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Pyrrolizidine alkaloid contamination in herbal medicinal products: Limits and occurrence

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ABSTRACT

Since 2013, a potential contamination of medicinal plant material with pyrrolizidine alkaloid-containing weeds e.g. *Senecio* has been discussed. The knowledge about such a risk of contamination induced suppliers of medicinal drugs and manufacturers of medicinal teas to investigate the situation regarding herbal drugs and teas and other medicinal products of plant origin. As due to worldwide cultivation/collection and season-dependent sourcing processes an immediate elimination or even reduction of PA contamination at all sourcing sites was considered impossible, manufacturers have taken action by application of their Code of Practice, by monitoring pyrrolizidine alkaloid contamination and by collection of data, by elimination of peak exposures as well as by participation in research projects. The Herbal Medicinal Products Committee at the European Medicines Agency recommended a transitional limit of 1.0 µg pyrrolizidine alkaloids per day related to the final product for three years which has recently been prolonged by a further two years. Against the background of the assessment of the European Food Safety Authority, the option of establishing a permanent limit of 1.0 µg per day should be taken into consideration during future discussions.

1. Introduction

Pyrrolizidine alkaloids (PA) are natural constituents of a large number of plants, parts of which are also used for medicinal purposes, e.g. *Symphytum, Petasites, Tussilago, Eupatorium*. As a regulatory measure with the aim to protect patients' and consumers from the intake of toxic PA through medicinal products, some health authorities set strict limits for the PA content in medicinal products during the 1990ies. E.g. the German health authority initiated a „graduated plan" in 1992 stating that the maximum daily exposure to PAs by medicinal products should not exceed 0.1 µg for internal use and 10 µg for external use. If the duration of application is limited to maximal 6 weeks per year, doses of up to 1 µg for internal and 100 µg for external use are accepted. Such higher doses are contraindicated for pregnant and breast-feeding women and, in case of topical use, should only be applied on intact skin (Bundesgesundheitsamt, 1992). Apart from the ongoing and new discussion on contamination with PA-containing plants, these measures are still valid for medicinal products produced from naturally PA-containing plants as listed by the German health authority (Bundesgesundheitsamt, 1992).

In the context of the elaboration of a monograph on *Symphytum*, the Herbal Medicinal Products Committee (HMPC) at the European Medicines Agency (EMA) issued a Public Statement on the use of herbal medicinal products containing toxic, unsaturated PA on 24 November 2014 (HMPC, 2014) which set an adult limit of 0.35 µg toxic unsaturated PA per day over a maximum of 14 days of consumption. This limit value lies within the order of magnitude at that time considered by the European Food Safety Authority (EFSA) as posing no risk to health in food at a lifetime daily intake of 0.42 µg PA per day (EFSA, 2005, 2011). Originally, the HMPC document was intended for assessment of naturally PA-containing plants, but products containing PA from other sources than biosynthesis in the plant were not excluded.

2. The issue of contamination

In July 2013, the German Federal Institute for Risk Assessment (BfR) published analysis results on the occurrence of pyrrolizidine alkaloids (PA) in 221 samples of herbal teas and some medicinal teas (BfR, 2013). From that time onwards, a potential contamination of medicinal plant material with PA-containing weeds e.g. *Senecio* was publicly discussed. The knowledge about such a risk of contamination induced suppliers of medicinal drugs and manufacturers of medicinal teas to investigate the situation regarding herbal drugs and teas and other medicinal products of plant origin, to evaluate the results and to

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A Very low or no problem of contamination

Based on data available it can be justified, that usually content of pyrrolizidine alkaloids in the finished medicinal products is ≤ 0.1 µg with respect to daily exposure. This classification can be accepted, if this limit is not exceeded for 90 % of samples analysed and no sample has a value higher than 0.35 µg with respect to daily exposure. For this category a skip testing can be accepted. The specific testing scheme must be derived from data available.

