14 November 2013
EMA/HMPC/280542/2013
Committee on Herbal Medicinal Products (HMPC)

Overview of comments received on the draft Public statement on the use of herbal medicinal products containing toxic unsaturated pyrrolizidine alkaloids (PAs) (EMA/HMPC/893108/2011)

Table 1: Organisations and/or individuals that commented on the first draft Public statement on the use of herbal medicinal products containing toxic unsaturated pyrrolizidine alkaloids (PAs) as released for public consultation on 25 October 2012 until 15 February 2013

	Organisations and/or individuals
1	AESGP (The Association of the European Self-Medication Industry)
2	BPI (Association of the German Pharmaceutical Industry)
3	ECHAMP (European Coalition on Homeopathic and Anthroposophic Medicinal Products)
4	ESCOP (European Scientific Cooperative on Phytotherapy)
5	Kooperation Phytopharmaka
6	NIMH (National Institute of Medical Herbalists)

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Table 2: Discussion of comments

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Interested party	**Comment and Rationale**	**Outcome**
AESGP	AESGP represents the manufacturers of non-prescription medicines of either chemical or herbal origin at European level. It counts 29 national associations and 25 associate members. Through its national and associate members, it represents many small and medium-sized companies operating in the self-care sector. AESGP agrees that the intake of pyrrolizidine alkaloids should be minimised due to their hepatotoxic and cancerogenic characteristics and welcomes the development of uniform European rules for the use of herbal medicinal products containing toxic 1,2-unsaturated pyrrolizidine alkaloids. However, for the reasons given below, prohibition of PA-containing drugs would be inadequate and we propose to apply internationally accepted guidelines which explain how limits of toxic impurities can be defined. By now, the regulatory status of PA-containing medicinal products differs Europe-wide. Taking into account a risk-benefit analysis, we welcome the German health authority's decision in the "Graduated Plan" (so-called "Stufenplan"), which allows the use of PA-containing herbal medicinal products under strict restrictions. **1. Introduction** The HMPC draft public statement concludes that herbal medicinal products containing herbal preparations with toxic unsaturated PAs (even in very low amounts) should not be used orally. An explanation is given by a presumed rather high exposition to PAs through the diet, especially by the intake of honey but also by other sources of PA-containing food (e.g. milk and other products which may contain PA traces). Consequently, according to the HMPC, the actual exposure cannot be assessed and the additional intake of PAs by medicinal products should be avoided completely [1]. In our opinion such an assessment is disproportionate. It neglects the fact that medicinal products in contrast to food products are taken in much lower amounts and in most cases for a restricted period of time. From our point of view, the HMPC's conclusion unfairly discriminates medicinal products against food products. Compared to the undefined and poorly controlled exposure to PAs by food, the impact through herbal medicinal products is of very low toxicological concern. Efforts should be concentrated on reducing PA contamination in food, as this is a source of uncontrolled and potentially very high intake. In this context, we welcome the work of the Codex Committee on Contaminants in Food (CCCF), a committee of the Codex Alimentarius	Generally, in the risk assessment of genotoxic carcinogens, the TD_{50} value (a measure of cancer potency) from the most sensitive species/tumour site is considered an appropriate point of reference for a linear down extrapolation to a "virtually safe dose", i.e. a dose corresponding to a theoretical excess cancer risk of either <1 in 100,000 (10^5) or <1 in 1,000,000 (10^6) over a lifetime of exposure. Linear extrapolation to a probability of 1 in 10^5 or 1 in 10^6 is achieved by simply dividing the TD_{50} by 50,000 or 500,000. A higher value of 1 in 10^6 could be used if risk-benefit considerations are regarded to justify such an adoption. Such a higher value situation would be adopted for (traditional) herbal medicinal products mainly because of the background-intake of PAs via food.

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	Commission, in establishing a code of practice for the prevention and reduction of contamination of food with PAs [2]. PAs are natural constituents of a high number of plant families. Banning all herbal medicinal products manufactured from plant species that may contain PAs (even in traces) would risk depriving patients of effective herbal drugs which are approved according to current scientific knowledge and for which positive monographs have been established. We will hereafter show that it is possible to define scientifically acceptable limits of daily intake of PAs by herbal medicinal products.	It has to be stressed that some of these alkaloids have been shown to be animal carcinogens and both IARC and EPA regard them possible carcinogenic to humans. Furthermore, mechanisms of actions related to carcinogenicity (metabolic activation, DNA binding, specific targets etc) operate similarly in experimental animals and humans.
	2. Accepted limits of PAs in medicinal products In Germany, herbal medicinal products prepared from PA-containing plants have been authorised by the German health authority and are subject to pharmacovigilance measures. Since 1992, the PA-content of herbal medicinal products has been regulated by a graduated plan. According to this, 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 [3]. The Swiss authority adopted the values for oral and parenteral use and describes these values in the actual law in the so-called KPAV (Komplementär- und Phytoarzneimittel-Verordnung) in the Annex which is a substance list [4]. The contraindication pregnancy and breast-feeding women is also mandatory on the labelling. Concerning the external application there are no limits set but a risk assessment is mandatory. This assessment has not to be done for homeopathics with an external application form over D4. For oral use in homeopathy PAs are not limited in dilutions over D6 and parenteral application including eye drops over D8. For special preparations or combinations a risk assessment has to be done. It is important to elucidate if the combination contains are other plants which may act lever protective. The limits set by the German graduated plan in 1992 and adopted of the Swiss can be regarded as safe and established. They are in the range of the value established by use of the Margin of Exposure (MOE) approach for food (see	The limits described in the German graduated plan are based on older studies which were available in that time. If such limits are safe cannot be concluded from usage because a higher cancer incidence will not be seen from the clinical usage. Studies would be needed to trace down such a risk or to exclude it. More recent studies with single PA exist. The $BMDL_{10}$ value 70 µg/kg/day - based on induction of liver haemangiosarcomas by lasiocarpine in male rats (EFSA 2011) - could be used instead of the TD_{50} value. For the calculation of a limit value for acceptable exposure via herbal preparations, this value is the lowest (i.e. most conservative) available, because lasiocarpine is one of the most potent pyrrolizidine alkaloids (e.g. the $BMDL_{10}$ value of riddelliine is 180 µg/kg/day). Thus the value for lasiocarpine covers less potent and inactive (e.g. saturated compounds) components in herbal preparations or even the combination of several PAs. To derivate a dose to cause tumours in 1 in 1,000,000 animals, divide by 100,000: 70 µg/kg/day ÷ 100,000 = 0.0007 µg/kg/day Generally for adults the calculation is done with an body weight of 50 kg. Therefore the daily dosage would be: 0.0007 µg/kg/day x 50 kg body weight =

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	2.1.), differing for short-term and long-term use. During the past 20 years after the establishment of this graduated plan, no basically new scientific knowledge concerning PAs has been published and no new information or signals relevant for assessment are available from pharmacovigilance that would necessitate further action. Furthermore, herbal medicinal products prepared from PA-containing plants which are on the German or Swiss marked have been authorized and are subject to pharmacovigilance measures, thereby their health risk is continuously controlled. For this reason, the limits set in the German graduated plan in 1992 are sufficient to guarantee the safety of PA-containing medicinal products. 2.1 Margin of Exposure approach '*The Margin of exposure (MOE) is the ratio between a defined point on the dose-response curve for the adverse effect (usually based on animal experiments in the absence of human data) and the human intake*' [5 p10]. For food products, the European Food Safety Authority (EFSA) Scientific Committee recommends the MOE approach for risk assessment of substances with both genotoxic and carcinogenic properties and states that '*in general a MOE of 10,000 or higher, if it is based on the $BMDL_{10}$* (the lower confidence limit on the benchmark dose associated with a 10% response) *from an animal study (and taking into account overall uncertainties in the interpretation) would be of low concern from a public health point of view and might be reasonably considered as a low priority for risk management actions*' [5 p 19]. As pyrrolizidine alkaloids are both genotoxic and carcinogenic, the MOE approach is well suitable for their risk assessment. According to this, the EFSA Panel on Contaminants in the Food Chain (CONTAM) applies the MOE approach to evaluate the health risk emanating from pyrrolizidine alkaloids in food and feed. And also the German Federal Institute for Risk Assessment (BfR), which previously endorsed the 'zero-tolerance principle' for the application or addition of genotoxic plant ingredients such as pyrrolizidine alkaloids in isolated form as well as for impurities in food, uses this approach in the field of PA contamination in food and feed [6-8]. In their respective Scientific Opinion, both conclude that, based on a margin of 10,000 and a $BMDL_{10}$ of 70 µg/kg b.w. per day for induction of liver haemangiosarcomas by lasiocarpine in male rats, a **daily intake of**	**0.035 µg/person/day** Hence, a daily life-long oral intake of 0.035 µg of lasiocarpine would correspond to a theoretical cancer risk of 10^{-6} and can be accepted as a virtually safe dose for adults. **Sensitive groups:** **Children** If children are included in the usage of certain products the daily amount of PA has to be adjusted to the body weight of the age group: e.g. body weight of 20 kg would lead to an acceptable daily intake of 0.014 µg PA/day. **Pregnant and nursing woman** Sensitive groups such as pregnant and breast feeding woman are also covered by the limit calculated above. If these limits are complied with, the chapter 4.6 of the SmPC of the products concerned should be phrased according to the 'Guideline on risk assessment of medicinal products on human reproduction and lactation: from data to labelling' (EMEA/CHMP/203927/2005).

