Pyrrolizidine alkaloids – Impact of the public statements made by EMA and national health authorities on the pharmaceutical industry

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ALARA	As low as reasonable achievable
ALB	LAV-Arbeitsgruppe "Lebensmittel, Bedarfsgegenstände, Wein und Kosmetika", LAV-working group “food products, consumer goods, wine and cosmetics”
ANZFA	Australian New Zealand Food Authority
API	Active pharmaceutical substance
BAH	Bundesverband der Arzneimittel-Hersteller e.V.; German Manufacturers’ Association
BASG	Bundesamt für Sicherheit im Gesundheitswesen; Austrian Federal Office for Safety in Health Care
$BMDL_{10}$	Benchmark dose lower confidence limit
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte; Federal Insitute for Drugs and Medical Devices
BfR	Bundesinstitut für Risikobewertung; Federal Institute for Risk Assessment
BPI	Bundesverband der Pharmazeutischen Industrie e.V.; German Pharmaceutical Industry Association
b.w.	Body weight
con	Concentration
CONTAM	EFSA Scientific Panel on Contaminants in the Food Chain
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
CYP	Cytochrome P450
DAB	Deutsches Arzneibuch; German pharmacopeia
DC procedure	Decentralised Procedure
DER	Drug-extract-ratio
DFA	Deutscher Fachausschuss für Arznei-, Gewürz- und Aromapflanzen; German Technical Committee for Medicinal, Aromatic and Perfumery Plants
DHP	dihydropyrrolizine
DHU	Deutsche Homöopathie-Union
EU	European Union
Eu	Europine

EuNO	Eurpine-N-oxide
DNA	Deoxyribonucleic acid
EFSA	European food safety authority
e.g.	For example
ELISA	Enzyme linked immunosorbent assay
EMA	European Medicines Agency
Em	Echimidine
EmNO	Echimidine-N-oxide
Er	Erucifoline
ErNO	Erucifoline-N-oxide
e.V.	Eingetragener Verein; registered assosiation
Fagg	Federaal agentschap voor geneesmiddelen en gezondheidsproducten; Federal agency for medicines and health products
FAH	Forschungsvereinigung der Arzneimittel-Hersteller e.V.; Research Association of Pharmaceutical Manufacturers
FAO	Food and Agriculture Organization of the United Nations
GACP	Good Agriculture and Collection Practice
GC-MS	Gas chromatography - mass spectrometry
GSH	Glutathione
He	Heliotrine
HeNO	Heliotrine-N-oxide
HMPC	Committee on Herbal Medicinal Products
HSOS	Hepatic sinusoidal obstruction syndrome
HVOD	Hepatic veno-occlusive disease
IARC	International Agency for Research on Cancer
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
Im	Intermedine
ImNO	Intermedine-N-oxide
IPCS	International Programme on Chemical Safety
Jb	Jacobine
JbNO	Jacobine-N-oxide

LAV	Länderarbeitsgemeinschaft Verbraucherschutz, German federal states working group on consumer protection
Lc	Lasiocarpine
LcNO	Lasiocarpine-N-oxide
LC-MS/MS	Liquid chromatography - tandem mass spectroscopy
LOD	Limit of detection
LOQ	Limit of quantification
LTL	Less-Than-Lifetime
Ly	Lycopsamine
LyNO	Lycopsamine-N-oxide
MOE	Margin of Exposure
MHRA	Medicines and Healthcare products regulatory agency
Mc	Monocrotaline
McNO	Monochroaline-N-oxide
MR procedure	Mutual recognition procedure
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NTP	National Toxicology Program of the U.S. Department of Health and Human Services
Nu	Nucleophile
OOS	Out of specification
PA	Pyrrolizidine alkaloid
PAH	Pulmonary arterial hypertension
PCBs	Polychlorinated biphenyl
PTDI	Provisional tolerable daily intake
REP	Relative potency factor
Re	Retrorsine
ReNO	Retrorsine-N-oxide
RIVM	Rijksinstituut voor Volksgezondheid en Milieu; Dutch National Institute for Public Health and the Environment
RTK	Real time kinematic
SmPC	Summary of product characteristics
SnNO	Senecionine-N-oxide

SPE-LC-MS/MS	Solid phase extraction liquid chromatography tandem mass spectrometry
Sp	Seneciphylline
SPNO	Seneciphylline-N-oxide
Sv	Senecivernine-N-oxide
SvNO	Senecivernine
Sk	Senkirkine
TDI	tolerable daily intake
TTC	Threshold of Toxicological Concern
Td	Trichodesmine
UHPLC-MS	Ultra performance liquid chromatography mass spectrometry
UK	United Kingdom
WHO	World Health Organization

1. Introduction

Pyrrolizidine alkaloids are secondary plant metabolites which are hepatotoxic in animals and humans. More than 660 pyrrolizidine alkaloids in over 6000 plants have been identified, corresponding to approximately 3 % of flowering plants. The ingestion of pyrrolizidine alkaloids can result in acute or chronic liver toxicity and genotoxicity. Acute poisoning is characterised by hepatic sinusoidal obstruction syndrome (HSOS) and can lead to liver cirrhosis and liver failure. Chronic toxicity can lead to abnormalities, mainly in the liver, lung and blood vessels, such as cell enlargement (megalocytosis), liver cirrhosis and carcinomas.

Herbal medicines derived from pyrrolizidine alkaloid producing plants (e.g. *Symphytum*) have been regulated in the different European countries for a long time. However, it was recently detected that herbal medicinal products, herbal teas and food supplements from non-pyrrolizidine alkaloid producing plants can also contain different amounts of pyrrolizidine alkaloids. These findings are usually attributed to cross-contamination of the herbal drugs with weeds containing pyrrolizidine alkaloids.
Therefore, different national and European legislations setting limits for pyrrolizidine alkaloids in herbal medicinal products have been enacted. This work discusses the recent regulatory developments and their effects on the pharmaceutical industry.

2. Pyrrolizidine alkaloids

2.1 Occurrence and structure of pyrrolizidine alkaloids

2.1.1 Occurence

Pyrrolizidine alkaloids are secondary plant metabolites, produced for protection against herbivores. The number of pyrrolizidine alkaloid producing plants is over 6000, which corresponds to approximately 3 % of all flowering plants (1). The following plant families are classified as the main sources: *Boraginaceae* (all genera), *Asteraceae* (subtribe *Senecioneae* and *Eupatorieae*), *Fabaceae* (subtribe *Crotalariaceae*, mainly genus *Crotalaria*) (1; 2). To date over 660 different pyrrolizidine alkaloids and N-oxide derivates have been identified (3).

(a) (b) (c)

Figure 1: (a) Family: *Boraginaceae*, Species: *Symphytum officinale spectabilis, from* (4); (b) Family: *Asteraceae*, Species: *Eupatorium cannabinum, from* (5); (c) Family: *Fabaceae*, Species: *Crotalaria Roth, from* (6)

Many of these plants grow as weeds and can contaminate fodder crops, as well as fields of cultivated herbal drugs, which are used to produce herbal medicinal products, food supplements or tea. Additionally, some of the pyrrolizidine alkaloid producing plants have been used as medicinal herbal products for many centuries, mainly *Senecio, Borago, Lithospermum, Heliotropium, Eupatorium, Tussilago and Symphytum* (2).

The biosynthesis of pyrrolizidine alkaloids can differ between the plant species but generally the pyrrolizidine alkaloids are produced in the roots of the plant and then transferred to leaves and flowers (7; 8). Analyses of *Senecio* species indicate that the flowers contain the highest amount of pyrrolizidine alkaloids, followed by the leaves. In the stem only low amounts of pyrrolizidine alkaloids could be found (9). Furthermore,

the pyrrolizidine alkaloid amount can over the life cycle of the plant and is influenced by environmental factors, such as weather, nutrition and water conditions (10).

2.1.2 Structure

Pyrrolizidine alkaloids consist of a necine base and a necine acid moiety, as shown in Figure 2. The necine base moiety is a 8 membered bicyclic heterocycle which can be saturated or unsaturated at the 1,2 position.

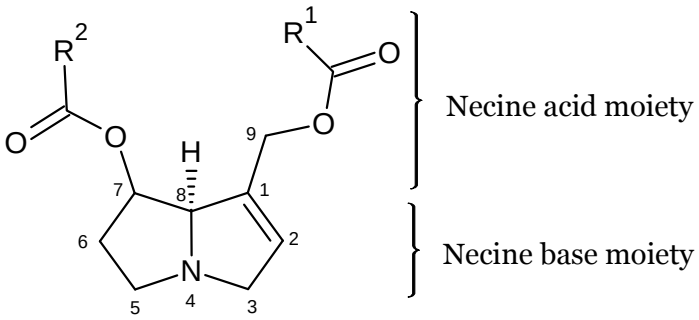

Figure 2: Structure of pyrrolizidine alkaloids

Three common types of the necine base can be distinguished, platynecine and its corresponding N-oxide, retronecine and its enantiomer heliotridine including their corresponding N-oxides and otonecine, see Figure 3 (11; 12).

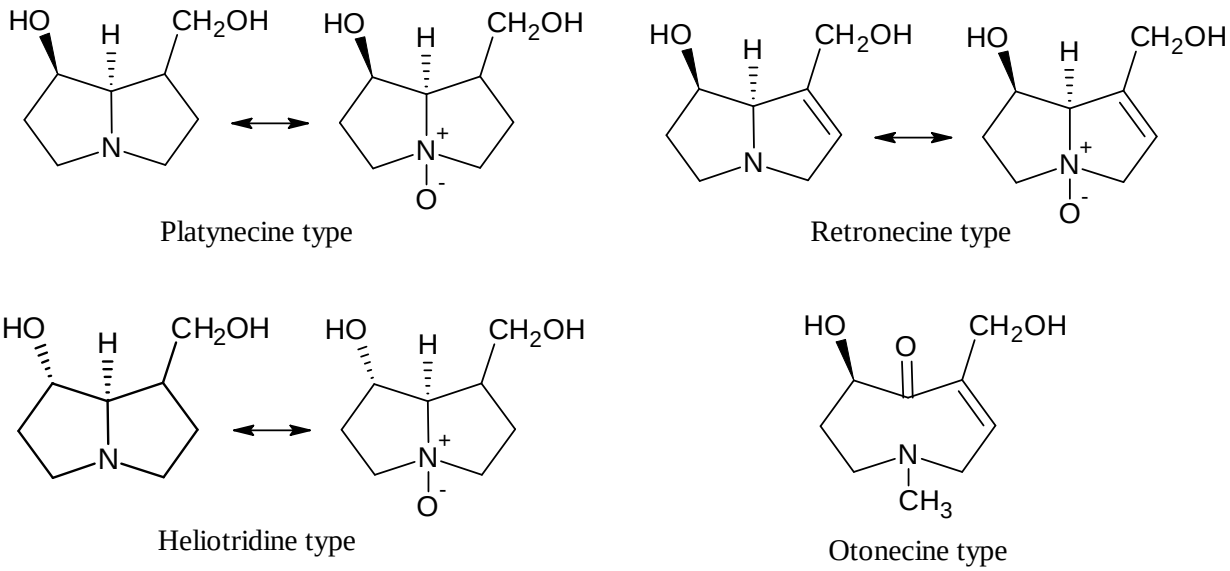

Figure 3: Platynecine, retronecine, heliotridine and otonecine type pyrrolizidine alkaloids and corresponding N-oxides

The necine acids can also be divided into different subtypes: monoester, open-chain diester and macrocyclic diester. Examples for the different types are displayed below (Figure 4).

