Scientific opinion on pirolizidine alkaloides in tea infusions on the Croatian market

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Andrea Gross-Bošković

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Background

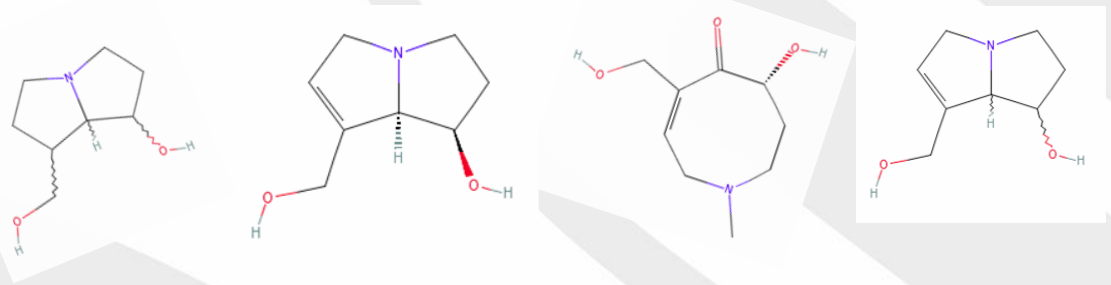
- In the Republic of Croatia (RH), monitoring of PA is not carried out thil las year and even now there is no laboratory that has the capability to implement analytical testing
- The Ministry of Health has supported the implementation of the research conducted in accordance with the recommendation of a risk assessment by the European Food Safety Authority (EFSA; EFSA, 2011)
- EFSA has twice issued a scientific opinion on the PA. For the first time in 2007 a risk assessment of the PA's in the feed, and in 2011 the assessment was expanded on food for human consumption. Due to insufficient data, it was restrict on honey and honey products
- Insufficient data are one of the main problems when it comes to the PA because of limited number of studies and available dana are insufficient for adequate and comprehensive assessment
- In addition to those researches, the German Federal Institute for Risk Assessment (BfR) also conducted research on PA by focusing it on plant products, (BfR, 2013)
- At the European Union (EU) level there is no legislation that regulates the PA nor establishes the maximum permissible concentration so those researches are aimed in gathering more data to which could finaly provide some limits

Aim

- To set a ground for any future researches and possible adoption of national legislation which would regulate this issue,
- To open opportunities for training of FBO's and education of consumers themselves.
- Analytical data obtained in this study will be submitted to the EFSA database and thus contribute to a common database on the PA at the EU level

Sources of exposure

- PA's are probably the most common types of natural toxins that affects wild and domestic animals and humans.
- over 6,000 plant species contains PA's
- though, direct poisoning of people and animals are associated with only a few species
- PA's are exclusively of plant origin (any food or feed, containing (parts of) plants with PA's, itself will also include the PA's)
- consumption of honey that were fed the toxic plant species, also considered possible route of exposure (but observed low levels and there were no recorded cases of intoxication)
- deliberate use of toxic plants as herbal tea or in traditional medicine. - another possible route of exposure and intoxication cases (well documented and known to have had a fatal outcome) (EFSA 2007; 2011; BfR, 2013).



Botanics

- known over 600 different PA's, and this number is growing steadily.
- also known to be present in over 6000 different plant species
- main source of family Boraginaceae (all species), Asteraceae (Asteraceae, of which Senecioneae and Eupatoriae) and Fabaceae (legumes, of which Crotalaria)
- some plant species may contain several different PA's, as well as some PA's are typical of a number of plant species
- toxins are usually concentrated in the seeds and flower parts of plants, while smaller amounts are possible in the leaves, stem and root




Methodology

- The analytical method for the determination of PAHs in tea was developed and validated "in - house" at the Federal Institute for Risk Assessment (BfR) as part of a research project (BfR, 2014)
- Method for determination of PA's is based on double treatment of plant material sample with ultrasound in an aqueous sulfuric acid solution to extract the PA's. After purification and extraction PA's are liberated from the cartridge by means of methanol. Eluate was evaporated to dryness and reconstituted in methanol / water (initial HPLC conditions).
- For chromatographic separation was used RP-HPLC column with binary gradient. The analytes are determined by a triple quadrupole mass spectrometry and quantification of PA's is achieved by corresponding calibration matrix
- Analysis was obtained at Quality Services International GmbH's (QSI) laboratory in Bremen
- 55 samples were analyzed (briar, green tea, black tea, chamomile, mint, cranberry, forest fruits, apricot)
- Tea samples are purchased in large retail chains, and selection of the type and amount was based on consumption habits data of the adult population of Croatia (HAH)

Exposure assessment

- based on the concentrations of PA's in all types of teas that were examined and data on consumption habits in Croatia
- all figures are based on the dry substance, not the finished beverage
- as the data on dietary habits, ie. consumption of tea, included information about the finished tea [ml], the same was to be converted to a dry matter basis using a special formula and certain assumptions, based on BfR's risk assessment (BfR, 2013)
- it was assumed that a filter bag tea contains 2 g of dried leaves, or that it takes 2 g of dried leaves for the preparation of 200 ml of the drink
- it was also assumed that 100% of these PA-migrates from the leaves into the liquid
- it is **important to note** that **there are no scientific studies that support the fact that indeed 100% PA's migrate into the liquid medium (hot water),** and this assumption entails a certain level of uncertainties

Exposure assessment (cont.)