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	0.007 µg/kg b.w. can be regarded as safe. For an adult of 60 kg b.w. a **daily intake of 0.42 µg** is reasonable [6,8]. Application of this $BMDL_{10}$ takes various safety factors in concern, as it is based on lasiocarpine, a pyrrolizidine alkaloid of high toxicity, and as it is the lower of two $BMDL_{10}$ which were determined by the National Toxicology Program (NTP) [6]. In order to investigate chronic toxicity of PAs, the NTP conducted the following comprehensive animal studies using riddelliine and lasiocarpine as representative PAs: A 2-year gavage study of riddelliine with 50 rats in each group [9], based on a subchronic toxicological study design from 1993 [10] resulted in a No-Observed-Adverse-Effect-Level (NOAEL) of 10 µg/kg b.w./day for non-neoplastic lesions (hepatocyte cytomegaly), whereas haemangiosarcoma were even not observed at 100 µg/kg b.w./day in female rats. Applying an uncertainty factor of 100 indicated that non-neoplastic-lesions would not be expected for doses up to 0.1 µg/kg b.w./day [11]. In another carcinogenicity study the effect of lasiocarpine was investigated on 24 rats in each group [12]. From this study a Benchmark Dose Lower Confidence Limit 10% ($BMDL_{10}$) of 73 µg/kg b.w./day has been calculated [11]. Based on a MOE of 10,000, corresponding doses of up to **0.007 µg/kg b.w./day** are practically not associated with any cancerogenic concern [11,6]. In spite of the HMPC guideline on the Assessment of Genotoxicity of Herbal Substances/Preparation [13], in which herbal medicinal products are excluded from the field of application of the MOE approach as carcinogenicity data are often missing for these products, the MOE approach has already been considered for some constituents of herbal medicinal products. For example the EMA uses this approach to evaluate the health risk of furocoumarins in *Angelica archangelica L.* [14]. Since a broad risk assessment on pyrrolizidine alkaloids in food and feed was presented by CONTAM [6], the MOE approach should be applicable for PA containing herbal medicinal products. According to this MOE approach **for adults a daily intake of maximal 0.42 µg** PAs can be regarded as safe. This supports the limits set in the German graduated plan, where 0.1 µg is the limit for internal use, and underlines that this limit is very conservative. With respect to the argument of the HMPC that '*familial susceptibility to PAs toxicity can also be expected*' [1 line 576] we wish to clarify that such	

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	uncertainties are already included in the applied safety factors. For instance, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) at the UK Food Standards Agency stated that possible interspecies variations are considered with a factor of 10 in the uncertainty factor of 100 which is applied on the assessed NOAEL [11]. Taking all together, we recommend applying the Margin of Exposure approach for pyrrolizidine alkaloids in herbal medicinal products. The calculations performed for contamination of food with pyrrolizidine alkaloids show that **daily doses of maximal 0.007 µg PAs/kg b.w**. are acceptable. The need to apply the 'zero-tolerance principle' on herbal medicinal products cannot be justified. Instead, reasonable, practically achievable limit values should be established. 2.2 TTC concept The limit for oral PA intake defined by the German graduated plan 1992 is also justifiable by the Threshold of Toxicological Concern (TTC) concept. According to the TTC concept, a **daily intake of less than 1.5 µg** of a compound of toxicological concern is regarded as associated with an acceptable health risk and no further testing or regulatory measures are necessary at this level. In general, the regulatory threshold value of 1.5 µg/day is applicable to all types of chemical compounds. For chemicals with a structural alert for genotoxicity a reduced threshold of 0.15 µg/day is suggested. However, a prerequisite for the application is a comprehensive knowledge of the chemical structure [15]. For compounds with specific structural alerts (i.e. aflatoxin-, azoxy-, N-nitroso-, dibenzodioxin- and dibenzofuran-like structures), which were identified as being of such high potency that intakes even below the TTC limits would be associated with a high probability of a significant carcinogenic risk, the TTC concept cannot be considered [16]. Pyrrolizidine alkaloids do not belong to these excluded substances but are known chemical structures that allow accurate analysis and application of the TTC concept. In general, the EMA Guideline on Genotoxic Impurities defines a threshold value of 1.5 µg/day for genotoxic impurities in pharmaceutical products [16]. This limit is in line with the specific guideline for herbal medicinal products which states that '*if a herbal preparation contains an identifiable genotoxic compound, the TTC approach could be applied*' [13 p 6].	**TTC** and other calculation models are not acceptable because PAs have to be considered as mutagenic compounds with positive carcinogenicity data. Therefore the TTC is not applicable. Although pyrrolizidine alkaloids do not officially belong to the mentioned high-potency class of compounds, their clear carcinogenicity at relatively low exposures indicate that they resemble the high-potency group.

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	This statement supports our opinion that a PA limit value for herbal medicinal products can be based on the TTC concept. It has to be emphasised that the threshold limits of the TTC concept derive from a very conservative model. Various elements of uncertainty and data for the most sensitive species and most sensitive site were used for their calculation. The TTC is a pragmatic risk management tool using a probabilistic methodology, i.e. there is a high probability that a 10^{-5} lifetime cancer risk (1 in 100,000) will not be exceeded if the daily intake of a genotoxic impurity with unknown carcinogenic potency is below the TTC value. Thus we wish to point out that even though the TTC concept cannot be interpreted as providing absolute certainty of no risk, it underlines that exposure to PAs as stipulated by the German graduated plan is associated with an acceptable risk as the intake is less than 1.5 µg/day [16].	It must be ensured that total PA concentration (free base and N-oxides) are measured.
	3. Analytics of PAs It can be shown that, using validated analytical methods, the requirements of the German graduated plan can be fulfilled with respect to the limits set. The requirements for such analytical procedures should be suitable and proportionate. In case PAs cannot be detected with validated analytical methods, this should be sufficient. An analytical 'zero' does not exist, since in every case, substances can only be detected up to the limit of detection. This approach is also applied e.g. in case of aflatoxins, for which the limit set in regulations or pharmacopoeias is not deduced from a toxicological assessment but from production conditions. Aflatoxins may also occur both in food and in herbal medicinal products and are genotoxic and carcinogenic. Even though their health risk is proven, the European Pharmacopoeia allows limit values for aflatoxin B_1 in herbal medicinal products [17]. Against this background it becomes obvious that zero-tolerance limits for PAs are inadequate and reasonable limits should be defined instead.	Even though that an extrapolation from animals to humans bear a lot of uncertainties, however it would be not ethical to conduct carcinogenicity studies in humans. Therefore all available data, regarding e.g. carcinogenicity studies in animals, genotoxicity data, and knowledge about metabolic steps together can lead to a estimation of risk for humans.
	4. Toxicological assessment Hepatotoxic and carcinogenic effects of PAs are evident and justify security measurements to minimise PA exposure. However, the progression of liver damage appears to be fairly unpredictable and can occur suddenly months or even years after PA exposure [18]. The	

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	reversibility of damage is also uncertain though there is a high rate of complete recovery (50% or more). Knowledge on toxicity of PAs is mostly based on studies in experimental animals. However, extrapolation of toxic findings in experimental animals to human beings concerning organ manifestation and time course of clinical findings as well as carcinogenicity is impossible. It is evident that toxicity of PAs varies between mammalian species. Some of these numerous factors, which are species-dependent and differ individually, are for example enzymatic metabolism to highly reactive didehydropyrrolizidines or detoxification by adduct-formation with glutathione. As far as carcinogenicity is concerned, though tumours have been observed in rats there are apparently no known reports of cancer in domestic animals caused by exposure to PA in their diet (grazing animals in particular may be exposed to high levels). The recovery rate in humans from both acute and chronic PA poisoning appears to be high (>50%). Prakash *et al*. believe that the human liver repairs damage more efficiently after PA poisoning compared to lower animals [19]. Furthermore, doses used in the experiments with animals are unrealistically high compared to medical treatment of humans with herbal medicinal products. For example, in an analysis of outbreaks of human poisonings up until 1983, Culvenor (see below [24]) reported that the dose range of alkaloids would have been in the range of 0.01-50 mg/kg/day. Additionally, doses used in experiments with animals cannot easily be transferred to humans because of a non-linear dose effect relationship. In our opinion, chronic toxicity in humans should be regarded with caution as chronic PA exposure to humans has not yet been systematically analysed. Several characteristics of PA toxicity that are listed by the HMPC in the current draft are not only known for PAs but are also common for a number of genotoxic carcinogens that are naturally included in traces or as impurities in the human diet, in medicinal products or packaging materials of food. Consequently, the source of PA toxification is hard to identify and most current PA intake is likely to be due to contamination of food or other confounding factors rather than due to herbal drugs. To underline the high toxicity of PAs the current HMPC public statement refers to some cases of single dose toxicity.	To be changed into "… has been described …"

