

# Pyrrolizidine Alkaloid Contamination in Medicinal Plants: Regulatory Requirements and Their Impact on Production and Quality Control of Herbal Medicinal Products

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## ABSTRACT

Against the background of potential contamination of medicinal plant materials with pyrrolizidine alkaloids caused by weeds, suppliers of herbal drugs and manufacturers of herbal medicinal products have taken action by establishing a Code of Practice by monitoring potential contamination and by collection of data. In August 2020, the Herbal Medicinal Products Committee, in its new draft public statement, proposed a daily intake of 1.0 µg of pyrrolizidine alkaloids per day for adults in general, also including contaminations of herbal medicinal products. Over the past years, the results of data collections showed a remarkable reduction of the pyrrolizidine alkaloid burden in herbal drugs and herbal extracts. Meanwhile, a stable situation has been achieved for herbal drugs, while further improvement can be observed for herbal extracts. The results indicate that the implemented measures have been efficient and contribute to a continuous and sustainable reduction of pyrrolizidine alkaloid contamination. A permanent limit of 1.0 µg of pyrrolizidine alkaloids per day is considered appropriate to guarantee sufficient availability of batches used for the production of herbal medicinal products. The new Ph.Eur. general chapter 2.8.26 describes, as an example, an analytical procedure suitable for the determination of target pyrrolizidine alkaloids.

## Introduction

The survey of the German Federal Institute for Risk Assessment (BfR) published in July 2013 [1] gave hints of potential contamination of medicinal plant material with pyrrolizidine alkaloids (PA)-containing weeds. This led to a number of immediate actions to avoid and/or reduce PA contamination as far as possible. Such measures consisted, e.g., in the establishment of a “Code of Practice” of manufacturers of herbal medicinal products in close cooperation with herb growers [2,3] and the collection of analytical data. In the following, these measures will be described in more detail. Their efficiency will be discussed in light of the most recent data on the occurrence of PAs in herbal drugs and herbal drug extracts and the current regulatory limits for the maximum daily intake.

## Guidance for Herb Growers and Manufacturers

The “Code of practice to prevent and reduce pyrrolizidine alkaloid contaminations of medicinal products of plant origin” [2,3] provides a framework for the implementation of individual measures starting along the entire process chain from agricultural production steps and ending up with the final product. As complete avoidance of PA contamination is considered impossible according to the current technical state of the art in agricultural and industrial production, the focus is mainly laid on preventing measures rather than removing potential sources of PA burden from cultivated plants.

The main principle of this guidance document is the identification of potential risks for each process step together with their probability of occurrence, the proposal for actions to be taken, and an assessment of these measures with regard to feasibility, time horizon, and efficiency as well as the allocation of the respec-

tive responsibilities. Within a risk analysis, these issues are examined along the entire process chain, comprising, e.g., selection of seeds, cultivation, harvesting, incoming goods inspection, and drug processing up to the release of the final medicinal product in the pharmaceutical company. Recommendations are given that are based on the experiences of herb growers and manufacturers of the respective products.

Some examples might elucidate during which production steps potential risks can exist and which options to insert influence can be utilised:

- In terms of the process step of cultivation planning, seeds of weeds that are already in the soil or weeds growing in fields in the neighbourhood may present a risk. Therefore, careful selection of fields for growing medicinal plants should take place, including measures such as mowing the field edges as well as appropriate use of herbicides where necessary.
- During harvesting the plant material, there is a risk of co-harvesting PA-containing weeds along with cultivated plants. The probability is high, depending on the species of the cultivated plant and the harvesting technology used. Potential measures can consist in the optimisation of the harvesting process, e.g., with regard to timing, technology, or cutting height.
- During incoming goods inspection, a risk-based selection of crude drugs that are subject to intensive testing for PAs takes place. This selection depends on the knowledge of the potential PA burden, e.g., from batch data or from the production process, since the probability of PA occurrence is higher for herbs harvested from a field than, e.g., for leaves collected from a tree.

