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Mini-review

Interim relative potency factors for the toxicological risk assessment of pyrrolizidine alkaloids in food and herbal medicines

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HIGHLIGHTS

- Pyrrolizidine alkaloids (PAs) are among the most potent natural toxins occurring in a broad spectrum of plant species used as food and herbal medicines.
- Risk assessment is currently based on the carcinogenicity of certain PAs after chronic application to rats using the sum of detected PAs as dose metric.
- Because of the well-documented large structure-dependent differences between sub-groups of PA congeners with respect to their genotoxicity and (cyto)toxicity this procedure appears inadequate.
- Based on a thorough analysis of the literature, we have derived interim Relative Potency (REP) factors for a number of abundant PAs for use in toxicological risk management.

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ABSTRACT

Pyrrolizidine alkaloids (PAs) are among the most potent natural toxins occurring in a broad spectrum of plant species from various families. Recently, findings of considerable contamination of teas/herbal infusions prepared from non-PA plants have been reported. These are obviously due to cross-contamination with minor amounts of PA plants and can affect both food and herbal medicines. Another source of human exposure is honey collected from PA plants. These findings illustrate the requirement for a comprehensive risk assessment of PAs, hampered by the enormous number of different PA congeners occurring in nature. Up to now, risk assessment is based on the carcinogenicity of certain PAs after chronic application to rats using the sum of detected PAs as dose metric. Because of the well-documented large structure-dependent differences between sub-groups of PA congeners with respect to their genotoxicity and (cyto)toxicity, however, this procedure is inadequate.

Here we provide an overview of recent attempts to assess the risk of PA exposure and the available literature on the toxic effects and potencies of different congeners. Based on these considerations, we have derived interim Relative Potency (REP) factors for a number of abundant PAs suggesting a factor of 1.0 for cyclic di-esters and open-chain di-esters with 7*S* configuration, of 0.3 for mono-esters with 7*S* configuration, of 0.1 for open-chain di-esters with 7*R* configuration and of 0.01 for mono-esters with 7*R* configuration. For *N*-oxides we suggest to apply the REP factor of the corresponding PA. We are confident that the use of these values can provide a more scientific basis for PA risk assessment until a more detailed experimental analysis of the potencies of all relevant congeners can be carried out.

During the last few years, one of the most unexpected findings regarding contaminants in food was the detection of relatively high amounts of pyrrolizidine alkaloids (PAs) in herbal infusions and teas not prepared from well-known ‘PA plants’ (Bodi et al., 2014). Based on the determined amounts, the German Federal Institute for Risk Assessment (BfR) concluded a risk for consumers, particularly children, who frequently drink large quantities of such teas/herbal infusions (BfR, 2013). Based on the outcome of risk assessments, the fact that teas/herbal infusions of several different types can contain substantial amounts of PAs has to be considered as a relevant food safety issue. Likewise, analyses in medicinal teas have revealed comparable PA levels in those (Schulz et al., 2015).

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PA producing plants grow widespread in nature and PA contaminations in herbal preparations have been reported. Cases of severe intoxication, including fatal cases, have been reported for developing countries due to the consumption of highly contaminated cereals (Tandon et al., 1976; Mohabbat et al., 1976). In Europe such intoxications are unlikely but chronic toxicity due to long-term consumption of food or herbal medicines containing trace contaminations with PAs has to be taken into account. Honey that (partially) originated from PA producing plants was estimated to be one of the main sources of PA exposure (Mulder et al., 2015), but the above mentioned findings also revealed unexpectedly high PA contents in teas/herbal infusions from the retail market. These findings were not only restricted to herbal infusions, but also more frequently consumed teas such as black and green teas exhibited relatively high PA concentrations. Obtained data revealed that numerous individual PAs of different structures were detected in teas and herbal infusions, while for instance open-chain PAs were mainly found in fennel infusion and rather cyclic PAs were detected in black tea (Bodi et al., 2014). As very few data on relative toxicity of PAs are available the sum-amounts of individual PAs are used for risk assessment and it is questionable if this procedure is adequate.

1. General aspects of PA toxicology

PAs are secondary metabolites which are exclusively biosynthesized by certain species belonging to plant families such as *Asteraceae, Boraginaceae* or *Leguminosae.* Up to now several hundreds of PAs have been identified (Hartmann and Witte, 1995; Wiedenfeld et al., 2008), whereas the exact number of naturally occurring PAs is not known. The chemical structures of PAs can be differentiated according to the type of the necine bases and their esterification mode leading to the formation of monoesters, open-chain or cyclic di-esters (Fig. 1).