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	We took a closer look to some papers mentioned in the HMPC public statement and do not agree with the conclusion of the HMPC. We have special annotations concerning the following statements: ▪ *'In some cases, a single episode of acute disease has been demonstrated to progress to cirrhosis (even in a period as short as 3 months from the acute phase), in spite of the fact that the patient has been removed from the source of toxic exposure and has been given symptomatic treatment [TANDON et al. 1977, STUART & BRAS 1957].'* [1, lines 362ff] This statement is not based on proven data but rather on speculations. The paper of **Tandon *et al*. 1977** describes epidemic intoxication in 25 persons. The morphological liver changes were further investigated in six patients. Acute hemorrhagic centrilobular, progressive sclerosis, veno-occlusive disease (VOD) and non-portal cirrhosis were observed [20]. Eleven of the 25 persons had ingested the same food, therefore '*ingestion of some hepatotoxic agent in the diet*' was '*suggested*'. The toxic agent was not defined, only suspected, and no estimation of the extent and duration of exposure was performed. In addition, no alternative causes have been assessed and excluded. '*The diet of patients and other members of the community concerned during this epidemic not being available for analysis, the exact toxic factor could not be identified. However, in a more recent outbreak of an identical epidemic in the same area involving 67 individuals, analysis of food has revealed the presence of significant amounts of the pyrrolizidine alkaloid, fulvine.'* Histological changes in liver biopsies in those patients '*showed many histological features identical to those described here'* (e.g. as the 6 patients described in the publication). '*Thus the liver damage discussed in the present paper can be attributed **presumably** to pyrrolizidine alkaloids*' [20]. The paper of **Stuart & Bras 1957**, is a review on veno-occlusive disease, in which a case series of 10 cases is investigated [18]. No evidence of PA ingestion was observed nor proven in any of the patients. Additionally, the clinical course of disease from 84 Jamaican patients with VOD was reviewed. Also in this	Even though that the source of poisoning could not be traced down the authors describe detailed the clinical observations and liver biopsies. In TANDON *et al*. (1976) ["Study of an epidemic of venoocclusive disease in India". Gut 17: 849-855] the same case is somehow described again and brought into connection with the poisoning with the seeds of a plant of the *Heliotropium species in Afghanistan. TANDON et al. (1976) describe that "there are many similarities in the clinical features and histopathology of the liver among the patients of the present series and those of the Afghanistan epidemic, it is likely that food contaminated with pyrrolizidine alakloids was responsible for this epidemic in Central India."* So even if not proven it is a bit more then speculation. More literature concerning the Jamaica-cases exist. In none of them a "hard" proof is given, that VOD was due to PA-containing plants. However, tests in animals and further investigations of the nature of "bush-teas" are there and support this theory. The sentence was changed (see above). The sentence starts now with "In literature it was postulated …" and a further reference [HUXTABLE RJ (1990) "Activation and pulmonary toxicity of pyrrolizidine alkaloids". Pharmac. Ther. 47(3):371-389] was added. This publication focussed on the role of monocrotalin in changes of the lung. Even though only

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	sample of patients, no evidence of PA ingestion was obtained. In the discussion it was mentioned that '*veno-occlusive disease is a form of toxic liver damage, and probably produced by local plant toxins'*. Mal-nourishment was mentioned as an additional risk-factor. The evidence that PAs are involved in the aetiology is indirect: four of the patients have ingested '*bush teas – infusions of herbs, seeds, and roots – used in Jamaica both medicinally and for food… more than 200 plants so used.[...] Further circumstantial evidence was obtained by botanical examination of a number of bushes sold in a large Jamaican market.[…] It was found that there is an extensive use, under a variety of local names, of species of known hepatotoxic plants, Senecio and Crotalaria* [21]. ▪ *'Thus, following dietary exposure to PAs, in vivo alkylation continues until the reservoir of labile tissue-bound adducts is eliminated, mainly as soluble conjugates (e.g. with GSH) in urine and bile. This may take many months so that even a single dietary exposure to PAs continues to produce silently progressing chronic diseases, which are unlikely to be attributed to PAs in food [EDGAR et al. 2011].'* [1, lines 368ff] No data provide evidence for the correctness of this statement, it is purely speculative based on possible consequences of known toxicological mechanisms. In **Edgar *et al*. 2011** the authors discuss potential health consequences of PA contamination of food [22]. They state and speculate that *'some staple and widely consumed food are sometimes contaminated by dehydroPAs and their N-oxides at levels that, while insufficient to cause acute poisoning, greatly exceed maximum tolerable daily intake and/or maximum levels determined by a number of independent risk assessment authorities. This suggests that there may have been cases of disease in the past not recognized as resulting from dietary exposure to dehydroPAs'. In the review they show that 'there are a number of reports of liver disease where either exposure to dehydroPA was **suspected** but **no source was identified'*** or '*was not considered but the symptoms and the pathology suggests their involvement*' [22]. Per definition, this is not an evidence-based proof of relevance for human toxicity with respect to the development of hepatic toxicity (veno-occlusive disease, cirrhosis), pulmonary hypertension (pulmonary VOD), cancer.	animal data are presented the mechanisms are described clearly. There it is said: "Following a single injection of Monocrotalin, metabolism and excretion are essentially complete within 24 hr. Increased lung mass, arterial medial hypertrophy and elevated pulmonary arterial blood pressure do not occur until 9-12 days later". To be more precise the wording was changed into: "In one study, a single dose has been carcinogenic [CULVENOR 1983]. In the study of Schoental & Magee [1957] a single dose of lasiocarpine provoked after ~13 months changes in the liver which were described as being very similar to those observed in the earlier stages of hepatic carcinogenesis due to several pyrrolizidine alkaloids after multiple dosing." In IPCS 1988 (quoted at the end of the sentence it is

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	The pathology of PA toxicity is not specific for PAs. For pulmonary VOD, different additional risk factors as infections, genetic factors, antineoplastic therapy, etc. are described [23].The multifactorial genesis of cancer development is self-evident. An evidence-based proof should be based on a single case analysis taking into account demographic (hereditary) factors, co-morbidity, co-medication, co-intoxication, thorough analysis of toxicant exposure (dose, duration, time-relationship) and other biasing factors. If possible, exposure has to be proven by blood or tissue concentrations. ▪ *'In rats, appropriately low repeated doses of several alkaloids have been shown to induce tumours. In some studies, a single dose has been carcinogenic. […] These dosages are roughly similar in magnitude to estimated intake rates (0.01-10 mg/kg b.w./day) in several episodes of human toxicity [CULVENOR 1983, IPCS 1988]' [1, lines 420ff].* **Culvenor 1983** describes one study in rats with a dose of 30 mg/kg (= LD_{50}) of retrorsine tumours developed up to 1 year after a single dose. '*The rates, which must be understood as very approximative, range from 0.01* ***to 50 mg/kg/day.***' Concentrations were in almost all cases very unsecure estimates. Furthermore, confounding with mycotoxin co-intoxication of humans by food had to be taken into account, as the author stated [24]. ▪ *'Comparison of the total intakes resulting in human toxicity with the total doses to death observed in the chronic toxicity studies on rats indicates that human beings are more susceptible and suggests that human beings may survive for sufficient time to develop cancer after only a brief exposure at this level or a longer exposure at a markedly lower level.' [1, lines 423ff]* There is no evidence that human beings are more susceptible in the cited paper. **5. Cutaneous Application** The HMPC draft on the use of herbal medicinal products containing pyrrolizidine	given: "Comparing the total intakes for human toxicity with the total doses up to death observed in the long-term administration of PAs to rats, 1.2-10.9 times the LD_{50} dose, equivalent to 360-3270 mg heliotrine/kg, it is evident that human beings are more susceptible to the acute and chronic effects of the alkaloids than rats, sometimes markedly so. It is to ensure that the daily intake of all PAs from the product is <0.035 µg/kg for adults. The use is restricted to intact skin. Higher contents of PA within the products would be possible if for the relevant product (means the relevant matrix, because absorption might be greatly influenced by the excipients, for instance essential oils as enhancers) low absorption rates (generated with modern analytical techniques; in animal species which are more comparable to human beings in relation to the skin or *in-vitro* human skin preparations) can be shown, not exceeding the daily intake of 0.035 µg PA/kg for adults. **Sensitive groups:** **Children** If children are included in the usage of certain products the daily amount of PA has to be adjusted to the body weight of the age group: e.g. body weight of 20 kg would lead to an acceptable daily intake of 0.014 µg PA/day. **Pregnant and breast feeding woman** Sensitive groups such as pregnant and breast feeding woman are also covered by the limit calculated above. If these limits are complied with, the chapter 4.6 of the SmPC of the products concerned should be phrased according to the 'Guideline on risk assessment of medicinal products on human reproduction and lactation: from data to labelling' (EMEA/CHMP/203927/2005).