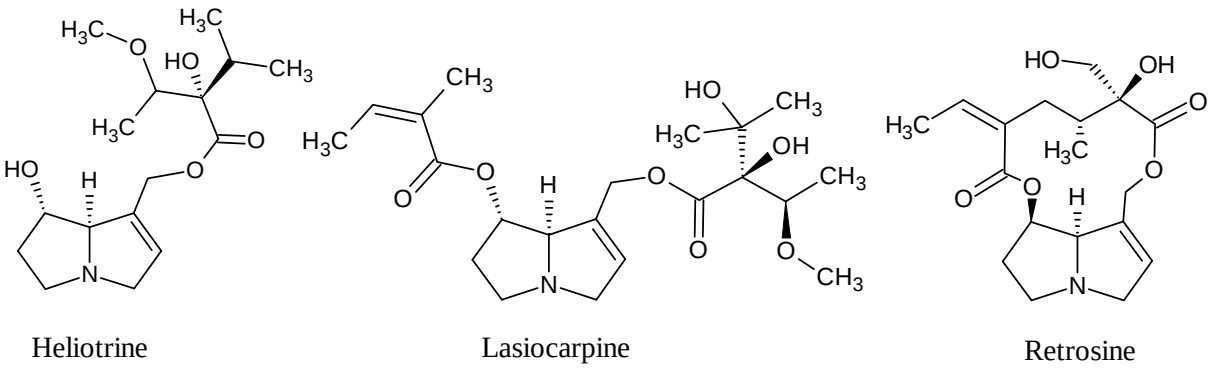

Figure 4: Structure of heliotrine (monoester), lasiocarpine (open-chain diester) and retrosine (macrocyclic diester)

Relevant for the toxic effects of pyrrolizidine alkaloids are the following three structural properties (13; 14):

- a double bond in the 1,2 position of the pyrrolizidine ring
- a hydroxymethyl substituent at the C-9 position, optionally additional at the C-7 position
- a branched necine acid moiety

Pyrrolizidine alkaloids of the platynecine type do not have a double bond in the 1,2 position and therefore cannot be metabolised into the toxic metabolite. Thus, in the remaining work the term pyrrolizidine alkaloids refers to 1,2 unsaturated pyrrolizidine alkaloids, which can be converted into the toxic metabolite.

2.2 Poisoning in humans

Two different poisoning patterns occur in humans, acute poisoning, caused by high doses of pyrrolizidine alkaloids and chronic poisoning, caused by small doses of pyrrolizidine alkaloids over a longer period.

Incidences of acute poisoning with pyrrolizidine alkaloids occur mainly in third world countries. The main cause here is the contamination of wheat with pyrrolizidine alkaloid containing plants. The wheat is mainly used for the production of bread. Different outbreaks have been reported in South Africa, India, Afghanistan and Tajikistan (15). Acute poisoning with pyrrolizidine alkaloids is accompanied by HSOS, formerly known as acute hepatic veno-occlusive disease (HVOD), and can lead to liver cirrhosis and, eventually, to liver failure. The main symptom of HSOS is abdominal pain, especially in the right hypochondrium, nausea and vomiting can also be present. Furthermore,

patients generally present with hepatomegaly, often combined with pleural effusion. The acute poisoning has a high mortality rate and survivors are likely to develop sub-acute or chronic disease (16; 15).

In developed countries the contamination of wheat is no concern. Here the main problem is the possible sub-acute or chronic intoxication from herbal remedies or food products, e.g. honey. This is especially relevant because the use of natural supplements is associated with a healthy lifestyle and thought to be harmless, in contrast to chemical supplements, for example. By ingesting contaminated herbal remedies, mostly small doses of pyrrolizidine alkaloids are ingested over a longer period of time, which can lead to sub-acute or chronic liver disease (14; 16).
An estimate of negative effects on the population is hard to make, as no epidemiological studies on this topic are available. Only few cases of hepatotoxic damage can be directly linked to the intake of herbal remedies, as the chronic disease can have a long latency period and is clinically similar to other toxic liver diseases like alcoholic liver disease (15). Furthermore, excretion studies in animals show that most of the un-metabolised pyrrolizidine alkaloids are excreted rapidly (approximately during the first 24 hours) (17; 18). This makes diagnosis more difficult, even if there is a suspicion of pyrrolizidine alkaloid intoxication. Recently, a UHPLC-MS method was developed to identify and quantify pyrrole-protein adducts, which can act as biomarker for pyrrolizidine alkaloid induced liver damage in human blood samples (19; 20). This may be a new approach to identify poisoning incidences with pyrrolizidine alkaloids.
Edgar et. all suggest that for all cirrhosis cases of unknown cause, the exposure to pyrrolizidine alkaloids should be considered. For this purpose, he established a list of six risk factors, which indicate a high likelihood of pyrrolizidine alkaloids induced liver cirrosis. Examples are latent or overt HSOS and pulmonary arterial hypertension (PAH) of unknown or uncertain cause or PAH accompanied by evidence of hepatotoxicity (21).
Additionally, many of the patients do not mention that they take any herbal remedies at all (22). Therefore, it is important to raise awareness for possible hepatic damage caused by herbal remedies within the medically trained staff, as well.

Approximately 20 cases of hepatotoxicity caused by pyrrolizidine alkaloids containing herbal remedies are known in the literature (15; 2). These cases are mainly acute to sub-acute cases, as here the connection to pyrrolizidine poisoning is easier to make. Two well-documented examples are stated below, one of a six-month-old girl and the second of a two-month-old boy, both consumed *Senecio longilobus* as herbal tea.
The girl (bodyweight of 6 kg) showed HSOS, which developed into extensive hepatic fibrosis after 2 month and further into liver cirrhosis after 6 month. In this case, the

herbal tea contained 1.3 % pyrrolizidine alkaloids and N-oxides based on dry weight. Over 2 weeks, the girl consumed approximately 70 - 147 mg of pyrrolizidine alkaloids or 12 - 25 mg/kg bodyweight, resulting in a daily dose of 0.8 - 1.7 mg/ kg bodyweight (15; 14; 23; 24). The boy (assumed body weight of 5.5 kg) developed haematemesis, jaundice, central nervous spasm, bradycardia and apnoea periods and died after 6 days. Here, the herbal tea contained 0.5 % pyrrolizidine alkaloids and 1.0 % N-oxide. Over 4 days he consumed approximately 66 mg pyrrolizidine alkaloids corresponding to 12 mg/kg bodyweight or 3 mg/kg bodyweight/day (24; 25).

Not only herbal remedies can be contaminated with pyrrolizidine alkaloids, also food products like honey, milk, meat and eggs. The contamination of food occurs when livestock ingest contaminated fodder or bees produce honey from pyrrolizidine alkaloid producing plants. The levels in these products are too low to cause any acute or sub-acute poisoning. However, when consuming contaminated products in high quantities, there might be a risk of chronic disease. Extensive studies on the contamination of honey have been conducted by the German Federal Institute for Risk Assessment (BfR) (26; 27; 24).

2.3 Metabolism and toxicity

2.3.1 Metabolism

As already mentioned, pyrrolizidine alkaloids consist of a necine base and a necine acid (Figure 2). The pyrrolizidine alkaloid itself is not toxic and needs to undergo metabolic activation in the liver in order to exhibit toxic effects. The assumed metabolic pathway for the retronecine, heliotridine and otonecine type pyrrolizidine alkaloids is shown below in Figure 5.

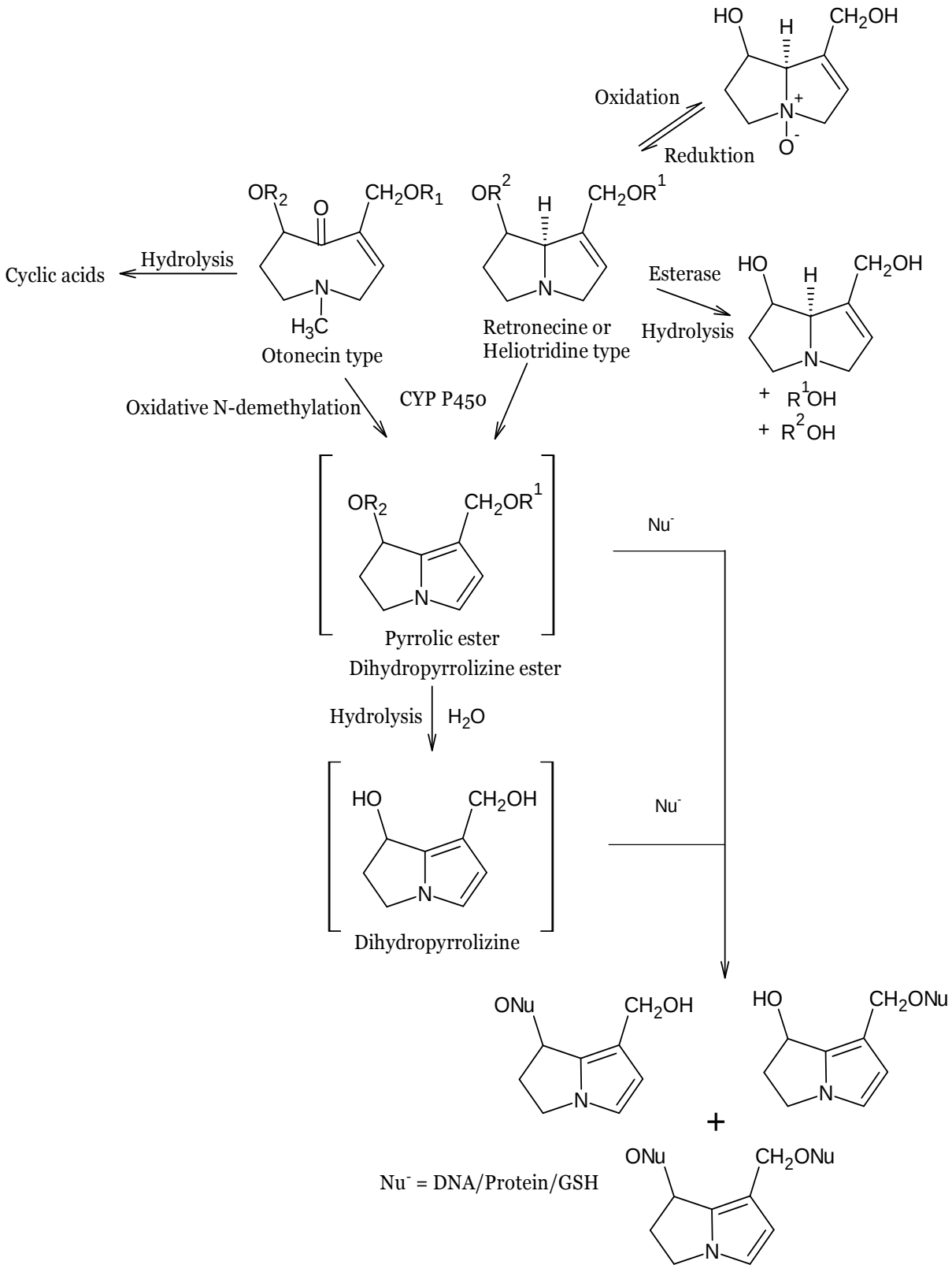

Figure 5: Metabolism of pyrrolizidine alkaloids, according to literature (12)

In general, three different metabolic pathways for the retronecine and heliotridine type pyrrolizidine alkaloids are possible, two lead to detoxification products and one to the toxic metabolite pyrrolic ester. The first detoxification pathway cleaves the ester groups at C7 and C9 via hydrolysis. The resulting necine acid and necine base are excreted readily. The second detoxification pathway leads to the corresponding pyrrolizidine alkaloid N-oxide via N-oxidation. The N-oxide is more water-soluble and can therefore be excreted more quickly. N-oxides can also reconvert to the corresponding pyrrolizidine alkaloid, however. The third pathway is the formation of the toxic metabolite pyrrolic ester via oxidation (toxification pathway), this step is catalysed by cytochrome P450, mainly CYP3A and CYP2B isoforms (12).