## Regulatory Limits

So far, the Herbal Medicinal Products Committee (HMPC) at the European Medicines Agency (EMA) recommended a transitional limit of 1.0 µg PA per day for PA contamination related to the final product in May 2016 [4]. As the problem of contamination was supposed not to be resolved immediately, the limit was set for a preliminary period of 3 years, later on prolonged to 5 years until May 31, 2021. After this period, in accordance with a former HMPC Public Statement on the use of herbal medicinal products containing toxic, unsaturated PAs dated 2014 [5], a daily limit of 0.35 µg PA should apply. During this time period, the producers of herbal medicinal products should take actions necessary to reduce the contamination to the lower level.

In August 2020, a revised version of the HMPC Public Statement of 2014 [5] was published as a draft for public consultation [6], which also included recommendations regarding contamination with PAs. Reference was made to the EFSA Panel on Contaminants in the Food Chain, which published a new assessment of the carcinogenic risks of PAs, including new occurrence data in honey, tea, herbal infusions, and food supplements in June 2017 [7]. The HMPC decided to follow this approach, which, in the context of updating the risk characterisation of PA, established a new Reference Point of 237 µg/kg body weight. On this basis, the HMPC deduced a daily intake of 1.0 µg PA per day for adults. For children, the daily amount of toxic, unsaturated PAs has to be adjusted to the body weight of the age group. Also, for contamina-

tion of herbal medicinal products with PAs, the same limit of 1.0 µg per day for adults applies [6].

The HMPC also mentions the German Code of Practice [2, 3] as a framework for the implementation of individual measures in pharmaceutical companies as well as for agricultural production steps. Moreover, it emphasises that available agricultural measures to reduce PA weeds by way of selective herbicides, manual weeding/sorting, seed cleaning, inspection of fields before harvesting, etc., need to be put in place to achieve the reduction of PA contamination.

For foodstuffs, against the background of the EFSA assessment [7], an amendment to Regulation (EC) No 1881/2006 as regards to maximum levels of PAs was published in December 2020 [8]. The Regulation sets maximum levels for certain food products of herbal origin and comprises, e.g., herbal infusions (200 µg/kg). Exemptions are foreseen for rooibos, anise, lemon balm, chamomile, thyme, peppermint, and lemon verbena (400 µg/kg). Moreover, specific maximum levels are set for food supplements and for fresh, frozen, and dried herbs. For herbal infusions, the product-related limit is comparable to the limit of 1.0 µg PA per day as recommended by the HMPC, since consumption of 4 cups of herbal tea, each containing 2 g of herbs, would lead to a daily intake of 1.6 or 3.2 µg PA, respectively. The limit shall apply from July 1, 2022. Respective foodstuffs that were lawfully placed on the market before July 1, 2022 may remain on the market until December 31, 2023.

## Extent of Testing

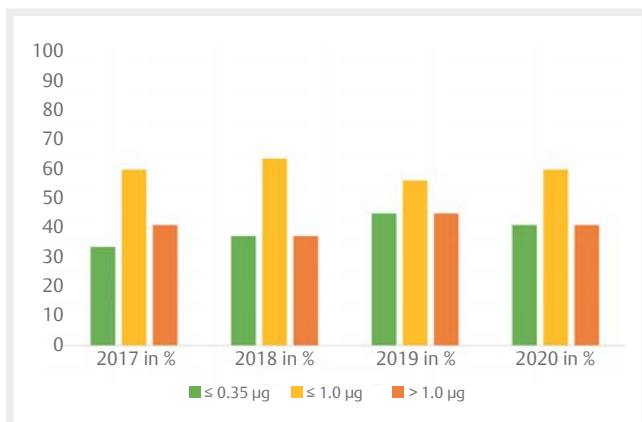
The HMPC Public Statement of 2016 [4] defines three categories for the frequency of testing herbal drugs and/or preparations on the potential occurrence of PAs. The allocation to one of these categories depends on the knowledge of potential contamination based on existing data:

- skip testing in case of a content of up to 0.1 µg PA per day in the finished product (category A)
- intensified skip testing in case of a content of up to 0.35 µg PA per day (category B)
- routine testing in case of more than 1.0 µg PA per day or in case no or insufficient data is available (category C).

