering this list, it should be taken into account that diverse PAs may co-elute with some of the PAs included. This is the case for instance of indicine and indicine-*N*-oxide that, however, are not relevant PAs in food. Under certain analytical conditions, these compounds may not be completely separate from the pair intermedine/lycopsamine and their respective *N*-oxides.

In addition to the proposed 17 PAs, recent analyses of tea samples (personal communication, Dr. Patrick Mulder, RIKILT) seem to indicate that other PAs could also have a relevant contribution to the levels of PAs in different foods. This refers mainly to integerrimine and echinatine together with their *N*-oxides which are not always chromatographically separated to baseline from the pairs senecionine-*N*-oxide/senecivernine-*N*-oxide and intermedine-*N*-oxide/lycopsamine-*N*-oxide, respectively. While echinatine is a structural isomer of lycopsamine and intermedine being a relevant PA in *Eupatorium* and *Cynoglossum* species, integerrimine has been described in *T. farfara* and *Senecio vulgaris* plants (El-Shazly and Wink, 2014; Nedelcheva et al., 2015).

Therefore, and based on standard availability, PAs other than those included in the proposed list of 17 PAs should be also monitored to better understand the occurrence of PAs in food.

Following the approach used in the 2016 EFSA scientific report (EFSA, 2016), an hypothetical scenario was built to assess what would be the dietary exposure in the European population if all results were below LOQ, based on the performance of current analytical methods for PAs (as provided in Table 12 of the 2016 EFSA scientific report). Estimates of dietary exposure to PAs were calculated assuming that all 17 PAs from the proposed list were below the LOQ, summing the 17 LOQs and combining the resulting value with the consumption of different food commodities (consumers only). Among the young population ('Infants', 'Toddlers' and 'Other children'), the maximum mean dietary exposure was estimated for 'Tea and herbs for infusions, unspecified' being 7.5 ng/kg bw per day, and a maximum 95th exposure of 10.1 ng/kg bw per day in the same food commodity. In the adult population ('Adults', 'Elderly' and 'Very elderly') highest mean exposure was estimated with the consumption of 'Tea and herbs for infusions, unspecified' being 5.2 ng/kg bw per day, while the maximum 95th exposure was estimated via the consumption of 'Tea unspecified, decaffeinated' to be 5.3 ng/kg bw per day. More details for the different food commodities and the range of chronic exposure estimates across the different dietary surveys is given in Appendix D. It can be noted that the application of the MOE approach to the exposure estimates reported in Appendix D and using the chronic RP of 237 μg/kg bw per day for the sum of 1,2-unsaturated PAs assuming equal potency, would result in MOEs above 10,000.

2.3.2. Feed

A total of 29,739 analytical results were available on different PAs, for a total of 524 samples. Samples were collected between 2006 and 2016, with 438 samples collected in the Netherlands and 86 in the Czech Republic. As compared to the situation at the moment of the publication of the 2011 CONTAM opinion, only few more samples (173) were available, 87 collected in the Netherlands and 86 in the Czech Republic. Samples collected in the Czech Republic were analysed for either four PAs (retrorsine, seneciphylline, senecionine and senkirkine, 37 samples) or five PAs (same PAs as before + monocrotaline, 49 samples).

All the samples collected in the Netherlands were analysed for 67 PAs, including 26 out of the 28 PAs provisionally selected by the European Commission (individual results for intermedine and its *N*-oxide were not reported). Following a clarification request to the data provider, it was confirmed that the analytical method was not able, at that time, to distinguish between intermedine/lycopsamine and intermedine-*N*-oxide/lycopsamine-*N*-oxide so the results were reported as lycopsamine and lycopsamine-*N*-oxide.

All samples from the Czech Republic were left-censored data; **Table 2**, therefore, shows the levels of PAs reported only for the samples collected in the Netherlands. Feed samples were classified according to the Catalogue of feed materials as described in Commission Regulation (EU) No 68/2013[4].

[4] Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials. OJ L 29, 30.1.2013, p. 1–64.

Table 2: Mean values of PAs reported for different types of feed samples collected in the Netherlands

		N	Number of LC	Mean concentration (μg/kg)		
				Lower bound	Middle bound	Upper bound
Cereal grains, their products and by-products	Wheat	1	0	23	171	320
	Maize	4	4	0	151	302
	Millet	4	4	0	151	302
	Oats	1	1	0	151	302
	Rice, broken	3	3	0	151	302
	Sorghum; [Milo]	2	2	0	151	302
Oil seeds, oil fruits, and products derived thereof	Palm kernel expeller	4	4	0	151	302
	Rape seed	4	1	9	159	308
	Toasted soya (beans)	46	37	3	153	303
	Sunflower seed	6	5	5	155	305
	Linseed	11	6	30	177	325
Legume seeds and products derived thereof	Peas	7	6	16	166	315
	Carob, dried	2	1	8	156	305
	Sweet lupins	4	4	0	151	302
Tubers, roots, and products derived thereof	Carrots	1	1	0	151	302
Other seeds and fruits, and products derived thereof	Other seeds and fruits, and products derived thereof	2	1	22	169	316
	Citrus pulp	3	2	12	161	311
Forages and roughage, and products derived thereof	Lucerne, alfalfa	149	18	368	503	637
	Grass, field dried, hay	152	117	174	322	470
Other plants, algae and products derived thereof	Other plants, algae and products derived thereof	32	12	290	435	580

N: Number of samples; LC: left-censored (samples with no PAs quantified).
The concentration in each sample was derived by summing the concentrations reported for each of the 67 PAs analysed.

The list of the 67 PAs analysed in the samples collected in the Netherlands is shown in **Table 3**. The sensitivity of the method (liquid chromatography with tandem mass spectrometry (LC–MS/MS)) was 4.5 μg/kg for all PAs, expressed as limit of detection (LOD). Out of the 438 feed samples, at least one PA was reported for 209 samples.

Regarding the 28 PAs that belong to the list provisionally selected by the European Commission, they were quantified at 803 occasions (7%), with seneciphylline (n = 129) and seneciphylline-*N*-oxide (n = 103), reported the most. For the 41 PAs that are not part of the Commission list, they were quantified in 579 occasions (3%) in 143 different samples. Among those quantified the most often occurring were: integerrimine (n = 66), integerrimine-*N*-oxide (n = 60), spartioidine (n = 60), spartioidine-*N*-oxide (n = 47), iso-acetylechimidine (n = 40), iso-echimidine (n = 41), riddelliine (n = 39) and riddelliine-*N*-oxide (n = 35).