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	alkaloids states that '*after the dermal application the excreted N-oxides in urine amounted to 0.1-0.4% of the dose. After oral dosage [it] was quoted as being 20-50 times greater*' [1 lines 202-204]. As the absorption rate after cutaneous application is lower than after oral application, medicinal products for external use must be discussed separately. Due to the lower absorption after application on intact skin, higher amounts of PAs should be accepted in medicinal products for external use. This is also in line with the German graduated plan where **daily limits for external application on intact skin are 10 µg PAs** (with unlimited treatment duration) and **100 µg PAs** (with restricted treatment duration of 6 weeks). In the summary report on *Symphyti radix* of the EMA Committee for Veterinary Medicinal Products, the external use of medicinal products containing *Symphyti radix* on intact skin is even assessed as being hazard free as no significant absorption could be shown on intact skin [25]. This recommendation for veterinary use can be transferred to humans, where absorption is of comparable low order. It supports our opinion that specific regulations are required for medicinal products for external use. It is evident that children are especially vulnerable to the effects of PAs. Thus these patients must be regarded with special caution. Nevertheless, cutaneous application can be considered for these patients. With respect to current scientific knowledge for safety reasons the potentially higher sensitivity of children against toxic substances in general compared to the sensitivity of adults is accounted with a factor of 10 [26]. Using the same amounts of a cutaneously applicated formulation and an application area of same size, the doses related to body weight are 1.5 to 1.2 fold higher in children (4-12 years old) compared to adults [26-30]. Based on these data a security factor (SF) of 15 (10*1.5) can be applied. Using the limit value for cutaneous application on intact skin of 10 µg as defined in the German graduated plan, a topic exposition of less than 0.7 µg PA/day (10 µg/SF 15) can be regarded as safe. **6. Homeopathic medicinal products** The public statement does not address homeopathic medicinal products. However, PA-containing drugs are also used in homeopathy. From our point of view, in case a risk assessment of homeopathic medicinal products is	The HMPC is not responsible for the assessment of homeopathic medicinal products. At the moment no regulatory measurements regarding PAs in food exist, even though the topic is discussed and European bodies (e.g. EFSA) are aware of the problems. As pointed out in the Public statement the intake of PAs from food cannot be avoided. Therefore the usage of PA containing medicinal products should be strongly assessed. For the derivation of a daily limit of PA intake via medicinal products see above.

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	performed, they should be treated in the same way as herbal medicinal products. Consequently, the same limit as set in the German graduated plan should apply. As the amount of original substance diminishes with each dilution of a homeopathic medicinal product, former national regulations on the use of PA-containing drugs include additional specific rules for this group of drugs. According to the German graduated plan of 1992, PA-containing homeopathic drugs with potency of **D6 for internal use** or **D4 for external use** are in general excluded from the restrictions without the necessity of PA content analysis [3]. The same exclusions are made in other European countries, e.g. Austria [30]. For the so-called "C potencies", limits should be set with C3 (=D6) and C2 (=D4), respectively. If product-specific analytical data indicate that potencies below D6 or D4 adhere to the limits given in the German graduated plan, such lower potencies are acceptable as well. **7. Health risk due to PAs in food compared to PAs in herbal medicinal products** Above all, we wish to outline that it is generally difficult to compare health risk due to contamination of food with the risk arising from the intake of herbal medicinal products containing such compounds. Pharmacovigilance measures in the sector of medicinal products ensure a constant quality, efficacy and safety. In addition, for herbal medicinal products, rules ensuring their quality and safety have been established, e.g. the EMA guideline on Quality of Herbal Medicinal Products/Traditional Herbal Medicinal Products [31] or the HMPC guideline on Good Agricultural and Collection Practice (GACP) [32]. Based on these regulations, the PA content of herbal medicinal products can be controlled and reduced to a sufficiently low level. Comparing contamination of food and the inherent occurrence of PAs in medicinal products, it should be kept in mind that the intake of medicinal products is always connected to a health benefit. Furthermore, PA intake via medicines is usually in terms of short-term use and in a defined dose. On the other hand, exposure by food is not predictable and might occur during a long period of time and in much higher doses. The daily amount of PA-intake via honey was pronounced to easily reach 10-	

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	100 µg PA/day, accompanied by an additional uptake via other dietary sources such as milk or meat [1]. Thus, the maximal daily amount of PAs from medicinal products permitted without further limitations [3] only makes up to 0.1-1%, which is almost negligible compared to the possible dietary intake. In this context the HMPC Guideline on the Assessment of Genotoxicity of Herbal Substances/Preparations states that *'a risk from administration of an HMP might be accepted if its contribution to the overall exposure through food is considered to be small'* [13]. This statement justifies the acceptability of the occurrence of PAs in herbal medicinal products at low levels. In contrast to the pharmaceutical sector PAs in food are not regulated. The Australian and New Zeeland Food Authority recommend that if the NOEL is 10 µg/kg/day an uncertainty factor of 10 should be applied for a provisional tolerable daily intake (PTDI) of 1 µg/kg/day. Then an acceptable intake for an adult weightening 70 kg would therefore be about 70 µg per day. These values can be supported only if the product is taken not for more than 4-6 weeks [33]. The EFSA concluded that 0.007 µg/kg (that means 0.42 µg/60 kg adult) as a daily intake of unsaturated PAs should not be exceeded [6]. However, as mentioned in the HMPC public statement on the use of herbal medicinal products containing pyrrolizidine alkaloids *'until now no limits for PAs in food exist within the EU'* [1 line 155]. It is important to emphasise that efforts must focus on establishing methods and regulations to minimise PA exposure by food. In the HMPC public statement, it is mentioned that *'(31% of) commercial bee pollen products purchased in Europe have been found to contain 1080 – 16350 µg PA/kg'* [1 lines 484f] and *'the daily amounts of PA-intake via honey can easily reach 10-100 µg PA/day'* [1 line 584] accompanied by an additional uptake via other dietary sources such as milk or meat [1]. Comparing these levels of exposure with the maximal annual intake of PAs of 36 µg (365 days * 0.1 µg/day) or 42 µg (6 weeks * 7 days/week * 1.0 µg/day) as set in the German graduated plan [3], the disproportion in the discussion about limits for PAs in food and drugs becomes obvious. In contrast to PA exposure by food, the exposure by herbal medicinal products can be regulated. In Germany, for example, it is minimised by the graduated plan. During the past 20 years, since the implementation of this regulation, it has proved its value as no proven cases of PA-induced hepatotoxicity or carcinogenicity due to	The limit value has been established on the basis of the

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	medicinal products became known. All published cases of PA-induced toxicity of the past 20 years are due to contamination of food and in the most severe cases contamination of grain causes disease [34 table 1]. The HMPC public statement does not solve this problem as the contribution of the PA content of herbal medicinal products to total PA exposure is only marginal. From pharmacovigilance data collected in Switzerland it can be derived that since 2007 more than 10'000 units of *Sympyhtum* preparations were sold and there was no serious adverse effect reported. For medicinal products of other PA containing plants as *Cineraria*, 26'000 units sold in 5 years, *Borrago*, more than 6'450 units sold, *Petasites*, more than 1 100 units sold, and *Eupatorium*, more than 100 units sold, results are analogous as no serious adverse effects have been reported. Four non-serious adverse effects were documented for external use. For this reason the limits set can be regarded as appropriate and safe. Efforts should be focused on reducing PA exposure in the food sector and not on tightening the existing scientifically based regulations for herbal medicinal products. In this context, the project of the Codex Committee on Contaminants in Food, a group of the Codex Alimentarius Commission, in establishing a code of practice for the prevention and reduction of contamination of food with PAs is a valuable approach. Efforts of this Committee are especially focused on intensifying weed control as various different methods are present to reduce this source of contamination [2]. Based on these findings we cannot agree with the argumentation of the HMPC Public statement that *'it seems important to accept that relative low and sometimes sporadic amounts of PA might be taken in by food'* [1, line 530f] and that therefore PA content of herbal medicinal products must be reduced on a 'zero-tolerance' level [1]. In our opinion such a conclusion is disproportionate and with regard to health risks especially for infants and children, the present high amounts of PAs in food cannot be accepted. **8. Conclusion** In summary, the need to apply the 'zero-tolerance principle' on herbal medicinal products cannot be justified. Instead, reasonable limit values should be established. It is therefore suggested to apply the existing limits of the German graduated plan [3]. In the past 20 years no further risks have been observed by	the risk assessment of genotoxic carcinogens, in which the TD_{50} value or a corresponding value (a measure of cancer potency) from the most sensitive species/tumour site is considered an appropriate point of reference for a linear down extrapolation to a "virtually safe dose" (see above). It should be noted that the limit value suggested by AESGP (0.42 µg/day) would be at the same general dietary exposure level of PA's estimated by EFSA and thus increase the burden of PA exposure to a significant extent, albeit for a duration of drug administration.