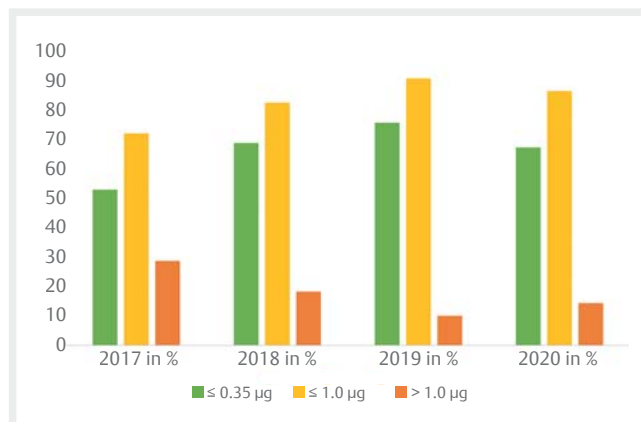
Herbal drugs leading to a PA content of more than 1.0 µg PA per day in the final product cannot be used for further production.

It is important that for medicinal products, the limit of 1.0 µg PA relates to the daily intake of the product. For this reason, the manufacturer has to specify the maximum PA content of the herbal drug taking into account the posology of the individual product for calculation as well as the drug extract ratio in the case of herbal extracts.

In order to define the individual extent of testing based on the probability of PA occurrence in the respective material, the availability of data is essential. For example, in case of many negative findings and additional consideration of the origin (e.g., tree or field) and/or the production process (harvesting technique), the probability of PA contamination can be regarded as low, resulting in the option of a less frequent skip testing. This has to be justified individually within the specification to be submitted to the health authorities.



► **Fig. 1** Changes in the allocation (%) to the classes  $\leq 0.35$ ,  $\leq 1.0$ , and  $> 1.0$   $\mu\text{g}$  PA per day for 27 herbal drugs with high market relevance (calculated using the maximum dose according to the HMPC monograph). Source: The figures were prepared by Dr. Heinz Dittrich, Bad Heilbrunner Naturheilmittel GmbH & Co. KG based on the mentioned data evaluation.



► **Fig. 2** Changes in the allocation (%) to the classes  $\leq 0.35$ ,  $\leq 1.0$ , and  $> 1.0$   $\mu\text{g}$  PA per day for 21 herbal extracts with high market relevance (calculated using the maximum dose according to the HMPC monograph). Source: The figures were prepared by Dr. Heinz Dittrich, Bad Heilbrunner Naturheilmittel GmbH & Co. KG based on the mentioned data evaluation.

## Procedure of Database Evaluations

During the past 7 years, approx. 50 pharmaceutical companies, mainly from Germany, established an extensive database that compiled results of analytical testing on PAs for herbal drugs, herbal extracts, and homoeopathic mother tinctures. Evaluations take place annually, each comprising data for the period May 1st of one year until April 30th of the following year. For the participating companies, the database allows a well-founded assessment of the overall development of PA occurrences and a continuous verification of the efficiency of the initiated measures. Moreover, it provides knowledge about the probability of contamination, which is relevant for defining the testing strategy.

