Pyrrolizidine alkaloids in herbal teas and teas

BfR Opinion No. 018/2013 of 5 July 2013

Pyrrolizidine alkaloids are secondary metabolites produced by a large number of plant species all over the world as protection against herbivores. The occurrence of pyrrolizidine alkaloids in plants varies widely, depending on the plant species and the part of the plant, and is also influenced by other factors (e.g. climate, soil properties). Due to their potentially harmful effects, 1,2-unsaturated pyrrolizidine alkaloids (PAs) in particular are undesirable in food and feed, as they can lead to acute liver damage when consumed in high doses. In animal studies, some PAs have proven to be genotoxic carcinogens.

The BfR is currently conducting a research project on the "Determination of Pyrrolizidine Alkaloids in Food and Feed". In the project 221 different commercially available herbal tea and tea samples as well as herbal drugs were analyzed for their PA content. Based on the analyses of the following herbal tea varieties the resulting exposure was estimated: baby fennel tea, fennel tea, chamomile tea, herbal tea, peppermint tea, nettle tea and melissa tea. The selection of tea varieties is not representative. The first data collected during the research project are to be verified, e.g. within the food monitoring programme.

According to the first results total PA contents ranging from 0 to 3.4 milligrams per kilogram of dry product in the tested herbal tea and tea samples have been determined. For the assessment of possible health risks the BfR used the MOE (Margin of Exposure) approach, which is internationally used to estimate the potential risk of substances with genotoxic and carcinogenic effects. The MOE is calculated from human exposure as a measure of the extent of contact with a substance relative to the effective dose established or calculated in animal tests for a given tumour incidence. It is assumed here that an MOE of 10000 or higher for genotoxic carcinogens poses little health risk.

Although unexpectedly high PA contents were measured in individual samples, it is improbable that short-term intake (up to 14 days) poses a health risk for adults and children. In view of considerable PA contents, conclusions regarding health impairment due to the regular consumption of highly contaminated tea infusions are subject to large uncertainties, as there are considerable variations in PA contents, also for the same tea variety. For consumers (adults and children) with an average consumption of amounts of herbal tea and tea who do not favour a specific variety, health impairment due to chronic intake of PAs is improbable, as the MOE values are above 10000. Based on the first results of the project the MOE value for the intake of PAs is considerably below 10000 for people who frequently drink large quantities of herbal tea and tea. Therefore, for longer-term consumption of products with high PA contents, there is a risk of health impairment, particularly in the case of children, pregnant women and breast feeding mothers.

The potential risk for the consumer can be reduced by following the general recommendation for variety and diversity in the choice of foods. This can prevent one-sided exposure to various potentially health-threatening substances that are expected to sporadically occur in low amounts in food. In particular parents are advised not to offer their children herbal teas and tea, exclusively. Pregnant and breastfeeding women should vary their consumption of herbal teas and tea with other beverages.

Due to the genotoxic and carcinogenic effects of PAs, the BfR is of the opinion that efforts should be made to minimise the PA contents in herbal teas and teas. This is also necessary since possible additional exposure to PAs from other food sources (e.g. honey) may occur. The BfR recommends analysis of PA contents in herbal tea and tea batches prior to market-

ing and research to uncover the cause of the high PA contents in the products in question by the economic operators. The food control authorities should analyze PA levels in tea and herbal tea available on the market.

BfR risk profile: Occurrence of pyrrolizidine alkaloids in herbal tea and tea varieties (Opinion no. 18/2013)					
A Affected group	General population, pregnant women, breastfeeding women, children 				
B Probability of health impairment due to the longer-term consumption of products with high contents	Practically Ruled out	Improbable	**Possible**	Probable	Certain
C Severity of health impairment due to the longer-term consumption of products with high contents	The severity of the impairment may vary.				
D Robustness of the available data	High: the most important data is available and is free of contradictions		**Average: some important data is missing or is contradictory**	Low: much important data is missing or is contradictory	
E Possibility of consumer control [1]	Control not necessary	**Can be controlled by taking precautionary measures**	**Can be controlled by refraining from consumption**	Not controllable	

Text fields with dark blue background highlighting characterise the properties of the risk assessed in this opinion (for more detailed information, please refer to the text in BfR Opinion No. [No./Year] dated [Day/Month/Year).

Explanations

The risk profile is designed to visualise the risk outlined in the BfR Opinion. It is not designed to permit risk comparisons. The risk profile should only be read in conjunction with the opinion.

Line E - Possibility of consumer control
[1] – The food producers are called upon to minimise contents of pyrrolizidine alkaloids (Consumers cannot control these contents.) Parents are advised not to exclusively offer their children herbal teas and tea. Pregnant and breastfeeding women should vary their consumption of herbal teas and tea with other beverages (precautionary measure).

The details in the line "Possibility of consumer control" are not to be seen as a recommendation of the BfR but are of descriptive character. The BfR has outlined recommendations for action in its Opinion.

FEDERAL INSTITUTE FOR RISK ASSESSMENT (BfR)

1 Subject of the assessment

The BfR is currently conducting a research project on the "Determination of Pyrrolizidine Alkaloids in Food and Feed". In the frame of this project 221 different commercially available herbal tea and tea samples[1] as well as herbal drugs (184 herbal tea and tea samples from food retailers and 37 "medicinal teas" from pharmacies) were analysed for the content of 1,2-unsaturated pyrrolizidine alkaloids (PAs)[2]. The samples were not collected by representative sampling of the corresponding products on the German market.

[1] The terms "herbal tea" and "tea" correspond to the definitions in the guidelines *(1)*
[2] In the following, PAs are used to designate pyrrolizidine alkaloids with 1,2-unsaturated base structure

Those PA which are commercially available as standard substances were determined by the used analytical method. The determined compounds are intermedine, lycopsamine, heliotrine, heliotrine-N-oxide, echimidine, lasiocarpine, lasiocarpine-N-oxide, monocrotaline, monocrotaline-N-oxide, retrorsine, retrorsine-N-oxide, trichodesmine, seneciphylline, seneciphylline-N-oxide, senecionine, senecionine-N-oxide and senkirkine. For each sample, the total PA content was calculated as the sum of measured individual contents.

The following herbal tea varieties were included in the estimation of exposure: baby fennel tea, fennel tea, chamomile tea, herbal tea, peppermint tea, nettle tea and melissa tea. Other tea varieties (e.g. green tea, rooibos tea, black tea) were not included in the exposure estimation due to the small number of samples.

2 Findings

Based on the first results of non-representative surveys within the framework of the BfR research project, total PA contents from 0 to 3430 µg/kg dry matter were measured in the herbal tea and tea samples. Although unexpectedly high PA contents were measured in individual herbal tea and tea samples, it is seen as improbable that this poses an acute health risk, even in high doses.

In view of the genotoxic and carcinogenic effects of PAs, efforts should be made to minimise the PA contents in herbal teas and teas and thus minimizing the potentially higher cancer risk of frequent consumers and in particular of children, whose higher sensitivity to PA-related effects should be taken into account. This is also necessary since possible additional exposure to PAs from other food sources may occur (e.g. honey).

In the assessment of long-term intake, the BfR continues to use the MOE approach (Margin of Exposure)[3], which is the international standard for estimating the potential risk of substances with genotoxic and carcinogenic effects. Based on animal studies, it is assumed that an MOE value of 10000 or higher for genotoxic carcinogens poses little health risk. According to the results of the BfR research project, the MOE value for PA intake by adults and children would be considerably below 10000 for frequent consumers of certain herbal tea and tea infusions.

Based on the available data, the BfR concludes that those adults and children who belong to the frequent consumers of herbal tea infusions are possibly at increased cancer risk, particularly if they consume products with high PA contamination over longer periods of time.

The BfR once again recommends that the total exposure of consumers to genotoxic and carcinogenic PAs from various food sources should be kept as low as possible.

In order to avoid the marketing of contaminated batches and to protect the consumer, it is recommended that herbal tea batches that are to be brought onto the market be checked for PA content beforehand.
Research activities to uncover the cause of the high PA content in the products in question by economic operators is regarded as essential. These include botanical analysis of the plant

[3] The MOE approach (Margin of Exposure) is a method used to describe the risk occurring due to exposure to carcinogenic and/or genotoxic substances in food. The MOE value is the ratio of the following two factors for a specific population: the smallest dose with which a minor but measurable negative effect is observed and the level of exposure to the substance in question. The higher the MOE value, the lower the potential health risk to the consumer *(2)*.

material to which the occurrence of the PAs found in the herbal tea and tea samples is attributed as well as a review of "good agricultural harvesting practice".

In general, the possibility of reducing PA contents through improved cultivation, harvesting and cleaning methods should be reviewed.

The BfR also recommends that the food control authorities should perform checks to determine the PA contents of herbal tea and tea samples. Furthermore, the gathering of representative data on PAs in herbal tea and tea samples as part of the food monitoring process is recommended.

3 Statement of reasons

3.1 Risk assessment

3.1.1 Agent

Pyrrolizidine alkaloids are secondary plant compounds and are undesirable substances in food and feed *(3-5)*. Over 500 different pyrrolizidine alkaloids and their N-oxides are known *(6)*, and their occurrence is expected in more than 6000 plant species based on chemotaxonomic considerations *(7)*. Plants containing pyrrolizidine alkaloids primarily belong to the *Asteraceae*, *Boraginaceae* and *Fabaceae (Leguminosae*) *(8)*.

Pyrrolizidine alkaloids are taken to mean esters from a 1-hydroxymethylpyrrolizidine (necine base) and aliphatic monocarbon or dicarbon acids (necine acids). Depending on the esterification of one or both hydroxyl groups, pyrrolizidine alkaloids can occur as monoesters or diesters. Esterification with two carboxyl groups of a dicarbon acid results in a cyclical diester. Depending on the structure of the necine base, a distinction is primarily made between pyrrolizidine alkaloids of the retronecine, heliotridine, otonecine or platynecine type (Figure 1). Pyrrolizidine alkaloids of the retronecine and heliotridine type are diastereomers at position C-7 *(9)*.