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	using the respective products. Thus the limits ensure a sufficient level of safety. Furthermore, the application of these values can be justified by the MOE approach and the TTC concept, two scientifically approved methods for establishing limits for toxic substances. To date, no new scientific findings necessitate stricter limits for PAs in herbal medicinal products. In order to reduce PA exposure, efforts should focus on the food sector as PA intake by food is much higher than intake by medicinal products. We cannot accept the argument that high exposure of PAs by food is unavoidable. In contrast there are many opportunities to reduce this source of intake as shown by the work of the CONTAM panel. Available data support that the use of herbal medicinal products containing PAs does not imply a significant health risk as long as the maximum daily exposure to PAs by herbal medicinal products does not exceed 0.1 µg for internal use and 10 µg for external use. These limits can be applied without any exception. With regard to a maximal duration of application of 6 weeks per year, and excluding pregnant and breast-feeding women, doses of up to 1 µg for internal and 100 µg for external use are accepted. In case of topical use, such higher doses should only be applied on intact skin. Consequently, we consider Europe-wide acceptance of these limits as justified.	
BPI	A great number of BPI's member companies produce herbal and homeopathic medicinal products. Especially in the latter product group potentially pyrrolizidine-containing herbs are being used. Therefore BPI is very interested in the HMPC Draft Statement on PA-containing medicinal products (EMA, 2012) of 25 October 2012. BPI principally agrees that the intake of pyrrolizidine alkaloids (PAs) should be restricted due to their (geno)toxic effects. However, a uniform regulation concerning the regulatory status throughout Europe would be advantageous. Moreover, a complete ban of PA-containing herbal medicinal products and, possibly in analogy, of homeopathic medicinal products seems not to be rational due to their benefits and the relatively small part in the total PA burden of consumers. The HMPC points out that "the so-called 'zero-tolerance principle' can be applied to herbal medicinal products containing PAs. This principle is used in cases where either no safe or tolerable level can be determined based on available, valid scientific data, or if insufficient toxicological data are available" (EMA, 2012, lines 128-130). The HMPC refers to the same recommendation given by the Bundesinstitut für Risikobewertung	To avoid misunderstandings the sentence and the two following sentences were removed.

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	(BfR) in Germany (line 131). However, in the respective position paper, the BfR endorses the 'zero-tolerance principle' for the application or addition of genotoxic plant ingredients such as pyrrolizidine alkaloids in isolated form as well as for impurities in food (BfR, 2007). In a more recent statement of the BfR on pyrrolizidine alkaloids, however, the 'zero-tolerance principle' has not been taken into consideration; instead, risk assessment based on a Benchmark Dose Lower Confidence Limit 10% ($BMDL_{10}$), resulting from an carcinogenicity bioassay (NTP, 1978), and the estimation of a Margin of Exposure (MOE) was considered suitable (BfR, 2010; BfR, 2011). The same conclusion was drawn by the EFSA Panel on Contaminants in the Food Chain (CONTAM) from their risk assessment on pyrrolizidine alkaloids in food and feed (EFSA, 2011). Therefore, we suggest considering existing restrictive regulations such as in Germany with fixed limits of daily PA intake via medicinal products instead of following a 'zero-tolerance principle'. The HMPC statement refers to already known pharmacokinetic and toxicological data of toxic pyrrolizidine alkaloids (PAs). Additionally, several characteristics of PA toxicity that are listed in the HMPC statement are not only known for PAs but are also common for a number of genotoxic carcinogens that are naturally included in traces or as impurities for example in the human diet or in medicinal products. Examples of such common modes of action are the possibility of cancerogenic effects by chronic exposure to low doses via interaction of toxic metabolites with DNA, as well as carcinogenic effects of single doses. For substances such as aflatoxins or other genotoxic constituents, limit values in food or medicinal products have been established. The main new aspect addressed in the HMPC statement is the emerging supposed high PA intake via food by certain consumer groups, which creates a certain background risk. The HMPC suggests that smallest amounts of PA-exposure result in fatal liver and other organ failures including malignity. Yet human PA-exposures are estimated up to 100 µg by honey and other foods. Therefore, according to HMPC, an additional intake of PAs by medicinal products should be prohibited. The high toxicity of PAs is underlined in the current draft by stating that "in some studies, a single dose has been carcinogenic" (line 421). According to EFSA, we found only one study, whose results revealed carcinogenicity following a single dose: "Rats receiving a sublethal dose of 30 mg/kg retrorsine showed irreversible hepatic lesions possibly leading to chronic liver disease and	The MOE approach was originally developed for variable, but continuous exposures such as via food. See the response to AEGSP above. See the response to AEGSP above.

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	eventually to hepatocellular carcinoma more than 13 months after administration" (EFSA, 2011). BPI understands the concern about the recent development in the food area, but from a scientific perspective the PA uptake from food and herbal medicines is not comparable at least due to the different daily quantities and different application periods. Additionally, the benefit of the herbal medicines has to be considered as it was done in the course of the German graduated plan (Bundesgesundheitsamt 1992). As it is said in the HMPC guideline on the assessment of genotoxicity of herbal substances / preparations (EMEA, 2008), *"A risk from administration of an HMP [herbal medicinal products] might be accepted if its contribution to the overall exposure through food is considered to be small".* Therefore, we see it as not proportionate to ban PA-containing herbal medicinal products. Even if the discussed PA limits for food will be implemented in the future, the low PA limit for oral use of herbal medicinal products given in the German graduated plan should remain in force respectively become valid throughout Europe. The adopted HMPC guideline on the assessment of genotoxicity of herbal substances / preparations (EMEA/HMPC/107079/2007) states that "the margin of exposure approach for the risk assessment of genotoxic and carcinogenic compounds […], which is recommended by the EFSA Scientific Committee on Food […], is probably not applicable for HMPs, because this approach is based on available carcinogenicity data, which is usually lacking in case of HMPs" (EMEA, 2008). However, if such data are available, the HMPC refers to the EFSA Committee, which is of the opinion that a compound with a calculated margin of exposure of 10,000 or higher would be of low health risk" (EMEA, 2008). Since a broad risk assessment on pyrrolizidine alkaloids in food and feed was presented by CONTAM (EFSA, 2011), such an approach should be possible for HMPs. The objection raised by the HMPC in the current draft concerning a need for research indicated by the BfR and the EFSA in their latest opinions on PAs in food (EMA, 2012, line 151) does not apply for HMPs, since both publication clearly refer to open points directly related with food and feed (BfR, 2011; EFSA, 2011).	See the response to AEGSP above.

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	As validated analytical methods exist to determine the amount of PAs in HMPs and as exposition data of pharmaceutical products can be predicted quite well, sufficient data is available to establish *practically achievable* limit values. Therefore, we suggest that the exposure of PAs to humans by herbal medicines is regulated by introducing a limit value considering the German graduated plan from 1992 (BGA, 1992). As is shown below, the limits fixed in this pharmacovigilance procedure from 1992 are still scientifically based and consistent with other current risk estimations/assessments. **Animal studies with PA and PA-containing herbal drugs** Hepatotoxicity and carcinogenicity is known from animal studies with pyrrolizidine alkaloids for decades. All toxic PAs share common structural features and similar metabolism / toxification. The common structural features are an unsaturated 1,2-necine-ring and an esterified hydroxymethyl group by a necic acid at C1 (monoester), diesters with a second group at the hydroxyl group of C7 and their macrocyclic diester varations belong to the group of PAs with comparable toxicity. This is verified by their common metabolism via pyrrolic derivatives, e.g. dehydroretronecine (DHR), which bind to DNA as a mechanism of genotoxicity / carcinogenicity. A representative overview but not overall data of dose effect relations to carcinogenic and organotoxic effects in animal studies is given in **ANNEX 1.** **Toxic doses of PA in animal studies** All these studies used relative high doses therefore it was not possible to calculate acceptable doses for human intake with herbal medicines. **Further studies with Riddelliine by the NTP (National Toxicology Program of the Department of Health and Human Services) USA** In 1993 the NTP started to evaluate the toxic potential of PAs for humans related with contaminated livestock products, e.g. milk and foodstuffs such as honey. Recognising the incomplete data of previous animal studies the NTP designed a test program with Riddelliine, a macrocyclic diester, and carried out repeated dose toxicity studies with mice and rats for 13 weeks. **Riddelliine is representative for other toxic PAs** Riddelliine is metabolised by CYP isoenzymes to Dehydroriddelliine and Dedydroretronecin (DHR). These 2 compounds are potent electrophiles which	For the derivation of the limit of intake it was focussed on the NTP study on lasiocarpine as done by EFSA and see the response to AEGSP above.

bind to the DNA. Studies confirm the basal toxification pathway of PAs via genesis of reactive pyrrols.
Xia *et al.* (2006) investigated the metabolism of Lasiocarpin, a Heliotridine-alkaloid. The reactive metabolite from liver microsomes of rats has been identified as (+/-)6,7-dehydro-1-hydroxymethyl-5H-pyrrolizine (DHP) = Dehydroretronecin (DHR). The same DHP-derived DNA-adducts have been found from Clivorine an Otonecine-alkaloid.
It was concluded that Riddelliine is representative for other toxic PA with the analogous structural features and these entire PA underlay basically the same metabolism by CYP isoenzymes to pyrrolic metabolites to form DNA-adducts which are responsible for cancerogenesis in low dose experiments.
In addition quantitative analysis of DNA-adducts of PA-metabolites in parenchymal liver cells and endothelial cells were carried out. In endothelial cells more DNA-adducts were found than in parenchyma cells. These findings correlate with the higher incidence of haemangioendothelial sarcoma than neoplasms in the liver parenchym. So the genotoxic mechanism of the covalent binding of electrophile pyrrol derivatives to the DNA would be confirmed.