B Low problem of contamination

Based on data available it can be justified, that usually content of pyrrolizidine alkaloids in the finished medicinal products is ≤ 0.35 µg with respect to daily exposure. This classification can be accepted, if this limit is not exceeded for 90 % of samples analysed and no sample has a value higher than 1.0 µg with respect to daily exposure. For this category an intensified skip testing is necessary. The specific testing scheme must be derived from data available.

C Relevant problem of contamination

If there is no data or based on data available a classification to category A or B is not possible, a routine testing is to be implemented into the release specification defining an upper threshold of 1.0 µg pyrrolizidine alkaloids with respect to daily exposure.

Fig. 1. Categories which determine the frequency of testing according to HMPC (2016).

initiate immediate actions in response.

Following a similar approach of the German Federal Institute for Drugs and Medical Devices (BfArM) on 1 March 2016 (BfArM, 2016) and other European health authorities, the HMPC issued a further Public Statement in May 2016 (HMPC, 2016), recommending a transitional limit of 1.0 µg PA per daydaily related to the final product for three years, since the problem of contamination was not considered to be resolvable immediately. The HMPC stated that after this period, a daily limit of 0.35 µg PA should apply, and during this time period the producers of herbal medicinal products should take actions necessary to reduce the contamination to the lower level. With regard to testing herbal drugs and/or preparations on potential occurrence of PA, the Public Statement also defines three categories for the frequency of testing: skip testing, intensified skip testing or routine testing. As shown in Fig. 1, the allocation to one of these categories depends on the knowledge of potential contamination based on existing data. In case no or insufficient data is available, routine testing has to be performed. 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.

As the limit of 1.0 µg PA relates to the daily intake of the product, the manufacturer has to specify the maximum PA content of the herbal drug using the posology of the individual product for calculation as well as the drug:extract ratio in case of herbal extracts.

This recommendation was implemented by national health authorities in Europe, but not in a uniform manner, i.e. the limit of 1.0 µg per day was not accepted by all countries.

3. Herbal medicinal products manufacturers‘ activities

After publication of the BfR study in 2013, it became evident that PA contamination is a serious problem and challenge for agriculture and horticulture in general, and for this reason interdisciplinary solutions along the entire process chain were needed. It was obvious that weeds including e.g. *Senecio* do not present a new phaenomenon, however, PA are nowadays detectable at trace levels by more sensitive analytical methods. From 2013 onwards, the German herbal medicinal products' industry initiated measures which were intended to avoid and/or reduce PA contamination as far as possible. Such measures consisted e.g. in causal research, in analytical testing in order to minimize the content of PA in herbal medicinal products, in the collection of data, in early discussions with health authorities, in participation in research projects and in the establishment of a „Code of Practice“ that was elaborated together with herb growers (Dittrich et al., 2016). Against the background that complete avoidance of PA contamination was not regarded as possible according to current technical state of the art, and therefore preventive measures, testing of batches and monitoring of the situation were considered essential, this document provides a framework for the implementation of individual measures in pharmaceutical companies as well as for the agricultural production steps. The main principle of the Code of Practice is the identification of potential risks for each process step along the entire process chain comprising e.g. cultivation, harvesting, incoming goods inspection, drug processing up to the release of the final medicinal product. For all these steps, the potential risks of PA-contamination are elucidated together with their probability, the proposal for actions to be taken and an assessment of these measures. E.g. for the process step of harvesting the plant material, the risk exists that PA-containing weeds are co-harvested 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 optimisation of harvesting technology (e.g. timing, technology, cutting height). The importance of these measures is assessed to be high, however, depending on various individual influencing factors, the feasibility might be limited.

4. Establishment of an industry database

Starting also in 2013, the German industry established an extensive

database compiling results of testing herbal drugs, herbal extracts and homoeopathic mother tinctures from currently almost 50 pharmaceutical companies. Summary reports are submitted to the German BfArM and to the HMPC on a regular basis, to further health authorities upon request. Evaluations take place annually, the most recent one being performed for the period 1 May 2017 until 30 April 2018. For the participating companies, the database allows a well-founded assessment of the situation and a continuous verification of the efficiency of the initiated measures. In case a large number of negative findings for a herbal drug indicates a low probability of PA burden and additionally the knowledge of the origin (e.g. leaves from trees) and the production process (e.g. harvesting technique) provides evidence for a low risk of contamination, such a herbal drug could be – considering the posology of the respective preparation/finished product – a candidate for a product-specific justification of skip testing with a rather low frequency.