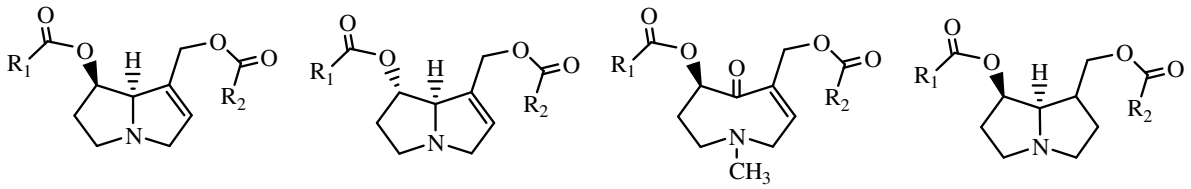

Figure 1: Structural formula of important necine bases

3.1.2 Risk potential

The current knowledge on toxicology, which is summarized in the following, forms the basis for the health assessment of pyrrolizidine alkaloids in food including herbal tea and also formed the basis for previous BfR opinions on this topic *(10, 11)*.

3.1.2.1 Structural chemical basics

The following structural features are characteristic for the typical toxic effects of pyrrolizidine alkalloids which are transmitted to the liver and partly also the lung *(3, 4, 12-14)*:

- Double bonding in the 1,2-position of the pyrrolizidine ring,
- Esterification of the hydroxymethyl group at C-1 of the ring system or - if present - the hydroxyl group at C-7,
- Branching of the alkyl side chain in at least one of the esterified carboxylic acids.

Pyrrolizidine alkaloids with 1,2-unsaturated necine structure esterified with at least one branched C_5-carboxylic acid are associated with hepatotoxic, carcinogenic and mutagenic effects *(13, 15)*. This includes the unsaturated pyrrolizidine alkaloids of the retronecine, heliotridine and otonecine type but not the saturated pyrrolizidine alkaloids of the platynecine type (Figure 1).

It is generally considered that the effects of esters of the hydroxymethyl group at C-1 (monoester) are enhanced if a second OH group is present in position C-7 of the necine. The effect is believed to be further strengthened if this OH group is also esterified (diester). The strongest toxic and carcinogenic effects are attributed to cyclic diesters *(12, 13, 16)*.
With the exception of pyrrolizidine alkaloids of the otonecine type, the plants in question generally contain combinations of the free unsaturated pyrrolizidine alkaloids with their N-oxides *(6)*. The oral toxicity of the latter substances is regarded as to be comparable to that of the reduced form of the alkaloids, to which they are metabolised in the colon by reductases.

3.1.2.2 Toxicokinetics and mode of action

In animal experiments, PAs are generally rapidly absorbed following peroral intake. Following partial metabolism, excretion occurs primarily via the kidney and to a lesser extent in the faeces *(14)*. The PAs remaining in the organism mainly take the form of metabolites bound to tissue components *(3)*.

No quantitative data is available on the oral bioavailability of PAs in humans. In vitro data suggests that both activation and detoxification take place.

The PAs are metabolised to pyrrole derivatives through hydrolysis, N-oxidation and dehydrogenation of the pyrrolizidine ring. The necine acids released during hydrolysis do not appear to be of toxicological relevance. Any N-oxide metabolites that are formed are highly soluble in water and are excreted extremely quickly in the urine, and this metabolic pathway is therefore seen as a detoxification pathway. The transformation of the uncleaved esters to toxic pyrrolic esters in the liver due to mixed-function oxidases, however, constitutes a toxification reaction. Pyrroles of this type are highly reactive alkylating agents that can react with nucleophilic groups of nucleic acids and proteins to form adducts and are considered active metabolites that are the cause of the hepatotoxic, hepatocarcinogenic and mutagenic effects of PAs *(4, 14, 16-21)*.
The mechanism behind the carcinogenic effect (direct or indirect DNA effects) is not yet known. The fact that PAs also cause extrahepatic lesions is attributed to the hydrolysis of the pyrrolic esters, which forms carcinogenic and developmental-toxic pyrrolic alcohols like (±)6,7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine (DHP) *(3, 14)*. The latter are less

short-lived and more readily water-soluble than the pyrrolic esters, which means that they are more easily distributed in the human body *(3, 14)*.

Species differences with regard to sensitivity to PA-induced toxicity are attributed to differences in the balance between detoxifying and toxic metabolism paths *(20)*. *In vitro* studies in which the metabolism of riddelliine to DHP and riddelliine-N-oxide occurred in the presence of human liver microsomes to a similar extent as with rat liver microsomes *(19)* are, like similar findings with retrorsine, retrorsine-N-oxide, riddelliine-N-oxide and monocrotaline-N-oxide *(18)*, seen as an indication that findings from rat experiments on tumour induction by PAs are relevant to humans.

Unsaturated PAs and its pyrrole derivatives cross the placenta and pass to breast milk. Rat foetuses and nursed male rats whose mother animals were feed with unsaturated Pas, exhibited e.g. acute and chronic liver damage *(3)*.

3.1.2.3 Toxicity in humans and animals

Various data on the toxicology of unsaturated PAs hve been published based on health impairments to humans and livestock observed around the world due to the intake of plant species containing unsaturated PAs (country-specific intake as food or medication, contamination of food or feed) as well as relevant feeding studies. For information on this, we refer primarily to the corresponding monographs and review studies*(3, 4, 14, 16, 22-24)*. Liver diseases with fatalities, some of them epidemic, have been observed in Pakistan, India and Afghanistan, which only recently saw another regional outbreak *(25)* following the consumption of grain contaminated with the seeds of Heliotrope (*Heliotropium* spp.) or *Crotalaria* varieties, and there have been cases of endemic poisoning in Jamaica from so-called "bush teas" containing parts of *Crotalaria* and Common groundsel (*Senecio*). Examples from livestock management include the frequent occurrence of liver cirrhosis in slaughtering cattle who have eaten Alpine ragwort (*Senecio alpinus*) with hay and silage as well as the senecioses associated with liver degeneration described in horses after the intake of *Senecio* varieties during grazing *(3, 16, 26-31)*.

The toxic effects of unsaturated PAs consumed in large doses over a short period of time are manifest in humans chiefly in liver in the form of veno-occlusive changes (veno-occlusive disease, VOD), and the occlusion of the central subobular liver veins is pathognomonic. The toxicity signs are frequently not observed until several days after exposure, which means it is often difficult to identify the cause of poisoning. The initial clinical signs of acute and subacute intoxication are increasing pain in the upper abdomen, the rapid development of ascites within the space of a few days and sometimes oliguria and oedemas at the feet. Accompanying symptoms may be nausea and vomiting, and more seldom jaundice and fever. Enlargement and hardening of the liver is generally observed after a few weeks, often in combination with massive pleural effusion. In addition to VOD, some PAs (e.g. monocrotaline) also induce pulmonary arterial hypertension, which can result in acute right-heart failure (Cor pulmonale) *(3, 4, 26)*.

The acute form of the disease has a high mortality rate, with death occurring anywhere between 2 weeks and over 2 years following exposure. Full recovery from hepatic VOD is possible. A chronic disease can develop among survivors of an acute hepatic VOD or with long-term intake of small quantities of unsaturated PAs and result in liver cirrhosis. This is non-specific, thus a causal diagnosis is also difficult in these cases. Children appear to be more sensitive than adults (see 3.1.2.3.1) *(4, 32-34)*. Similar findings were documented in experiments on rats *(14)*. In pathological terms, acute intoxication with unsaturated PAs is charac-

terised by lobule-central toxic liver cell necrosis *(3, 4, 16)*. Values for the LD 50 of PAs following intraperitoneal (i.p.) administration can be found in Mattocks *(4)*. Studies have been conducted with retrorsine-N-oxide after peroral administration in which an LD 50 of 48 mg/kg body weight (bw) (administration over the course of seven days) was observed in rats *(4)*.

Enlarged hepatocytes with big hyperchromatic cell nuclei are typical for the chronic liver toxicity of unsaturated PAs in animals. This is seen as a morphological manifestation of the antimitotic effect of unsaturated PAs and is not found in humans *(3)*. In a chronic rat study in which riddelliine was administered by gavage (treatment on 5 days per week for 105 weeks), a no-observed-adverse-effect-level (NOAEL) of 0.01 mg/kg bw/day (hepatocytomegaly at 0.033 mg/kg bw/day) was found for non-neoplastic changes *(24)*.

Animal experiments give clear evidence of the carcinogenic effect of certain unsaturated PAs (lasiocarpine, monocrotaline, riddelliine) and a corresponding risk is considered to exist for humans *(3, 16, 22-24, 35, 36)*. In the case of other unsaturated PAs, the animal experiments using the compound itself or its active metabolites also indicate a carcinogenic effect (e.g. isatidine, jacobine, retrorsine, seneciphylline, senkirkine, petasitenine), but the data are incomplete *(3, 22, 36, 37)*. In many cases, compounds that showed themselves to be carcinogenic in animal experiments also had positive results for mutagenicity testing *(3, 38)*. The lowest BMDL10 (benchmark dose lower confidence limit 10%) of 0.073 mg/kg bw/day was derived from a carcinogenicity study using lasiocarpine based on findings in male rats *(35, 39)*. In this study, doses of 7, 15, and 30 mg lasiocarpine/kg (equivalent to 0.35, 0.75 and 1.5 mg/kg bw/day) was administered orally to Fischer 344 rates over 104 weeks. Liver angiosarcomas were observed in 13 out of 23 male and 2 out of 23 female rats in the highest dose, 11 out of 23 male and 7 our of 24 female rats in the medium dose and 5 out of 24 male and 8 out of 22 female rats in the lowest dose *(35)*.

The embryotoxic effect of certain PAs is known from animal experiments, but the data is scarce and there are no insights into possible developmentally related toxic effects in humans *(3, 4)*.

3.1.2.3.1 Poisoning in humans

Based on the description of poisoning cases following the intentional or accidental intake of plants containing PAs, information is scarce allowing only limited conclusions with regard to the doses of unsaturated pyrrolizidine alkaloids that can lead to toxicity in humans following short or long-term exposure *(3, 5)*.