Table 3: List of PAs analysed in samples collected in the Netherlands

Acetylheliosupine	Acetylheliosupine-*N*-oxide	Acetylechinatine	Acetylechinatine-*N*-oxide	Acetylerucifoline	Acetylerucifoline-*N*-oxide
Acetylseneciphylline	Acetylseneciphylline-*N*-oxide	Acetyllycopsamine	Acetyllycopsamine-*N*-oxide	Acetylechimidine	Acetylechimidine-*N*-oxide
Doronine	Desacetyldoronine	Dehydrojaconine	Echinatine	Echinatine-*N*-oxide	**Echimidine**
Echimidine-*N*-oxide	Echiumine	Echiumine-*N*-oxide	**Europine**	**Europine-*N*-oxide**	**Erucifoline**
Erucifoline-*N*-oxide	Florosenine	Floridanine	**Heliotrine**	**Heliotrine-*N*-oxide**	Heliosupine
Heliosupine-*N*-oxide	Heleurine-*N*-oxide	Integerrimine	Integerrimine-*N*-oxide	**Jacobine**	**Jacobine-*N*-oxide**
Jacoline	Jacoline-*N*-oxide	Jaconine	Jaconine-*N*-oxide	Jacozine	Jacozine-*N*-oxide
Lasiocarpine	**Lasiocarpine-*N*-oxide**	**Monocrotaline**	**Monocrotaline-*N*-oxide**	**Lycopsamine**	**Lycopsamine-*N*-oxide**
Otosenine	Onetine	**Retrorsine**	**Retrorsine-*N*-oxide**	Riddelliine	Riddelliine-*N*-oxide
Senecionine	**Senecionine-*N*-oxide**	**Senecivernine**	**Senecivernine-*N*-oxide**	Spartioidine	**Seneciphylline**
Seneciphylline-*N*-oxide	**Senkirkine**	Spartioidine-*N*-oxide	**Trichodesmine**	**Trichodesmine-*N*-oxide**	Usuramine
Usaramine-*N*-oxide					

Those PAs included among the 28 provisionally selected by the European Commission are in bold (intermedine/lycopsamine and intermedine-*N*-oxide/lycopsamine-*N*-oxide were reported as lycopsamine and lycopsamine-*N*-oxide, respectively, as they were not resolved by the analytical method used).

Some further assessments for the contribution of the different analysed PAs were focused on the two feed categories best represented: '*Lucerne (alfalfa)*' and '*Grass, field dried (hay)*', since they were the only feed groups with a relatively high number of samples quantified (n = 131 and n = 35, respectively). The feed group 'Other plants, algae and products derived thereof' (n = 32) covered a very heterogeneous number of samples, with seventeen different types of plants and six samples reported as 'Herbal mix' without further information. In most of the cases, only one or two samples were available for each type of plant, making any interpretation either on the PA levels or on the profile of PAs reported difficult (see **Table 4**).

Table 4: Samples of 'Other plants, algae and products derived thereof' collected in the Netherlands

		Mean concentration (µg/kg)				
		N	Number of LC	Lower bound	Middle bound	Upper bound
Other plants, algae and products derived thereof	Herbal mix	6	0	353	492	630
	Herbal mix, artichoke	1	0	2,252	2,385	2,517
	Herbal mix, camomile	2	1	35	184	334
	Herbal mix, dandelion	2	1	663	793	924
	Herbal mix, fennel	2	1	1,592	1,732	1,871
	Herbal mix, ginseng	1	0	5	154	302
	Herbal mix, goldenrod	2	0	18	165	312
	Herbal mix, knotweed	1	0	97	241	385
	Herbal mix, leek	1	1	0	151	302
	Herbal mix, marigold	1	1	0	151	302
	Herbal mix, milk thistle	1	0	12	161	309
	Herbal mix, mint	2	2	0	151	302
	Herbal mix, nettle	5	3	16	165	314
	Herbal mix, oregano	1	0	89	235	381
	Herbal mix, parsley	1	1	0	151	302
	Herbal mix, rose hip	1	1	0	151	302
	Herbal mix, rosemary	1	0	5	154	302
	Herbal mix, verbena	1	0	18	164	310

N: Number of samples; LC: left-censored (samples with no PAs quantified).
The concentration in each sample was derived by summing the concentrations reported for each of the 67 PAs analysed.

Grass, hay

In a total of 35 samples among the 152 analysed, at least one PA was quantified (23%). In almost half of these samples (17), the PAs from the Commission list represented 100% of the total concentration, while in 10 samples (29%) they represented below 60% of the total. On average, the PAs from the European Commission list represent 78% of the PA levels reported in hay. When looking at the potential contribution of the 17 PAs suggested to be monitored in food, the average contribution in the 35 samples was 69% of the total, in 15 samples representing 100%.

There was one sample where none of the PAs from the Commission list was quantified; the only PAs quantified was acetylerucifoline-*N*-oxide. Overall, the other 41 PAs were identified in total in 88 occasions (18 samples), with no PA standing up among the others in number of occasions reported as quantified (acetylerucifoline, n = 6).

Lucerne, alfalfa

In a total of 131 samples among the 149 analysed, at least one PA was quantified (88%). In 26 samples (20%), the PAs from the Commission list represented 100% of the total concentration, while in only 32 (24%) they represented less than 60% of the total (**Table 5**). On average, the PAs from the Commission list represented 72% of the PA levels reported. In these 32 samples, the most important PAs outside those from the Commission list were acetylheliosupine and heliosupine that represented on average 23% and 16% of the total concentration, respectively.

Concerning the list of 17 PAs suggested to be monitored in food, they represented, on average, 68% of the total PA levels, in 23 of the samples representing 100% and in another 70 samples above 60%.

Overall, the 41 PAs not on the European Commission list were quantified in 449 occasions (118 samples); those that were more often reported as quantified were integerrimine (n = 58), spartioidine (n = 55) and integerrimine-*N*-oxide (n = 52). It is also worth mentioning that riddelliine and riddelliine-*N*-oxide were reported as quantified in 34 and 32 occasions, respectively (**Table 6**).