The results of the most recent database evaluation (2019/2020) in comparison to the past two evaluations will be presented and discussed in the following. They represent an update of the results recorded up to 2018, which were published earlier [9]. In the 2020 evaluation, altogether, 7739 samples consisting of 6806 samples from 272 different herbal drugs and 933 samples from extracts of 92 herbal drugs and 80 extraction solvents were recorded. Analytical data were generated during quality control of the manufacturers and determination of PAs in the respective samples being performed using validated methods, e.g., LC-MS/MS technologies such as the established BfR PA-Tee-2.0/2014 procedure [10]. For each of the 27 important herbal drugs and 21 important extracts with the highest market relevance (altogether 4522 samples, consisting of 4030 samples for herbal drugs and 492 samples for herbal extracts), the percentage of values allocated to each of the three abovementioned categories [A ( $\leq 0.1$   $\mu\text{g}$ ), B ( $\leq 0.35$   $\mu\text{g}$ ), C ( $\leq 1.0$   $\mu\text{g}$ )] was calculated. In order to establish a relation between these limits and the PA content of the herbal drug/extract, the maximum daily dose according to the respective HMPC monograph was used for calculation. This might demonstrate a worst-case scenario for all products, although actual daily doses of many products are much lower.

## Results and Discussion

The development of the analytical findings related to the PA content for the most important herbal drugs and herbal extracts between 2017 and 2020 is shown in ► **Figs. 1** and **2**. The percentage values each represent cumulative values from the abovementioned categories A + B ( $\leq 0.35$   $\mu\text{g}$  per day) or A + B + C ( $\leq 1.0$   $\mu\text{g}$  per day), respectively.

For herbal drugs as well as for extracts, a shift from the columns representing higher PA contents ( $> 1.0$   $\mu\text{g}$  per day) to those representing lower contents ( $\leq 1.0$  or  $\leq 0.35$   $\mu\text{g}$  per day, respectively) can be seen between 2017 and 2018, and a more or less stable situation was achieved between 2018 and 2020.

For herbal drugs, the proportion of samples with a *higher* PA content ( $> 1.0$   $\mu\text{g}/\text{day}$ ) after a reduction to 37% in 2018 is relatively stable with 41% in 2020. For herbal extracts, the proportion of samples with a higher PA content is much lower than for herbal drugs and also stable with 14% in 2020. The generally lower PA content of herbal extracts can be explained by an increased selection of herbal drugs that are used for extraction.

In the same period, for herbal drugs, the proportion of samples with *lower* PA contents is also stable with 59% ( $\leq 1.0$   $\mu\text{g}$  per day) or 41% ( $\leq 0.35$   $\mu\text{g}$  per day), respectively, in 2020. For herbal extracts, the proportion of samples with lower PA contents has increased to 86% ( $\leq 1.0$   $\mu\text{g}$  per day) or 67% ( $\leq 0.35$   $\mu\text{g}$  per day), respectively, in 2020.

With regard to the ability of herbal drugs and herbal extracts to keep the proposed limits, the 2020 evaluation demonstrates that only 41% of the most important 27 herbal drugs can keep the limit of 0.35  $\mu\text{g}$  PA per day (2018: 37%, 2019: 44%), whereas 59% can keep the limit of 1.0  $\mu\text{g}$  PA per day (2018: 63%, 2019: 56%). With regard to the most important 21 herbal extracts, 67% can keep the limit of 0.35  $\mu\text{g}$  PA per day (2018: 68%, 2019: 75%), whereas 86% can keep the limit of 1.0  $\mu\text{g}$  PA per day (2018: 82%, 2019: 90%). All in all, the percentages of herbal drugs

and herbal extracts allocated to the different categories – except slight oscillations – have not changed between 2018 and 2020. Slight increases might be caused by a higher testing frequency of those herbal drugs and herbal extracts considered as potentially problematic, or additional testing in case of equivocal results.

Thus, it can be shown that in many cases, the daily limit of 1.0 µg PA related to the final product can be kept. By annual evaluations of the collected data, the efficiency of the performed measures according to the Code of Practice [2, 3] can be verified. Although over the past years an overall reduction of the total PA burden can be seen and a stable situation has been achieved now, the data evaluation demonstrates that a general reduction to 0.35 µg PA per day cannot be achieved but apparently follows an asymptotic function.