In the 2018 database evaluation, altogether 7251 samples from 264 herbal drugs and 820 samples from extracts of 117 herbal drugs and 86 extraction solvents were included. Analytical data were generated during quality control of the manufacturers, determination of PA in the respective samples was performed using validated methods. For each of the 27 herbal drugs (4446 samples) and the 22 herbal extracts (425 samples) with the highest market relevance, the percentage of values allocated to each of the 3 above-mentioned classes A (≤0.1 µg), B (≤0.35 µg), C (≤1.0 µg) 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 HMPC monograph was used for calculation, thus demonstrating a worst-case scenario for all products, although actual daily doses of many products are much lower.

5. Results of the database evaluation

Tables 1 and 2 show the results of the database evaluation between 2015 and 2018 for the most important 27 herbal drugs and 22 herbal extracts. For herbal drugs as well as for extracts a shift from the columns representing higher PA contents (> 1.0 µg/day) to those representing lower contents (≤1.0 µg/day or ≤ 0.35 µg/day, respectively), can clearly be seen (2017 vs. 2016 and 2018 vs. 2017). The comparison between 2016 and 2015 appears to disclose a negative trend – however, this is an artefact due to the changed testing scheme from “BfR 17” to “BfR 28”, the official method of the German BfR (BfR, 2014), which has extended the coverage of the method significantly from 17 to 28 alkaloids.

For herbal drugs the tables demonstrate that between 2016 and 2018 the proportion of samples with a higher PA content (> 1.0 µg/day) has been reduced successively from 64% to 41% and to 37%, and for herbal extracts from 64% to 29% and to 18%. In the same period, for herbal drugs the proportion of samples with lower PA contents (≤1.0 µg/day or ≤ 0.35 µg/day, respectively) increased from 36% to 59% and to 63% and from 24% to 33% and to 37%, respectively, for herbal extracts from 62% to 71% and to 82% as well as from 43% to 52% and 68%, respectively.

The development of the PA content of herbal drugs and herbal extracts over the past few years is also shown in Figs. 2 and 3. As an overall result, a continuous improvement can clearly be seen for both herbal drugs and herbal extracts. From the presented data is becomes also obvious that only 37% of the herbal drugs can meet the limit of 0.35 µg/day, whereas 63% can meet the limit of 1.0 µg/day.

The data collection shows that in many cases the daily limit of 1.0 µg PA related to the final product can be kept. The results confirm that by collection of data and by annual evaluations, the efficiency of the performed measures according to the Code of Practice (Dittrich et al., 2016; Anon., 2016) can be verified. Over the past few years a clear reduction of the total PA burden of herbal medicinal products can be seen. However, the data also demonstrate that a general reduction to 0.35 µg PA cannot be achieved in the near future but follows an asymptotic function.

Table 1
Percentage of the most relevant 27 herbal drugs related to classes.

	≤ 0.35 µg	≤ 1.0 µg	>1.0µg
2015 in %	44	44	56
2016 in %	24	36	64
2017 in %	33	59	41
2018 in %	37	63	37

Table 2
Percentage of the most relevant 22 herbal extracts related to classes.