Acutely toxic doses

Only two cases are described in the literature that contain robust details about the dose regarding poisoning following short-term exposure(4 to 14-day). The cases in question are the disease of a 6 month-old girl (body weight: 6 kg) and a 2 month-old boy (body weight: unknown), both of Mexican-American origin, who were given *Senecio longilobus* as a herbal tea *(40-42)*. The girl initially exhibited ascites and pleural effusion, followed by sinusoidal liver fibrosis after 2 months and liver cirrhosis 6 months later. The boy suffered from haematemesis, developed jaundice with pronounced hepatomegaly, exhibited central nervous cramps, bradycardia and apnoea periods and died after 6 days.

Referring to their dry weight, the herbal teas contained 0.3% PAs (mainly riddelliine) and 1% N-oxides (mainly of retrorsine, in lesser amounts from seneciphylline and senecionine) in the first case and 0.5% PAs and 1% N-oxides in the second. Based on the findings of analysis of

the herbal tea infusions and the administration schedule, it was calculated that the girl weighing 6 kg ingested a total of between 70 and 147 mg, equivalent to 12 - 25 mg/kg bw over a 2-week period, while the boy (assumed body weight: 5.5 kg) *(3)* ingested a total of approx. 66 mg, equivalent to 17 mg/kg bw of PAs over a period of 4 days. This supplies an estimated daily dose of 0.8 - 1.7 mg/kg bw for the girl and 3 mg/kg bw for the boy for a mixture of PAs with riddelliine and retrorsine-N-oxide as the main components.

Toxic doses with medium and longer-term exposure

The literature describes a case of VOD following four-month consumption of a preparation of comfrey leaves (no details of the *Symphytum* species) containing up to 0.27 g of alkaloids/kg. In addition, a herbal tea containing PAs had been consumed over a lengthy period of time. The authors estimated that a dose of 15 µg alkaloids/kg bw/day was consumed over a period of six months (main alkaloid: echimidine) *(3, 39, 43)*. This assumption is subject to uncertainty, however, as the person in question consumed PAs from different sources *(3, 39)*.

VOD - ending fatally in one case - was also diagnosed in four Chinese women following consumption of a herbal tea based on *Heliotropium lasiocarpum* over a period of 19 to 46 days. It is estimated that the women ingested a daily dose of 0.59, 0.49 (fatal course), 0.60 or 0.71 mg of PAs (heliotrine)/kg bw/day over a period of 45, 46, 19 or 21 days *(3, 44-46)*.

In two Indian cases of VOD following 20 to 50-day consumption of *Heliotropium eichwaldii* for medicinal purposes, exposure was calculated as 3.3 mg of PAs (heliotrine)/kg bw/day *(3, 47)*.

In frequent cases of VOD in Afghanistan and India due to the consumption of grain contaminated with seeds containing PAs, it was estimated that a daily dose of 0.033 or 0.66 mg of PAs/kg bw/day were ingested over periods of six or two months, and the main alkaloids were assumed to be heliotrine or crotananine and crotaburmine *(3, 48-50)*.

3.1.2.3.2 Existing assessments of the BfR and other national and international bodies

The WHO determined back in 1988 that the toxic effects of PAs are cumulative. This is why low chronic exposure can also pose a health risk*(3)*. The predominant long-term effects in humans are liver cirrhosis and the development of tumours. To date, however, there have been no robust follow-up or epidemiological studies investigating a dose-effect relationship over longer periods of time. With regard to the aforementioned case of liver disease after PA consumption through comfrey leaves *(43)*, in which the daily intake was in the order of 15 µg PA (main alkaloid: echimidine)/kg bw, this dose was converted into a heliotrine equivalent. As PAs from *Symphytum officinale* have a weaker effect, the ratio of LD 50 values (i.p. application) was used to calculate a dose equivalent of 10 µg heliotrine/kg bw and day, of which it was assumed that it would still lead to diseases in humans.

In 1992, maximum levels not to be exceeded were stipulated for pyrrolizidine alkaloids with a 1,2-unsaturated necine structure and their N-oxides in medications in Germany (“Stufenplanverfahren”), and these maximum levels are still in force. In the case of medications with recognised applications in line with monographs pursuant to section 25 para. 7 of the German Medicinal Products Act (AMG), it is stipulated that the daily exposure with maximum dose may not exceed the following levels: 100 µg PA/person for external use, 1 µg PA/person for internal use, 10 µg PA/person with the use of coltsfoot leaves as a tea infusion. Moreover,

the recommended duration of use is to be limited to a maximum 6 weeks a year for these medications, and they should not be used for non-topical application during pregnancy and nursing. The exceptions to this stipulation - alongside certain homeopathic medications - are medications containing PAs where the maximum dose does not result in daily exposure exceeding 0.1 µg PA/person for internal application and 10 µg PA/person for external application *(15)*.

In 2001, the "Australia New Zealand Food Authority" (ANZFA) primarily looked at the "non cancer effects" and proposed a "tentative NOAEL" for all PAs of 10 µg/kg body weight (bw)/day on the basis of the data from the aforementioned case description. Using an uncertainty factor of 10, this would supply a PTDI (provisional tolerable daily intake) of 1 µg/kg bw/day. In addition, it was emphasised that there was no evidence for the carcinogenicity of PAs in humans *(51)*.

In 2002, the Senate Commission on Food Safety (SKLM) of the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) came to the conclusion that the available data on PA contents in honey obtained from plants containing PAs (e.g. *Echium spp*. honey or *Senecio spp*. honey) and on consumer exposure to PAs is to be considered insufficient. The data basis on the toxicology of these kinds of PAs and on their metabolism in humans was also seen as being incomplete, with the result that it was not possible to publish a conclusive risk assessment. The SKLM also recommended that special attention be primarily paid to products made using pollen from plants containing PAs. It said that these products were brought onto the market as food supplements and probably consumed in large quantities *(52)*.

In 2005, the "Dutch National Institute for Public Health and the Environment" (RIVM) assessed "non-cancer effects" and also included data from experiments on animals. Based on the NOAEL of 0.01 mg/kg bw and day for non-neoplastic changes from the chronic rat study using riddelliine *(24)* and an uncertainty factor of 100, a "tolerable daily intake" (TDI) of 0.1 µg/kg bw/day was calculated. With regard to the risk assessment of a carcinogenic effect of the PAs, the RIVM calculated a so-called "virtually safe dose" (VSD) for PAs of 0.00043 µg/kg bw and day based on the same study. This is the dose that is expected to still result in a cancer risk of one to one million *(53)*.

In 2008, the "Committee on Toxicity of Chemicals in Food, Consumer Products and Environment" (COT) in the UK also assessed "non-cancer effects". From the rat study with riddelliine, applying an uncertainty factor of 100 and a NOAEL of 0.01 mg/kg bw/day, it was concluded that "non-cancer effects are not to be expected below a dose of 0.1 µg riddelliine/kg bw/ day. The COT recommended that the ratio of LD 50 values could be used to convert other PAs into riddelliine equivalents. With regard to their carcinogenic effect, the COT regarded PAs as a group of substances with cumulative effect. The lowest BMDL10 of 0.073 mg/kg bw/day derived from the study of lasiocarpine and male rats *(35)* was used to estimate the MOE (Margin of Exposure) for individual PAs or their sum. A MOE of 10000 was assumed in line with the EFSA (European Food Safety Authority) guidelines *(2)*. On this basis, COT concluded that "MOEs of 10000 and above, corresponding to doses of up to 0.007 µg /kg b.w./day, would be unlikely to be of concern." *(39)*.

A "Discussion paper on pyrrolizidine alkaloids" of the Codex Committee on Contaminants in Food published in 2011 summarises the available assessments, regulations and analytical methods as well as data on the occurrence and toxicity of pyrrolizidine alkaloids and provides recommendations for a future assessment by JECFA (Joint FAO/WHO Expert Committee on

Food Additives) as well as for the establishment of a code of practice for the avoidance and reduction of contamination with PAs *(5)*.

Up to 2011, the BfR based its risk assessment of PAs in food on the assessment approach agreed with the medicinal product sector, an approach that was established in 1992 in connection with the aforementioned “Stufenplanverfahren” for medications containing PAs. As was the case with certain medications, the BfR also called for PA levels in foods to remain below a maximum daily intake of 0.1 µg PA/person wherever possible *(15)*. In its opinion dated 11 August 2011 *(10)* the BfR referred to the outcome of an expert meeting on 4 March and recommended that the total exposure of consumers to genotoxic and carcinogenic 1,2-unsaturated pyrrolizidine alkaloids (PAs) in various foods be kept as low as possible. Based on the MOE approach, which is standard in the EU, and in line with the COT assessment, the BfR also recommended that consumers should as possible not exceed a daily intake of 0.007 µg of unsaturated PA/kg body weight (bw). This corresponds to compliance with an MOE of at least 10000. This assessment is still valid and is explained in the "Risk characterisation" section (see 3.1.4). Following an evaluation of analysis data on the occurrence of PAs in honey, the BfR said efforts were necessary to reduce PA contents in honey in order to minimise the potential risks to frequent consumers of honey and in particular children.

In the opinion on the assessment of pyrrolizidine alkaloids as undesired substances in feed in 2007, the Panel on Contaminants in the Food Chain of the European Food Safety Authority (CONTAM Panel) outlined gaps in knowledge with regard to exposure and pointed out that the occurrence of PAs in honey merits particular attention. It also recommended that analytical investigation of occurrence in feed be focused on selected PAs: senecionine, seneciphylline, erucifoline, monocrotaline, trichodesmine, heliotrine, indicine, intermedine and lycopsamine *(54)*. In 2011, the CONTAM Panel of EFSA published a new expert opinion on the risk assessment of pyrrolizidine alkaloids in food and feed *(55)*. This expert opinion also chose the MOE approach to estimate the potential cancer risk of PA exposure in food, also using the BMDL10 of 0.073 mg/kg bw/day from the study with lasiocarpine and male rats *(35)* as a point of reference. Although it also considered other potential sources of PAs, the CONTAM Panel was unable to quantify exposure from further foodstuffs other than honey due to lack of data. In the case of infants and children, who consume large amounts of honey, the panel, like the BfR, concluded that the MOE value could be considerably below 10000, which would indicate possible health risks.

3.1.3 Analysis

3.1.3.1 Analytical concept

An analytical method for the determination of PAs in herbal tea and tea samples was developed and validated in-house at the Federal Institute for Risk Assessment (BfR) as part of a research project. This method was used to analyse herbal tea and tea samples from German retailers for 17 PAs. The total PA content of each sample was calculated as the sum of the measured individual contents and was used to assess the health risk due to the occurrence of PAs in herbal tea/tea varieties.