Table 5: Presence of the PAs from the Commission list in different samples of '*Lucerne, alfalfa*' and '*Grass, field dried (hay)*') quantified for at least for one PA

	Lucerne; (alfalfa)				Grass, field dried, (hay)			
	N[(a)]	Contribution			N[(a)]	Contribution		
		Quantified[(b)]	Average	Max		Quantified[(b)]	Average	Max
Echimidine	131	1	0.1	14.0	35	0	0.0	0.0
Echimidine-*N*-oxide	131	6	1.0	86.0	35	1	0.0	0.1
Europine	131	0	0.0	0.0	35	0	0.0	0.0
Europine-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Heliotrine	131	0	0.0	0.0	35	0	0.0	0.0
Heliotrine-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Erucifoline	131	10	0.3	25.7	35	8	2.3	25.7
Erucifoline-*N*-oxide	131	6	0.8	100.0	35	5	2.5	27.2
Jacobine	131	15	1.6	100.0	35	6	1.8	17.1
Jacobine-*N*-oxide	131	4	0.8	100.0	35	3	2.5	77.8
Lasiocarpine	131	0	0.0	0.0	35	0	0.0	0.0
Lasiocarpine-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Lycopsamine[(c)]	131	29	2.0	38.5	35	8	5.9	67.3
Lycopsamine-*N*-oxide[(c)]	131	10	1.2	100.0	35	8	8.5	100.0
Monocrotaline	131	0	0.0	0.0	35	0	0.0	0.0
Monocrotaline-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Retrorsine	131	78	7.3	43.9	35	6	1.4	17.5
Retrorsine-*N*-oxide	131	71	7.6	43.2	35	8	4.5	100.0
Senecionine	131	81	7.9	100.0	35	8	2.0	15.8
Seneciphylline	131	107	20.1	100.0	35	19	26.3	100.0
Senecionine-*N*-oxide	131	66	5.2	33.3	35	10	4.7	100.0
Seneciphylline-*N*-oxide	131	84	13.8	100.0	35	14	15.6	100.0
Senecivernine	131	34	0.8	7.8	35	2	0.1	3.5
Senecivernine-*N*-oxide	131	19	0.6	11.1	35	0	0.0	0.0
Senkirkine	131	2	0.8	100.0	35	1	0.0	0.5
Trichodesmine	131	0	0.0	0.0	35	0	0.0	0.0

(a): Number of samples with at least one PA quantified.
(b): Number of times quantified.
(c): Intermedine/lycopsamine and intermedine-*N*-oxide/lycopsamine-*N*-oxide were reported as lycopsamine and lycopsamine-*N*-oxide respectively as they were not resolved by the analytical method used.

Table 6: Presence of PAs other than those from the Commission list in different samples of '*Lucerne, alfalfa*' and '*Grass, field dried (hay)*') quantified for at least for one PA

	Lucerne; (alfalfa)				Grass, field dried, (hay)			
	N[(a)]	Contribution			N[(a)]	Contribution		
		Quantified[(b)]	Average	Max		Quantified[(b)]	Average	Max
Acetyllycopsamine	131	10	2.2	82.3	35	3	1.0	26.0
Acetyllycopsamine-*N*-oxide	131	1	0.0	1.5	35	3	0.4	10.1

	Lucerne; (alfalfa)				Grass, field dried, (hay)			
	N[(a)]	Contribution			N[(a)]	Contribution		
		Quantified[(b)]	Average	Max		Quantified[(b)]	Average	Max
Acetylechimidine	131	0	0.0	0.0	35	0	0.0	0.0
Acetylechimidine-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Acetylerucifoline	131	4	0.1	5.3	35	6	1.5	40.0
Acetylerucifoline-*N*-oxide	131	0	0.0	0.0	35	4	3.2	100.0
Acetylseneciphylline	131	1	0.2	22.7	35	1	0.1	3.2
Acetylseneciphylline-*N*-oxide	131	0	0.0	0.0	35	1	0.1	5.2
Dehydrojaconine	131	0	0.0	0.0	35	1	0.0	1.2
Desacetyldoronine	131	0	0.0	0.0	35	3	0.3	5.8
Doronine	131	0	0.0	0.0	35	1	0.1	4.8
Echiumine	131	7	0.1	4.7	35	2	0.3	5.5
Echiumine-*N*-oxide	131	2	0.0	2.5	35	3	2.9	65.7
Floridanine	131	0	0.0	0.0	35	1	0.2	7.0
Florosenine	131	1	0.0	0.4	35	2	0.5	9.2
Heleurine-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Integerrimine	131	58	2.5	13.6	35	5	0.5	5.4
Integerrimine-*N*-oxide	131	52	2.2	20.0	35	4	0.5	8.2
Acetylheliosupine	131	35	6.2	62.7	35	4	2.3	36.0
Acetylheliosupine-*N*-oxide	131	22	1.3	21.5	35	2	0.6	18.4
Acetylechinatine-*N*-oxide	131	0	0.0	0.0	35	2	0.1	2.5
Acetylechinatine	131	2	0.0	2.1	35	2	0.2	5.5
Heliosupine	131	32	4.3	50.0	35	5	1.4	20.0
Heliosupine-*N*-oxide	131	19	1.1	22.2	35	1	0.2	7.8
Echinatine	131	4	0.3	30.6	35	1	0.1	2.5
Echinatine-*N*-oxide	131	1	0.0	0.7	35	0	0.0	0.0
Jacoline	131	0	0.0	0.0	35	1	0.1	1.8
Jacoline-*N*-oxide	131	0	0.0	0.0	35	1	0.0	0.7
Jaconine	131	11	0.3	22.7	35	4	1.0	15.2
Jaconine-*N*-oxide	131	0	0.0	0.0	35	1	0.1	4.2
Jacozine	131	2	0.0	3.4	35	1	0.0	0.2
Jacozine-*N*-oxide	131	0	0.0	0.0	35	1	0.0	0.4
Onetine	131	0	0.0	0.0	35	2	0.2	8.7
Otosenine	131	1	0.0	0.3	35	3	1.1	20.0
Riddelliine	131	34	1.0	12.2	35	5	0.3	5.8
Riddelliine-*N*-oxide	131	32	0.9	19.0	35	2	0.1	1.7
Spartioidine	131	55	2.9	21.4	35	4	0.4	9.8
Spartioidine-*N*-oxide	131	42	2.2	45.2	35	4	2.0	41.7
Trichodesmine-*N*-oxide	131	0	0.0	0.0	35	0	0.0	0.0
Usaramine-*N*-oxide	131	10	0.2	9.5	35	1	0.0	0.4
Usuramine	131	12	0.1	2.1	35	1	0.0	0.0

(a): Number of samples with at least one PA quantified.
(b): Number of times quantified.