For otonecine type pyrrolizidine alkaloids only two different pathways are possible, due to the different chemically structure. As a detoxification pathway, the otonecine type can also undergo hydrolysis to form cyclic acids, these are very water-soluble and can be excreted readily. The second pathway is the formation of the toxic metabolite pyrrolic ester via oxidative N-demethylation and several intermediate steps (12).

Pyrrolic ester is a highly reactive electrophile and can react with various nucleophiles. The toxicity results from the reaction of pyrrolic ester with sulphhydryl-, amino- or hydroxyl groups of DNA or proteins to form DNA-protein crosslinks, DNA-DNA crosslinks or DNA adducts. Pyrrolic ester can also react with water to form dihydropyrrolizine (DHP), which is a less reactive electrophile but can still react with DNA or proteins (28). DHP has a higher water solubility and can be excreted more readily than pyrrolic ester. Pyrrolic ester can also react with glutathione (GSH), which is an endogenous antioxidant, to give another detoxification product.

Due to its high reactivity and short half-live pyrrolic ester could not be isolated in vitro or in vivo so far (21).

As various metabolic pathways are involved in the metabolism of pyrrolizidine alkaloids, the most important factors influencing the toxicity are the metabolic kinetics of these pathways in relation to each other. Great differences between different pyrrolizidine alkaloids and species exist here (26).

In general, humans, rats, mice, cattle and horses are more susceptible to pyrrolizidine alkaloids than sheep, goats, rabbits and guinea pigs. Infants and young children, as well as young animals, are more susceptible than adults (12; 26). In mice, the metabolic activation, and thus the amount of pyrrolic ester formed, is greater for retronecine type

than for otonecine type pyrrolizidine alkaloids. Therefore, it can be assumed that the necine base has an influence on the toxicity of the pyrrolizidine alkaloid (11). The necine acid moiety also influences the metabolic activation, for example through steric hindrances or ester substitutions. The necine acids can be classified according to their metabolic activation or their toxicity respectively. Cyclic diesters have a higher potency than diesters, followed by monoesters (17; 14; 16).

2.3.2 Toxicity

The main target of the toxicity of pyrrolizidine alkaloids is the liver, particularly the sinusoidal endothelial cells, as the pyrrolic ester react rapidly, after their formation. More stable compounds, such as DHP or pyrrolic ester adducts, can reach other target organs, like the lungs and blood vessels (21).

Acute poisoning with pyrrolizidine alkaloids in human cases and experimental animals is characterised by HSOS and can lead to liver cirrhosis and liver failure (21; 16; 18).

The intake of pyrrolizidine alkaloids at lower levels over a longer period, sub-acute or chronic, mainly damages the liver, lungs and blood vessels. In the liver, chronic doses can lead to cell enlargement (megalocytosis) and fibrosis, which can also result in liver cirrhosis. HSOS may not necessarily develop. Consequently, liver cirrhosis caused by chronic pyrrolizidine alkaloid intake cannot be distinguished from liver cirrhosis caused by other substances, like alcohol or aflatoxins (21; 16; 18).

Some pyrrolizidine alkaloids (e.g. monocroteline, fulvine) also cause pulmonary arterial hypertension (PAH) in experimental animal studies. The lowest effective dose to induce PAH was found to be lower than the dose needed for HSOS. This could be an explanation for the occasional development of PAH without liver abnormalities (21; 16; 18).

Pyrrolizidine alkaloids are genotoxic. This effect is based on the reaction of pyrrolic esters with DNA to form DNA adducts, DNA-DNA crosslinks or DNA-protein crosslinks, as well as DNA strand breakage, chromosomal damage and mutations. In animal studies and studies with human cell cultures, pyrrolizidine alkaloids showed carcinogenic effects. Mainly liver tumours were detected, but also lung, kidney and bladder tumours (16; 17; 18).
For example riddelliine has been shown to be carcinogenic in rats. Furthermore, the metabolism of riddelliine in rats and in human microsomes are found to be comparable. Therefore, it can be assumed that the carcinogenic effect is also an issue for humans,

even though no epidemiological data on the carcinogenic effects of pyrrolizidine alkaloids in humans is available (29; 21; 16).

The International Agency for Research on Cancer (IARC) evaluated six pyrrolizidine alkaloids, namely isatidine, lasiocarpine, monocrotaline, retrorsine, riddelline and senkirkine, on their carcinogenicity to humans based on the available animal studies. For isatidine, retrorsine and senkirkine only limited evidence exists and they were hence classified as “not classifiable as to its carcinogenicity to humans (class 3)”. Lasiocarpine, monocrotaline and riddelline were classified as “possibly carcinogenetic to humans (class 2B)” (30; 31).

In experimental animal studies it was also shown that pyrrolizidine alkaloids are teratogenic and foetotoxic, as they have the ability to cross the placenta. Pyrrolizidine alkaloids can also be transferred via mother’s milk to the infant (18).

Another effect that adds to the toxicity of pyrrolizidine alkaloids is the ability to detached from weak nucleophiles and react with another, stronger nucleophile (21). This means that pyrrolizidine alkaloids have a long lasting toxic effect.

As already mentioned, one detoxification pathway is the pyrrolic ester binding with GSH. If the GSH is consumed by the pyrrolic ester, it is no longer available as an antioxidant to bind other potentially harmful substances, which can then damage, the liver, for example (32).

3 Regulatory measures

3.1 Pyrrolizidine alkaloids in herbal medicinal products and food

The finding that pyrrolizidine alkaloids can have hepatoxic effects was published first in the 1950th by Schoental and Cook (33; 34). Recognition of this issue did not start until the 1980th, however. Smith and Convenor's explanation for this is the coincidence with the first risk evaluations of mycotoxins (1).
The following section shows the historical risk assessments of competent authorities for pyrrolizidine alkaloids in medicinal products and food, in chronological order.

World Health Organization – International Programme on Chemical Safety (WHO-IPCS) (1988, 1989a)
The intake of low and chronic doses of pyrrolizidine alkaloids can cause health problems, especially liver cirrhosis and tumours. Evaluation of literature data led the IPCS to the assessment that a dose equivalent to 10 µg heliotrine/kg b.w. (body weight) per day may lead to disease in humans. It was not possible to evaluate the human cancer risk caused by pyrrolizidine alkaloids due to a lack of epidemiological data (18).

BfArM (Federal Institute for Drugs and Medical Devices – Bundesinstitut für Arzneimittel und Medizinprodukte) - Germany (1992)
The graduated plan regarding pyrrolizidine alkaloid containing plants in medicinal products was published by the BfArM. Namely for the following medicinal plants: *Alkanna, Anchusa, Borago, Brachyglottis, Cineraria, Cynoglossum, Erechthites, Eupatorium (except E. perfoliatum), Heliotropium, Lithospermum, Petasites, Senecio, Symphytum and Tussilago.*
The exposure of pyrrolizidine alkaloids should not exceed the following limits:
- 100 µg/day for topical applications (maximum 6 weeks per year)
- 1 µg/day for internal use (maximum 6 weeks per year)
- 10 µg/day for teas made from *Tussilago* (maximum 6 weeks per year)
- 0.1 µg/day for internal or 10 µg/day for topical medicinal products without therapeutic indications or without restriction of intake to 6 weeks (35).

BASG (Austrian Federal Office for Safety in Health Care - Bundesamt für Sicherheit im Gesundheitswesen) - Austria (1994)

In the finished medicinal products of pyrrolizidine alkaloid producing plants (e.g. *Cynoglossum L.*, *Petasites Mill.*, *Senecio L.*, *Symphytum L.*, *Eupatorium cannabinum L.* and *Tussilago farfara L.*) no pyrrolizidine alkaloids should be contained. This should be proven with a state of the art analytical method (36).

Fagg – (Federal agency for medicines and health products - Federaal agentschap voor geneesmiddelen en gezondheidsproducten) - Belgium (2000)

In Belgium, the following pyrrolizidine alkaloid producing plants are forbidden in medicinal products for internal use: *Borago officinalis*, *Eupatorium cannabinum*, *Petasites officinalis*, *Senecio jacobeae*, *Senecio vulgaris*, *Symphytum officinale*, *Tussilago farfara*, all *Aristolochia spp.*, *all Asarum spp.*. (37; 38).

ANZFA (Australian New Zealand Food Authority) – Australian/New Zealand (2001)

The ANZFA carried out a risk assessment on pyrrolizidine alkaloids. Major effects of pyrrolizidine alkaloids are hepatocellular injury, liver cirrhosis and HVOD. As evidence for pyrrolizidine alkaloid induced cancer was lacking in humans, a no-observed-effect level (NOEL) for HSOS of 10 µg/kg b.w. and a provisional tolerable daily intake (PTDI) of 1 µg/kg b.w. of pyrrolizidine alkaloids was recommended for food stuff (39).

RIVM (Dutch National Institute for Public Health and the Environment - Rijksinstituut voor Volksgezondheid en Milieu) - Netherlands (2005)

A tolerable daily intake (TDI) of 0.1 µg/kg b.w./day for non-cancer effects was recommended for food stuff, derived from a study on rats receiving riddelliine for 105 weeks (40).

Swissmedic – Switzerland (2006)

The same regulations for phytopharmaceuticals as in Germany were enacted (41).

CONTAM Panel (EFSA Scientific Panel on Contaminants in the Food Chain) (2007)

The CONTAM panel published a statement on the content of pyrrolizidine alkaloids in feed and the subsequent exposure. There was not enough data available for evaluation. Further test for feed should concentrate on the following pyrrolizidine alkaloids: senecionine, seneciophylline, erucifoline, monocrotaline, trichodesmine, heliotrine, indicine, intermedine and lycopsamine (42).

COT (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment) - UK (2008)

The COT carried out a risk assessment on pyrrolizidine alkaloids in food. It assumed that pyrrolizidine alkaloids are genotoxic carcinogens. A NOAEL of 10 µg/kg b.w./day was derived from a study in rats for hepatocyte cytomegaly. Using an uncertainty factor of 100, a non-cancer effects dose of 0.1 µg riddelliine/kg b.w./day was established.

The COT recommends to asses all pyrrolizidine alkaloids as a cumulative group and establish an adequate Margin of Exposure (MOE). On the basis of a 2 year carcinogenicity study of lasiocarpine in rats, a $BMDL_{10}$ (benchmark dose lower confidence limit) of 0.073 mg/kg b.w./day was established. Originating from an MOE of at least 10,000, a non-cancer effects dose of 0.007 µg/kg b.w./day was established (43).

BfR (Federal Institute for Risk Assessment - Bundesinstitut für Risikobewertung) - Germany (2007)

The BfR assessed leaves and blossoms of *Senecio vulgaris* as contaminants of mixed salad. Acute to sub-acute liver damage as a consequence of consumption of the contaminated salad could not be ruled out (44).

BfR (Federal Institute for Risk Assessment - Bundesinstitut für Risikobewertung) - Germany (2011)

The BfR reported on analytics and toxicity of pyrrolizidine alkaloids as well as risk assessment of the occurrence of pyrrolizidine alkaloids in honey. The total exposure should be as low as possible and should not exceed an intake of 0.007 µg/kg b.w./day (24).