3.1.3.2 Analytical method

Detection of PAs is based on acidic extraction, clean-up and enrichment by means of solid phase extraction followed by liquid chromatographic separation and mass spectrometric detection (LC-MS/MS). Using this method those 17 PAs are determined, which are currently commercially available as standard substances (echimidine, heliotrine, heliotrine-N-oxide,

intermedine, lasiocarpine, lasiocarpine-N-oxide, lycopsamine, monocrotaline, monocrotaline-N-oxide, retrorsine, retrorsine-N-oxide, senecionine, senecionine-N-oxide, seneciphylline, seneciphylline-N-oxide, senkirkine and trichodesmine). Identification of these PAs in the samples is accomplished by comparing of retention times with those of the standard substances as well as detection of at least two transitions of the molecular ion in question to substance-specific fragment ions.

In Table 1a the calculated limits of detection (LOD) and limits of quantification (LOQ) of the method for the 17 analytes are listed.

Table 1a: Limits of detection (LOD) and limits of quantification (LOQ) based on DIN ISO 32645 *(56)* in herbal tea and tea (results of in-house validation)

Analyte	LOD (µg PA/kg tea)	LOQ (µg PA/kg tea)
Echimidine	0.9	2.9
Heliotrine	0.9	2.9
Heliotrine-N-oxide	5.3	13.3
Intermedine	1.5	4.7
Lasiocarpine	1.1	3.4
Lasiocarpine-N-oxide	5.4	12.2
Lycopsamine	1.2	3.9
Monocrotaline	1.0	3.2
Monocrotaline-N-oxide	2.0	6.2
Retrorsine	49.1	151.8
Retrorsine-N-oxide	16.5	48.7
Senecionine	22.1	64.1
Senecionine-N-oxide	15.8	46.8
Seneciphylline	2.4	7.8
Seneciphylline-N-oxide	5.6	14.3
Senkirkine	1.7	5.3
Trichodesmine	1.1	3.5

Table 1b shows the number of samples (in absolute numbers and in percent) of each herbal tea variety in which at least one of the 17 tested PAs was determined above the limit of detection (LOD). In the case of herbal tea 88% of samples contained at least one PA in concentrations above the LOD. In the case of fennel tea in 57% of samples at least one PA is determined in concentrations above the LOD.

Table 1b: Percentage of samples per tea variety exceeding the limit of detection in %

Tea variety	n	%> LOD
Herbal tea	42	88.1 %
Peppermint tea	29	86.2 %
Chamomile tea	31	87.1 %
Fennel tea	30	56.7 %
Baby fennel tea	9	100.0 %
Nettle tea	12	91.7 %
Melissa tea	8	100.0 %

3.1.3.2 Evaluation of measured PA contents

3.1.3.2.1 Qualitative confirmation

The specificity of LC-MS/MS methods is considered very high. The identification of the 17 individual PA substances in the samples was based on the retention times and the determination of two substance-specific mass fragments in compliance with the performance criteria for mass-spectrometric analytical methods according to Decision (EC) 2002/657/EC *(57)*. With the method used here, a PA is consired to be identified with certainty if the retention time and the molecular mass correspond to the respective PA standard and at least the two fragment ions are formed from the molecular ion in the sample in a ratio that is comparable to that in the respective PA standard. Additional confirmation of the identity of the PAs was achieved by the standard addition approach. Selected samples containing PAs were spiked with defined quantities of PA standards and the increase in the mass-spectrometric signal of the PA in question was verified. In addition, product-ion spectra of the relevant analytes were recorded in selected samples. This means that the entire fragmentation pattern of the PA molecular ions was documented and compared with the patterns of the standard substances.

3.1.3.2.2 Quantitative evaluation

Selected samples with particularly high PA contents were diluted in several steps and the resulting reduction in the PA signal was checked for linearity. It was therefore possible to exclude signal enhancements or suppressions due to a matrix-based enhancement or suppression of the ionisation rate and the resulting overestimation or underestimation of PA contents for the PAs that were present as standard substances *(58)*.

3.1.3.3 International method validation study

The developed and in-house validated method for the determination of PAs in herbal tea/tea is current being validated by the BfR in an international collaborative trial based on the ISO/IUPAC/AOAC protocol *(59)*. The aim is to standardise the method. A further subject of the method validation study is a method to determine PAs in honey.
The familiarisation round for the validation of the methods is currently in progress with 24 international participants.

3.1.4 Measured total PA contents in herbal tea and tea

The evaluation of PA contents in different herbal tea and tea varieties is based on data from a total of 221 commercially off-the-shelf samples (184 from food retailers and 37 medicinal teas from pharmacies) purchased in the Berlin region between October 2012 and March 2013. The samples were not obtained by way of representative sampling of the corresponding products available on the German market. The focus was on herbal teas like fennel tea, chamomile tea, peppermint tea and herbal tea blends. Other teas including black, green and rooibos tea were also analysed.
Table 2 shows the PA contents in the tea samples purchased from retailers. The PA sum was calculated for each sample from the individual contents of the 17 available PAs: intermedine, lycopsamine, heliotrine, heliotrine-N-oxide, echimidine, lasiocarpine, lasiocarpine-N-oxide, monocrotaline, monocrotaline-N-oxide, retrorsine, retrorsine-N-oxide, trichodesmine, seneciphylline, seneciphylline-N-oxide, senecionine, senecionine-N-oxide and senkirkine. The contents and calculations listed below always refer to the PA sum. Due to the uncertainties arising with a high number of values below the limit of detection (LOD) or limit of quantification (LOQ), the evaluation of content data was based on the approach developed for dioxin analysis *(60)* according to lower bound (LB; values < LOD => 0 or < LOQ = LOD)[4], medium bound (MB; values < LOD or LOQ are set to half LOD or LOQ) and upper bound (UB; values < LOD or LOQ are set to full LOD or LOQ).
It becomes apparent that sometimes the contents do not only vary widely in the same tea varieties, indicating a major difference between the minimum and maximum values, but that PA contents also sometimes vary considerably depending on the tea variety. Fennel tea (including baby fennel tea) and green tea tend to have lower contents on average [mean(UB) = 181 or 197 µg/kg] compared to the other tea varieties, for example. In contrast chamomile and melissa tea show the highest average PA levels [mean(UB) = 554 or 763 µg/kg].

Table 2: PA contents in different herbal tea and tea varieties by LB[1], MB[2], UB[3] in µg/kg of dry product

Tea varieties		n	Min	Mean	Median	P95	Max
Baby fennel tea	LB	9	0.9	50.8	58.1	.*)	94.5
	MB	9	68.8	116.3	122.7	.*)	157.4
	UB	9	135.8	182.5	186.4	.*)	226.1
Fennel tea	LB	30	0.0	47.9	1.4	527.5	904.2
	MB	30	67.8	114.9	69.2	590.6	965.5
	UB	30	133.8	181.0	136.3	652.3	1026.0
Chamomile tea	LB	31	0.0	420.8	256.1	2556.1	3428.8
	MB	31	67.8	478.9	310.2	2597.0	3435.0
	UB	31	133.8	553.7	379.0	2661.2	3441.2
Herbal tea	LB	42	0.0	137.2	24.5	770.7	1469.8
	MB	42	67.8	199.0	89.8	809.8	1530.5
	UB	42	133.8	270.3	160.4	888.8	1589.6

[4] Depending on the individual sample: if the value is stated as < LOD, the value was set to = 0 and used for the evaluation. If the value is stated as < LOQ, the LOD was used.

Peppermint tea	LB	29	0.0	127.2	67.8	620.2	766.1
	MB	29	67.8	188.0	131.8	671.6	827.5
	UB	29	133.8	258.5	206.9	735.6	887.2
Nettle tea	LB	12	0.0	197.6	91.6	.*)	1140.9
	MB	12	67.8	263.4	158.5	.*)	1205.6
	UB	12	133.8	329.0	224.2	.*)	1276.1
Melissa tea	LB	8	32.1	646.5	649.5	.*)	1499.1
	MB	8	95.1	692.0	688.7	.*)	1528.0
	UB	8	169.3	763.2	752.1	.*)	1558.7
Data for tea varieties that were not included in the exposure assessment							
Green tea	LB	8	0.0	63.9	8.1	.*)	384.1
	MB	8	67.8	128.3	74.9	.*)	435.8
	UB	8	133.8	196.5	141.5	.*)	486.9
Rooibos tea	LB	7	79.8	199.8	140.4	.*)	409.6
	MB	7	139.3	250.8	198.8	.*)	448.4
	UB	7	214.9	316.0	295.6	.*)	498.6
Black tea	LB	8	1.2	170.7	33.2	.*)	1001.7
	MB	8	69.1	234.0	100.5	.*)	1054.9
	UB	8	136.4	306.2	166.9	.*)	1155.5

*) It was not possible to calculate a 95th percentile (P95) due to the low number of tested samples.
[1]Lower bound (values < limit of detection are set to 0; values < limit of quantification are set to limit of quantification); [2]Medium bound (values < limit of detection/limit of quantification are set to half the limit of detection/limit of quantification); [3]Upper bound (values < limit of detection/limit of quantification are set to the full limit of detection/limit of quantification).

3.1.5 Exposure

Exposure estimates are based on the herbal teas[5] listed in section 3.1.4 and Table 2 and the consumption data outlined in the following section 3.1.5.1. All figures on contents and consumption refer to the dried herbs. Consumption figures based on the consumption of infusions were converted to the dry form using the formula listed in section 3.1.5.3.

Exposure estimates are calculated for adults and children. However, the consumption of herbal tea infusions does not refer to the different tea varieties but to all tea infusions taken together, as no differentiated consumption data are available. This method results in a slight (and desired) overestimation of exposure. This applies in particular to the tea varieties that tend to have lower contamination levels. The uncertainties inherent in this method have already been outlined. The results are shown for tea and herbal tea. The term "herbal tea" is used to describe all types of herbs declared as such that are used to make tea infusions. Tea (overall) means all varieties and not teas defined in more detail.