Further attention was also paid in highly contaminated samples to the contribution of the PAs quantified the highest number of times among the 41 PAs not included in the Commission list. The focus was put on 'Lucerne, alfalfa' where a total of 131 samples were reported with at least one PA quantified (see **Table 6**); among them the 50 samples with the highest levels were selected. In these samples, heliosupine and, above all, acetylheliosupine contributed significantly to the total levels of PAs. Acetylheliosupine was quantified in 18 out of these 50 samples, in several occasions with

contribution above 40% (max = 57%); heliosupine was also quantified in 18 samples, in several with a contribution above 20% of the total PA levels (max = 27%).

The CONTAM Panel is of the opinion that a very limited number of feed samples are available to carry out a comprehensive evaluation of the PAs most typically present in feed. Furthermore, they come from one country and may be locally produced (grass, alfalfa). As a result, specific weeds present in these products may not be representative for those growing in other parts of the EU, like in the South or at higher altitudes. Based on this, it is difficult to conclude on which PAs should be monitored when analysing feed samples. Overall, a recommendation is given to analyse, at least, the 17 PAs proposed for food. Likewise, and as proposed for food, PAs other than those included in the proposed list should be also monitored to better understand the occurrence of PAs in feed.

2.4. Uncertainty analysis

Uncertainties associated to the estimates of dietary exposure to PAs have been already described (EFSA, 2016). In brief, the main uncertainties refer 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, there is uncertainty on how analytical methods (extraction) represent the different ways consumers prepare tea and herbal infusions. In addition, the fact that many other PAs, not routinely monitored or not yet identified, could also be present in food may lead to an underestimation of the exposure levels. Overall, the dietary exposure to PAs calculated was likely to overestimate the exposure levels of the European population.

There are also uncertainties linked to the assessment of the PAs present in feed; the number of samples was very limited and collected in just one country so they may not be representative especially considering the role of weeds growing specifically in certain parts of Europe.

Regarding the hazard characterisation, the CONTAM Panel confirmed the uncertainties already identified in the 2011 opinion (EFSA CONTAM Panel, 2011), and noted additional uncertainties in particular related to the data sets used for the dose–response analysis for the characterisation of the carcinogenic hazard (see Section 2.1). However, the Panel confirmed that the main uncertainties remain considering the lack of toxicological data on most of the PAs of relevance for food and feed contamination. As already concluded in 2011, the CONTAM Panel confirmed that the carcinogenic potency of many PAs present in food is expected to be lower than the potency of the two PAs with available long term studies, lasiocarpine and riddelliine. Therefore, basing the cumulative risk assessment of PAs on an RP derived from riddelliine without correcting for individual potencies is considered as a conservative approach. In relation to the acute risk assessment, the CONTAM Panel noted substantial uncertainties in the available human data hindering the possibility to establish an ARfD.

3. Conclusions

- In view of the updated guidance of the EFSA Scientific Committee on the use of Benchmark dose in risk assessment, the CONTAM Panel updated the BMD analysis of the available long-term studies on lasiocarpine and riddelliine performed in its previous risk assessment. Using model averaging, the Panel calculated the BMD confidence interval and selected the $BMDL_{10}$ of 237 μg/kg bw per day for increase in the incidence of liver haemangiosarcoma in female rats exposed to riddelliine as the RP for chronic risk assessment.
- The CONTAM Panel updated the risk characterisation performed in its scientific opinion published in 2011 considering the updated RP and most recent exposure levels calculated in the EFSA report of 2016 considering data in honey, teas, herbal infusions and food supplements.
- In line with its previous opinion, considering the genotoxic and carcinogenic nature of PAs, the CONTAM Panel applied a MOE approach to the cumulative chronic exposure levels of PAs. The EFSA Scientific Committee concluded that, for substances that are both genotoxic and carcinogenic, an MOE of 10,000 or higher, based on a $BMDL_{10}$ from an animal study, and taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view.

