1. Problem/Starting point

Pyrrolizidine alkaloids (PA) are natural toxins that many plants produce as a defence mechanism against insect herbivores. The decomposition products in the liver are a health risk for people and animals. To date, the presence of PA has been verified in more than 350 types of plant. In accordance with the details of the Code of Practice of the German Food Association [Lebensmittelverband Deutschland] [1] the plant families of the boraginaceae (borage or boraginaceous plants), the aster family (Asteraceae) and the legume family (fabaceae). Our domestic plants with high pyrrolizidine contents include various types of groundsel and the Common Blue Thistle. Other plants containing PA and commonly occurring here include types from the genus forget-me-not, such as field forget-me-not, True Comfrey or coltsfoot. Due to the Pa-forming beneficial weeds harvested at the same time, or their components (especially their seeds and fine filaments), these can make their way into the crop harvested from teas and herbal teas, salad crops, herbs and spices. This was established by various institutions once new analytical methods at the Bundesinstitut für Risikobewertung (BfR) [2] led to the discovery in July 2013 of PA in herbal teas and teas. PA occurs in the form of its free compound (PA) and as an N oxide (PANO). There is a total of more than 660 types of PA and their PANO oxides [1]. PA is also known to find its way into honey, especially if the bees fly to plants that contain PA to collect their nectar.

2. Toxicology

Further to the statement by the BfR in June 2018 [3] and the European Food Safety Agency (EFSA) [7], 1,2-unsaturated PAs are undesirable in foods because they can cause liver damage and, in animal trials, have been found to have mutagenic and carcinogenic side-effects. The 1,2-unsaturated Pas with similar toxicological properties include the
four main types: the retronecine, heliotridine, otonecine and platynecine types. Of these, the International Agency for Research on Cancer (IARC) [4] has classified three of these Pas as “potentially carcinogenic for humans” (K 2B): lasiocarpine, monocrotaline and riddelliine. The active strength of individual PAs is variable and depends on their structure. There are monoesters, diesters and cyclical diesters. The last of these are found to have the highest level of toxicity and carcinogenic impact. [3] There is a range of open questions in relation to this.

High concentrations of PA have an acutely toxic impact whereas the ingestion of lower concentrations has a chronically toxic action. Humans ingest PAs primarily from contaminated foodstuffs. For this reason, the quantities of PA ingested needs to be restricted as far as possible.

The BfR extrapolated the level that constitutes acute toxicity from case studies of human subjects across a dosage range of 1-3 mg/kg of body weight per day.

The BfR agrees with the opinion of the EFSA [7] from 2017 on the chronic risk assessment of 1,2-unsaturated PAs by basing the Margin of Exposure (MOE) approach with a BMDL10 (benchmark dose lower confidence limit 10%) as a reference point. The primary objective of the BMD calculation is to use it to estimate a daily exposure level for the human population (including vulnerable sub-groups) that probably does not constitute any significant risk of adverse effects in the course of a lifetime. As a rule, this is derived from chronic animal studies. It is advisable to use the lower confidence limit of 10% for the benchmark dose.

Accordingly, the

$$BMDL_{10} \text{ with a value of 237 µg/kg body weight and day}$$

is defined as a reference point for calculating the Margin of Exposure (MOE).

This margin of exposure (MOE) is the ratio of BMDL10 value to the forecast or estimated dose for people. According to the EFSA, the MOE should measure ≥ 10,000 for an assessment of the mutagenic and carcinogenic substances like 1,2-unsaturated Pas in foodstuffs. Then the foodstuff investigated is always classified as low-risk, “Health impairments due to an overall exposure level to 1,2-unsaturated Pas of this kind should therefore be viewed as unlikely to arise” [8]:

$$\text{MOE} = \frac{BMDL_{10}}{\text{exposure}}$$

$$\text{Exposure} = \frac{BMDL_{10}}{10,000}$$

It follows from this that for the entirety of the 21 BfR/EFSA pyrrolizidine alkaloids - until further notice classified in terms of their toxicity as a group of equi-potent substances with cumulative impact - a daily dosage of

$$0.024 \text{ µg/kg body weight and day}$$

should not be exceeded.
3. Pathways

Unintended intake: Due to the incidental harvesting of Pa-forming beneficial weeds (and/or their constituent elements), these may find their way into the harvested crop of, by way of example, teas and herbal teas, salad vegetables, salad mixtures, herbs, spices and leaf vegetables, and from there into food supplements. Incidental harvesting of just 6 of these PA plants with a high PA content such as Common Groundsel in a field measuring 1 hectare and a planting density of 60,000 plants can render the entire crop unusable. This can also affect honey if the bees visit Pa-forming plants. The seeds of these plants can also be transported by adhering to agricultural implements or by sticking to the pelt of animals. Planting seed can also contain the seeds of plants containing PAs.

