Pyrrolizidine Alkaloids in Bee Pollen

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Pyrrolizidine Alkaloids (PAs) are produced by plants as secondary plant metabolites for protection against herbivores. It has been estimated that more than 6000 plant species contain PAs, many of them contributing to the production of honey and bee pollen.

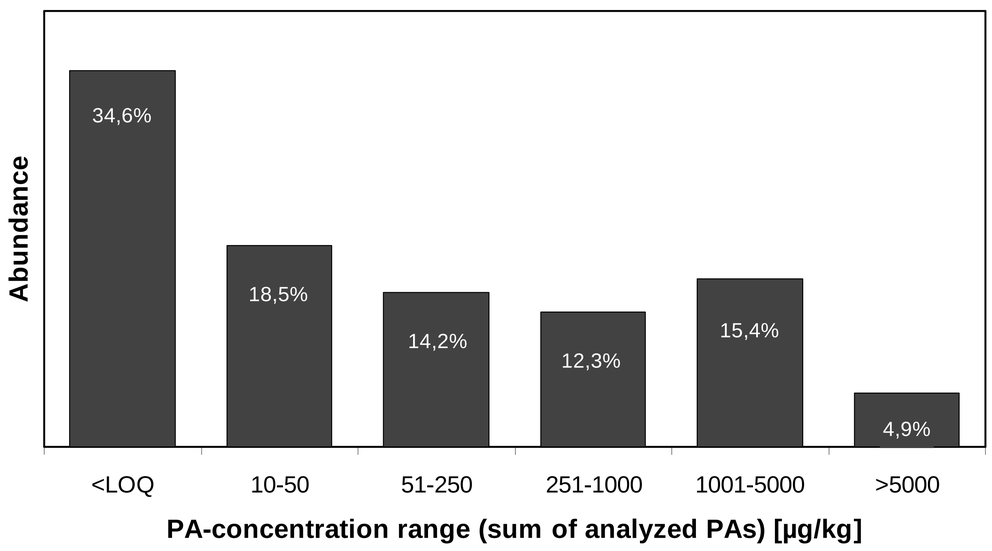
In August 2011, the Federal Institute for Risk Assessment (BfR) in Germany remcommended a maximum daily uptake of PAs of 0.007 µg/kg body weight (Statement No. 038/2011) in order to prevent an increase in cancer risk. Thus, it is worthwhile to have a look on the PA-concentrations in commercial bee pollen.

In this study, 162 bee pollen samples were analyzed. The results show strong variations between <LOQ (10 µg/kg) and up to several mg/kg of PAs (average PA-concentration in honey is below 0.06 mg/kg). Lycopsamine and isomers thereof as well as echimidine were the most common PAs.
The reason for the strong variation of the PA-concentrations are the different botanical origins of bee pollen. E.g. some bee pollen samples contain high amounts of *Echium* bee pollen grains. As *Echium* is a well known PA plant, bee pollen mixtures containing *Echium* pollen are likely to contain high amounts of PAs, especially echimidine, which can be regarded as marker PA for the genus *Echium*.

Many people eat bee pollen grains on a daily basis as food supplement. The maximum measured PA-concentration was nearly 38 mg/kg (Dübecke et al. 2011). The consumption of only 5 g of those pollen would lead to and 450-fold exeedance of the recommended value of 0.007 µg/kg bodyweight and day.