Investigations with Riddelliine in rats are relevant for humans

8 DNA-adducts were detected from female rats treated with Riddelliine for 3 or 6 month. *In-vitro* studies with Riddelliine in the presence of human liver microsomes and calf thymus DNA resulted in the same 8 DNA-adducts.

Repeated dose toxicity studies with Riddelliine

In 1993 the NTP carried out repeated dose toxicity studies with mice and rats up to 13 weeks. The study design and results are presented in the following table.

Table 1: NTP USA 1993 for the evaluation of the toxic potential of Riddelliine

Riddelliine	2-week-study: 0; 0.33, 1.0, 3.3, 10, and 25 mg/kg body weight (b.w.) (by gavage)	2-week-study: 5 x per week, for a total of 12 doses	2-week-study: groups of 5 female / 5 male rats (F344/N) and mice ($B6C3F_1$)	2-week-study: dose-related hemorrhagic centrilobular hepatic necrosis, hepato-cytic karyomegaly and cyto-logic alterations, pulmonary hemorrhage and/or edema, splenic

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			13-week-study: 0, 0.1, 0.33, 1.0, 3.3, or 10 mg/kg b.w. (rats) and 0, 0.33, 1.0, 3.3, 10 or 25 mg/kg b.w. (mice) (by gavage)	13- week-study: 5 x per week for 13 weeks	13- week-study: groups of 20 female / 20 male rats (F344/N) and mice (B6C3F1)	extramedullary hematopoiesis, and pancreatic edema. 13- week-study: **NOAEL** (for histopathologic changes): **mice:** **3.3 mg/kg b.w.** **rats:** **0.1 mg/kg b.w.**	Riddelliine. Administered by gavage to F344/N rats and B6C3F$_1$ mice

NTP working group (1993); National Toxicology Program Toxicity Report Series, 27; 106p. Toxicity studies of Riddelliine. Administered by gavage to F344/N rats and B6C3F$_1$ mice

Further details, see NTP Technical Report on Toxicity Studies of Riddelline (CAS No. 23246-96-0) Administered by Gavage to F344/N Rats and B6C3F$_1$ Mice. NIH Publication 94-3350. December 1993
(available from: http://www.ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox027.pdf).

This is the first repeated dose toxicity study with a representative toxic PA resulting in a NOAEL. These are 3.3 mg/kg for mice and 0.1 mg/kg for rats indicating that rats are more sensitive than mice. However the NTP went on to conclude that these studies failed to calculate the risk for low level and long term intake of PAs by humans and the carcinogenic risk would equally not be evaluated by these study results.

Two year studies with Ridelliine in rats and mice
Based on the subchronic toxicological study design from 1993 the NTP followed up the study with Riddelliine in mice and rats in 2003. Especially the question of low dose effects below 3.3 mg/kg in mice and 1 mg/kg in rats were investigated over 2 years. The aim of this study is to consider the dose response curve for carcinogenicity rather than to focus on the identification of a carcinogenic response itself.

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Table 2: NTP USA 2003, 2-Year gavage study with Riddelliine in rats and mice, number of animals from 50 animals in each group – selected incidences (animals per group) of neoplasms of the liver

	vehicle	**~~0.01mg/kg~~**	**~~0.033mg/kg~~**	**~~0.1mg/kg~~**	**~~0.33mg/kg~~**	**~~1mg/kg~~**
male rats						
Haemangiosar-coma	0	n.a.	n.a.	n.a.	n.a.	43
First incidence (days)		n.a.	n.a.	n.a.	n.a.	307
Hepatocellular adenoma		n.a.	n.a.	n.a.	n.a.	4
First incidence		n.a.	n.a.	n.a.	n.a.	398
female rats						
Haemangiosar-coma	0	0	0	0	3	38
First incidence (days)					524	350
Hepatocellular adenoma	1	0	0	0	1	7
First incidence					729 (T)	426
Hepatocellular carcinoma	0	0	0	0	1	1
	vehicle	**0.1mg/kg**	**0.3mg/kg**		**1mg/kg**	**3mg/kg**
male mice						
Haemangiosar-coma	2	1	0		2	31
First incidence (days)	729 (T)	729 (T)	n.a.		729 (T)	550
Hepatocellular adenoma	16	18	14		5	0
Hepatocellular carcinoma	23	21	19		20	3
First incidence (adenoma and carcinoma)	475	542	567		566	590
female mice						
Haemangiosar-coma	0	n.a.	n.a.		n.a.	1
First incidence (days)		n.a.	n.a.		n.a.	
Hepatocellular	9	n.a.	n.a.		n.a.	0

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	adenoma Heptocellular carcinoma 8 n.a. n.a. n.a. 0 n.a. = not applicable because the doses hadn't been selected for the animal group due to small amounts of available substance T = terminal sacrifice For further details with statistic information, see NTP Technical Report on the Toxicology and Carcinogenesis Studies of Riddelliine (CAS No. 23246-96-0) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). NTP TR 508, NIH Publication No. 03.4442, May 2003. (available from: http://*ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr508.pdf)* The study confirmed the known response of haemangioendothelial sarcoma after exposition of PA to rats and mice, see table 2. Neoplasms were detected at low doses of 1 mg/kg in male rats after 398 days, in female rats at 0.33 mg/kg after 729 days and at 1 mg/kg after 426 days. In the tested concentrations below 0.33 mg/kg, no neoplasm in female rats were observed until the terminal sacrifice (male rats were not studied). Male mice showed neoplasms at 3 mg/kg after 590 days. A dose dependent increase for haemangiosarcoma in the liver in all animal groups is related to Riddelliine. Nevertheless a decrease in hepatocellular neoplasms is noted in male and female mice. Up to 0.1 mg/kg no haemangioendothelial sarcoma are detected in female rats. It is adequate to accept this dose as the relevant no effect dose of PAs to assess the carcinogenic potential and safety margin for humans. The reasons for this view are summarized as follows: • Riddelliine is representative for all toxic PAs as described above • Riddelliine is metabolised by the same way in rats and humans • Rats are more sensitive than mice • Liver blood vessels are the primary target organ of Riddelliine demonstrated by quantitative DNA adduct analysis Due to the consistent data of the NTP studies it seems reasonable to accept haemangioendothelial sarcoma as the key lesion and 0.1 mg/kg should be taken as basis to evaluate the human PA-exposure in herbal medicines and food for assessing the carcinogenic potential. **PA-intake with Herbal Medicines** The only national health authority, who yet accepted and fixed a limit value for	See the response to AEGSP above.

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	the intake of PAs via herbal medicines has been the BfArM respectively the BGA in 1992 (BGA, 1992). The respective graduated plan is also valid for homeopathic and anthroposophic medicinal products. The BGA limited the intake of PAs with the upper daily dose of the concerned herbal medicine. These limits are dependent on the duration of use • 1 µg total PAs per day with a duration limit of 6 weeks. (Additionally there is a contra-indication in pregnancy and lactation.) • 0.1 µg total PAs per day without limit for the duration of the use. These 2 limits refer to all PAs with a 1-2 unsaturated necin moiety with at least 1 ester group by an acid. The carcinogenic potential of the PA has been the reason for the legal measures in this pharmacovigilance procedure which resulted in the limits given above. **Accepting the limit value of 0.1 µg PA in the maximum daily dose of herbal medicines the safety margin to the free dose of 0.1 mg/kg in rats is 5 x 10^4 for a 50 kg weight human (0.002 µg PA/kg bw).** **PA-intake with Food** Food Agencies in Europe and New Zealand propose throughout higher limit values, e.g. BfR: 0.007 µg/kg/day and COT (UK): 0.1 µg/kg/day (non-cancer unlikely) 0.007 µg/kg/day (cancer unlikely). 0.007 µg/kg/day result in 0.35 µg per day for a 50 kg weight human. **Even considering a high PA-intake from food as 10 µg per day the safety margin is 5 x 10^2 compared to 0.1 mg/kg for a 50 kg weight human.** **Topical use** Since experiments suggest a 20-50-fold lower percutaneous absorption compared to oral intake (EMA, 2012, lines 202-204) a respective higher PA limit would be possible for herbal medicinal products for topical use. Such an approach was also implemented by the German graduated plan of 1992. We see the values given there as appropriate. **Conclusions** The suggestion of HMPC to apply a 'zero-tolerance principle' on PA-containing	See the response to AEGSP above. See the response to AEGSP above. See the response to AEGSP above.