EFSA (European food safety authority) (2011)

The EFSA panel on contaminants in the food chain (CONTAM) evaluated the pyrrolizidine alkaloid content in honey. The panel concluded that 1,2 unsaturated pyrrolizidine alkaloids may act as genotoxic carcinogens in humans and therefore decided to apply the MOE approach recommended by the COT. Based on the available data, possible health concerns for toddlers and children who consume large quantities of honey cannot be ruled out (16).

BfR (Bundesinstitut für Risikobewertung) - Germany (2013)

The BfR analysed different herbal teas and their herbal drugs with regard to their pyrrolizidine alkaloid content. For the evaluation of the results, a MOE of 10,000 was applied. Despite some high levels of pyrrolizidine alkaloids, an acute danger to health is unlikely. However, long-term consumption can still be a health risk, especially for children, breastfeed infants and unborn children. The content in herbal teas and drugs should be as low as possible to avoid any health risk (45).

FAO/WHO (Food standard programme - Codex Committee on Contaminants in Foods) (2014)

The FAO/WHO proposed a draft code of practice for weed control to prevent and reduce pyrrolizidine alkaloid contamination in food and feed. General instructions and information are given to prevent or reduce the occurrence of pyrrolizidine alkaloid producing plants in field and meadow (46).

HMPC (Committee on Herbal Medicinal Products – EMA) (2014)

The exposure to pyrrolizidine alkaloids should be as low as practically achievable. Assuming a 50 kg person and a permitted daily pyrrolizidine alkaloid intake of 0.007 µg/kg b.w./day from all sources, a daily intake of 0.35 µg from herbal medicinal products might be acceptable. If children are allowed to use the product, the amount of pyrrolizidine alkaloids has to be calculated according to the body weight of the age group. The intake of the product should last no longer than 14 days (17).

EFSA (European food safety authority supporting publication - occurrence of pyrrolizidine alkaloids in food) (2015)

Various samples of animal and plant derived products from across Europe were tested by the EFSA. Animal derived samples occasionally revealed low levels of pyrrolizidine alkaloids. 91 % of the herbal tea samples and 60 % of the herbal food supplement samples contained one or more pyrrolizidine alkaloid. More information is needed on the occurrence of pyrrolizidine alkaloids in herbal tea and herbal food supplements (47).

BfArM (Bundesinstitut für Arzneimittel und Medizinprodukte) – Germany (2016)

The BfArM implemented a risk assessment on the contamination of herbal medicinal products with pyrrolizidine alkaloid producing plants, resulting in three risk categories.

Category A – very low or no contamination problem

In 90 % of the obtained data, the amount of pyrrolizidine alkaloids is below 0.1 µg per day and no amount is higher than 0.35 µg per day.

Skip testing is acceptable.

Category B – low contamination problem

In 90 % of the obtained data, the amount of pyrrolizidine alkaloids is below 0.35 µg per day and no amount is higher than 1.0 µg per day.

Skip testing with shorter periods is acceptable.

Category C – relevant contamination problem

If no data is available or an allocation to categories A and B is not possible, the control test has to be included in the release specification with an upper limit of 1.0 µg per day (48).

It was later clarified, that these categories and the limit of 1.0 µg pyrrolizidine alkaloids per day are only intended for a transition period of three years. After that, in 2019 the stricter limits proposed by the HMPC (0.35 µg per day) should apply (49; 50).

BASG (Bundesamt für Sicherheit im Gesundheitswesen) –Austria (2016)

The following 10 herbal drugs were identified as having a high risk of contamination: *Hyperici herba, Passiflorae herba, Matricariae flos, Alchemillae herba, Liquiritiae radix, Melissae folium, Menthae piperitae folium, Salviae folium, Taraxaci herba cum radice and Thymi herba*. For these drugs, the risk classification of the BfArM was adopted. Homeopathic preparations starting from D6 for internal and D4 for external application are excluded from the obligatory analyses (51).

MHRA (Medicines and Healthcare products regulatory agency) - UK (2016)

The MHRA informed the holders of traditional herbal medicine registrations that a limit of 1.0 µg pyrrolizidine alkaloids per day is acceptable for a transition period. The limit should be included in the release specification and analytical data of three batches should be provided (50; 52).

HMPC (Committee on Herbal Medicinal Products – EMA) (2016)

Contamination of herbal medicinal products with pyrrolizidine alkaloids should be as low as reasonably achievable (ALARA-principle). An upper limit of 1.0 µg pyrrolizidine alkaloids per day is acceptable for a transitional period of three years. For the transition period, the three risk classes proposed by the BfArM are adopted. After that, the limit of 0.35 µg per day should apply (50).

3.2 Derivation of the pyrrolizidine alkaloid limit

Pyrrolizidine alkaloid containing plants have been regulated for a long time in medicinal products in different EU countries.

Only the investigation of herbal teas, including medicinal teas, by the BfR in 2013 determined that pyrrolizidine alkaloids can also be detected in teas from non-pyrrolizidine alkaloid producing plants. This is likely caused by cross-contamination with pyrrolizidine alkaloid producing plants during the harvesting process.

Taking this into account, thresholds for pyrrolizidine alkaloids for all herbal medicinal products were established.

Even though various risk assessments on the contamination of food and feed with pyrrolizidine alkaloids exist, no regulation for food products has been established so far. Only recommendations, for example by the EFSA, exist.

The limit of 0.007 µg/kg b.w./day established by the COT is obligatory for herbal medicinal products and recommended for food stuff.

The limit is derived from a 2 year carcinogenicity study with lasiocarpine on rats performed in 1978 by the NTP (National Toxicology Program of the U.S. Department of Health and Human Services). The endpoint of this study was the formation of liver haemangiosarcomas. The COT assessed all pyrrolizidine alkaloids as a cumulative group using the MOE approached established by the EFSA in 2005. The MOE is used to consider possible safety concerns from both genotoxic and carcinogenic food and feed. It is defined as the ratio between the dose at which small but measurable harmful effects occur and the exposure level of the substance (53).

$$MOE = \frac{BMDL_{10}}{Exposure}$$

The $BMDL_{10}$ (benchmark dose lower confidence limit, lowest dose with a 95 % certainty that the cancer incidence doesnot exceed 10 %) is calculated with a value of 0.073 mg/kg b.w./day, based on the lasiocarpine study on rats. A MOE of 10,000 or higher is considered to be of low carcinogenic risk. Therefore, the exposure to humans with pyrrolizidine alkaloids should be not more than 0.007 µg/kg b.w./day (43).

Nevertheless, differences in the limits for herbal medicinal products and food products exist. The recommended daily limit for food products is based on an average adult body weight of 60 kg and is therefore 0.42 µg/day. For herbal medicinal products the HMPC calculated with an average adult body weight of 50 kg and therefore the limit is 0.35 µg/day. Additionally, the herbal medicinal product should not be used more than 14 days in a row.

3.3 Analytical procedure for testing pyrrolizidine alkaloids

According to the HMPC Guideline at least 28 pyrrolizidine alkaloids listed below in Table 1 should be quantified to establish the content of pyrrolizidine alkaloids in a herbal medicinal products. The HMPC recommends to use the SPE-LC-MS/MS method developed by the BfR (BfR-PA-Tea-2.0/2014) to determine the pyrrolizidine alkaloid content, other validated method are also acceptable (50).

Table 1: Pyrrolizidine alkaloids to be determined according to HMPC Guideline (50)

1. Echimidine (Em)	11. Jacobine (Jb)	21. Senecionine (Sn)
2. Echimidine-N-oxide (EmNO)	12. Jacobine-N-oxide (JbNO)	22. Senecionine-N-oxide (SnNO)
3. Erucifoline (Er)	13. Lasoicarpine (Lc)	23. Seneciphylline (Sp)
4. Erucifoline-N-oxide (ErNO)	14. Lasoicarpine-N-oxide (LcNO)	24. Seneciphylline-N-oxide (SpNO)
5. Europine (Eu)	15. Lycopsamine (Ly)	25. Senecivernine (Sv)
6. Europine-N-oxide (EuNO)	16. Lycopsamine-N-oxide (LyNO)	26. Senecivernine-N-oxide (SvNO)
7. Heliotrine (He)	17. Monocrotaline (Mc)	27. Senkirkine (Sk)
8. Heliotrine-N-oxide (HeNO)	18. Monocrotaline-N-oxide (McNO)	28. Trichodesmine (Td)
9. Intermedine (Im)	19. Retrorsine (Re)	
10. Intermedine-N-oxide (ImNO)	20. Retrorsine-N-oxide (ReNO)	

4 Pyrrolizidine alkaloid content in herbal products

To evaluate the pyrrolizidine alkaloid contamination problem various studies on the content in herbal teas were conducted over the past years. In the following table, the mean content of pyrrolizidine alkaloids in six studies for different herbal teas and some food supplements are summarised.

Table 2: Mean amount of pyrrolizidine alkaloids in different tea samples

Tea sample / Typ of Study	Mixed herbal		Peppermint		Chamomile		Fennel		Melissa	
	PA [µg/kg]	PA [µg/cup*]	PA [µg/kg]	PA [µg/cup*]	PA [µg/kg]	PA [µg/cup*]	PA [µg/kg]	PA [µg/cup*]	PA [µg/kg]	PA [µg/cup*]
BfR Study[1] (45)	199.0	0.348	188.0	0.367	478.9	0.957	114.9	0.229	692.0	1.384
Bodi et al.[2] (54)	151.4	0.302	134.2	0.268	439.7	0.879	51.7	0.103	649.6	1.299
Mathon et al.[3] (55)	2.00	0.004	--**	--	190.5	0.381	0	0	--**	--
Schulz et al.[4] (56)	253.4	0.506	8.9	0.017	4.6	0.009	5.7	0.011	202.9	0.405
Shimshoni et al.[5] (57)	315	0.630	261	0.520	564	1.130	n.d.	--	n.d.	--
Mulder et al.[6] (47)	439.4	0.878	496.2	0.992	273.8	0.547	--**	--	--**	--

* Assumes one tea bag containg 2g of herbal drug, brewed in 200 ml of water
** type of tea not analysed
1) 17 PAs and N-oxides tested, medium bound mean value stated, LOQ = 2.9-64.1 µg/kg, retrorsine: 151.8 µg/kg (2013; Germany)
2) 17 PAs and N-oxides tested, LOQ = 1.7-6.4 µg/kg (2014; Germany)
3) 9 PAs and N-oxides tested, LOQ = 1-5 ng/ml (2014; Swiss)
4) 23 PAs and N-oxides tested, LOQ = 10 µg/kg; only registered medicinal tea samples (2015; Germany)
5) 34 PAs and N-oxides tested, LOQ = 1-50 µg/kg (2015; Israel)
6) 28 PAs and N-oxides tested, LOQ = 1.7-6.4 µg/kg (2015; Europe)

Even though the analytical methods and LOQs, as well as the number of pyrrolizidine alkaloid reference substances differ, the results are mostly within the same range. Overall, chamomile and melissa teas are the most contaminated tea samples. Only fennel tea meets the limit of 0.35 µg/kg pyrrolizidine alkaloids in all studies for one cup of tea per day. The results by Schulz et al. and Mathon et al. are lower compared with the results of the other studies. For Schulz et al. this could be due to the higher LOQ of 10 µg/kg for all tested pyrrolizidine alkaloids or because, unlike in the other studies, only medicinal teas were analysed (56). The reason the study of Mathon et al. shows low levels could also be the relatively high LOQ and the fact that only 8 pyrrolizidine alkaloids and one N-oxide were tested (47). According to the results of Bodi et al. and Mulder et al. approximately 2/3 of the amount of pyrrolizidine alkaloids are present as

N-oxides (54; 47). That means the study of Mathon et al. probably only captured a fraction of the pyrrolizidine alkaloids contained.
Furthermore, concentrations of pyrrolizidine alkaloids can vary a lot between different tea samples for the same herbal drug. This can be verified by calculating the standard deviation for the pyrrolizidine alkaloid content in the samples. For example in the study of Mulder et al. the mean content of pyrrolizidine alkaloids in chamomile tea is 273 µg/kg with a standard deviation of 337 µg/kg.