- MOEs considering chronic mean exposure levels in all consumers ranged from > 10,000,000 to about 4,900 (min LB–max UB across dietary surveys and age classes) in the younger age classes and from > 1,000,000 to 5,700 in adults. When considering high (95th percentile) consumption, MOEs ranged from > 10,000,000 to about 1,100, and from > 200,000 to about 1,900 for the younger and adult age classes, respectively.
- In the case of the chronic mean exposure levels calculated in consumers only of different types of teas and herbal infusions, MOEs ranged from > 1,000,000 to 4,300 and from > 1,000,000 to 1,000 for the adult and young population, respectively. MOEs calculated at 95th percentile of consumption ranged from 395,000 to 1,500 and from 43,000 to 770 for the adult and young population, respectively. Lower MOEs were calculated for the consumption of camomile flowers in particular in the young population, and for rooibos leaves both for the adult and young population.
- In the case of the chronic mean exposure levels calculated in consumers only of retail honey, MOEs ranged between > 1,000,000 and 32,000 and between 593,000 and 13,500 for adults at mean and 95th percentile consumption, respectively. For the younger groups of the population, MOEs ranged between 790,000 and 8,800 and between 339,000 and 7,600 for mean and 95th percentile consumption, respectively.
- Overall, the CONTAM Panel concluded that the MOEs calculated for all consumers in the mean and high (95th percentile) consumption scenarios indicate a possible concern for human health. In particular, a concern was expressed for frequent and high consumers of teas or herbal infusions.
- The CONTAM Panel assessed also the acute/short-term risks, considering the dietary acute exposure levels estimated in the 2016 EFSA report and the lowest known dose range of 1–3 mg PA/kg bw per day, at which acute/short-term adverse effects have been reported in humans.
- Acute exposure considering the simultaneous presence of high contamination levels in all the different food commodities ranged from 1 to 300 ng/kg bw per day and from 6 to 170 ng/kg bw per day for mean consumers in the younger age classes (infants–adolescents) and adults, respectively. Exposure for the 95th percentile consumption levels was well below 1 μg/kg bw per day in all age classes. In view of the margin of more than three orders of magnitude between these exposure levels and the lowest known dose range associated with human acute/short-term adverse effects, the CONTAM Panel concluded that there is a low risk related to acute dietary exposure to PAs through the consumption of teas, herbal infusions and honey.
- Acute or short-term exposure to PAs related to the consumption of food supplements was estimated to vary considerably depending on the type of supplement. Consumption of PA producing plant extracts to be consumed following infusion led to exposure levels as high as 890 ng/kg bw per day. Ingestion of one tablet/capsule based on PA-producing plants corresponded to estimates of acute/short-term exposure levels of about 800 or 1,800 μg/kg bw per day. Acute/short-term exposure through the consumption of pollen-based supplements showed much lower exposure estimates in the range of 3–44 ng/kg bw per day.
- The CONTAM Panel concluded that the consumption of herbal food supplements based on PA-producing plants could reach acute/short-term exposure levels in the range of doses associated with severe acute/short-term effects in humans. In view of the uncertainty on the possible toxicity levels of PAs in humans and of the severity of the effects, the CONTAM Panel concluded that exposure levels less than 100 times lower than the aforementioned dose range of 1–3 mg PA/kg bw per day may be associated with the risk of acute/short-term effects.
- Consumption of pollen-based supplements is not considered to pose acute risks to human health.
- Based on the current data set, the CONTAM Panel proposed a set of 17 PAs to be monitored in food, namely: intermedine/lycopsamine, intermedine-*N*-oxide/lycopsamine-*N*-oxide, senecionine/senecivernine, senecionine-*N*-oxide/senecivernine-*N*-oxide, seneciphylline, seneciphylline-*N*-oxide, retrorsine, retrorsine-*N*-oxide, echimidine, echimidine-*N*-oxide, lasiocarpine, lasiocarpine-*N*-oxide, and senkirkine.
- The CONTAM Panel acknowledged that the number of feed samples was very limited to carry out a comprehensive evaluation of the PAs most typically present in feed. However, while expecting to have more representative data in the future, the Panel considered appropriate to monitor at least the 17 PAs proposed for food also in feed.

- The list of PAs proposed for monitoring in food and feed is not expected to cover all possible PAs that may be present in the different commodities, but to include the most relevant PAs considering both their contribution to the total levels and their possible toxicological potencies. This approach is expected to facilitate the monitoring of PAs without compromising a high level of consumer protection.

4. Recommendations

- There is a need for toxicological data relating to the PAs most commonly found in food. In particular information on the toxicokinetics, metabolic activation and carcinogenic potency of the individual PAs would allow substantial refinement of the risk assessment.
- Ongoing efforts should continue to collect analytical data on the occurrence of PAs in relevant food and feed commodities, as well as in herbal food supplements.
- Based on standard availability, PAs other than those included in the proposed list of 17 PAs should be also monitored to better understand the occurrence of PAs in food and feed.
- More sensitive and selective analytical methods should be developed to assess the presence of PAs in food and feed and to decrease the uncertainties in the exposure assessment.

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Abbreviations

AIC	Akaike information criterion
ARfD	acute reference dose
bw	body weight
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDU	benchmark dose upper confidence limit
BMR	benchmark response
CI	confidence interval
CONTAM	EFSA Panel on Contaminants in the Food Chain
HPLC–MS/MS	high-performance liquid chromatography – tandem mass spectrometry
HVOD	hepatic veno-occlusive disease
IARC	International Agency for Research on Cancer
i.p.	intraperitoneal
i.v.	Intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives and Contaminants
LB	lower bound
LC–MS/MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median
LOD	limit of detection
LOQ	limit of quantification
MOE	Margin of Exposure
NTP	National Toxicology Programme
PA	pyrrolizidine alkaloids
PANO	pyrrolizidine alkaloid-*N*-oxide
RP	reference point
RPF	relative potency factor
s.c.	subcutaneous
THIE	Tea & Herbal Infusions Europe
UB	upper bound

Appendix A – Benchmark dose modelling of incidence of liver haemangiosarcoma in male rats exposed to lasiocarpine (NTP, 1978)

A. Data description

As already outlined in the previous EFSA opinion (EFSA CONTAM Panel, 2011), reported lasiocarpine levels administered in the diet were converted to doses by considering average body weight and daily food intake of 400 g and 20 g, respectively. This corresponds to the default conversion factor of 0.05 recommended by the EFSA Scientific Committee guidance (2012) for chronic rat studies.

Dose (μg/kg bw per day)	Incidence liver haemangiosarcoma	N
0	0	23
350	5	24
750	11	23
1,500	13	23

N: number of animals; bw: body weight.

B. Selection of benchmark response

A default benchmark response (BMR) of 10% (extra risk compared with the background risk) and a two-sided 90% confidence interval of the BMD were selected as recommended by EFSA Scientific Committee (2017). Additional calculations were performed applying a BMR of 30% for comparing carcinogenic potencies of lasiocarpine and riddelliine.

C. Software used and specifications

- Fitting benchmark dose models was based on the R-package proast61.3.
- Averaging results from multiple fitted benchmark dose models was based on the methodology in Wheeler and Bailer (2008).
- The default set of fitted models was applied as recommended by EFSA Scientific Committee (2017)
- Selection of the BMD confidence interval and the BMDL was carried out following the flow chart of EFSA Scientific Committee (2017)

D. Results

Model	Number of parameters	Log-likelihood	AIC	BMD_{10}[(a)]	$BMDL_{10}$[(a)]	$BMDU_{10}$[(a)]	Converged	Accepted AIC
Null	1	−57.71	117.42	NA	NA	NA	Yes	
Full	4	−43.95	95.90	NA	NA	NA	Yes	
Logistic	2	−48.17	100.34	392.86	301.85	510.82	Yes	No
Probit	2	−47.89	99.78	388.11	281.08	489.20	Yes	No
Log-logistic	3	−44.25	94.50	134.32	4.81	297.70	Yes	Yes
Log-probit	3	−44.23	94.46	151.06	7.36	309.78	Yes	Yes
Weibull	3	−44.34	94.68	103.34	1.70	271.73	Yes	Yes
Gamma	3	−44.36	94.72	99.41	0.65	283.22	Yes	Yes
Two-stage	3	−44.50	95.00	157.76	117.10	218.95	Yes	Yes