## Analytical procedures

The new Ph.Eur. general chapter 2.8.26 Contaminant Pyrrolizidine Alkaloids, which was published as a draft in December 2019 [11] and adopted by the Ph.Eur. Commission in November 2020, describes, as an example, an analytical procedure suitable for the determination of target PAs that corresponds to the established BfR procedure [10]. As the procedure had been validated for specific matrices, the chapter permits the use of any procedure consisting of chromatography coupled with mass spectrometry if specific validation requirements are met. For this purpose, at least one representative matrix from several matrix groups is used. When the validation requirements are met for a representative matrix (e.g., dried peppermint leaf), the procedure is assumed to be valid for any other matrix belonging to the same matrix group (e.g., leaf). In order to demonstrate that the analytical procedure remains valid during routine analysis, verification has to be performed in accordance with specific requirements.

With regard to, e.g., sample preparation, it is important to ensure that the sample has a uniform particle size and a homogeneous distribution of PA or PA-containing material, against the background that inhomogeneity might be caused by spot contamination. The extent of testing in accordance with Ph.Eur. chapter 2.8.26 comprises 28 substances, which are listed in ► **Table 1**.

The amendment to Regulation 1881/2006 [8] explains, for the food area, that the possible maximum levels listed for 21 PAs are lower bound concentrations, i.e., they are calculated based on the assumption that all the values of the different individual alkaloids below the limit of quantification are equal to zero. An additional 14 PAs known to coelute with one or more of the mentioned 21 alkaloids shall be quantified and included in the sum in case they can be individually and separately identified with the used method of analysis.

## Overall Conclusion

The HMPC draft Public Statement [6] recommends, as a strategy for risk management, that the main approach for risk management should be according to the concept of ALARA, i.e., as low as reasonably achievable. It explains that in principle, contamination of herbal substances with PA-containing weeds should not occur at all for reasons of requirements on pharmaceutical product quality and compliance with GACP/GMP. However, as shown

► **Table 1** Substances listed in Ph.Eur. chapter 2.8.26 [11].

- Echimidine
- Echimidine *N*-oxide
- Erucifoline
- Erucifoline *N*-oxide
- Europine
- Europine *N*-oxide
- Heliotrine
- Heliotrine *N*-oxide
- Intermedine
- Intermedine *N*-oxide
- Jacobine
- Jacobine *N*-oxide
- Lasiocarpine
- Lasiocarpine *N*-oxide
- Lycopsamine
- Lycopsamine *N*-oxide
- Monocrotaline
- Monocrotaline *N*-oxide
- Retrorsine
- Retrorsine *N*-oxide
- Senecionine
- Senecionine *N*-oxide
- Seneciphylline
- Seneciphylline *N*-oxide
- Senecivernine
- Senecivernine *N*-oxide
- Senkirkine
- Trichodesmine

by the industry's data collections over the past years [9] and stated in the Code of Practice [2, 3], complete elimination is not possible. Contamination of medicinal plants with PAs continues to be a challenge for herb growers and medicinal product manufacturers although numerous activities have been undertaken, e.g., by application of a Code of Practice, by data collection, and by elimination of peak exposures. The results of the data collection clearly demonstrate that after a period of continuous reduction of PA contamination, a rather stable situation has been achieved now for herbal drugs, while a further improvement can be observed for herbal extracts. The results indicate that the implemented measures have been efficient and contribute to a continuous and sustainable reduction of PA contamination. For herbal drugs and herbal extracts, in many cases, the applicable daily limit of 1.0 µg PA per day related to the final product can be kept like in 2018 and 2019. Thus, a permanent limit of 1.0 µg PA per day is considered appropriate to guarantee sufficient availability of batches used for the production of herbal medicinal products.

## Contributors' Statement

Conception and design of the work, analysis and interpretation of the data, drafting the manuscript, critical revision of the manuscript: B. Steinhoff.

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## Conflict of Interest

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The author is an employee of the German Medicines Manufacturers Association.

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