Bodi et al. and Mulder et al. also analysed the pyrrolizidine alkaloid patterns in the different herbal teas, in order to obtain information on the contaminating weed species (54; 47).
Bodi et al. subdivided the pyrrolizidine alkaloids according to their necine acid into monoesters, open-chain diesters and cyclic diesters, as *Senecioneae* species mainly produce cyclic diesters, *Eupatorieae* mainly monoesters and *Boraginaceae* family mainly open-chain diesters and monoesters. In peppermint, mixed, chamomile and melissa tea all three types of necine acids were found, therefore it can be concluded that these teas were contaminated by several different weeds. In fennel tea only monoester and open-chain diester were found, therefore *Senecioneae* species can be ruled out as the contaminating weed (54).
For mixed tea, Mulder et al. found that senecionine- and heliotrine-type pyrrolizidine alkaloids had the highest concentration (over 90 % of the pyrrolizidine alkaloid amount), indicating that the contamination probably occurred through *Senecio* and *Heliotropium* species. For chamomile, senecionine-type pyrrolizidine alkaloids, followed by lycopsamine-type, had the highest percentage, suggesting that *Senecio*, *Heliotropium* and *Boraginaceae* species contaminated chamomile tea. In peppermint tea, primary senecionine-type pyrrolizidine alkaloids are found and only small amount of heliotrine- and lycopsamine-type, therefore the most important weed contributing to the contamination is from the *Senecio* species (47).
Even though some of the families and species can be ruled out or are primarily accountable for the contamination, according to the pyrrolizidine alkaloid pattern, it is not possible to say which individual plant is responsible for the contamination. That is because the pyrrolizidine alkaloid pattern is similar for one species and can vary, for example through weather and nutritional conditions, even in the same plant (2; 58).

Mulder et al. also tested herbal food supplements from non-pyrrolizidine alkaloid producing plants. Here, 107 samples were analysed, 63 % of the samples contained pyrrolizidine alkaloids over the limit of detection (LOD). The mean pyrrolizidine alkaloid content over all samples was 317.6 µg/kg. 14 food supplements containing St. John's

wort (*Hypericum perforatum*) were analysed. In 13 of them, pyrrolizidine alkaloids could be detected with a mean concentration of 991.7 µg/kg, reflecting the high contamination problem of this herbal drug (47).

In summary, food supplements contained comparable levels of pyrrolizidine alkaloids to herbal teas. As with the teas, the amount of pyrrolizidine alkaloids is highly variable between different supplements and batches.

In February 2016, six batches containing St. John's wort tablets were recalled by the MHRA, as the limits for pyrrolizidine alkaloids were exceeded (59). Up to now, this is the only recall of a medicinal product by a health authority due to pyrrolizidine alkaloids.

Especially cultivated plants, which are harvested at the same height as pyrrolizidine alkaloid producing weeds, are affected by the contamination problem, for example melissa, chamomile and St. John's wort.

Even though most studies are conducted on non-medicinal products, the results reflect the need for action to reduce the pyrrolizidine content in medicinal products as well as food products.

5 Challenges and problems for the industry

The limits for pyrrolizidine alkaloids determined by the competent authorities are a challenge for growers of herbal drugs, manufacturer of herbal active substances and for manufacturers of the herbal finished product.

The major difference between the limits for certain other substances (e.g. aflatoxins or mycotoxins) and the limits for pyrrolizidine alkaloids is, that the limits for pyrrolizidine alkaloids need to be set and calculated dose-dependently and individually for each herbal medicinal product.

The first question when implementing the limits for pyrrolizidine alkaloids is whether to test the herbal medicinal product or the herbal drug/preparation. Testing the herbal drug/preparation is generally favourable, as the herbal drug/preparation can be used in different products and therefore only one analysis is necessary. Furthermore, the matrix of the herbal medicinal product is presumably more complex than that of the herbal drug/preparation. Finally, the concentration in the herbal medicinal product is even more diluted, which makes analysis more difficult. In case of multi-combinational medicinal products, the pyrrolizidine alkaloids can be clearly attributed to the different herbal drugs/preparations, which is another advantage. Testing the herbal preparation is more favourable than testing the herbal drug, as the contamination with pyrrolizidine alkaloids is a spot-contamination. The chance of inhomogeneous samples, which can lead to false analytic results, is therefore higher in herbal drug samples than in samples of the herbal preparation.

When deciding how to test and calculate the limit for the herbal preparation, the second decision would be whether to calculate the pyrrolizidine limit with the native herbal preparation or “as is” with additives. Here, the calculation for the preparation with additives is more suitable, as this is easier to handle and no further conversion of the analytical results is necessary. For the calculation, it must be taken into account, that the patient leaflet and summary of product characteristics always states the dosage of the native extract and therefore must be converted to the preparation “as is” with additives.

The calculation of the limits was even more problematic during the period between March 2016 and May 2016, when different limits were valid in the European countries.

For Germany, Austria and the UK the classification into three risk classes, as proposed by BfArM, was applicable, whereas for the remaining European countries the limit set by the HMPC in 2014 had to be applied.

In the following, the effects of the different limits during the transition period is calculated for a fictional herbal cough syrup containing thyme liquid extract according to DAB (DER 1:2-2.5) as the active substance. The cough syrup is registered according to the HMPC community herbal monograph on *Thymus vulgaris* L. und *Thymus zygis* L., herba as a traditional herbal medicinal product with a maximal daily dose of 14 g extract for adolescents, adults and the elderly (60). The limit for pyrrolizidine alkaloids can be calculated according the following formula:

$$Limit_{PA}API = \frac{L_{PAHealth} \cdot W_{PAHealth}}{D_{max}}$$

Limit $_{PA}$API = limit of pyrrolizidine alkaloids in the herbal drug or herbal preparations [µg/g]
$L_{PAHealth}$ = maximal daily intake of pyrrolizidine alkaloids per day and kg [0.007 µg/kg/day]
$W_{PAHealth}$ = bodyweight (50 kg for adults) [kg]
D_{max} = maximal daily dose of the herbal drug or preparation according to SmPC [g/day]

According to the HMPC recommendation from 2014, the following limit can be calculated for an adult (50 kg):

$$Limit_{PA}API = \frac{0.007 \mu g/kg/day \cdot 50\ kg}{14\ g/day} = \frac{0.35\ \mu g/day}{14\ g/day} = 0.025\ \mu g/g\ extract$$

This gives a maximal limit of 25 µg pyrrolizidine alkaloids per kg thyme liquid extract.
The calculation for category C according to the BfArM statement can be performed accordingly.

$$Limit_{PA}API = \frac{1.0\ \mu g/day}{14\ g/day} = 0.071\ \mu g/g\ extract$$

Here, a maximal concentration of 71.4 µg pyrrolizidine alkaloids per kg thyme liquid extract is permitted.
This means that for the finished product a thyme liquid extract with a maximum concentration of 71.4 µg pyrrolizidine alkaloids per kg could be used, if the finished product is sold in Germany, Austria and the UK, but not for the remaining European countries. Here, the maximum permitted level is 25 µg pyrrolizidine alkaloids per kg for the same thyme liquid extract and finished product.
If the finished product is marketed in different European countries, the manufacturer of the finished product has three main options. The first is to establish the lowest limit for all countries. Here a possibility exists, especially with high risk herbal drugs or preparations, that the limits cannot always be fulfilled, which can cause bottlenecks in the supply of the active substance. In the worst-case scenario, this may result in the product being out of stock. The second option is to implement both limits in the incoming goods specification, so that herbal drugs or preparations above the lower limit

but below the higher limit can be used to produce the bulk of the finished product for Germany, Austria and the UK, for example. Here, the implementation of such a system is problematic, as two different limits are not supported by most electronic systems for incoming goods and manual control would be needed, which is cumbersome and time consuming. The third option would be to completely separate the incoming goods specification for the herbal drug or preparation according to the limits of the countries. These two incoming goods specifications would then be handled like two different materials. Consequentially, the bulk of the finished product has to be produced separately, which could increase production costs.
Which option would be the most suitable depends on the risk of pyrrolizidine alkaloid contamination. For a herbal drug or preparation with a low risk of contamination, option one would be the best. For a herbal drug or preparation with a high risk of contamination options two or three, depending on manufacturing quantities and marketed countries, would be better choices.

Furthermore, during the transition period parallel imports of herbal medicinal products could lead to problems. If a product is imported from Germany, Austria or the UK, where the higher limits for pyrrolizidine alkaloids are valid, into another European country, where the lower limits are valid, this product would not be marketable, if the limit of 0.35 µg/day cannot be fulfilled. However, this would only affect a very small number of high revenue herbal medicinal products. Furthermore, Germany, Austria and the UK are rather high-priced countries, which makes parallel imports from them less profitable.

Fortunately, the requirements in the European countries have been harmonised by the "Public statement on contamination of herbal medicinal products/traditional herbal medicinal products with pyrrolizidine alkaloids" of the HMPC in May 2016. For a transition period of three years, the three risk classes implemented by the BfArM are valid for all European countries. After the transition period in 2019, the limit for the pyrrolizidine alkaloid content in herbal medicinal products will be lowered to 0.35 µg/day.

Another challenge are combinational products with two or more different herbal drugs or preparations. Here, the limit for the content of pyrrolizidine alkaloids has to be differentiated between the different herbal substances contained in the herbal medicinal product.
As an example, a fictional cough syrup with thyme liquid extract DAB (DER 1:2-2.5) and soft primula root extract (DER 1-2:1; extraction solvent ethanol 55 % V/V) is chosen. The

finished product is registered according to the HMPC European Union herbal monograph on *Thymus vulgaris* L. and *Thymus zygis* L., herba and *Primula veris* L. and *Primula elatior* (L.) Hill, radix as a traditional herbal medicinal product with a fixed single dose combination of 1.16 g thyme liquid extract and 0.17 g soft primula root extract, maximum daily dose: 4 times (61). Therefore, the calculated maximum dose for both extracts for an adult is 5.32 g/day. The following calculation can be made according to the formula above.

For category A:

$$Limit\ PA_{total} = \frac{0.35\ \mu g/day}{5.32\ g/day} = 0.065\ \mu g/g\ extract$$

For category C:

$$Limit\ \ PA_{total} = \frac{1.0\ \mu g/day}{5.32\ g/day} = 0.187\ \mu g/g\ extract$$

The most feasible approach for setting the limits for the extracts would be to implement the limit of 65 µg/kg or 187 µg/kg for both extracts, to ensure compliance with the pyrrolizidine alkaloid limits.