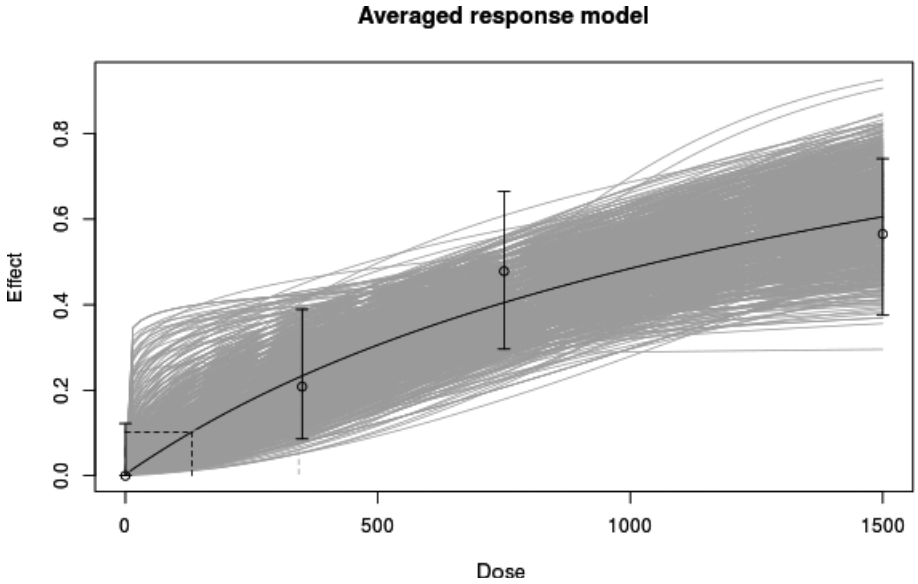
AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.
(a): Results expressed as μg/kg bw per day.

	Logistic	Probit	Log-logistic	Log-probit	Weibull	Gamma	Two-stage
Estimated model weights	0.01	0.02	0.21	0.22	0.19	0.19	0.16

Using the parametric bootstrap with a total of 1,000 generated data sets, the BMDL and the BMDU were the 5th and 95th percentile of all parametric bootstrap BMD values, respectively.

Estimates in μg/kg bw per day based on the model averaging (see EFSA Scientific Committee, 2017):

BMD_{10}	$BMDL_{10}$	$BMDU_{10}$
131.38	8.34	343.32



When applying a BMR of 30%, the following results were obtained

Model	Number of parameters	Log-likelihood	AIC	BMD_{30} [(a)]	$BMDL_{30}$ [(a)]	$BMDU_{30}$ [(a)]	Converged	Accepted AIC
Null	1	−57.71	117.42	NA	NA	NA	Yes	
Full	4	−43.95	95.90	NA	NA	NA	Yes	
Logistic	2	−48.17	100.34	880.86	711.16	1,166.91	Yes	No
Probit	2	−47.86	99.72	857.49	698.66	1,153.10	Yes	No
Log-logistic	3	−44.25	94.50	470.78	158.26	705.30	Yes	Yes
Log-probit	3	−44.23	94.46	470.49	162.65	695.34	Yes	Yes
Weibull	3	−44.34	94.68	469.42	133.92	726.08	Yes	Yes
Gamma	3	−44.36	94.72	473.39	119.38	727.37	Yes	Yes
Two-stage	3	−44.50	95.00	534.05	396.43	741.21	Yes	Yes

AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.
(a): Results expressed as μg/kg bw per day.

	Logistic	Probit	Log-logistic	Log-probit	Weibull	Gamma	Two-stage
Estimated model weights	0.01	0.02	0.21	0.22	0.19	0.19	0.16

Using the parametric bootstrap with a total of 1,000 generated data sets, the BMDL and the BMDU were the 5th and 95th percentile of all parametric bootstrap BMD values, respectively.

Estimates in μg/kg bw per day based on the model averaging (see EFSA Scientific Committee, 2017):

BMD_{30}	$BMDL_{30}$	$BMDU_{30}$
490.88	210.5	810.85

Appendix B – Benchmark dose modelling of incidence of liver haemangiosarcoma in female rats exposed to riddelliine (NTP, 2003)

A. Data description

As already discussed in the previous EFSA opinion (EFSA CONTAM Panel, 2011), reported riddelliine doses were corrected by a factor of 5/7 to account for the exposure regime applied in the study (5 days of exposure per week) were converted to doses by considering average bw and daily food intake of 400 g and 20 g, respectively. This corresponds to the default conversion factor of 0.05 recommended by the EFSA Scientific Committee guidance (2012) for chronic rat studies.

Dose (μg/kg bw per day)	Incidence liver haemangiosarcoma	N
0	0	50
7	0	50
24	0	50
71	0	50
236	3	50
714	38	50

N: number of animals.

B. Selection of benchmark response

A default benchmark response (BMR) of 10% (extra risk compared with the background risk) and a 90% interval around the BMD were selected as recommended by EFSA Scientific Committee (2017). Additional calculations were performed applying a BMR of 30% for comparing carcinogenic potencies of lasiocarpine and riddelliine.

C. Software used and specifications

- Fitting benchmark dose models was based on the R-package proast61.3.
- Averaging results from multiple fitted benchmark dose models was based on the methodology in Wheeler and Bailer (2008).
- Default set of fitted models were applied as recommended by EFSA Scientific Committee (2017)
- Selection of BMDL was carried out following the flow chart of EFSA Scientific Committee (2017)

D. Results

Model	Number of parameters	Log-likelihood	AIC	BMD_{10}[(a)]	$BMDL_{10}$[(a)]	$BMDU_{10}$[(a)]	Converged	Accepted AIC
Null	1	−119.66	241.32	NA	NA	NA	Yes	
Full	6	−38.90	89.80	NA	NA	NA	Yes	
Logistic	2	−40.32	84.64	362.77	298.90	430.74	Yes	Yes
Probit	2	−39.63	83.26	327.91	270.55	385.59	Yes	Yes
Log-logistic	3	−38.95	83.90	278.32	216.29	345.24	Yes	Yes
Log-probit	3	−38.90	83.80	269.90	215.09	323.21	Yes	Yes
Weibull	3	−39.00	84.00	290.30	218.19	366.26	Yes	Yes
Gamma	3	−38.92	83.84	277.13	215.62	336.89	Yes	Yes
Two-stage	3	−41.12	88.24	207.97	182.26	239.53	No	No