The capability of PAs to cause acute toxic effects in animals has already been observed in the 19th century in draft animals that often had to graze on waysides without the possibility of free foraging. Toxic effects in animals are characterized by slow emaciation and weakness (Williams and Molyneux, 1987; Stegelmeier et al., 1999; Woolford et al., 2014). *Post mortem* examination revealed hepatocellular necrosis among others. These toxic effects were attributed to the ingestion of PA producing plants of genera of *Heliotropium*,*Senecio* or *Crotalaria.* Intoxication in humans was also observed, e.g. in Uzbekistan, Pakistan, Afghanistan or India where up to 6000 people fell victim to intoxications after consumption of weed contaminated with PA-containing seeds of *Heliotropium spp*. or *Crotalaria* (Stewart and Steenkamp, 2001).

Chronic treatment of rodents with PAs caused liver cell carcinoma and haemangio-endothelial sarcoma and in some instances, tumors in extra-hepatic tissues (lung, pancreas, intestine).

PAs themselves are pro-toxins as they are biologically and toxicologically inactive and require metabolic activation to exert toxic effects. Bioactivation occurs primarily in the liver by cytochrome P450 (CYP) monooxygenases, in particular CYP3A and, to a minor extent, CYP2B isoforms (Prakash et al., 1999; Reed et al., 1992; Huan et al., 1998; Fu et al., 2004; Chung et al., 1995; Lin et al., 2003). This metabolically mediated toxification process includes an oxidation step to yield the corresponding dehydropyrrolizidine (pyrrolic ester) derivatives (Jago et al., 1970; Mattocks, 1971). These pyrrolic alkaloids possess an allylic structure which promotes an increase in their reactivity. Formed pyrroles can rapidly bind to nucleophilic centers in DNA, proteins, amino acids etc. (Fu et al., 2004; Chen et al., 2010). It has been shown (Kedzierski and Buhler, 1985) that liver microsomal metabolism of retronecine-type and heliotridine-type PAs all resulted in the formation of the racemic DHP [(+/−)-6,7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine], rather than the optically active 7R-enantiomer DHR (dehydroretronecine) or the 7S-enantiomer DHH (dehydroheliotridine). Due to their high reactivity, pyrrolic ester metabolites can also react readily with water or glutathione, resulting in the formation of additional products (Fig. 2).

Alternative metabolic reactions may also occur: Ester bonds of PAs may be saponified by hydrolases in the intestinal tract towards the corresponding necic acid and necine base. Secondly, the nitrogen of the pyrrolizidine ring can be oxidized. Any of those formed metabolites are highly soluble in water, relatively stable, and excreted quickly *via* the urine. Therefore, these metabolic pathways are considered as mechanisms of detoxification (Fig. 2).

Obviously, all types of PAs require a metabolic activation in order to exert toxicity and although the metabolic pathway described in the literature seems to be essentially the same for all PAs regardless of their structure, the results of conducted toxicity studies revealed a marked variation in the toxicity of different PAs, with the cyclic di-esters apparently having a higher potency in comparison to open-chain di-esters or monoesters.

2. Regulations and previous risk assessment(s)

The International Agency for Research on Cancer (IARC) concluded that there was, depending on the PA congener, limited or sufficient evidence for the carcinogenicity of several PAs in experimental animals. Consequently, some PAs, namely monocrotaline, retrorsine and lasiocarpine, were classified as “possibly carcinogenic to humans” (IARC, 2002; NTP, 2008). Later on, experiments revealed that the metabolic pattern and DNA adduct profiles produced by certain PAs when incubated with DNA and human liver microsomes were similar to those formed in rat liver *in vitro* and *in vivo.* The authors concluded from these finding that the results of studies with experimental rodents are relevant to humans (Yan et al., 2008).

In Austria only a few PA-containing plants are authorized for herbal remedies. These plants or preparations thereof can only be marketed if they are analyzed by a state of the art detection

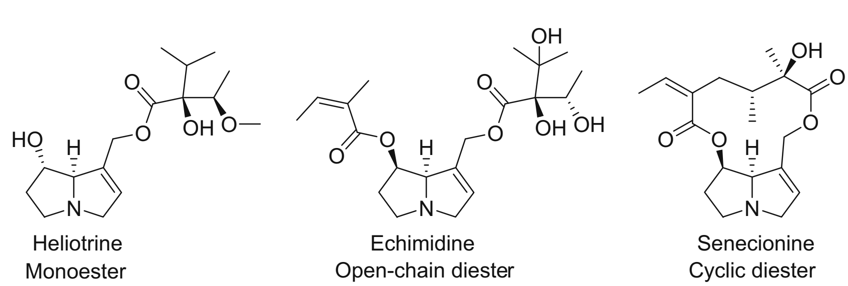

Fig. 1. PA categories – according to the type of necine bases and their type of esterification.