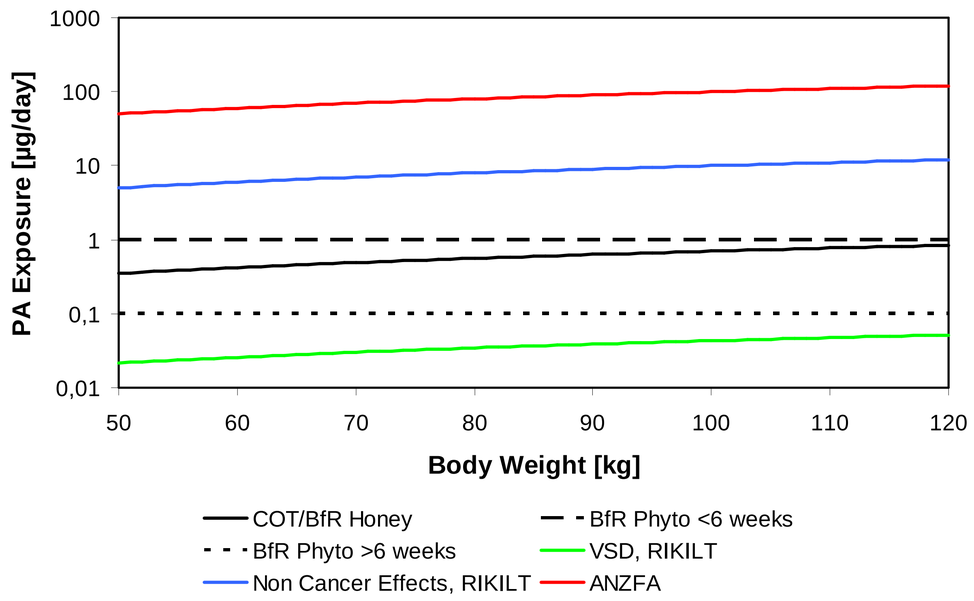
Those pollen mixtures containing high numbers of bee pollen grains from PA-plants are likely to lead to an uptake of PAs, that is higher than the limits recommended by the German Federal Institute for Risk Assessment (and also other institutions) and thus may enhance the risk to develop cancer.

PA-Concentration distribution in pollen samples

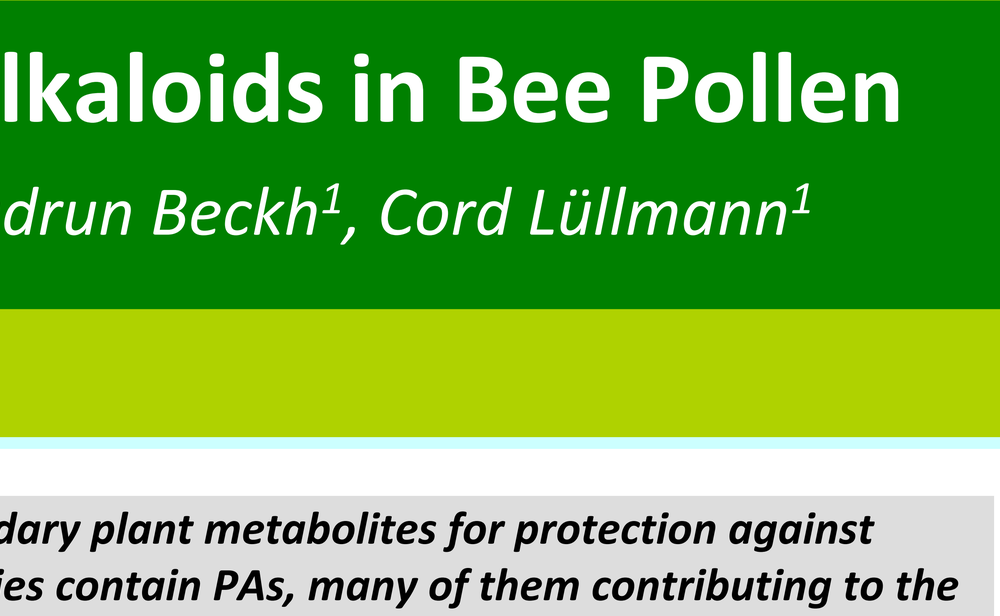


PA-concentration distribution in the analyzed data set of bee pollen samples. <LOQ = below Limit of Quantitation, which is 10 µg/kg for the used method. In about one third of the samples, PA-concentrations were below LOQ. ~45% of the samples were below 1 mg/kg and only 5 % of the bee pollen samples contained very high amounts of PAs of more than 1 mg/kg. The highest measured concentration was almost 38 mg/kg.