3.1.5.1 Consumption studies

NVS II National Food Consumption Study

[5] Also see footnote 1 on the use of the terms "tea" and "herbal tea"

The data for consumption of tea infusions by young people and adults was take from the NVS II National Food Consumption Study conducted by the Max Rubner Institute (MRI). The NVS II is currently the most recent representative study on consumption by the German population. The study, during which around 20,000 people aged between 14 and 80 were asked about their dietary habits using three different survey methods (dietary history, 24h recall and weighing protocol) took place throughout Germany between 2005 and 2006 *(61)*. The evaluations are based on the data from the two independent 24h recalls in the NVS II collected in a computer-assisted interview using "EPIC-SOFT" *(61, 62)*. The data of 13,926 people for whom both interviews were available were evaluated. Due to the presence of consumption data for individual days, the 24h recall method is suitable for exposure estimates for both acute and chronic risk. The individual body weights of respondents were factored into the intake estimates.

VELS

The data for consumption of tea infusions by children was based on consumption data from the VELS study (food consumption survey to determine food intake by infants and small children for the estimation of the acute toxicity risk from pesticide residues) *(63, 64)*. The study was conducted in 2001 and 2002 and covered 816 infants and small children aged between 6 months and less than 5 years all over Germany. The parents kept two 3-day diet logs for each child listing all consumed foods. Intake was calculated from the consumption data of the children between 2 and 5 years of age with an average body weight of 16.15 kg. Due to the availability of consumption data for individual days, the 2x3-day diet logs are suitable for exposure estimates for both acute and chronic risks, although the use of a low number of single-day measurements to calculate a lifelong intake is subject to uncertainties, particularly with regard to statements on detailed food groups or estimates with a high percentage of non-consumers.

3.1.5.2 Data basis

The content data for baby fennel tea, fennel tea, chamomile tea, herbal tea, peppermint tea, melissa tea and nettle tea from Table 2 were taken into account for the calculation of exposure and summarised on the basis of the individual data as the group "herbal tea" (see Table 3).

Table 3: PA contents in the "herbal tea" group by LB, MB, UB[1] in µg/kg of dry product

		N	Min	Mean	Median	P95	Max
Group "herbal tea"	LB	161	0.0	**198.4**	54.5	**901.5**	3428.8
	MB	161	67.8	**259.9**	120.6	**960.7**	3435.0
	UB	161	133.8	**330.0**	190.0	**1027.6**	3441.2

*Values in bold were included in the exposure assessment
[1]Lower/Medium/Upper bound: see section 3.1.4 and Table 2.

3.1.5.3 Evaluation of consumption data

Adults, 14 – 80 years of age

The food groups "tea (overall)" and "herbal tea" were taken into consideration for evaluation of the consumption data of the NVS II for the adult German population. Instant teas were not included. Long-term consumption quantities based on all respondents (including non-consumers) and short-term maximum consumption quantities based on consumers of herbal tea and tea were calculated for the overall population. Separate categorisation of the herbal

tea varieties is not possible due to the lack of data in the NVS II. The calculated consumption quantity as beverage in ml was converted on the assumption that a teabag contains 2 g of dry product of herbal tea or tea prepared with 200 ml of hot water. It is assumed that 100% of the PAs migrated from the herbal tea and tea to the finished beverage. The data used for the exposure estimation are listed in Table 4 in consumption quantity in grams per day relative to body weight. The figures show that, over the long term, the adult population consumes an average 0.03 g of tea per kg body weight (bw), equivalent an amount of 3 ml tea infusion or 0.15 g tea per kg body weight for the frequent consumers (95th percentile), equivalent to 15 ml of tea infusion. If herbal tea is taken on its own, the consumed quantity decreases to around 0.02 g tea per kg bw (mean) or 0.09 g tea per kg bw for the frequent consumers.

There is no breakdown of consumption by gender and age group, as these parameters are not included in the exposure estimation. What can be said, however, is that the consumption amounts for herbal tea and tea are slightly higher among women than men. Moreover, there are indications that people drink increasing amounts of tea with increasing age.

Table 4: Consumption of herbal tea and tea (overall) by adults acc. to the NVS II National Food Consumption Study (24h recall), based on dry product

	Share of consumers	Long-term consumption (all respondents)		Short-term consumption (consumers only)
		Mean (g/kg bw/d)	P 95 (g/kg bw/d)	P95 (g/kg bw/d)
Tea (overall)	43%	0.032	0.153	0.291
Herbal tea	22%	0.015	0.093	0.269

Children, 6 months to < 5 years of age

The consumption data for long and short-term intake by children (from 6 months to under 5 years of age and no longer being nursed) based on the 3-day diet logs from the VELS study were evaluated for the food group "tea (overall)" as well as the scenario "herbal tea + tea not specified in more detail (nns)". The evaluation is based on the conservative assumption that all tea varieties not specified in more detail are herbal teas. As with the adults, the calculated consumption quantity was converted into beverage in ml on the assumption that a teabag contains 2 g of dry product of herbal tea or tea prepared with 200 ml of hot water. The figures show that, over the long term, children consume an average 0.06 g of tea per kg body weight (bw) and day, equivalent to an amount of 6 ml tea infusion or 0.23 g tea per kg body weight and day for the frequent consumers (95th percentile), equivalent to 23 ml of tea infusion. In the scenario "herbal tea + tea nns", we find average daily consumption of 0.03 g tea per kg bw, while the frequent consumers among children consume 0.14 g tea per kg bw daily. The maximum consumption quantity of consumers from the diet logs was used to calculate the short-term consumption (acute perspective). It is seen that children in the 95th percentile consume a daily 0.76 g per kg bw of "tea (overall)" and 0.64 g per kg bw of "herbal tea + tea nns" (see Table 5).

Table 5: Long-term and short-term consumption of herbal tea and tea (overall) by children (0.5 - <5 years of age) based on the VELS study

	Share of consumers	Long-term consumption (all respondents)	Short-term consumption (consumers only)

		Mean (g/kg bw/d)	P 95 (g/kg bw/d)	P95 (g/kg bw/d)
Tea (overall)	56%	0.055	0.231	0.763
Herbal tea + Tea nns	33%	0.026	0.137	0.637

Detailed analysis of individual tea varieties is not possible due to the mainly low number of consumers. Fennel tea (with 32%) is the only herbal tea that shows a higher percentage of consumers and comparatively significantly higher consumption quantities. By comparison, chamomile and peppermint tea are represented by only 1% of consumers, and the consumption quantities are lower than those for fennel tea.

No significant differences can be determined based on age group.

3.1.5.4 Exposure estimation

For the purpose of estimating exposure to PAs via the consumption of herbal tea and tea, the food groups "tea (overall)" and "herbal tea" were evaluated for both adults and children using various consumption scenarios and taking into account the calculation approaches "lower bound", "medium bound" and "upper bound". The content data basis is made up of the PA sums of all PAs measured in the tea varieties fennel, chamomile, herbal, peppermint, melissa, nettle and baby fennel tea from retailers in the aforementioned special research project (see Table 3).

Long-term intake – chronic contamination

Table 6 and Table 7 show the long-term PA intake of adults and/or children for the following consumption scenarios.

Scenario 1 (average consumption (mean) and average content (mean)) portrays the average consumer who sometimes drinks tea varieties with higher contamination levels and sometimes tea varieties with lower contents (no brand loyalty).

Scenario 2 (high consumption (95th P.) and average content (mean)) portrays the frequent consumer who sometimes drinks tea varieties with higher contamination levels and sometimes tea varieties with lower contents (no brand loyalty).

Scenario 3 (average consumption (mean) and high content (95th P.)) portrays the average consumer who only drinks tea varieties with higher contamination levels.

Scenario 4 (high consumption (95th P.) and high content (95th P.)) portrays the frequent consumer who only drinks tea varieties with higher contamination levels.

Adults

Table 6 shows that adults in particular who belong to the group of frequent consumers can exhibit higher daily intakes of PAs; it should be noted that scenario 4 is based on the worst case situation, in which someone drinks high amounts of a tea every day and in which the tea in question also has high PA contents.

It is to be assumed that the intake for "tea (overall)" is overstated, as it includes other tea varieties apart from herbal tea (green and black tea, among others) that tend to have lower PA contents.

Table 6: Long-term intake of PAs (µg/kg bw/d) via consumption of herbal tea and tea (overall) by adults for different calculation models and consumption scenarios

		PA intake (µg/kg bw/d)	
Consumption scenarios	**Calculation method[1]**	**Tea (overall)**	**Herbal tea**
Scenario 1: Average consumer without brand loyalty	**LB**	0.006	0.002
	MB	0.008	0.003
	UB	0.010	0.003
Scenario 2: Frequent consumer without brand loyalty	**LB**	0.030	0.018
	MB	0.039	0.023
	UB	0.050	0.030
Scenario 3: Average consumer with brand loyalty to highly contaminated herbal teas	**LB**	0.027	0.009
	MB	0.029	0.010
	UB	0.031	0.010
Scenario 4: Frequent consumer with brand loyalty to highly contaminated herbal teas (worst case)	**LB**	0.135	0.081
	MB	0.144	0.086
	UB	0.154	0.092

[1]Lower/Medium/Upper bound: see section 3.1.4 and Table 2.

The exposure estimation was based on the consumption data for the adult population without taking account of age and gender differences. It can be assumed, however, that men and the younger age groups ingest slightly lower quantities of PAs through the consumption of herbal tea and tea than the overall population.

Children (0.5 months - <5 years of age)

Table 7: Long-term intake of PAs (µg/kg bw/d) via consumption of herbal tea and tea (overall) by children (0.5 months - <5 years of age) for different calculation models and consumption scenarios

		PA intake (µg/kg bw/d)	
Consumption scenarios	**Calculation method[1]**	**Tea (overall)**	**Herbal tea + tea nns**
Scenario 1: Average consumer without brand loyalty	**LB**	0.011	0.005
	MB	0.014	0.007
	UB	0.018	0.009
Scenario 2: Frequent consumer without brand loyalty	**LB**	0.046	0.027
	MB	0.060	0.036
	UB	0.076	0.045
Scenario 3: Average consumer with brand loyalty to highly contaminated herbal teas	**LB**	0.050	0.023
	MB	0.053	0.025
	UB	0.057	0.027
Scenario 4: Frequent consumer with brand loyalty to highly contaminated herbal teas (worst case)	**LB**	0.208	0.124
	MB	0.222	0.132
	UB	0.237	0.141

[1]Lower/Medium/Upper bound: see section 3.1.4 and Table 2.