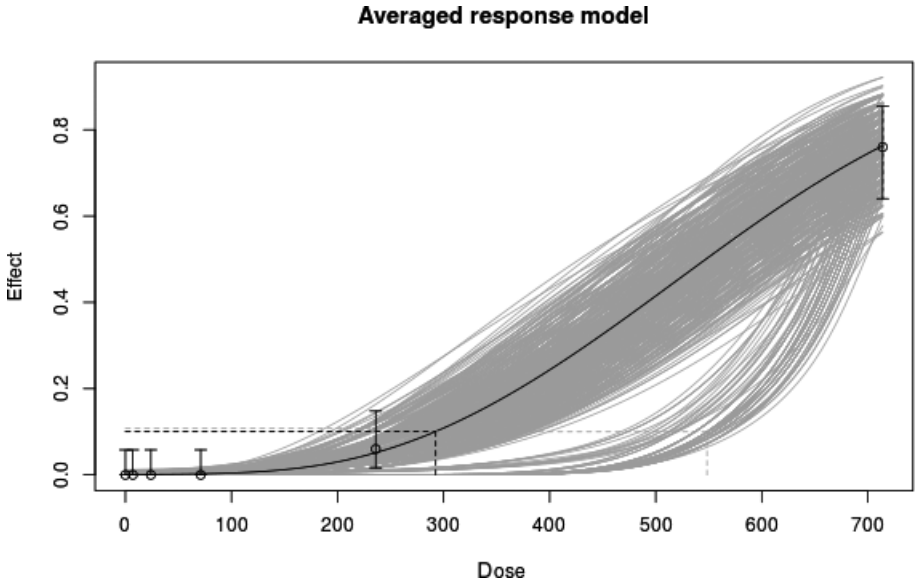
AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.
(a): results expressed as μg/kg bw per day.

	Logistic	Probit	Log-logistic	Log-probit	Weibull	Gamma
Estimated model weights	0.11	0.23	0.16	0.17	0.16	0.17

Using the parametric bootstrap with a total of 1,000 generated data sets, the BMDL and the BMDU were the 5th and 95th percentile of all parametric bootstrap BMD values, respectively.

Estimates in μg/kg bw per day based on the model averaging (see EFSA Scientific Committee, 2017):

BMD_{10}	$BMDL_{10}$	$BMDU_{10}$
292.53	236.58	548.31



When applying a BMR of 30%, the following results were obtained

Model	Number of parameters	Log-likelihood	AIC	BMD_{30} [a]	$BMDL_{30}$ [a]	$BMDU_{30}$ [a]	Converged	Accepted AIC
Null	1	−119.66	241.32	NA	NA	NA	Yes	
Full	6	−38.90	89.80	NA	NA	NA	Yes	
Logistic	2	−40.32	84.64	501.48	447.86	553.76	Yes	Yes
Probit	2	−39.63	83.26	473.57	423.56	525.77	Yes	Yes
Log-logistic	3	−38.95	83.90	406.60	344.26	472.04	Yes	Yes
Log-probit	3	−38.90	83.80	390.94	335.64	447.35	Yes	Yes
Weibull	3	−39.00	84.00	442.09	375.50	506.25	Yes	Yes
Gamma	3	−38.92	83.84	411.12	353.44	468.78	Yes	Yes
Two-stage	3	−41.12	88.24	382.65	335.35	440.72	No	No

AIC: Akaike information criterion; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; BMDU: benchmark dose upper confidence limit.
(a): results expressed as μg/kg bw per day.

	Logistic	Probit	Log-logistic	Log-probit	Weibull	Gamma
Estimated model weights	0.11	0.23	0.16	0.17	0.16	0.17

Using the parametric bootstrap with a total of 1,000 generated data sets, the BMDL and the BMDU were the 5th and 95th percentile of all parametric bootstrap BMD values, respectively.

Estimates in μg/kg bw per day based on the model averaging (see EFSA Scientific Committee, 2017):

BMD_{30}	$BMDL_{30}$	$BMDU_{30}$
434.91	373.01	622.37

Appendix C – Margin of Exposure tables

Table C.1: All consumers exposure levels calculated in the EFSA report on dietary exposure assessment to pyrrolizidine alkaloids (PAs) (EFSA, 2016), using occurrence data set from THIE (Scenario B, see Section 1.3.2), and related Margin of Exposure (MOEs) using the Reference Point of 237 μg/kg bw per day for the sum of all 1,2-unsaturated PAs

Age class		Mean dietary exposure (ng/kg bw per day)						MOEs Mean dietary exposure					
		Lower bound[a]			Upper bound[a]			Lower bound			Upper bound		
	N	Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
Infants	6	0.00	0.60	5.50	0.00	3.60	26.60	(> 1,000,000)	395,000	43,091	(> 1,000,000)	65,833	8,910
Toddlers	10	0.00	1.00	6.10	0.00	4.60	29.80	(> 1,000,000)	237,000	38,852	(> 1,000,000)	51,522	7,953
Other children	18	0.20	1.20	4.40	1.00	5.20	23.70	1,185,000	197,500	53,864	237,000	45,577	10,000
Adolescents	17	0.20	0.70	3.40	0.50	4.40	18.10	1,185,000	338,571	69,706	474,000	53,864	13,094
Adults	17	0.10	1.20	3.70	0.40	8.10	22.60	2,370,000	197,500	64,054	592,500	29,259	10,487
Elderly	14	0.70	1.80	5.40	3.40	9.80	31.60	338,571	131,667	43,889	69,706	24,184	7,500
Very elderly	12	0.90	1.80	5.70	4.30	10.90	33.40	263,333	131,667	41,579	55,116	21,743	7,096

Age class		95th percentile dietary exposure[b] (ng/kg bw per day)						MOEs 95th percentile dietary exposure					
		Lower bound[a]			Upper bound[a]			Lower bound			Upper bound		
	N	Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
Infants	5	0.00	_[c]	19.00	0.00	_[c]	106.20	(> 1,000,000)		12,474	(> 1,000,000)		2,232
Toddlers	7	0.00	7.60	23.30	0.00	45.60	131.30	(> 1,000,000)	31,184	10,172	(> 1,000,000)	5,197	1,805
Other children	18	1.30	7.00	14.30	6.30	26.70	77.00	182,308	33,857	16,573	37,619	8,876	3,078
Adolescents	17	0.80	3.70	13.10	2.40	18.50	64.90	296,250	64,054	18,092	98,750	12,811	3,652
Adults	17	0.90	5.40	14.70	1.90	33.70	78.10	263,333	43,889	16,122	124,737	7,033	3,035
Elderly	14	3.00	6.70	14.70	15.90	37.20	78.80	79,000	35,373	16,122	14,906	6,371	3,008
Very elderly	9	4.00	8.20	15.90	18.20	33.90	76.90	59,250	28,902	14,906	13,022	6,991	3,082

bw: body weight.
(a): Estimates were rounded to one decimal figure.
(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may be not statistically robust (EFSA, 2011). Those estimates were not included in the table.
(c): A minimum number of six dietary surveys is required to estimate a statistically robust median (EFSA, 2011).