- exposure assessment was calculated for the adult population 18 to 64 years due to results of consuption habits study on national level
- consumption refers to teas in general, and not to any other particular, because of the small number of dana recorded by consumers
- for the calculation of exposure to concentrations of PA's kg body mass of consumers were used related to the sum of all 30 investigated individual PA's.
- four different scenarios for each type of tea are made related to the method of calculating

Metoda izračuna	Number of samples	Min	Mean value	Median	P95	P99	Max
1 scenario	55	0	414,2	0	260,3	9 664	20 633
2. scenario	55	165,7	576,5	165,7	410,6	9 813,3	20 774
3. scenario	55	265	673,5	265	501,1	9 902,5	20 858
4. scenario	55	530	932,8	530	730	10 141	21 083

	Consumers of population	Long term consumption (all consumers) [g/kg t.m./dan]		Short term consumption (tea consumers only) [g/kg t.m./dan]
		Srednja vrijednost	P95	P95
Tea total	18%	0,0061	0,036	0,11

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Exposure assessment

✓ To estimate long-term exposure, were used 6 different scenarios:

Scenariji konzumacije	Metoda izračuna*	Izloženost PA-ovima [µg/kg t.m./dan]
1) scenarij	1. scenarij	0
	2. scenarij	0,0010
	3. scenarij	0,0016
	4. scenarij	0,0032
2) scenarij	1. scenarij	0
	2. scenarij	0,0060
	3. scenarij	0,0095
	4. scenarij	0,0191
3) scenarij	1. scenarij	0,0016
	2. scenarij	0,0025
	3. scenarij	0,0031
	4. scenarij	0,0045
4) scenarij	1. scenarij	0,0590
	2. scenarij	0,0599
	3. scenarij	0,0604
	4. scenarij	0,0619
5) scenarij	1. scenarij	0,0094
	2. scenarij	0,0148
	3. scenarij	0,0180
	4. scenarij	0,0263
6) scenarij	1. scenarij	0,3479
	2. scenarij	0,3533
	3. scenarij	0,3565
	4. scenarij	0,3651

✓To estimate short-term exposure, were used 3 different scenarios:

Scenariji konzumacije	Metoda izračuna*	Izloženost PA-ovima [µg/kg t.m./dan]
1) scenarij	1. scenarij	0
	2. scenarij	0,0182
	3. scenarij	0,0292
	4. scenarij	0,0583
2) scenarij	1. scenarij	0,0286
	2. scenarij	0,0452
	3. scenarij	0,0551
	4. scenarij	0,0803
3) scenarij	1. scenarij	1,0630
	2. scenarij	1,0795
	3. scenarij	1,0893
	4. scenarij	1,1155

Conclusions

- for substances that show carcinogenic effects MOE approach is commonly used for isk assessment
- EU agreed that the values obtained by MOE's which are over 10 000, indicate a low concern (risk)
- in order to achieve a value of at least 10 000, consumer should not consume more than 0,007 mg PA / kg PP values, because all above that could increase the risk of cancer in terms of effects
- using the MOE approach, in this way is calculated and PDD value
- all scenarios indicates low or moderate concern
- as was expected, the worst case scenario [6] that represents the highest consumption (P95) and extremely high concentration of PA's (P99), has the lowest MOE values.
- However, it should be noted that this scenario (and the like), where for a person that on a daily basis, over a longer period of time consumes in high quantities of tea that contains high and extremely high concentrations of PA's, which is unlikely to happen in reality

Conclusions

- In general, various uncertainties have to be taken into account when assessing input PA's.
- First, in this assessment, there are no differences between the consumption of different types of tea which can lead to overestimation or underestimation of the consumption of certain types of tea.
- On the other hand, it can be assumed that there are no major differences in the amount of tea consumed, especially in the case of frequent consumers.
- Second, although an attempt to sample a representative sample, can not be known with certainty whether the measured concentrations illustrate the real picture of the distribution of PA's in teas on the Croatian market, especially given the relatively small number of samples.
- The content of 1,2-unsaturated PA's measured in teas from different series is significantly different both qualitatively as well as quantitatively.
- One possible reason is that the plants, some of which are true teas, grow together with a variety of wild plant species containing PA's whose parts contaminate the others

Recommendations and next steps

- additional commitment to reduce the content of PA's in teas like to reducing the supposed greater risk of cancer among frequent consumers are required
- based on the review of BfR's risk assessment, it can be concluded that children are particularly vulnerable population, but since Republic of Croatia is still do not have dietary habits for children, the same will be done in the future.
- in addition, a risk is not only of teas, but also from the other food products, such as eggs, honey (often consumed in combination with the tea) and other
- Therefore, important to point out to FBO’s the harmful effects of PA's which so far have not been systematically examined.
- a decrease of PA's in food products can be obtained by analytical testing of botanical materials (it is well known in which plant species may be the highest concentration), improved methods of growing, harvesting, purification and separation procedures.
- as this was the first study of PA's in Croatia, it is necessary to continue to monitor levels of PA’s due to findings of initial study because of possible extremely high levels of PA's which is not possible to ignore as a risk to human health