If one of the extracts exceeds this limit, the maximal permissible level for the second extract can be calculated according to the following formula:

$$Limit\ PA_{total} = \frac{D_{API1}}{D_{total}} \cdot con\ PA_{API1} + \frac{D_{API2}}{D_{total}} \cdot con\ PA_{API2}$$

With regard to the traditional herbal medicinal product above the following example for category A is calculated. The thyme liquid extract has a pyrrolizidine concentration of 0.070 µg/ g extract, therefore the equation is:

$$0.065\ \mu g/g = \frac{4.64g}{5.32g} \cdot 0.070 \mu g/g + \frac{0.68g}{5.32g} \cdot con\ PA_{Primula}$$

$$<=> con\ PA_{Primula} = \frac{0.065\ \mu g/g - 0.061\ \mu g/g}{0.127} = 0.031\ \mu g/g\ extract$$

That means if the pyrrolizidine alkaloid content for the thyme liquid extract is 0.070 µg/g extract, the content in the soft primula root extract can only be 0.031 µg/g extract.

This calculation can be a feasible approach for special cases. For example, if a herbal drug or preparation is already purchased and exceeds the set limits for pyrrolizidine alkaloids. Other cases would be, if one herbal drug or preparation generally has a higher contamination with pyrrolizidine alkaloids, so that the limits cannot be fulfilled. A third case would be a product at risk of running out of stock, where no herbal drug or

preparation within the set limits is available. This approach is not suitable for the day to day business, however, as the limit has to be re-calculated for each extract.
If the finished product is authorised for the use in children, the allowed amount of pyrrolizidine alkaloids has to be calculated according to the limit 0.007 µg/kg b.w./day with the appropriate weight of the different age groups.
For example, the already mentioned mono cough syrup with thyme liquid extract (DER 1:2-2.5) can be used for children from 4 years onwards. The single daily dose for children is 0.5 - 0.9 ml 3 to 5 times. When calculating with a density of 1.1 g/ml, the maximum daily dose for a 4 year old is 4.59 g thyme liquid extract. According to the child growth standard of the WHO, the average weight of a 4 year old is 16 kg (62). The limit of pyrrolizidine alkaloids can now be calculated as follows:

$$Limit_{PA}API = \frac{0.007\ \mu g/day/kg \cdot 16kg}{4.59\ g/day} = 0.024\ \mu g/g\ extract$$

Therefore, the actual limit for pyrrolizidine alkaloids in the thyme liquid extract is 24 µg/kg extract, which is a lower limit than the one calculated for adolescents and adults with 25 µg/kg extract. For this example, the absolute difference between both limits is only small, but for other products, it can be more significant.

The use of a single herbal drug or preparation in different herbal medicinal products is another challenge.
Here, the limits of pyrrolizidine alkaloids need to be calculated separately for all products and then, as there is usually only one incoming goods specification, the lowest limit is implemented in the specification. Again, this is no problematic case for herbal drugs or preparations with a low risk of contamination but can be problematic for the ones with a high risk of contamination and low limits, for example due to indications in children. Here, the same problem arises as during the transition period with the different limits in European countries.
Since the specification for pyrrolizidine alkaloids needs to be calculated and implemented in all herbal medicinal products, this is particularly challenging for small to medium sized companies, due to the needed resources and money. As pyrrolizidine alkaloids are potentially genotoxic, the implementation of the limits cannot be submitted as a type IA variation according to B.I.b.1c) or B.II.d.1c) of the variation guideline, as one condition for this variation is, that the impurity is not genotoxic. It must be submitted as a type IB by default. Furthermore, herbal medicinal products are mostly registered or authorised nationally and not per MR- or DC procedures. Therefore, a separate variation needs to be submitted for every country.

Another issue are changes in the category limit, from a limit according to category C to category B or A, or the other way around. If a herbal drug or preparation is classified into category A but new results do not allow this classification, a Type II variation needs to be submitted to switch the approved category to the next higher category.

Furthermore, the analysis of pyrrolizidine alkaloids is a challenge, as a high sensitivity and selectivity of the analytical method is needed. Due to the low limit of determination required, techniques like GC-MS or LC-MS/MS are particularly suitable. The quantitative analysis of pyrrolizidine alkaloids can be conducted via two different principles. One way is to reduce all pyrrolizidine alkaloids and N-oxides in the given sample to the same general necine base (retronecine/heliotridine) and then determine the content of the general necine base structure. The advantage is that the amount of all contained pyrrolizidine alkaloids is detected. The disadvantage is that no conclusions on the identity of the pyrrolizidine alkaloids can be made (24).
The second method is to determine the individual pyrrolizidine alkaloids. Here, the limitation is that corresponding reference substances are needed. As approximately 660 different pyrrolizidine alkaloids exist and only a fraction can be obtained as reference substances, not all pyrrolizidine alkaloids can be analysed (up to now only 35 reference substances are commercially available) (63). Furthermore, it cannot be guaranteed that the available reference standards for pyrrolizidine alkaloids cover the major pyrrolizidine alkaloids contained in the given sample. The advantage is that the occurring pyrrolizidine alkaloids and patterns can be identified and thereby conclusions on the contaminating species can be made.
For testing pyrrolizidine alkaloids in herbal medicinal products and food products, the second alternative is used, because pyrrolizidine alkaloid patterns can be identified.
First comparisons of both analytical methods show similar results, even though further studies should be conducted (64; 65).
According to the publication by the BfArM and public statement of the HMPC, an adequate sampling and sampling preparation should be conducted. Both refer to the Commission Regulation 401/2006 EC “methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs” for establishing the sample plan (66).

Another problem are the sometimes very complex matrixes of the herbal drugs or preparations and herbal medicinal products. As herbal substances are multi-component mixtures, a lot of interfering factors exist. Therefore, a verification of the method is recommended for every herbal drug or preparation, to completely exclude matrix effects, even if a validated method for determination of pyrrolizidine alkaloids exists. The

verification is a matrix-specific validation and includes the following parameters: specificity, accuracy and linearity (63).

The HMPC recommends the LC-MS/MS method developed by the BfR. This method has been validated through an international ring trial (67). The 28 analysed pyrrolizidine alkaloids (Table 1) are selected from the commercially available reference substances based on their occurrence and relevance.

Table 3 Main pyrrolizidine alkaloids according to plant species (16; 54)

Plant species	Structural features	Pyrrolizidine alkaloids
Senecioneae (Asteraceae family)	mainly cyclic diesters	erucifoline, jacobine, retrosine, senecionine, seneciphylline, senecivernine, senkirkine
Crotalaria spp. (Fabaceae family)	mainly cyclic diesters	erucifoline, jacobine, retrosine, senecionine, seneciphylline, monochrotaline, trichodesmine
Eupatorieae (Asteraceae family)	mainly mono- and open chain diesters	echimidine, lycopsamine, intermedine
Boraginaceae family	mainly mono- and open chain diesters	echimidine, lycopsamine, intermedine, europine, heliotrine, lasiocarpine

In the cultivation of herbal drugs, the pyrrolizidine alkaloid problem also poses new challenges. The measures of the good agriculture and collection practice (GACP) alone are not sufficient to avoid contamination of herbal drugs with pyrrolizidine alkaloid producing plants. Point 11.6 of the GACP Guideline of the HMPC states that “during harvesting, care should be taken to ensure that no toxic weeds mix with harvested medicinal plants” (68; 69). However, the main challenge is that one pyrrolizidine alkaloid producing plant in a field can lead to analytically detectable traces of pyrrolizidine alkaloids. For example, *Senecio* species can contain between 1 - 10 g pyrrolizidine alkaloids per kg dry weight, therefore one weed in one hectare medicinal plants would lead to detectable pyrrolizidine alkaloid concentrations (7). This problem, the possibility of contamination through slightest traces, is not considered in the GACP Guideline. According to the general monograph “Herbal Drugs” in the European Pharmacopoeia, a contamination of up to 2 % weight of foreign matter is permitted (69). This reflects the huge effort that must be undertaken by the grower, collector and manufacturer of the herbal drug or herbal preparation as well as by the manufacturer of the herbal finished product to minimise the content of pyrrolizidine alkaloids.

6 Measures of the pharmaceutical industry

After the BfR publication on cross-contamination of herbal teas with pyrrolizidine alkaloid producing weeds in 2013, the pharmaceutical industry took first control measures. In Germany the German Medicines Manufacturers' Association (Bundesverband der Arzneimittel-Hersteller e.V., BAH), the German Pharmaceutical Industry Association (Bundesverband der Pharmazeutischen Industrie e.V., BPI) and the German Technical Committee for Medicinal, Aromatic and Perfumery Plants (Deutscher Fachausschuss für Arznei-, Gewürz- und Aromapflanzen, DFA) established a Code of Practice with the aim to reduce the cross-contamination of herbal drugs/preparations with pyrrolizidine alkaloid producing weeds as far as possible. The proposed measures affect all steps along the process chain. In the following, the risks of contamination with pyrrolizidine alkaloids and the corresponding possible measures are illustrated according to the Code of Practice:

Table 4 Contamination risks along the process chain according to literature (69)

Process step	Risks	Probability	Possibility to exert influence	Evaluation (feasibility, time horizon, efficiency)	Responsi-bility
Cultivation: Cultivation planning	Weed seeds already in the soil, possible impact from the neighbourhood (such as agrobiodiversity areas, field margins to be kept free of crop protection agents, hedgerows, transfer of pollen containing PA)	High	Selection of fields without corresponding weed populations, observance of crop rotation (closing herbicidal efficacy gaps over preceding crops and soil preparation as well as adjacent surfaces / vegetation along field edges, mowing the field edge, attention to cleanliness of the equipment after changing fields)	Great importance, feasible, medium to long-term action, need for research on the effectiveness of actions	Grower and research institutions
Cultivation: seeds	Cultivated seeds contaminated with weed seeds	low to high depending on the plant species	Selection of seeds under consideration of their purity, harvest of seeds after inspection of the field (intensive weed control), attention to cleanliness of harvesting and processing equipment	The technology for cleaning seeds is currently fully exploited; need for further research (seed-cleaning project in planning stage)	Seed supplier

Process step	Risks	Probability	Possibility to exert influence	Evaluation (feasibility, time horizon, efficiency)	Responsi-bility
Cultivation: course of cultivation	Non-recognition of weeds containing PA	High	Dissemination of scientific findings in agriculture	Great importance, weed database project	FAH, research institutions and consultants
	weed infestation	High	Cultivation method and species-specific actions in weed management, selective crop protection measures (also treating sub-areas and areas between the crop rows), application technologies	Great importance, medium to long-term extension of chemical crop protection and simplification/accelera tion of approval processes are required	Growers, approval authorities
			Intensification of manual/mechanic al weed control	Great importance, feasible short to medium-term, necessity of research and innovation for weed control in the crop row	Growers and research institutions
Harvest	Harvesting weeds along with cultivated plants	Low to high depending on species and harvesting technology	Optimisation of harvesting technology (among other things timing, technology, cutting height)	Great importance, limited feasibility depending on various influencing factors	Growers
Wild collection	Harvesting weeds along with cultivated plants	Low to high depending on species and collection technology (e.g. hand-picking versus (partly) mechanised collection)	Risk analysis including the drug, collecting/harvesti ng technology, site/accompanyin g flora, training, earliest possible visual inspection of the collected material	Great importance, central starting point: training, short-term feasibility	Supplier, collecting organisation
Drying	Cross-contamination via dust	Low	careful cleaning of the drying equipment	Slight importance	Drying operation, drug supplier
Incoming goods inspection of crude drug	Non-detection of a PA burden caused by weeds	Low to high depending on the drug and inspection method	Risk-based selection of crude drugs subject to intensive testing for PA, definition of acceptance criteria for processible crude drug qualities	Great importance, feasible to implement tests in the short term, high analytical effort and expense, question of suitability of the inspection method, long-term common database of manufacturers	Manufacturer
Drug processing	Transfer of weeds containing PA	Low to high depending on the drug	Cleaning options, e.g. sorting small quantities of harvested crops	Great importance, feasible in the short term, but high effort and expense for personnel and technology	Processor, supplier

Process step	Risks	Probability	Possibility to exert influence	Evaluation (feasibility, time horizon, efficiency)	Responsi-bility
Drug processing and all further process steps	Cross-contamination with weeds containing PA	Low	Careful cleaning of the process equipment	Slight importance	Processor, supplier, manufacturer
Manufacture of active substance	Transfer of PA to the active substance	Low to high depending on the crude drug and the extraction method	Development of extraction methods to deplete PA	Slight importance, high technical and regulatory effort and expense	Manufacturer
Release of active substance	Non-recognition of a PA burden	Low to high depending on the crude drug and the extraction method	Risk-based selection of active substances subject to intensive testing for PA, definition of acceptance criteria for the active substance	Great importance, feasible to implement tests in the short term, high analytical effort and expense, question of suitability of the inspection method, long-term common database of manufacturers	Manufacturer
Manufacture of medicinal products	Transfer of PA to the finished medicinal product	Low to high depending on the PA burden of the active substance	None		Manufacturer
Release of finished medicinal product	Non-recognition of a PA burden	Low to high depending on the PA burden of the active substance	Risk-based selection of finished medicinal products subject to intensive testing for PA, definition of acceptance criteria for the medicinal product	Great importance, feasible to implement tests in the short term, high analytical effort and expense, question of suitability of the inspection method, long-term common database of manufacturers	Manufacturer

Measures of high importance are, for example, the generation of databases, the weed management, the closing of herbicide gaps or the seed cleaning project.