Natural occurrence: These can occur on roadside verges and embankments, alongside the edges of paths and ditches, at railway embankments on derelict land, in gravel pits, fallow meadows and at the borders of woodland. Because they grow so quickly, some types of groundsel are sown on roadside embankments to prevent erosion.

Many plants containing PA are part of an intact eco-system and are important for sustaining biodiversity.

Targeted application: Individual PA plants are also used in German cuisine. For example, borage is used as an ingredient in the Seven Herb Mixture for Frankfurt Green Sauce [1]. Certain phytopharmaceuticals used in a targeted manner also contain PA naturally: celandine (e.g. in Iberogast), butterbur, coltsfoot etc.

4. Analytical aspects

PA detected in vegetable crops originates from ‘spot contaminations’ where harvesting can accidentally take in plants that produce PA, the constituent elements of plants and their seed. Due to this spot contamination issue, there is no definitive way to extract samples in a reliable and representative way. At best, it is only an approximation. As a rule, sampling strategies are adopted from (statutory) mykotoxin analysis. The issues of local contamination are comparable there. However, this sampling method is very resource-intensive. Consequently, many companies tend to employ individual procedures based on their own experience. Determination of the presence of individual PAs involves the use of HPLC-MS/MS. In the past in Germany, most investigations involved the “BfR 28 Spectrum” [5]. It includes 15 PA and 13 PANO [1].

For EFSA [7] an investigation spectrum of 17 PA/PANO is recommended. This was supplemented by the EU Commission with four further substances (europine, europine-N-oxide, heliotrine, heliotrine-N-oxide) taking the total number of PA/PANO substances to 21. In 2015, this ‘spectrum’ was adopted by the BfR.
The analytical spectrum now used throughout the EU, comprising 21 PA/PANO or ‘marker substances’ including co-eluting isomers (14 other PA/PANO substances), is covered by the term **BfR/EFSA 21**.

At this time, there are still no statutory analytical methods for PA in foodstuffs. To date, the BfR has validated the *BfR-PA-Tee-2.0/2014* [5] method and this is the one usually practised by laboratories. This assures a high level of comparability between laboratory results. The comparative laboratory investigations now on offer enable analysis laboratories to check the quality of their PA determinations.

5. **Legal aspects**

On 3 January 2021, COM Regulation 2020/2040 of 11 December 2020, amending Regulation (EC) No. 1881/2006 as regards the maximum levels of PAs in various foodstuffs entered into force [9]. **A transitional period** (sell-off period) of 18 months is foreseen, i.e. all products manufactured and legally placed on the market before 1 July 2022, may then continue to be marketed until 31 December 2023.

For each individual PA, the limit of determination from the total of PAs should be 5 µg/kg for dry and solid products, and 2 µg/kg for honey.

Previously on the revised statements by the BfR back in 2018 [3], in agreement with the EFSA, the BMDL\textsubscript{10} value is 237 µg/kg body weight and day. In an announcement on 1 March 2016 the *Bundesinstitut für Arzneimittel und Medizinprodukte* (BfArM) [6] advised that in each medicinal product that contains vegetable substances and/or vegetable preparations, the limit for each product must not exceed a maximum of 1.0 µg PA in respect to the daily dosage, in accordance with Memorandum No. 002/2016 of the BfR, replaced by the updated risk assessment [3] on the contents of 1,2-unsaturated PAs in foodstuffs, Statement No. 020/2018 of the BfR on 14 June 2018. At present, the assessment of findings involves the monitoring of toxicological reference values with their associated levels of uncertainty in respect of a uniform approach across the various federal states in Germany and other countries.
The listed maximum levels of PAs will come into force as of 01.07.2022.⁹