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	herbal medicinal products is, in the view of the above presented data, neither appropriate nor justified. Instead, the result of the German pharmacovigilance procedure in 1992 with tolerable 0.1 µg PAs in the maximum daily dose of herbal medicine for oral use without restrictions is requested to be confirmed by the HMPC considering the discussed results of studies in rats and the unequal higher estimated intake of PAs with foods. This limit for herbal medicines ranges below the limit values for foods proposed by different authorities. It is also below the TTC value of 1.5 µg/day acceptable for genotoxic impurities in medicinal products as well as genotoxic compounds in general in herbal medicinal products acc. to the HMPC Guideline on the assessment of genotoxicity of herbal substances / preparations (EMEA, 2008). These comparisons show that the limit fixed by the German authority in 1992 is still scientifically sound and sufficiently safe. The topical use can also be regulated by fixing a respective limit for daily use taking into account the lower absorption through the skin. Here fore we also refer to the German graduated plan (BGA,1992). Specific regulations for pregnant women are not necessary since the respective safety factors are included. For children a weight adaption seems to be advisable.	See the response to AEGSP above. For pregnant and breast feeding woman and children see the response to AEGSP above.

Annex 1: Toxic doses of PA in animal studies

Substan-ce	**Dose**	**Duration of treatment**	**Animals**	**Effect**	**Literature**
Fuchsisene-cionine (FS), Senecio-nine (S)	8 mg/kg b.w. (FS) resp. 40 mg/kg b.w. (S) (by gavage)	5 x per week for 114 weeks	male and female Sprague-Dawley rats	during the 2nd year of age pre-dominantly in female rats dose-related neoplastic changes in the liver.	**Habs *et al*. 1982** Carcinogenic and mutagenic activity of an alkaloidal extract of *Senecio nemorensis* ssp. Fuchsii. Arzneim.-Forsch. 32 (I) 144-148.
Symphytine	13 mg/ kg	2 x per week	male ACI-rats	4 rats with liver	**Hirono *et al*.**

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			b.w. (ip injections)	for 4 weeks; then once a week for 52 weeks	(20 per group)	tumors, 3 rats with hemangio-endothelial sarcomas and 1 rat with liver cell adenoma. <u>Control group:</u> no liver tumors	**(1979).** Induction of hepatic tumors in rats by senkirkine and symphytine. J. Natl. Cancer Inst. 63, 469-471	
		Senkirkine	22 mg/kg b.w. (ip injections)	2 x per week for 4 weeks; then once a week for 52 weeks	male ACI-rats (20 per group)	9 rats with liver cell adenoma.	**Hirono *et al*. (1979)** Induction of hepatic tumors in rats by senkirkine and symphytine. J. Natl. Cancer Inst. 63, 469-471	
		Mono-crotaline	5 mg/kg b.w (sc injections)	biweekly for 1 year ⇒ correspondent to a daily dose of 0.36 mg/kg b.w.	60 male Sprague-Dawley rats	pulmonary adenocarcinoma (17%), hepatocellular carcinoma (8%), rhabdomyo-sarcoma (7%), acute myelogenous leukemia (5%), adrenal adenoma (13%) <u>Control group:</u> 2 adrenal adenomas	**Shumaker *et al*. (1976).** Neoplastic transformation in tissues of rats exposed to monocrotaline or dehydroretronecine. J. Natl. Cancer Inst. 56, 787-790 **Allen *et al*. (1975)** Dehydroretronecine-induced activity of an alkaloid, heliotrine. Cancer res. 35 (4), 997-1002	
		Dehydro-retronecine	20 mg/kg b.w. for 4 months, then 10 mg/kg b.w. for 8 months	biweekly for 1 year (s.c. injections)	75 male Sprague-Dawley rats	51.6% of the animals with rhabdomyo-sarcomas at the site of injection	**Allen *et al*. (1975)** Dehydroretronecine-induced activity of an alkaloid,	

GENERAL COMMENTS								
						heliotrine. Cancer res. 35 (4), 997-1002		
	Heliotrine	230 mg/kg b.w.	2 doses (by stomach tube)	12 male rats	Islet-cell tumors, transitory cell papillomas of the urinary bladder, interstitial testicular tumors, hepatoma.	**Schoental (1975)**. Pancreatic Islet – cell and other tumors in rats given Heliotrine, a monoester pyrrolizidine alkaloid, and nicotinamid. Cancer Res. 35, 2020-2024		
	Lasiocarpine	7.8 mg/kg b.w. (ip injection)	2 x weekly for 4 weeks and once a week for additional 52 weeks	25 male Fischer 344 rats	of 18 rats that survived, 16 with tumors mainly hepatocellular carcinomas (11) and squamous cell carcinomas of the skin of the back (6), 2 with adenocarcinoma of the small intestine and 1 with chol-angiocarcinoma and adenomyoma of the ileum. <u>Control group</u>: 2 adenomas in the lung	**Svoboda and Reddy (1972)** Malignant tumors in rats given lasiocarpine. Cancer Res. 32, 908-912		
	Intermedine / Lycopsamine	up to1500 mg/kg b.w (oral)	single doses	15 male rats	adenoma (1) and adenocarcinoma of the islet-cells (1) and adenoma of the exocrine pancreas (1) in rats given a single dose of 500 to 1500 mg/kg b.w.	**Schoental *et al*. (1970)** Islet cell tumors of the pancreas found in rats given pyrrolizidine alkaloids from Amsincka intermedia Fisch and Mey and from Heliotropium		

GENERAL COMMENTS		
	supinum L. Cancer Res. 30, 2127-2131	
ECHAMP	The HMPC paper does not address homeopathic and anthroposophic medicinal products directly; the topic is highly important to our member companies because a large part of these medicinal products is of plant origin and a range of important potentially PA-containing plants is used for a long time in homeopathy as well as in anthroposophic medicine. In both therapies, most of these drugs are especially intended for oral use. The safety assessment of homeopathic and anthroposophic medicinal products of plant origin is mainly based on the herbal literature and existing regulations of herbal medicinal products. If a complete prohibition of PA-containing herbal medicinal products for oral use will be followed by respective measures for homeopathic and anthroposophic medicinal products, this would result in the loss of a large range of established and safe homeopathic and anthroposophic medicinal products.	See the response to AEGSP above.
ESCOP	From our point of view, the intention of the HMPC to reduce the content of PAs in herbal medicinal products to zero is disproportionate as it discriminates against herbal medicinal products compared to food. In contrast to food, herbal medicinal products are strongly regulated. They have to fulfil all legal requirements with regard to quality, efficacy and safety according to the respective EU Directives and EMA/HMPC guidelines, and are monitored by pharmacovigilance systems like all other medicinal products. Furthermore, herbal medicinal products are consumed in much lower quantities than foods and in most cases for a restricted period of time. Thus a potential health impact through herbal medicinal products is of very low toxicological concern as compared to the uncontrolled exposure to PAs by food. For this reason, ESCOP as an organization representing phytotherapy on a European level strongly pleads against this discrimination.	See the response to AEGSP above.
KOOP PHYTO	Kooperation Phytopharmaka, a German scientific organisation, would like to comment on this HMPC draft public statement on the use of herbal medicines containing toxic, unsaturated pyrrolizidine alkaloids (PAs). In Europe, modern HMPs derived from plants holding PAs contain these compounds only in traces below toxicological relevance. Their legal status of well-established use, based on a proof of efficacy with high levels of evidence according to Evidence Based Medicine, or of traditional use, showing an excellent risk benefit ratio, and belonging to the best established medicines in their indication, indicates already that such products are well observed and also discussed concerning toxicological aspects. The issue of a draft public statement on PAs is therefore in principle welcomed	

GENERAL COMMENTS		
	by Kooperation Phytopharmaka, as traces of PAs may be contained in some important European phytomedicines, but by now successful definitions of limits have been established only on a national level, e.g. in Germany. In the following, detailed remarks are given.	
NIMH	We agree that exposure to pyrrolizidine alkaloids (PA) should be minimized. However we argue that the benefit of certain herbs used by herbal practitioners, with recognized precautions, means that their usage entails an acceptable risk.	

SPECIFIC COMMENTS ON TEXT			
Section number and heading	**Interested party**	**Comment and Rationale**	**Outcome**
2.1 Regulatory/ legal status of PAs or PA-containing products	ESCOP	Aware of the current undefined PA exposure by food the EFSA and the BfR apply the Margin of Exposure (MOE) approach, and state that for 1,2-unsaturated PAs a daily intake of 0.007 µg/kg may be attributed with only a neglible risk [EFSA 2011, BfR 2011]. This limit value is already very low compared to the actual risk of most PAs as it is based on studies with lasiocarpine, a PA of high toxicity which was shown to have the lowest $BMDL_{10}$ when screening various PAs [EFSA 2011]. Additionally, efforts of the Codex Alimentarius Committee show that PA exposure by food can be reduced [Codex Alimentarius Commission 2012]. Against this background, that the MOE approach is already quite pragmatic and PA exposure by food is reducable, limit values for PAs in herbal medicinal products should be established.	See the response to AEGSP above.
Sections 2.4. and 2.5	KOOP PHYTO	The draft of this public statement on pyrrolizidine alkaloids seems not to be complete, as important publications, e.g. Ridker 1985 and Coulombe 2003, are not mentioned. However, this additional literature is helpful to understand that food and medicine should be considered as different sources of PA-intake. Reporting toxicological studies without giving information on NOAELs/NOELs does make it difficult to draw reasonable conclusions based on the available scientific evidence.	Ridker 1985 and Coulombe 2003 were taken into the AR and reference list, even though they did not add any new knowledge. Dose considerations and appropriate level values have been reported whenever they are clearly needed for argumentation.
2.5	ESCOP	It is evident that PAs are toxic agents. However, evaluating	See the response to AEGSP above.