Children (0.5 months - <5 years of age) sometimes also have not inconsiderable intake quantities of PAs due to the long-term consumption of herbal tea and tea (overall). Due to the higher consumption quantities relative to body weight, the PA intake of children is higher than that of adults. It must be taken into account, however, that (in contrast to the situation with adults) the food group "herbal tea" in this case also includes quantities of tea whose variety was not exactly listed in the diet logs. The conservative assumption was made that all these teas are herbal teas.

Short-term intake – acute contamination

Table 8 shows the short-term PA intakes of adults and children for two consumption scenarios. In the first scenario, the data are based on the consumption of herbal tea and tea (overall) with average PA contents. This means that there is no preference for a specific tea variety (without brand loyalty (without brand loyalty). In the second scenario, it is assumed that the consumed herbal tea and tea (overall) has high contents; in other words, that there is a preference for certain tea varieties (with brand loyalty).

Table 8: Short-term intake of PAs (µg/kg bw/d) via consumption of herbal tea and tea by adults and children for different calculation models and consumption scenarios

		PA intake (µg/kg bw/d)			
		Adults		Children	
Consumption scenarios	Calculation method[1]	Tea (overall)	Herbal tea	Tea (overall)	Herbal tea + tea nns
Without brand loyalty: 95.P. consumption* Mean content	LB	0.060	0.060	0.151	0.126
	MB	0.078	0.078	0.198	0.166
	UB	0.099	0.099	0.252	0.210
With brand loyalty: 95.P. consumption* 95.P. content (worst case)	LB	0.270	0.270	0.688	0.574
	MB	0.288	0.288	0.733	0.612
	UB	0.308	0.308	0.784	0.655

[1]Lower/Medium/Upper bound: see section 3.1.4 and Table 2.

As with long-term intake, children show a far higher burden than the adult population when to comes to short-term PA intake (see Table 8). Moreover, the intake quantities with assumed brand loyalty are 3 to 5 times higher than in the scenario without brand loyalty.

One-time consumption of a herbal tea infusion with high contamination levels is certainly a realistic proposition. Based on the available data, however, it is difficult to make any statement on the probability of longer-term consumption of highly contaminated herbal tea and tea (overall), as the content data sometimes varies widely even within the same tea variety (see Table 2). It can therefore be assumed that the consumed herbal tea and tea (overall) also has differing PA contents and therefore also leads to differing intake amounts among consumers with brand loyalty.

What remains to be taken into account is that the content data for the individual PAs shows a high number of values below the limit of detection and the limit of quantification, and this is why the PA sums are mainly determined by the various limits of detection and limits of quantification of the 17 PAs. The actual exposure of the consumer is basically in between the LB and UB calculation approaches, with LB being an underestimation and UB an overestimation.

Due to the low number of cases supplying the content data and given the low number of consumers, it is not possible to provide any detailed overview of PA intake through individual tea varieties. Consequently, the exposure estimation was only possible on the level of an overall perspective of herbal tea or tea (overall), and this is associated with uncertainties that result in overestimation of intake levels.

3.1.6 Risk characterisation

In general, statements on the necessity to limit PA intake through food are primarily directed towards avoiding the risk of cancer and were based on estimations of chronic consumption of the foods in question. In addition, it is also necessary to assess potential risks of acute toxicity, e.g. in cases of possible high short-term exposures.

With regard to the question of whether PAs have a carcinogenic effect in humans, there are no robust epidemiological studies or follow up surveys for cases of poisoning over longer periods of time. Based on the entirety of experimental findings on metabolism, activation mechanism, DNA adduct formation, genotoxicity and carcinogenicity, it should be considered that PAs can also have a carcinogenic effect in humans.

At a BfR expert meeting on 4 March 2010, however, basic agreement was reached to give preference to the MOE approach for the risk assessment of substances with genotoxic and carcinogenic properties over the previous practice based on the assessment approach used in the field of medicinal products*(2)*. The corresponding assessment proposed by COT in the UK in 2008, in which PAs are considered as a group with cumulative effect with regard to their carcinogenic effect was seen as being suitable. In this approach, the derived BMDL10 (Benchmark Dose Lower Confidence Limit, 10%) for lasiocarpine of 0.073 mg/kg bw/day is used as the basis for the MOE estimation. The precondition for this assessment is that a carcinogenic potency equivalent to that of lasiocarpine is assumed for all detectable PAs. This "cumulative assessment group approach" might, however, overstate the actual risk. In view of the lack of data, comparative statements on the carcinogenic effect of individual PAs are currently not possible. For substances with genotoxic and carcinogenic effects, it is currently accepted within the EU that the exposure levels can be regarded as being of low concern if the MOE is 10000 or above *(2)*. This means that daily doses of 0.007 µg PA/kg bw are viewed as posing little risk in terms of carcinogenic effects. The BfR therefore recommends that the PA intake from all sources should not constitute an MOE of less than 10000. However, total exposure to PAs from all food sources should generally be kept as low as possible.

3.1.6.1 Long-term intake of PAs via teas including herbal teas

Adults

Table 9 shows that, among adults, the MOE values are only above 10000 for average consumers of herbal tea and tea (overall) (LB) who do not prefer a certain variety. All other calculation approaches result in MOE values below 10000 for the adult population and in particular for frequent consumers with brand loyalty. It is to be assumed, however, that the intake figures for herbal tea and tea (overall) are on the high side, as they include consumption amounts of other tea varieties apart from herbal tea that tend to have lower PA contents.

Table 9: Long-term intake of PAs (µg/kg bw/d) via consumption of herbal tea and tea by adults for different calculation models and consumption scenarios

		PA intake (µg/kg bw/d)		MOE (based on a BMDL10 of 0.073 mg PA/kg bw/day)	
Consumption scenarios	**Calculation method[1]**	**Tea (overall)**	**Herbal-tea**	**Tea (overall)**	**Herbal-tea**
Scenario 1: Average consumer without brand loyalty	**LB**	0.006	0.002	12268	36803
	MB	0.008	0.003	9362	28085
	UB	0.010	0.003	7373	22118
Scenario 2: Frequent consumer without brand loyalty	**LB**	0.030	0.018	2454	4089
	MB	0.039	0.023	1872	3121
	UB	0.050	0.030	1475	2458
Scenario 3: Average consumer with brand loyalty to highly contaminated herbal teas	**LB**	0.027	0.009	2699	8098
	MB	0.029	0.010	2533	7599
	UB	0.031	0.010	2368	7104
Scenario 4: Frequent consumer with brand loyalty to highly contaminated herbal teas (worst case)	**LB**	0.135	0.081	540	900
	MB	0.144	0.086	507	844
	UB	0.154	0.092	474	789

[1]Lower/Medium/Upper bound: see section 3.1.4 and Table 2.

Children (0.5 months - <5 years of age)

Table 10: Long-term intake of PAs (µg/kg bw/d) via consumption of herbal tea and tea by children (0.5 months - <5 years of age) for different calculation models and consumption scenarios

		PA intake (µg/kg bw/d)		MOE (based on a BMDL10 of 0.073 mg PA/kg bw/day)	
Consumption scenarios	**Calculation method[1]**	**Tea (overall)**	**Herbal tea + tea nns**	**Tea (overall)**	**Herbal tea**
Scenario 1: Average consumer without brand loyalty	**LB**	0.011	0.005	6691	14155
	MB	0.014	0.007	5106	10802
	UB	0.018	0.009	4021	8507
Scenario 2: Frequent consumer without brand loyalty	**LB**	0.046	0.027	1593	2686
	MB	0.060	0.036	1216	2050
	UB	0.076	0.045	957	1614
Scenario 3: Average consumer with brand loyalty to highly contaminated herbal teas	**LB**	0.050	0.023	1472	3114
	MB	0.053	0.025	1382	2923
	UB	0.057	0.027	1292	2732
Scenario 4: Frequent consumer with brand loyalty to highly contaminated herbal teas (worst case)	**LB**	0.208	0.124	351	591
	MB	0.222	0.132	329	555
	UB	0.237	0.141	308	519

[1]Lower/Medium/Upper bound: see section 3.1.4 and Table 2.

With children as well (0.5 months - <5 years), MOE values above 10000 are only calculated for average consumers of herbal tea (LB, MB) who do not favour a specific variety. Due to the higher consumption amounts relative to body weight, the PA intake of children is higher than that of adults in the various scenarios. It must be taken into account, however, that (in

contrast to the situation with adults) the food group "herbal tea" in this case also includes quantities of tea whose variety was not exactly listed in the diet logs. The conservative assumption was made that all these teas are herbal teas.

Overall, therefore, the MOE values in scenario 4 (worst case), in which frequent consumers exclusively consume herbal teas/teas with higher levels of contamination, are considerably below 10000 for both children and adults. It is to be assumed that the intake for "tea (overall)" is overstated, as it includes other consumption amounts of other tea varieties apart from herbal tea (green and black tea, among others) that tend to have lower PA contents. The MOE values for herbal tea infusions are in a range from 900-789 (LB-UB) for adults (Table 9) and a range of 591-519 (LB-UB) for children (Table 10).

With regard to the induction of non-neoplastic damage, a value of 0.1 µg PA (riddelliine)/kg bw/day was derived at which no non-neoplastic damage is yet expected *(39, 53)*. Levels are above this value for children and adults based on the assumptions of scenario 4.

At the same time, however, this exposure scenario where a person drinks a high amount of highly contaminated tea/herbal tea on a daily basis over a long period of time is highly improbable in practice.

3.1.6.2 Short-term intake of PAs in teas including herbal teas

As with long-term intake, children show a far higher PA exposure per kg body weight than the adult population whith regard to short-term PA ingestion (see Table 8). One-time consumption of a herbal tea infusion with high contamination levels is certainly a realistic proposition.