	≤ 0.35 µg	≤ 1.0 µg	>1.0µg
2015 in %	56	56	38
2016 in %	43	62	64
2017 in %	52	71	29
2018 in %	68	82	18

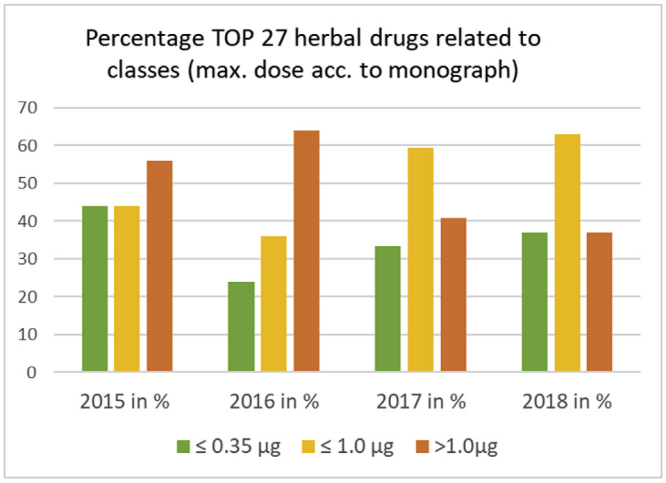

Fig. 2. Changes in the allocation to classes for 27 herbal drugs with high market relevance.

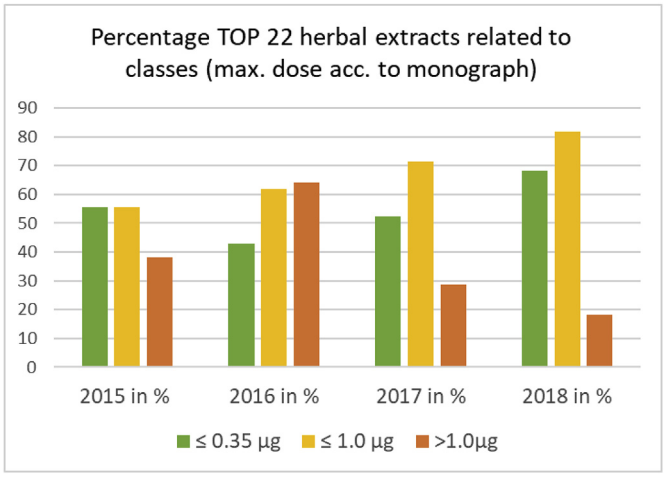

Fig. 3. Changes in the allocation to classes for 22 herbal extracts with high market relevance.

6. Analytical challenges

For the determination of PA nowadays LC-MS/MS technologies (combination of liquid chromatography and mass spectrometry) are used which allow the detection and quantitative determination of PA in a range substantially below 1 mg/kg. Based on the complexity of the analytical questions and the required comparability of the results, LC-MS/MS methods such as the BfR PA-Tee-2.0/2014 method (BfR, 2014) with a scope of testing of 28 substances are today considered state-of-the-art technology.

The method of the BfR is based on the MRM technique (multiple reaction monitoring transitions) and is characterised by a high specificity and sufficient sensitivity. This tandem mass spectrometry is a long-established analysis technology in residue analysis and is also suited for the determination of PA, since measurements are made in the same concentration range (1 µg/kg to 3 mg/kg). Other validated LC-MS/MS methods used in practice are also suitable for this purpose which ensure that the alkaloids are identified and quantified with sufficient sensitivity (Dittrich et al., 2016; Anon., 2016).

A specific European Pharmacopoeia (Ph.Eur.) method for determination of PA in herbal drugs and herbal extracts does not yet exist. However, manufacturers are asked by health authorities to submit detailed specifications as well as the description of the analytical methods used including validation data and reference substances. For this reason, the development of a framework for a quantitative method in the European Pharmacopoeia (Ph.Eur.) is considered extremely useful. An expert group was founded at the European Directorate for the Quality of Medicines and Healthcare (EDQM) who is currently preparing a respective draft for publication and consultation with interested parties.