The project by the Research Association of Pharmaceutical Manufacturers (Forschungsvereinigung der Arzneimittel-Hersteller e.V., FAH) should evaluate location- and plant-specific weeds, in particular pyrrolizidine alkaloid producing weeds, in cultivated medicinal plant fields. For all weeds, the amount of pyrrolizidine alkaloids should be determined in an interactive database and pooled together with pictures and descriptions of the weeds. In 2015, 68 different fields of chamomile, melissa, parsley and peppermint were sampled and tested. The amount of pyrrolizidine alkaloid producing weeds was relatively low, 7 out of 143 weeds. *Senecio vulgaris* had the highest incidence with 0.7 % followed by *Myosotis arvensis* with 0.1 %. Because of the low percentage of pyrrolizidine alkaloid producing weeds it is even more difficult to detect and control these weeds. Since only few pyrrolizidine alkaloid-producing weeds have been tested so

far, a general statement of the pyrrolizidine alkaloid amount cannot be made. The project will be continued in 2016 and 2017 to include more samples (70).
Another project, which is also incorporated in the project of the FAH, is the evaluation of the uptake of pyrrolizidine alkaloids from non-producing plants through the soil, also known as horizontal transfer. First results confirm the assumption that pyrrolizidine alkaloids, which have penetrated into the soil from rotten pyrrolizidine alkaloid-containing plants, can be absorbed by other plants. For this experiment, 1 g of ragwort (*Senecio jacobaea*) was mulched into the soil of melissa, peppermint, chamomile and parsley. Around 0.1 % of the 3.2 mg pyrrolizidine alkaloids present in the mulch were found in the acceptor plants after one week. After two weeks, the amount of pyrrolizidine alkaloids in the cultivated plants significantly declined. Further questions about the uptake of pyrrolizidine alkaloids need to be answered, for example the distribution in soil and acceptor plants and the speed of degradation. However, the essential fact is that pyrrolizidine alkaloids can be distributed through soil and therefore pyrrolizidine alkaloid producing-plants should not only be uprooted but also removed (70).

Two other database projects by Phytolab and the Deutsche Homöopathie-Union (DHU) have been started to evaluate the content of pyrrolizidine alkaloids in herbal drugs and preparations. For this purpose approximately 40 companies enter their analytical results for all tested herbal drugs and herbal preparations into the corresponding database (70). On this basis herbal drugs and preparation with a high risk potential can be identified and prevention methods and resources can be used more effectively. According to the first evaluations the following herbals drugs can be considered higher risk: *Hyperici herba, Passiflorae herba, Matricariae flos, Alchemillae herba, Liquiritiae radix, Melissae folium, Menthae piperitae folium, Salviae folium, Taraxaci herba cum radice and Thymi herba* (50).
Another major contamination possibility is through seeds, therefore a seed-cleaning project has been drafted to prevent the contamination of seeds with weeds. At first the percentage of seeds from pyrrolizidine alkaloid producing weeds in cultivated plant seeds should be evaluated. Based on these results, further aspects, such as technical feasibility and requirements, can be assessed. The objective is to detect a minimum of 10 weed seeds in the sowing quantity for 10 hectares (71).
Furthermore, a project for the development of an immunological screening method for the determination of pyrrolizidine alkaloids in herbal teas and fodder was started in 2016. The enzyme linked immunosorbent assay (ELISA) can be used to give a quick, specific and cost-effective result. The test could easily be performed by herbal drug/preparation manufacturers or finished product manufacturers. This approach would simplify and speed up the detection procedure of pyrrolizidine alkaloids (70).

Another project is the optimisation of non-chemical weed control, a follow up project of the „Status-quo-analysis to weed control in the ecological medicinal and aromatic plant cultivation“. Here, modern real time kinematic (RTK)- control techniques, ultrasonic and camera techniques are being considered. These techniques could be used to automatically control and examine fields for weeds and so take control measures as early as possible (69).
Furthermore, herbicide gaps, especially concerning *Senecio* and *Myosotis arvensis*, should be investigated and closed. A test program will be conducted during the next years to evaluate herbicides in representative plants and the corresponding residue data. However, herbicides should only be used as last opportunity, if all other measures are exhausted. The problem of herbicide residues should not be underestimated (70).
Finally, the consultation, information and training of growers is of high importance, so that pyrrolizidine alkaloid producing weeds can be recognised and eliminated (manually or mechanically) before harvesting (69).

Many measures have either been implemented or are in research, however the outcome of these cannot be determined for the next one or two years, as the cultivation of plants is an annual process and the measures mentioned above need time to be effective. Consequently, some of the high risk plants that cannot comply with risk category A of the HMPC statement at the moment, will probably do so after the transition period of three years, when the measures take effect.

7 Alternative Risk Assessment Approaches

The risk assessment for pyrrolizidine alkaloids according to the MOE approach, performed by the COT and adopted by the EFSA and HMPC, evaluates the pyrrolizidine alkaloids as a cumulative group. As a reference for the toxicity potential of the group, one of the most toxic pyrrolizidine alkaloids, lasiocarpine, is used. The COT acknowledged that this approach is very conservative. In the following, other possible concepts to evaluate genotoxic and mutagenic substances are examined.

7.1 Threshold of Toxicological Concern (TTC) concept

As an alternative approach for determining the limit for pyrrolizidine alkaloids, the Threshold of Toxicological Concern (TTC) concept was proposed by different associations during the commentary phase of the HMPC "Public statement on the use of herbal medicinal products containing toxic unsaturated pyrrolizidine alkaloids (PAs) (EMA/HMPC/893108/2011)" (72).

According to the ICH M7 Guideline on Genotoxic Impurities and the former EMA Guideline on Genotoxic Impurities, a threshold of 1.5 µg/day is acceptable for a genotoxic and mutagenic substance without any available data on its carcinogenicity. This threshold is based on a lifelong exposure and an acceptable lifetime cancer risk of 10^{-5} (73).

Furthermore, the ICH M7 introduces a Less-Than-Lifetime (LTL) approach. Here, the duration of treatment with the medicinal product is taken into account when calculating the threshold, as few medicinal products need to be taken lifelong (73).

These guidelines and concepts cannot be applied for two reasons. First, the ICH M7 Guideline excludes herbal medicinal products for formal reasons. Second, this concept is only applicable for genotoxic and mutagenic substances with an unknown carcinogenic potential. For pyrrolizidine alkaloids, carcinogenic studies are available and therefore, according to the ICH M7 Guideline, a compound specific acceptable limit for the substance should be evaluated.

7.2 Relative potency (REP) factors

Merz and Schrenk propose a different approach via relative potency (REP) factors for risk assessment of pyrrolizidine alkaloids. This approach is established for polychlorinated dioxins, furans, PCBs and in pharmacology for synthetic glucocorticoids. The REP factors describe the relative toxic potency of the substance in comparison to the most toxic substance of the group, here the value of 1.0 corresponds to 100 % toxic. Merz and Schrenk evaluated experimental studies that compare three or more pyrrolizidine

alkaloids. Here, studies on the genotoxic potential in *Drosophila*, cytotoxicity in mammalian cell cultures and acute toxicity data in rodents were considered as well as structural features of the pyrrolizidine alkaloids. This data was used to estimate the provisional REP factors. Furthermore, the following assumptions are made: the mode of action is the same for all pyrrolizidine alkaloids and the N-oxides have the same toxicity as the corresponding pyrrolizidine alkaloid (74).
They estimated the following provisional REP factors:

Table 5: REP factors for pyrrolizidine alkaloids according to Merz and Schrenk (74)

Structure element of pyrrolizidine alkaloids	REP factor	Pyrrolizidine alkaloid examples
Cyclic diesters	1.0	Erucifoline, Erucifoline-N-oxide, Jacobine, Jacobine-N-oxide, Monocrotaline, Monocrotaline-N-oxide, Retrorsine, Retrorsine-N-oxide, Senecionine, Senecionine-N-oxide, Seneciphylline, Seneciphylline-N-oxide, Senecivernine, Senecivernine-N-oxide, Senkirkine, Trichodesmine
Open chain diesters with 7S configuration	1.0	Lasoicarpine, Lasoicarpine-N-oxide
Monoester with 7S configuration	0.3	Europine, Europine-N-oxide, Heliotrine, Heliotrine-N-oxide
Open chain diesters with 7R configuration	0.1	Echimidine, Echimidine-N-oxide
Monoester with 7R configuration	0.01	Lycopsamine, Lycopsamine-N-oxide, Intermedine, Intermedine-N-oxide

7.3 Exemplary application of the REP factor

Only little literature data, which states the individual amount of every pyrrolizidine alkaloid tested in herbal drugs or preparations, is available. Therefore, the influence of the risk assessment suggested by Merz and Schrenk will be exemplarily calculated for two cases. One example is the pyrrolizidine alkaloid analysis in tea, herbal drugs and honey from Bodi et al. (54). Although only the mean amounts of 17 pyrrolizidine alkaloids and N-oxides over all 274 tea samples were stated, the information is suitable for an exemplary calculation. The analysis was performed on the herbal tea drug and the amount of pyrrolizidine alkaloids are specified in µg/kg.

Table 6: Mean concentration of pyrrolizidine alkaloids with and without REP factor

Pyrrolizidine alkaloid	Mean concentration of PA [µg/kg]*	REP Factor	Mean effective concentration of PA including REP factor
Echimidine	16	0.1	1.6
Heliotrine	7	0.3	2.1
Heliotrine-N-oxide	15	0.3	4.5
Intermedine	28	0.01	0.28
Lasoicarpine	3	1.0	3
Lasoicarpine-N-oxide	9	1.0	9
Lycopsamine	14	0.01	0.14
Monocrotaline	0	1.0	0
Monocrotaline-N-oxide	0	1.0	0
Retrorsine	15	1.0	15
Retrorsine-N-oxide	34	1.0	34
Senecionine	24	1.0	24
Senecionine-N-oxide	58	1.0	58
Seneciphylline	9	1.0	9
Seneciphylline-N-oxide	15	1.0	15
Senkirkine	1	1.0	1
Trichodesmine	0	1.0	0
Sum	248		176.62

*Data of mean concentration of PA from Bodi et al. (54)

To calculate the exposure, the consumption data used and calculated by the BfR for tea (overall) was adopted, as in both cases herbal teas and non-herbal teas were analysed. Furthermore, it is assumed that one tea bag contains 2 g of tea and is brewed in 200 ml of water and 100 % of the pyrrolizidine alkaloids transfers into the tea water (45).