<table>
<thead>
<tr>
<th>Product Description</th>
<th>µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>In each case for dried and instant products</td>
<td></td>
</tr>
<tr>
<td>Herbal tea – rooibos, aniseed, lemon balm, camomile, thyme, peppermint, lemon verbena</td>
<td>400</td>
</tr>
<tr>
<td>Other herbal teas</td>
<td>200</td>
</tr>
<tr>
<td>Tea and aromatised tea (Camillia sinensis)</td>
<td>150</td>
</tr>
<tr>
<td>Tea (Camellia sinensis) and herbal teas, all dried and instant products for babies and infants</td>
<td>75</td>
</tr>
<tr>
<td>Tea (Camellia sinensis) and herbal teas, in liquid form for babies and infants</td>
<td>1.0</td>
</tr>
<tr>
<td>Nutritional supplement with herbal ingredients</td>
<td>400</td>
</tr>
<tr>
<td>Pollen-based nutritional supplement</td>
<td>500</td>
</tr>
<tr>
<td>Pollen and pollen products</td>
<td></td>
</tr>
<tr>
<td>Dried herbs except for borage, lovate, marjoram and oregano, dried *</td>
<td>400</td>
</tr>
<tr>
<td>Borage (fresh and frozen)*</td>
<td>750</td>
</tr>
<tr>
<td>Cumin seed (seed spice)</td>
<td>400</td>
</tr>
<tr>
<td>(*) If necessary, the member states can issue even stricter regulations.</td>
<td></td>
</tr>
</tbody>
</table>

6. Recommendations / Summary

The ingestion of pyrrolizidine alkaloids through food should be kept as minimal as possible because of their potentially adverse health implications. There are however gaps in knowledge in relation to toxicity and the relative active strength of individual PAs. Furthermore, at present there are no statutory analytical methods. There is no uniform procedure for sampling. In the cultivation of useful plants, precautions and measures should be taken to prevent the introduction and spread of PA plants to arable land and to the edges of those cultivated areas. With the planting seed, care must be taken to ensure that it contains no seed from PA plants. In the Code of Practice of the German Food Association [Lebensmittelverband] [1], there is a comprehensive report on the avoidance and reduction of PA in foodstuffs and animal feed. For bees, a sufficient number of alternative food sources needs to be available to prevent bees from visiting flowering plants that produce PAs. To exclude plants containing Pas from certain cultures such as teas and herbal teas, salad vegetables, herbs and spices, it is important for the producers to have a comprehensive knowledge of these plants. This is the only way to detect them in fields accurately and to remove them reliably. In organic agriculture, beneficial weed populations can only be controlled using mechanical methods and by applying meticulous crop production principles. Results from a research project into the cultivation of herbs and medicinal plants [10] show that the beneficial weed flora is similar in
conventional and organic cultivation contexts. One of the points to emerge from this project was that, in conventional plant populations, the Common Groundsel was more prevalent than in organically managed populations. Presumably, organic farming entails a higher level of awareness in relation to PA plants. If plants (or their parts) containing PA enter a harvested crop, it is much more resource-intensive to remove these or parts of plants from it. On the other hand, these plants are members of the domestic plant communities here, and form part of an intact eco-system. A high degree of finesse is therefore needed when checking for plants that contain PAs.

7. Literature and references

[1] Code of Practice to prevent and reduce the contamination of foodstuffs with pyrrolizidine alkaloids issued by the German Food Association [Lebensmittelverband Deutschland] in 2019;


[3] Updated risk assessment on the contents of 1,2-unsaturated PA in foodstuffs, statement no. 020/2018 by the BfR dated 14 June 2018


[6] Announcement by the German federal institute of pharmaceuticals and medical products [Bundesinstitut für Arzneimittel und Medizinprodukte] for checking the content of pyrrolizidine alkaloids to assure the quality and safety of pharmaceuticals containing plant matter and/or plant-based preparations or homoeopathic preparations made of vegetable raw materials as active ingredients dated 01.03.2016

[7] Pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements - European Food Safety Authority (EFSA), 21 June 2017

[8] BfR - Updated risk assessment on the contents of 1,2-unsaturated pyrrolizidine alkaloids (PA) in foodstuffs (statement no. 026/2020 by the BfR on 17 June 2020)


[10] Research projects: Recording of location-dependent and cultivated plant-specific beneficial weed flora in pharmaceutical residues taking particular account of weeds
containing pyrrolizidine alkaloids, published as a conference paper in “Gülzower Fachgespräche Band 56” on 20 – 21 June 2017 in Schweinfurt for the Fachagentur Nachwachsende Rohstoffe e.V. (FNR)

Association of Organic Food Processors (AöL) information
The Association of Organic Food Processors [Assoziation ökologischer Lebensmittelhersteller (AöL)] is a consortium of more than 110 companies involved in the food business. Its European members generate annual bio-sales revenues in excess of four billion euro. Their work focuses on the representation of their interests at a political level and the promotion of dialogue and cooperation between its members.

This information was produced with the collaboration of the Scientific Committee of the AöL.

Contact:
Brunhard Kehl
Assoziation ökologischer Lebensmittelhersteller e.V.
Untere Badersgasse 8 | 97769 Bad Brückenau | Tel: +49 (0)9741 - 938 733 - 0
brunhard.kehl@aoel.org | www.aoel.org