7. Research projects

In addition to the individual measures and activities undertaken by all involved responsible persons on the respective process steps from cultivation/collection of the plant material up to the release of the final product, research projects play an important role with respect to the solution of the extremely complex PA issue. The following projects might serve as examples for numerous activities in the areas of sourcing, processing and analytical testing:

- Data collection on weed flora in cultivated medicinal plants with specific view to PA-containing weeds („weed database“), public funding with participation of FAH (Forschungsvereinigung der Arzneimittel-Hersteller e.V., an association of pharmaceutical companies involved in research). The project also contains investigations on PA uptake from the soil (FAH, 2019a).
- Preparation of „weed characteristics“ („Unkrautsteckbriefe“) for use in cultivation and collection, describing the species *Crotalaria, Echium, Heliotropium, Myosotis, Senecio*) (FAH, 2019b). The project was established by 19 member companies of FAH.
- Weed management in conventional and organic cultivation by selection of suitable herbicides or non-chemical methods.
- Development of methods for identification and mechanical separation of foreign matter from harvested material.
- Development of an immunological screening method to determine toxicologically relevant levels of pyrrolizidine alkaloids in herbal teas and related matrices, public funding.
- In vitro testing of hepatotoxicity and genotoxicity of selected PA congeners representing different structural classes (lasiocarpine, riddelliine, retrorsine, senecionine, seneciphylline, heliotropine, echimidine, lycopsamine, europine, indicine) with the objective of verifying the hypothesis that different PA have significantly different toxicological potencies (Merz and Schrenk, 2016).

8. Further regulatory guidance

On 21 June 2017, the EFSA Panel on Contaminants in the Food Chain (CONTAM) published a new assessment of the carcinogenic risks of PA including new occurrence data in honey, tea, herbal infusions and food supplements (EFSA, 2011). In the context of updating the risk characterisation of PA, the CONTAM Panel established a new Reference Point of 237 µg/kg body weight per day. When applying the rationale used by HMPC, this new approach which includes a factor of 3 would result in a limit dose 3×0.35 µg PA/day and thus be close to the transitional HMPC limit of 1.0 µg PA per day.

On 1st February 2019, the EMA published on its website the Report of the 86th HMPC meeting held on 14–16 January 2019 (HMPC, 2019) which includes an update to the Public Statement on Contamination of 2016 (HMPC, 2016). Against the background of the new EFSA risk assessment (EFSA, 2011) and the activities of the Ph.Eur. to establish a general method for testing PA, the HMPC had agreed by consensus to extend the transitional period for products with levels up to 1.0 µg PA per day for a further two years, i.e. until 31 May 2021. Moreover it was stated that *„whilst difficulties for manufacturers of herbal medicinal products to implement measures to reduce PA contamination are acknowledged, manufacturers should continue to take appropriate actions including implementation of enhanced GACP* (for explanation: Good Agricultural and Collection Practice) *to ensure daily intake does not exceed 1.0 µg PA/day“*. Revision of the Public Statements (HMPC, 2014, 2016) was announced in order to provide guidance for industry and national health authorities.

9. Overall conclusion

Contamination of medicinal plants with PA is a challenge for growers and manufacturers with regard to the precise identification and quantification of the contaminants and to their reduction. Due to worldwide cultivation/collection and season-dependent sourcing processes a complete elimination of PA contamination at all sourcing sites is impossible. The herbal medicinal products manufacturers have taken action by application of their Code of Practice, by monitoring PA contamination and by collection of data, by elimination of peak exposures as well as by participation in research projects. All these measures contribute to a continuous and sustainable reduction of PA contamination and guarantee a further production of medicinal products which are safe and of high and consistent quality.

The results of the data collection show a remarkable decrease of the PA burden in herbal drugs and extracts used for the production of herbal medicinal products that has been achieved over the past few years. However, it has become clear that the problem of PA contamination cannot be completely solved in the near future. According to the current data evaluation, manufacturers are able to keep the limit of 1.0 µg PA per day for most products, but reduction to 0.35 µg per day is not realistic. Taking into account the EFSA risk assessment (EFSA, 2011) as well as the Ph.Eur. activities and having acknowledged that difficulties for manufacturers to implement PA-reducing measures exist, the HMPC has extended the transitional period for the limit of 1.0 µg PA per day until 31 May 2021. For future discussions on a potential permanent limit, again the EFSA assessment and in addition the results of ongoing research activities should be taken into consideration.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.05.026.

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