Table 7: Consumption data of tea (45)

Long-term consumption	Mean value of tea consumption [g/kg b.w./day]	Frequent users* tea consumption [g/kg b.w./day]
Adults	0.032	0.153
Children 6 month to <5 years	0.055	0.231

*95th percentile

For an adult of 50 kg this equals a mean consumption of 0.8 tea cups per day and for frequent users a tea consumption of 3.82 tea cups per day.

The uptake of pyrrolizidine alkaloids can be calculated as follows for the different groups:

Table 8: Uptake of pyrrolizidine alkaloid content with and without REP factors

	Uptake of PAs [µg/kg b.w./day]*	Uptake of PAs calculated including the REP factor [µg/kg b.w./day]*
Adults mean value of tea consumption	0.0079	0.0056
Adults frequent users of tea	0.0379	0.0270
Children 6 month to <5 years mean value of tea consumption	0.0136	0.0097
Children 6 month to <5 years frequent users of tea	0.0572	0.0407

*limit = 0.007 µg/kg b.w./day

Alternatively, the uptake of pyrrolizidine alkaloids in one cup of tea can be calculated as well.

Table 9: Uptake of pyrrolizidine alkaloid content in one cup of tea with and without REP factors

Uptake of PAs in one cup of tea [µg/cup]*	Uptake of PAs in one cup of tea calculated including the REP factor [µg/cup]*
0.496	0.353**

*limit = 0.35 µg/day for adults (50 kg)
**meets limits according to rounding rules laid down in ICH Guideline Q3B(R2) Impurities in New Drug Substances (75)

The results are within the same range calculated by the BfR, which indicates that these results, even though based on average values, are realistic. For adults consuming the mean amount of tea, the limit for pyrrolizidine alkaloids would not be exceeded when calculating with the REP factors but without the REP factors the limit cannot be met, even for this group. For one cup of tea per day, the limit of 0.35 µg/day for adults would just be met, when calculating with the REP factor and would be well above the limit without REP factors.

In the second source, Mathon et al. tested 9 pyrrolizidine alkaloids and one pyrrolizidine-N-oxide in 70 different tea samples purchased on the Swiss market (55). In contrast to Bodi et al. (54), the testing was not performed on the herbal drug but on the brewed tea itself. A tea bag of 2 g was brewed in 200 ml of water and then analysed. For the calculations with REP factors only the results for chamomile tea were used. In 7 out of 10 tested chamomile tea samples pyrrolizidine alkaloids could be quantified. Even though 10 pyrrolizidine alkaloids including the N-oxide were analysed, Mathon et al. tabulated the results for only 8 of the pyrrolizidine alkaloids (55). As only echimidine (Em), lycopsamine (Ly), senecionine (Sn) and seneciphylline (Sp) were detected in chamomile tea, in the following table only these results are summarised.

Table 10: Mean concentration of pyrrolizidine alkaloids in chamomile tea with and without REP factors

Pyrrolizidine alkaloids / Tea samples		Mean concentration of PA [µg/cup] for sample*				
		Em	Ly	Sn	Sp	Sum
1	without REP factor	0.040	0.148	0.061	0.044	0.292
	with REP factor	0.0040	0.00148	0.061	0.044	0.11046
2	without REP factor	0.098	0.153	0.086	0.084	0.420
	with REP factor	0.0098	0.00153	0.086	0.084	0.18133
3	without REP factor	0.214	0.180	0.044	0.037	0.474
	with REP factor	0.0214	0.00180	0.044	0.037	0.1042
4	without REP factor	0.054	0.111	0.216	0.114	0.495
	with REP factor	0.0054	0.00111	0.216	0.114	0.33651
5	without REP factor	0.038	0.258	0.187	0.114	0.597
	with REP factor	0.0038	0.00258	0.187	0.114	0.30738
6	without REP factor	0.411	0.301	--	0.036	0.747
	with REP factor	0.0411	0.00301	--	0.036	0.08011
7	without REP factor	0.217	0.367	0.118	0.085	0.788
	with REP factor	0.0217	0.00367	0.118	0.085	0.22837

Data of mean concentration of PAs from Mathon et al. (55).
*limit = 0.35 µg/day for adults (50 kg)

Here, differences between the calculation without and with REP factors can be seen as well. When calculating the pyrrolizidine alkaloid content without the REP factors, 6 out of the 7 samples were over the category A limit of 0.35 µg/day. If the calculation includes the REP factors, none of the 7 samples exceed the limit of 0.35 µg/day.

Risk assessment of pyrrolizidine alkaloids with REP factors could be a feasible approach to distinguish the toxic potencies of the individual pyrrolizidine alkaloids and therefore give a more realistic estimate of the toxic potential.

8 Discussion

Contamination of herbal medicinal products and foodstuff with pyrrolizidine alkaloids is a serious problem, as pyrrolizidine alkaloids are hepatotoxic and likely carcinogenic in humans. The contamination problem is recognised by health authorities and the pharmaceutical industry. As already mentioned, various measures by the growers, herbal active substance manufacturer and finished product manufacturer to reduce and prevent the contamination of herbal medicinal products with pyrrolizidine alkaloid producing weeds have been implemented or are in research. Most of these measures need time to become effective as the time for cultivating herbal plants is one to three years. According to a presentation by Maximilian Wittig, chairman of the German Tea Association (Deutscher Teeverband e.V.), held at the "16. BfR - Forum Verbraucherschutz Pyrrolizidinalkaloide", the concentration of pyrrolizidine alkaloids in peppermint has been reduced by more than half from 2013 to 2015 (76). This shows that the implemented measures are effective and should be pursued in the long-term. These first results indicate that after the transition period of 3 years in 2019, all herbal medicinal drugs and preparations can probably fulfil the limit of 0.35 µg/day.

Nevertheless, risk assessments and limits for pyrrolizidine alkaloids in herbal medicinal products should be discussed. At the moment, the limit in herbal medicinal products is 0.007 µg/kg b.w./day (0.35 µg/day for an average adult of 50 kg) with a maximal duration of use of 14 days. This is a more strict limit than the limit for food products proposed by the EFSA with 0.007 µg/kg b.w./day (0.42 µg/day for an average adult of 60 kg) for lifelong use. The argumentation of the HMPC is that herbal medicinal products are consumed in addition to other pyrrolizidine alkaloid sources and are therefore an additional risk to the consumer. Consequentially, the duration of use should be restricted. However, the HMPC does not consider the additional benefit of herbal medicinal products. Furthermore, the different assumptions of the average body weight for adults are inconsistent and difficult to explain to consumers. As the BfR and EFSA did not provide information on the source of the average adult weight, it is difficult to discuss which weight is more appropriate. Overall, one limit, valid for food and for herbal medicinal products, would be more transparent for the consumer.

Furthermore, the risk assessment of pyrrolizidine alkaloids performed by the COT assessed the pyrrolizidine alkaloids as a cumulative group, with lasiocarpine one of the most toxic pyrrolizidine alkaloids as reference. Pyrrolizidine alkaloids are a very diversive group of substances with large differences in toxicity values. However, the evaluation according to REP factors proposed by Merz and Schrenk would therefore be a more realistic approach to evaluate pyrrolizidine alkaloids and include their actual toxic

potency. Even though the REP factors proposed are only provisional and further verification and examinations need to be conducted, especially on the toxicokinetic of pyrrolizidine alkaloids, the approach seems more reasonable than assessing all approximately 660 pyrrolizidine alkaloids in the same manner.

Based on the two databases maintained by Phytolab and DHU (70), different categorisations for herbal drugs/preparations could be derived according to their risk of contamination. Herbal drugs/preparations, which have a low risk of contamination due to their growth and harvesting technique (e.g. nutmeg, crataegus and eucalyptus) and whose results in the databases were continuously negative could be excluded from the mandatory testing of pyrrolizidine alkaloids, for example. Furthermore, skip testing frequency for medium-risk herbal drugs/preparation could be derived from the obtained data of defined numbers of batches. This would allow concentrating on the high-risk herbal drugs/preparations, for example melissa, chamomile and St. John's Worth.

Furthermore, health authorities could further reduce the fees for the submission of grouped variations to include the limits of pyrrolizidine alkaloids in the specification, as long as the submitted documentation (reference standards, validation of analytical method) is the same for the herbal medicinal products. An article 5 variation for the change in the risk categories in the specification, for example from classification A to B, could be introduced. Then the variation could be submitted as a typ IB and not as a typ II variation.

These two measures would reduce the costs for analytical testing and variations and would be a relief especially for medium and small companies, which own the majority of herbal medicinal products.

Another question is what happens with herbal medicinal product batches, which are released based on the category B and C before the transition period ended and are still on the market after the transition period. From the formal point of view, these batches would then be OOS, even though a recall of these batches would probably not be proportional. Pharmaceutical companies could be tempted to release high-risk batches just before the transition period ends, to bypass the lower limits in the first years after the transition period. Here moral considerations should prevent pharmaceutical companies from using such a procedure.

A further ethical aspect would be the marketing of herbal medicinal products in non-EU countries. While the limits for pyrrolizidine alkaloids are not valid for these countries, the same limits as for EU countries should be applied, since pyrrolizidine alkaloids are a health risk to the consumer.

Another important issue is educating consumers of herbal medicinal products as well as tea and honey, the main contaminated food products. Consumers should be aware of the potential health risks of pyrrolizidine alkaloids but should also be able to estimate the risks. For example tea consumers should know that the risk of an acute intoxication is only an issue for consumers of large quantities and that they can minimise the risk of excess contamination by varying the source and type of tea. This would be more appropriate than inadequate information resulting in alarmism. A study of Öko-Test tested herbal medicinal products against urinary tract infection (77). In this study, values of pyrrolizidine alkaloids from 0.175 - 0.35 µg/day, which is below the category A limit, resulted in down ranking of the product, for example. This leads to uncertainty in consumers and also damages the image of herbal medicinal products.

The limits for pyrrolizidine alkaloids will only protect the consumer, if these limits apply for herbal medicinal products and for food. A different approach for both categories, as it is today, is not reasonable.
For food in Germany, the LAV-Arbeitsgruppe "Lebensmittel, Bedarfsgegenstände, Wein und Kosmetika" (ALB) proposed a limit of 0.1 µg/kg b.w./day, derived from the NOAEL of the lasiocarpine study, for example (78). Only if this limit, which is 5 to 14 times higher than the limits set for herbal medicinal products, is exceeded the food product is considered not to be safe. Below the limit of 0.1 µg/kg b.w./day risk management measures should be undertaken, if the MOE is significantly under 10.000. Even though the ALB also refers to the ALARA principle, the set limits are much higher than for herbal medicinal products.

In summary, the limit of 0.007 µg/kg b.w./day, derived from the study of lasiocarpine in rats according to the MOE approach, should be valid for herbal medicinal products and food products. However, a common reference value for the weight for an average adult should be discussed, so that a consistent limit for both product types can be established. Furthermore, the implementation of REP factors should be discussed, so that the relative toxicity of the different pyrrolizidine alkaloids can be taken into account.

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Declaration:

Hiermit erkläre ich an Eides statt, die Arbeit selbständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.