Comparison of (suggested) PA-Limits in different countries

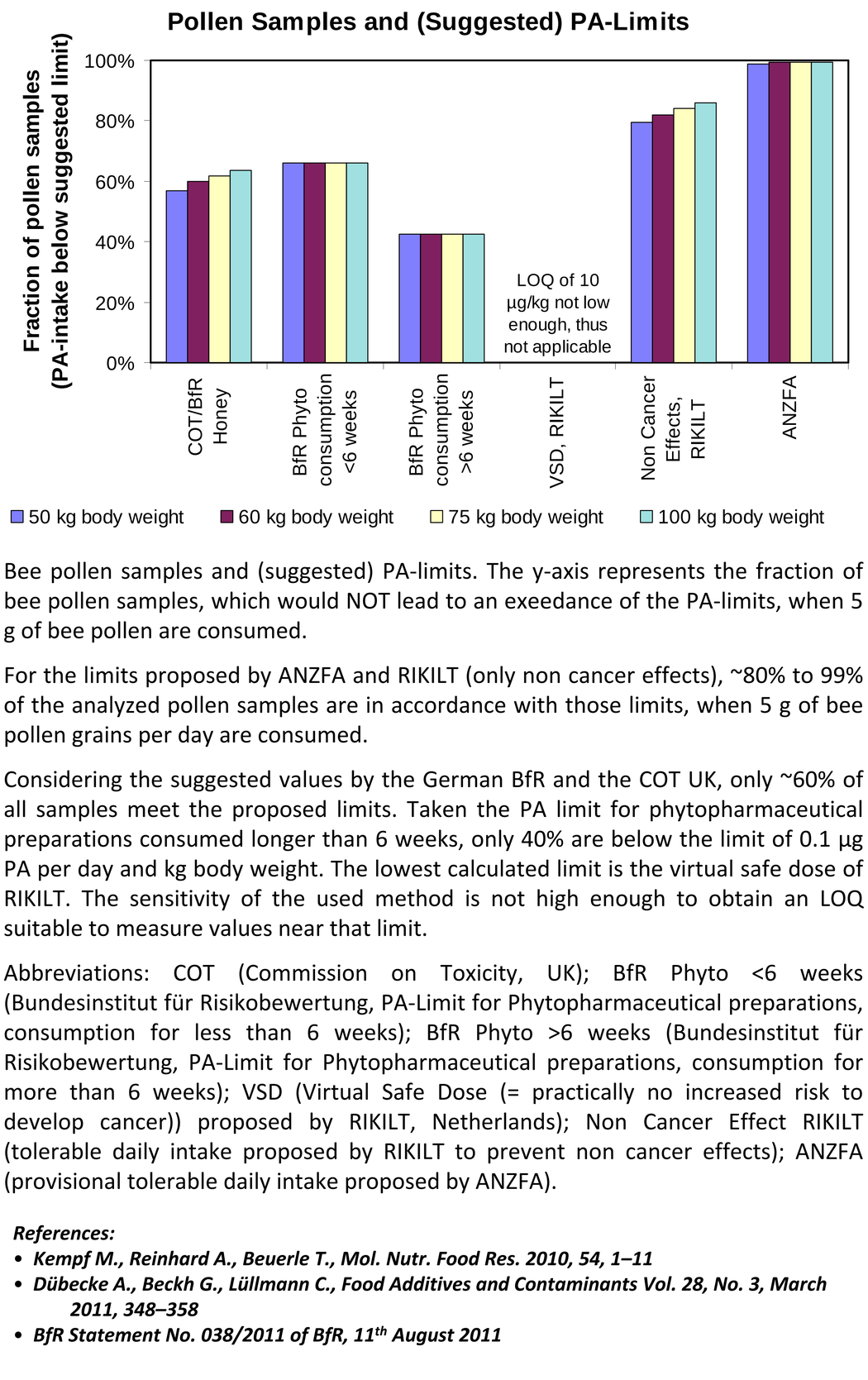


Comparison of suggested PA-limits in different countries. Note the logarithmic scale on the y-axis. The German Federal Institute for Risk Assessment (BfR) set limits for PAs in phytopharmaceutical preparations in 1992 (1 µg PA per day, when consumed for at most 6 weeks or 0.1 µg PA per day when consumed for a longer period). In 2001, ANZFA (Australia New Zealand Food Authority) suggested a provisional tolerable daily intake of 1 µg PA/kg bodyweight and day. In 2007, the RIKILT (Netherlands) proposed a virtual safe dose of 0,00043 µg/kg body weight and day (Kempf et al. 2010). Below that value, practically no increased cancer risk is expected. In the same study, a limit for non cancer effects of was proposed as well (tolerable daily intake of 0.1 µg PA/kg bodyweight and day). The commission on toxicity (UK) proposed 0,007 µg PA/kg body weight and day in 2008, which was adopted by the German BfR in its statement on PAs in Honey in August 2011.



Extraction of PAs from bee pollen grains was done according to the depicted scheme. First, the pollen grains were shredded in a blender. Sulfuric acid (0.05 M) was added and the samples were put on a shaker for 1 hour. After 10 minutes of centrifugation, the samples were filtered over night. The filtrate was applied onto Varian SCX 500 mg/3 ml cartridges (strong cation exchanger). Elution was done by using ammoniated methanol. The samples were evaporated to dryness under a gentle flow of air and finally reconstituted in water. Analysis was done by LC-MS/MS (API 4000 QTRAP, Shimadzu HPLC, Thermo Hypersil Gold 2.1 x 50 mm, 1.9 µm particles). The MS was operated in positive MRM-mode (ESI) and 1 quantifier and 2 qualifiers were measured. The LOQ of this method was 10 µg/kg.