SPECIFIC COMMENTS ON TEXT			
Acute and chronic toxicity in humans		their single dose-toxicity with the studies of Stuart & Bras 1957 and Edgar et al. 2011 is not correct as these studies only presume a correlation of single-dose toxicity and PA intake, and are not evidence-based proved [Stuart et al. 1957, Tandon et al. 1977]. Severe cases of PA poisoning became known after intake of high amounts of PAs and are mostly due to PA exposure by contaminated food [Wiedenfeld et al. 2011]. Lowest known doses associated with acute toxicity in humans are 3 mg PA/kg b.w. per day and 0.8-1.7 mg PA/kg b.w. per day. [EFSA 2011] It is unlikely that the intake of small amounts of PAs for a short period of time would result in such severe poisoning, and limit values for PAs in herbal medicinal products are justifiable from a toxicological point-of-view.	Most concerns do not exist connected to acute toxicity but to cancerogenicity.
2.5 Acute and chronic toxicity in humans	NIMH	While accepting the argument that adverse events resulting from herbal medicines can go undetected, we note that a recent paper on Comfrey reported 5 cases (Mei et al., 2010), one more than the original four cases which drew Comfrey to the attention of the regulatory authorities (Denham, 1996). The fifth case is a detailed case report but a complicated and unclear case (Zuckerman, Steenkamp & Stewart, 2002). Recently in Germany, three cases of VOD have been identified in neonates (Rasenack, Müller, Kleinschmidt, Rasenack & Wiedenfeld, 2003). In the case report, maternal consumption of a cooking herb mix containing comfrey and helioptropium was mentioned. The mixture was shown to contain lycopsamine (diester found in comfrey) but also the macrocyclic diester, integerrimine. Without wanting to minimized the significance of this tragic case, it may indeed have been related to consummtion of macrocyclic diesters. Taking the argument that exposure to PA should be minimized, then the relative benefits of usage of the root and the leaf of Symphytum spp. required consideration. The levels of PA have been shown to be higher in S. x uplandicum and suppliers should ensure that only S. offficinale is used. This can be achieved by correct identification of growing plant materials (Denham, 1996). The levels of PA in the root have been shown to be higher (Couet, Crews & Hanley, 1996). However, analysis of these compounds presents challenges (Liu et al., 2009; Mei et al., 2010; Wuilloud, Gratze,	

SPECIFIC COMMENTS ON TEXT			
		Gamble & Wolnik, 2004), and further work would be required to prepare recommendations for internal usage.	See the response to AEGSP above.
Section 2.7	KOOP PHYTO	The PA-intake by food is indeed unregulated. The uncertainty about the amount of PA intake by food should not lead to insufficient conclusions about well-regulated products like medicines. Medicinal drugs are sources for very limited amounts of PAs or even only traces, and they are used for a limited time period only to treat defined indications and complaints. Therefore, medicinal products are associated with different, and overall not relevant, risk levels for humans. Details on acute and chronic toxicology of pyrrolizidine alkaloids and their derivates and in particular the pathology in humans related to single PAs are missing just as a correlation between available animal data and their relevance for humans.	'Limited' or 'traces' are inadequate expressions in terms of quantitative risk assessment, because potency matters with toxic substances. Quantitative measures are needed for argumentation. Medicinal products are used by individuals and consequently potential consequences affect individuals who should be protected by appropriate science-based limits to exposure. In most of the cases no direct comparison can be done: due to missing intake-data from humans, other isolated PAs used etc.
2.7 Human exposure to PA by food	NIMH	You do not explain that these disastrous events occurred by the combination of contamination of grain and poor nutrition: high intake was associated with a possibly reduced ability to form glutathione conjugates. However, these events are a warning that grain sources should be actively monitored.	Not always – see Schoental R (1954) Senecio alkaloids and liver cancer. Br Med J 1(4857): 335-336, which describes that (at least in some cases) the children were in quite good nutritional state, even though that a lot of cases worldwide are connected with poor nutritional state.
2.7 Human exposure to PA by food	NIMH	If carcinogenicity is taken as a major risk factor, then to set minimum standards is problematic. However, to propose a blanket ban is unrealistic as people can cultivate and consume medicinal plants for themselves. A problem with the recommendations for maximum levels in foods is that determination of the concentration of PAs poses technical challenges. It has been shown that the concentration of PAs found in plant specimens depends on the methodology used, and that care must be taken to ensure that the water-soluble N-oxides are reduced so that the measurement accounts for the total PA concentration (Cao, Colegate & Edgar, 2008; Oberlies et al., 2004).	See the response to AEGSP above.
Section 3	KOOP	By now 350 PAs were described which showed a very different	See the response to AEGSP above.

SPECIFIC COMMENTS ON TEXT			
	PHYTO	level of toxicity. Against this background, a conclusion to avoid any PAs down to the zero-level would not be reasonable. Instead of this the definition of an acceptable limit of PAs would be much more helpful and appropriate. Such a limit has been established in countries like Germany, where it has proven to be suitable regarding the safety of registered medicinal drugs.	
Section 3., Recommenda-tions	KOOP PHYTO	Finally, in the recommendation section of the public statement on HMPs, it is stated that the ALARA-principle should be used. For substantiating this recommendation, IPCS 1988, EFSA 2007 and BfR 2007 are cited. IPCS 1998, which is the 25 years old Inchem-report of WHO, is a good review of the scientific knowledge on PAs at that time, and contains some important general remarks like "there is a need to create awareness", which have in the meantime found attention by EFSA, COT, BfR and other regulatory bodies, which have given recommendations by now. The EFSA recommendation of 2007 is related to the content of PAs in animal food and does not directly contribute to a definition of an acceptable level of intake in humans. With regard to the view of the EFSA regarding PAs, the EFSA-paper from 2011, where Margins of Exposure (MOEs) are presented, seems to be more relevant. In this paper, the existing studies on chronic toxicity and carcinogenicity are rated relevant for the assessment of the toxicity in humans. The MOEs in adults are rated as likely of low concern. It is taken reference on the assessment of the CONTAM-panel, which sees a reason of concern only in toddlers and children consuming large amounts of honey, when doses of up to 114 ng/kg b.w. are consumed. Such doses are orders of magnitude higher than those maximally to be expected from herbal medicines. In the cited paper of BfR 2007, only one short paragraph is related to PAs, where, before the background of the uncertainties of the exact height of exposition from food and feed, it is recommended to eat some food items containing PAs only in limited amounts, and not to consume them regularly in large amounts. A total restriction is only recommended for the addition of gentoxic phytochemical compounds in isolated form to food. This paper therefore does not support a zero tolerance	See the response to AEGSP/BPI above. See the response to AEGSP above.

SPECIFIC COMMENTS ON TEXT			
		approach, but rather contradicts it. The same is true for the second paper of BfR cited in the public statement, which also does not support a zero-tolerance-level for PAs, but defines acceptable levels. Such an approach should also be used to warrant a safe usage of medicinal products. From the BfR report from 2011, it can be concluded, that HMPs when underlying a maximally acceptable level like those in the market in Germany, do not contribute in a toxicologically relevant way to the total exposure of the population to PAs. At the same time, a risk-benefit assessment is possible for these HMPs, which is clearly positive. Therefore, with regard to the HMPC public statement, we would like to recommend to clearly establish the recommendation that the use of the ALARA principle in HMPs means that herbal medicines within well-established limits of PA content are of no relevant toxicological concern, so that the result of a risk benefit assessment of these medicines is clearly positive.	
Recommenda-tions	NIMH	We argue that the benefits of certain herbs used by herbal practitioners, for fixed time periods, is an acceptable risk. Contraindications would include pregnancy, the possibility of pregnancy, persons aged under 18, and concomitant use of any drug or herb which induces CYP450 3A4. We argue that the internal usage of Sympytum officinale is acceptable as it contains diesters only.	The meaning of this sentence is not clear. The benefit-risk ratio for traditional herbal medicines is currently not a scientific concept, because the acceptability of the use of traditional herbal medicines is based solely on the length of the use for a specified indication, not on pharmacological evidence of efficacy.
Recommenda-tions	NIMH	We support the recommendation that there should be more testing on safe usage of external preparations. As regards Symphytum spp, Comfrey, there are variations in PA level between root and leaf, and according to species and time of harvesting. It would be useful to learn more about the relative levels and thus choice of sources for plant material for external preparations.	
Recommenda-tions	NIMH	We support the recommendation to completely restrict oral usage in children, adolescents, pregnant women and nursing mothers. We do not support the recommendation to restrict external use of products containing Symphytum spp. as this would deny the use of an effective remedy for injuries such as bruising to children and adolescents.	See the response to AEGSP above.