A comparison with the poisoning cases in children outlined in 3.2.1.3.1 *(40-42)* provides a posibility for dose-related risk estimation. The estimated intake of an alkaloid mixture with riddelliine and retrorsine-N-oxide as the main components led to death in a 2 month-old boy in doses of 3 mg/kg bw/day (= 3000 µg/kg bw/day) over a period of 4 days, while doses of 0.8-1.7 mg/kg bw/day (= 800-1700 µg/kg bw/day) over a period of 14 days resulted in ascites and pleural effusion in a 6 month-old girl, followed by a liver fibrosis 2 months later, which finally developed a liver cirrhosis after 6 months.

The intake for "tea (overall)" might result in an overestimation of exposure, as it includes other tea varieties apart from herbal tea (green and black tea, among others) that tend to have lower PA contents. The "worst case" is therefore assumed to be high consumption of herbal tea with high PA content, where a child would consume 0.655 µg PA/kg bw/day via a herbal tea infusion (Table 8). This supplies a differential of factor 4580 plus for the boy relative to the lethal dose and a differential of between 1221 and 2595 for the girl relative to the dose that caused liver cirrhosis. Therefore, acute damage to health from short-term intake (e.g. over a period of 14 days) of PAs in herbal tea/tea infusions in doses corresponding to the scenarios outlined in Table 7 is considered to be improbable.

In general, various uncertainties have to be taken into account when estimating PA intake. First, there was no differentiation between the consumption of different tea varieties, for example, and this can lead to overestimation or underestimation of the consumption of individual tea varieties. It can be assumed, however, there are no major differences in the amounts of tea consumed, particularly in the case of frequent consumers. It is also not known whether the measured contents reflect the actual distribution of PAs in herbal teas on the German market.

The content of 1,2-unsaturated pyrrolizidine alkaloids measured in the herbal tea and tea samples from different batches vary considerably in both qualitative and quantitative terms. One possible reason is that the plants used to make the herbal teas grew together with various wild herbs containing PAs and that components from the latter contaminated the herbal teas. This appears plausible, as in the past specific salade has been contaminated with plant components containing PAs, namely blossoms and leaves of the common groundsel (*Senecio vulgaris* L.). The BfR published a corresponding risk assessment in 2007 *(11)*. The increasing spread of *Senecio* species containing PAs in moderate climate zones was already known *(5, 11)*, and back then the BfR recommended that horticultural and agricultural products be checked for contamination with corresponding plants. At the time, there were good reasons to focus these efforts on lettuce and leaf vegetables as well as herbs, and it was pointed out that it appeared necessary to train harvesting personnel accordingly. It must be taken into account that other wild herbs unrelated to *Senecio* species containing PAs may contaminate plant material used for food production.

3.2 Conclusions, framework for action and measures

Based on the entirety of experimental findings on metabolism, activation mechanism, DNA adduct formation, genotoxicity and carcinogenicity, it should be considered that PAs can also have a carcinogenic effect in humans. However, the incomplete data basis does not permit any robust statements on the differences between the individual PAs in terms of their carcinogenic potential and their toxicity.

The use of equivalence factors based on the ratio of the LD 50 values measured for i.p. administration of the individual PAs to rats is not considered suitable for assessment of the longer-term exposure to PAs and the resulting risks (in particular carcinogenic effects), as they do not take into account possible differences between individual PAs in terms of toxicokinetics following oral administration and in terms of toxicodynamics following longer-term exposure. The BfR therefore regards PAs as a group of substances with cumulative effect with respect to their carcinogenic effect. It is possible, however, that this "cumulative assessment group approach" overestimates the risk. Using the MOE approach for the risk assessment of substances with genotoxic and carcinogenic properties, an approach which is currently standard in the EU *(2)*, the derived BMDL10 (Benchmark Dose Lower Confidence Limit, 10%) for lasiocarpine of 0.073 mg/kg bw/day is used as the basis for the MOE estimation. It is assumed that the exposure levels can be seen as being of low concern if the MOE is 10000 or above *(2)*. The BfR therefore recommends that considering the PA intake from all sources, an MOE should not fall below 10000. However, total exposure to PAs from all foods should generally be kept as low as possible.

In view of the genotoxic and carcinogenic effects of PAs, efforts should be made to minimise the PA contents in herbal teas and teas in order to minimise the putative higher cancer risk of frequent consumers and in particular of children, whose higher sensitivity to PA-related effects must be taken into account. This measure is also deemed necessary, because possible additional exposure to PAs may occur from other food sources like honey.

Although unexpectedly high PA contents were measured in individual herbal tea and tea samples, an acute health risk is regarded as improbable, even if tea was brewed up with high doses.

Based on the available data, the BfR concludes that the adults and children who belong to the frequent consumers of herbal tea infusions are possibly at increased risk of impaired

health due to cancer, particularly if they consume products with high PA content over longer periods of time.

It is considered to be a matter of urgency that the economic operators investigate the causes of the contamination. This includes botanical analysis of the plant material to which the occurrence of the PAs found in the herbal tea and tea samples is attributed. The companies in question should evaluate ways to reduce PA content by improving cultivation, harvesting and cleaning methods.

In order to avoid the marketing of contaminated batches and to protect the consumer, it is recommended that herbal tea batches should be checked for PA content prior to marketing.

The BfR also recommends that the food control authorities should analyze the PA content of herbal tea and tea samples. The Institute also recommends the gathering of representative data on PAs in herbal tea and tea samples as part of the food monitoring programme.

The measured data for PA content for the non-representative tea varieties rooibos tea, green tea and black tea is based on only small samples and should be followed up; if necessary, the corresponding measures must be extended to include these tea varieties as well.

The BfR recommends that the Federal Institute for Drugs and Medical Devices should be informed, since the findings may also be of relevance for medicinal teas.

This assessment is based on the data currently available to the BfR. The risk assessment is to be considered preliminary due to the non-representative data sample and the significant gaps in knowledge.

Due to the existing uncertainties discussed, the BfR is currently not able to deduce or even specify a limit value for PAs in herbal tea/tea. The available knowledge on the causes of PA contamination is insufficient, and data on actual consumption of the individual varieties is incomplete. It has not yet been possible to adequately determine any variation between different batches of a product.

References

1. Leitsätze für Tee, teeähnliche Erzeugnisse, deren Extrakte und Zubereitungen vom 02.12.1998 (BMELV) (BAnz. Nr. 66a vom 09.04.99, GMBl. Nr. 11 S. 228 vom 26.04.99).
2. EFSA Gutachten des Wissenschaftlichen Ausschusses auf Ersuchen der EFSA in Bezug auf einen harmonisierten Ansatz für die Risikobewertung von Substanzen mit genotoxischen und karzinogenen Eigenschaften. *The EFSA Journal* **2005,** *282*, 1-30.
3. WHO IPCS International Programme on Chemical Safety. *Environmental Health Criteria 80* **1988**.
4. Mattocks, A. R. Chemistry and Toxicology of Pyrrolizidine Alkaloids. *Academic Press* **1986**.
5. JOINT FAO/WHO Discussion Paper on Pyrrolizidine Alkaloids. *Food Standard Programme* **2011**.
6. Wiedenfeld, H.; Roeder, E.; Bouaul, T.; Edgar, J. Pyrrolizidine Alkaloids - Structure and Toxcity. *V&R Uni Press, Bonn University Press* **2008**.
7. Teuscher und Lindequist *Biogene Gifte;* Wissenschaftliche Verlagsgesellschaft Stuttgart: 2010.
8. Smith, L. W.; Culvenor, C. C. Plant sources of hepatotoxic pyrrolizidine alkaloids. *J Nat. Prod.* **1981,** *44* (2), 129-152.
9. Hartmann, T.; Witte, L. Chemistry, biology and chemoecology of pyrrolizidine alkaloids. *Alkaloids: chemical and Biological Perspectives* **1995,** *9*, 155-233.
10. BfR-Stellungnahme vom Aug. 11, 2011 Risikobewertung von Pyrrolizidinalkaloiden in Honig. http://www.bfr.bund.de/cm/343/analytik-und-toxizitaet-von-pyrrolizidinalkaloiden.pdf
11. BfR-Stellungnahme Nr. Salatmischung mit Pyrrolizidinalkaloid-haltigem Greiskraut verunreinigt. http://www.bfr.bund.de/cm/343/salatmischung_mit_pyrrolizidinalkaloid_haltigem_geiskraut_verunreinigt.pdf
12. Teuscher, E.; Melzig, M. F.; Lindequist, U. *Biogene Arzneimittel;* Wissenschaftliche Verlagsgesellschaft mbH Stuttgart: 2007; Vol. 6.
13. Roeder, E. pyrrolizidinalkaloidhaltige Arzneipflanzen. *Deutsche Apotheker Zeitung* **1992,** *45* (132), 2427-2435.
14. National Institutes of Health *TP Technical Report on the Toxicology and Carcinogenesis studies of Riddelliine in F344/N rats and B6C3F1 mice*;NIH Publication No. 03-4442; May, 03.
15. Pyrrolizidin-Alkaloide, Stufe II Abwehr von Arzneimittelrisiken. Bekanntmachung über die Zulassung und Registrierung von Arzneimitteln. 111 ed.; 1992; p 4805.
16. Danninger, T.; Hagemann, U.; Schmidt, V.; Schönhöfer, P. S. Zur Toxizität Pyrrolizidinalkaloidhaltiger Arzneipflanzen. *Pharmazeutische Zeitung* **1983,** *128* (6), 289-303.
17. Xia, Q.; Chou, M. W.; Kadlubar, F. F.; Chan, P. C.; Fu, P. P. Human liver microsomal metabolism and DNA adduct formation of the tumorigenic pyrrolizidine alkaloid, riddelliine. *Chem Res Toxicol* **2003,** *16* (1), 66-73.
18. Wang, Y. P.; Yan, J.; Fu, P. P.; Chou, M. W. Human liver microsomal reduction of pyrrolizidine alkaloid N-oxides to form the corresponding carcinogenic parent alkaloid. *Toxicol Lett* **2005,** *155* (3), 411-420.
19. Xia, Q.; Chou, M. W.; Edgar, J. A.; Doerge, D. R.; Fu, P. P. Formation of DHP-derived DNA adducts from metabolic activation of the prototype heliotridine-type pyrrolizidine alkaloid, lasiocarpine. *Cancer Lett* **2006,** *231* (1), 138-145.