Table C.2: Consumers only exposure levels calculated in the EFSA report on dietary exposure assessment to pyrrolizidine alkaloids (PAs) (EFSA, 2016), using occurrence data set from Article 36 project and EU Member States (Scenario A, see Section 1.3.2), and related Margin of Exposure (MOEs) using the Reference Point of 237 μg/kg bw per day for the sum of all 1,2-unsaturated PAs

	Adult consumers															
	Mean exposure				P95 exposure				MOEs (Mean exposure)				MOEs (P95 exposure)			
	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB
Tea and herbs for infusions, unspecified	0.2	39.6	0.2	54.7	0.6	114.4	0.8	158.1	1,185,000	5,985	1,185,000	4,333	395,000	2,072	296,250	1,499
Tea, unspecified	0.9	22.2	1.5	37	6	53.7	10	89.5	263,333	10,676	158,000	6,405	39,500	4,413	23,700	2,648
Tea unspecified, decaffeinated	0.5	2.5	2.3	12.6	6.3	6.3	31.5	31.5	474,000	94,800	103,043	18,810	37,619	37,619	7,524	7,524
Black tea, infusion	1.9	32.2	2.5	42.6	15.9	70.3	21.1	93.1	124,737	7,360	94,800	5,563	14,906	3,371	11,232	2,546
Green tea, infusion	2.4	15.4	4.8	30.7	15.4	41.8	30.7	83.5	98,750	15,390	49,375	7,720	15,390	5,670	7,720	2,838
Camomile flowers	1.9	14.1	2.7	19.6	39.9	39.9	55.7	55.7	124,737	16,809	87,778	12,092	5,940	5,940	4,255	4,255
Peppermint	0.7	34	0.8	42					338,571	6,971	296,250	5,643				
Rooibos leaves	11	36	12.6	41.3	32.9	96.4	37.8	110.6	21,545	6,583	18,810	5,738	7,204	2,459	6,270	2,143
Tea for infants and young children	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Honey	0.1	3.9	0.3	7.4	0.4	9.3	0.8	17.6	2,370,000	60,769	790,000	32,027	592,500	25,484	296,250	13,466
	Young consumers															
	Mean exposure				P95 exposure				MOEs (Mean exposure)				MOEs (P95 exposure)			
	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB
Tea and herbs for infusions, unspecified	0.6	165	0.8	228	5.5	222.2	7.6	307	395,000	1,436	296,250	1,039	43,091	1,067	31,184	772
Tea, unspecified	0.6	33.9	1	56.5	14.7	93	24.5	155	395,000	6,991	237,000	4,195	16,122	2,548	9,673	1,529
Tea unspecified, decaffeinated	0.4	2	2.1	9.9					592,500	118,500	112,857	23,939				
Black tea, infusion	1.5	41.4	2	54.9	44.4	64.4	58.8	85.3	158,000	5,725	118,500	4,317	5,338	3,680	4,031	2,778
Green tea, infusion	1.2	11.5	2.4	23					197,500	20,609	98,750	10,304				
Camomile flowers	8.4	32.3	11.7	45.1					28,214	7,337	20,256	5,255				
Peppermint	0.7	29.9	0.8	37	61.9			74.6	338,571	7,926	296,250	6,405	3,829			3,177

	Young consumers															
	Mean exposure				P95 exposure				MOEs (Mean exposure)				MOEs (P95 exposure)			
	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB	Min LB	Max LB	Min UB	Max UB
Rooibos leaves	13.4	70.2	15.4	80.5					17,687	3,376	15,390	2,944				
Tea for infants and young children	0.2	10.6	0.4	24.8					1,185,000	22,358	592,500	9,556				
Honey	0.3	14.2	0.6	27	0.7	16.4	1.4	31.1	790,000	16,690	395,000	8,778	338,571	14,451	169,286	7,621

LB: lower bound; UB: upper bound.

Appendix D – Hypothetical chronic exposure estimates to PAs across different dietary surveys considering consumers only

	Concentration of PAs[d] (μg/kg per μg/L)	Young population[a]		Adult population[b]	
		Mean exposure	95th exposure[c]	Mean exposure	95th exposure[c]
		ng/kg bw per day		ng/kg bw per day	
Tea and herbs for infusions, unspecified	19/0.25	0.03–7.5	0.25–10.1	0.01–1.8	0.03–5.2
Tea unspecified	19/0.25	0.05–2.8	1.2–7.8	0.08–1.9	0.5–4.5
Tea unspecified, decaffeinated	19/0.25	0.35–1.7	–	0.38–2.1	5.3
Black tea, infusion	19/0.25	0.10–2.8	3.0–4.4	0.13–2.2	1.08–4.8
Green tea, infusion	19/0.25	0.13–1.2	–	0.25–1.6	1.60–4.4
Camomile flowers	19/0.25	0.55–2.1	–	0.13–0.9	2.6
Peppermint	19/0.25	0.03–1.1	2.3	0.03–1.3	–
Rooibos leaves	19/0.25	0.55–2.9	–	0.45–1.5	1.35–4.0
Tea for infants and young children	19/0.25	0.08–4.4	–	–	–

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, 2011). Those estimates were not included in this table.
(d): Hypothetical concentration of PAs assuming that the 17 selected PAs were all left-censored data and the analytical method used reported the lowest LOQs as provided in Table 12 of the 2016 EFSA scientific report on dietary exposure to PAs. Levels in μg/L for tea/herbal infusions are obtained using 2 g of dry product in 150 mL of water.