Pollen Samples and (Suggested) PA-Limits



Bee pollen samples and (suggested) PA-limits. The y-axis represents the fraction of bee pollen samples, which would NOT lead to an exeedance of the PA-limits, when 5 g of bee pollen are consumed.

For the limits proposed by ANZFA and RIKILT (only non cancer effects), ~80% to 99% of the analyzed pollen samples are in accordance with those limits, when 5 g of bee pollen grains per day are consumed.

Considering the suggested values by the German BfR and the COT UK, only ~60% of all samples meet the proposed limits. Taken the PA limit for phytopharmaceutical preparations consumed longer than 6 weeks, only 40% are below the limit of 0.1 µg PA per day and kg body weight. The lowest calculated limit is the virtual safe dose of RIKILT. The sensitivity of the used method is not high enough to obtain an LOQ suitable to measure values near that limit.

Abbreviations: COT (Commission on Toxicity, UK); BfR Phyto <6 weeks (Bundesinstitut für Risikobewertung, PA-Limit for Phytopharmaceutical preparations, consumption for less than 6 weeks); BfR Phyto >6 weeks (Bundesinstitut für Risikobewertung, PA-Limit for Phytopharmaceutical preparations, consumption for more than 6 weeks); VSD (Virtual Safe Dose (= practically no increased risk to develop cancer)) proposed by RIKILT, Netherlands); Non Cancer Effect RIKILT (tolerable daily intake proposed by RIKILT to prevent non cancer effects); ANZFA (provisional tolerable daily intake proposed by ANZFA).

References:

- ***Kempf M., Reinhard A., Beuerle T., Mol. Nutr. Food Res. 2010, 54, 1–11***
- ***Dübecke A., Beckh G., Lüllmann C., Food Additives and Contaminants Vol. 28, No. 3, March 2011, 348–358***
- ***BfR Statement No. 038/2011 of BfR, 11th August 2011***