20. Fu, P. P.; Xia, Q.; Chou, M. W.; Lin, G. Detection, hepatotoxicity, and tumorigenicity of pyrrolizidine alkaloids in Chinese herbal plants and herbal dietary supplements. *Journal of Food and Drug Analysis* **2007,** *15* (4), 400-415.
21. v Borstel, K.; Witte, L.; Hartmann, T. Pyrrolizidine alkaloid patterns in population of Senecio vulgaris, Senecio vernalis and their hybrids. *Phytochemistry* **1989,** *28* (6), 1635-1638.
22. WHO Evaluation of Carcinogenic Risk of Chemicals to man. *IARC Monographs* **1976,** *10*.
23. WHO Evaluation of Carcinogenic Risks to Humans. *IARC Monographs* **2002,** *82*.
24. NTP Technical Report Toxicity Studies of Riddelliine administered by gavage to F344/N rats and B6C3P1 mice. NIH Publication No. 94-3350, 1992.
25. Kakar, F.; Akbarian, Z.; Leslie, T.; Mustafa, M. L.; Watson, J.; van Egmond, H. P.; Omar, M. F.; Mofleh, J. An outbreak of hepatic veno-occlusive disease in Western afghanistan associated with exposure to wheat flour contaminated with pyrrolizidine alkaloids. *J Toxicol* **2010,** *2010*, 313280.
26. Roeder, E. Wie verbreitet und wie gefährlich sind Pyrrolizidinalkaloide? *Pharmazie in unserer Zeit* **1984,** *13* (2).
27. Pohlenz, J.; Lüthi, J. Auftreten einer Pyrrolizidinalkaloid-Zirrhose beim Rind nach Aufnahme von Senecio alpinus (Alpenkreuzkraut). *Wochenzeitschrift* **1981,** *111* (24).
28. Kim, H. Y.; Stermitz, F. R.; Li, J. K.; Coulombe, R. A., Jr. Comparative DNA cross-linking by activated pyrrolizidine alkaloids. *Food Chem Toxicol* **1999,** *37* (6), 619-625.
29. Brown, P. H. Seneciosis or grass staggers of horses in Basutoland. *Bull. epiz. Dis. Afr.* **1956,** *4*, 285.
30. Bull, L. B.; Culvenor, C. C.; Dick, A. T. The pyrrolizidine alkaloids. Their chemistry, pathogenicity and other biological properties. *North Holland Publishing Company* **1968**.
31. Sedlmaier, H.; Dahme, E.; Schiefer, B. Frühveränderungen an der Rattenleber nach Fütterung von Senecio vulgaris (Kreuzkraut) und von p-Dimethylaminoazobenzol (Buttergelb). *Zbl. Vet. Med.* **1959,** *6*, 854-871.
32. Poison Information, senecio vulgaris. 2013.
33. Altaee, M. Y.; Mahmood, M. H. An outbreak of veno-occlusive disease of the liver in northern Iraq. *Eastern Mediterranean Health Journal* **1998,** *4* (1), 142-148.
34. Huxtable, R. J. Herbal teas and toxins: novel aspects of pyrrolizidine poisoning in the United States. *Perspect Biol Med* **1980,** *24* (1), 1-14.
35. NTP, 1. Bioassay of Lasiocarpine for possible carcinogenicity. NTP Technical Report 39, 1-66.
36. WHO; 1983 IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans; Volume 31.
37. WHO; 1987 IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volumes 1-42, Supplement 7.
38. Fu, P. P.; Xia, Q. S.; Lin, G.; Chou, M. W. Pyrrolizidine alkaloids - Genotoxicity, metabolism enzymes, metabolic activation, and mechanisms. *Drug Metabolism Reviews* **2004,** *36* (1), 1-55.
39. COT Statement on Pyrrolizidine Alkaloids in Food; 2008. Food Standard Agency.
40. Huxtable, R. J. Herbal Teas and Toxins - Novel Aspects of Pyrrolizidine Poisoning in the United-States. *Perspectives in Biology and Medicine* **1980,** *24* (1), 1-14.
41. Fox, D. W.; Hart, M. C.; Bergeson, P. S.; Jarrett, P. B.; Stillman, A. E.; Huxtable, R. J. Pyrrolizidine (Senecio) Intoxication Mimicking Reye Syndrome. *Journal of Pediatrics* **1978,** *93* (6), 980-982.

42. Stillman, A. E.; Huxtable, R.; Consroe, P.; Kohnen, P.; Smith, S. Hepatic Veno-Occlusive Disease Due to Pyrrolizidine (Senecio) Poisoning in Arizona. *Gastroenterology* **1977,** *73* (2), 349-352.
43. Ridker, P. M.; Ohkuma, S.; Mcdermott, W. V.; Trey, C.; Huxtable, R. J. Hepatic Venocclusive Disease Associated with the Consumption of Pyrrolizidine-Containing Dietary-Supplements. *Gastroenterology* **1985,** *88* (4), 1050-1054.
44. Culvenor, C. C. J.; Edgar, J. A.; Smith, L. W.; Kumana, C. R.; Lin, H. J. Heliotropium-Lasiocarpum Fisch and Mey Identified As Cause of Venoocclusive Disease Due to A Herbal Tea. *Lancet* **1986,** *1* (8487), 978.
45. Kumana, C. R.; Ng, M.; Lin, H. J.; Ko, W.; Wu, P. C.; Todd, D. Hepatic Veno-Occlusive Disease Due to Toxic Alkaloid in Herbal Tea. *Lancet* **1983,** *2* (8363), 1360-1361.
46. Kumana, C. R.; Ng, M.; Lin, H. J.; Ko, W.; Wu, P. C.; Todd, D. Herbal Tea Induced Hepatic Veno-Occlusive Disease - Quantification of Toxic Alkaloid Exposure in Adults. *Gut* **1985,** *26* (1), 101-104.
47. Datta, D. V.; Khuroo, M. S.; Mattocks, A. R.; Aikat, B. K.; Chhuttani, P. N. Herbal Medicines and Veno-Occlusive Disease in India. *Postgraduate Medical Journal* **1978,** *54* (634), 511-515.
48. Tandon, B. N.; Tandon, R. K.; Tandon, H. D.; Narndranathan, M.; Joshi, Y. K. Epidemic of Veno-Occlusive Disease of Liver in Central India. *Lancet* **1976,** *2* (7980), 271-272.
49. Krishnamurthi, D.; Krishnaswamy, K.; Nagarajan, V.; Krishnamachari, K. A. V. R.; Bhat, R. V. Aetiopathogenesis of endemic ascites in Surguja district of Madhya Pradesh. *Indian Journal of Medical Research* **2013,** *65* (5), 672-678.
50. Mohabbat, O.; Srivastava, R. N.; Younos, M. S.; Merzad, A. A.; Sediq, G. G.; Aram, G. N. Outbreak of Hepatic Veno-Occlusive Disease in Northwestern Afghanistan. *Lancet* **1976,** *2* (7980), 269-271.
51. ANZFA (Australia New Zealand Food Authority); 2001. Pyrrolizidine Alkaloids in Food. A Toxicological Review and Risk Assessment. Technical report series no.2. Canberra, Australia (http://www.foodstandards.gov.au/_srcfiles/TR2.pdf).
52. DFG – Senatskommission zur Beurteilung der gesundheitlichen Unbedenklichkeit von Lebensmitteln (SKLM), Stellungnahme zu Pyrrolizidinalkaloiden in Honigen, Imkereierzeugnissen und Pollenprodukten, Beschluss vom 8. Nov. 2002 (URL: http://www.dfg.de/aktuelles_presse/reden_stellungnahmen/2003/download/sklm_pa_honig_170403end.pdf ; Stand 24.09.2009).
53. RIVM 2005. Advisory report on pyrrolizidine alkaloids in herb preparations. 2013.
54. EFSA Opinion of the Scientific Panel on Contamiants in the Food Chain on a request from the European Commission related to Pyrrolizidine Alkaloids as undesirable substances in Animal Feeds. *The EFSA Journal* **2007,** *447*, 1-51.
55. EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on Pyrrolizidine alkaloids in food and feed. *The EFSA Journal* **2011,** *9(11):2004*, 134.
56. DIN ISO 32645:1994 Chemical Analsysis; Decision limit, Detection limit and determination limit, Estimation in case of repeatability, terms, methods, evaluation. *Deutsches Institut für Normung DIN* **1994**.
57. European Commission Commission Dicision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of the European Communities* **2002,** *L221*, 8-36.

58. Stahnke, H.; Kittlaus, S.; Kempe, G.; Alder, L. Reduction of Matrix Effects in Liquid Chromatography-Electrospray Ionization-Mass Spectrometry by Dilution of the Sample Extracts: How Much Dilution is Needed? *Analytical Chemistry* **2012,** *84* (3), 1474-1482.
59. AOAC Guidelines for collaborative study procedures to validate characteristics of a method of analysis. *AOAC International* **2002**.
60. EFSA European Food Safety Authority, 2010. Scientific Report of EFSA. Results of the monitoring of dioxin levels in food and feed. *The EFSA Journal* **2010,** *2010* (8(3):1385), 36.
61. MRI (Max Rubner-Institut) 2008: Nationale Verzehrsstudie II (NVS II), Ergebnisbericht 1, 2 http://www.was-esse-ich.de/.
62. Krems, C.; Bauch, A.; Götz, A.; Heuer, T.; Hild, A.; Möseneder, J.; Brombach, C. Methoden der Nationalen Verzehrsstudie II. *Ernährungsumschau* **2006,** *53* (2).
63. Banasiak, U.; Heseker, H.; Sieke, C.; Sommerfeld, C.; Vohmann, C. Abschätzung der Aufnahme von Pflanzenschutzmittel-Rückständen in der Nahrung mit neuenVerzehrsmengen für Kinder. *Bundesgesundheitsblatt Gesundheitsforsch. Gesundheitsschutz* **2013,** *48* (1), 84-89.
64. Heseker, H.; Oeppining, A.; Vohmann, C. Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln (VELS). Forschungsbericht im Auftrag des Bundesministeriums für Verbraucherschutz, Ernährung und Landwirtschaft, Universität Paderborn. 2003.