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REVIEWS

Honey from Plants Containing Pyrrolizidine Alkaloids: A Potential Threat to Health

JOHN A. EDGAR,*,† ERHARD ROEDER,‡ AND RUSSELL J. MOLYNEUX§

CSIRO Livestock Industries, PB 24, Geelong, Victoria 3220, Australia; Pharmazeutisches Institut der Universitat Bonn, An der Immenburg 4, D-53121 Bonn, Federal Republic of Germany; and Western Regional Research Center, ARS, USDA, 800 Buchanan Street, Albany, California 94710

Following scientific risk assessments, several countries have imposed strict regulations on herbal medicines containing 1,2-dehydro-pyrrolizidine alkaloids. Using published data on the plants used in honey production, pyrrolizidine alkaloid-containing plants are shown in this review to represent a significant source of honey worldwide. This observation, honey consumption data, reported levels of pyrrolizidine alkaloids in honeys, and consideration of tolerable exposure levels determined for pyrrolizidine alkaloids in herbal medicines, leads to the conclusion that some honey is a potential threat to health, especially for infants and fetuses, and further investigation is warranted.

Keywords: Honey; pyrrolizidine alkaloid; food safety; hepatotoxicity

INTRODUCTION

The International Programme on Chemical Safety (IPCS), a joint Programme of the United Nations Environment Programme, the International Labor Organization, and the World Health Organization, has evaluated 1,2-dehydro-pyrrolizidine ester alkaloids (pyrrolizidine alkaloids) and determined that their presence as contaminants in foods is a threat to human health and safety (*1, 2*). In particular, grains contaminated by pyrrolizidine alkaloids have been the cause of very significant episodes of acute human poisoning with high mortality (*1, 3*−*7*). Herbal medicines containing pyrrolizidine alkaloids have also caused liver damage and deaths (*8*−*12*).

In addition to causing acute poisoning, pyrrolizidine alkaloids are also considered to produce delayed, progressive chronic effects, including hepatic cirrhosis, following brief, low level exposure (*1*). Although they are not proven human carcinogens (*13, 14*), pyrrolizidine alkaloids are genotoxic and mutagenic, and they cause cancer in rats (*1, 13*). They may also act synergistically with aflatoxins and hepatitis viruses in causing human liver cancers (*1, 15*).

In keeping with their sweeping distribution across numerous plant families and genera, pyrrolizidine alkaloids exhibit broad structural diversity (*16*). Nevertheless, they can be integrated structurally through the presence of a bicyclic necine base moiety, the most common of which is retronecine (**1**), possessing a hydroxymethyl substituent at position 1, a hydroxyl group at position 7, and unsaturation between positions 1 and 2 (**Figure 1**). Other necine bases may have different bridgehead and substituent stereochemistries, possess different substitution patterns or additional hydroxy groups, or have a saturated double bond. These complexities are further elaborated by esterification of the hydroxyl groups with a variety of acid moieties, known as necic acids.

The largest group of pyrrolizidine alkaloids subclasses is the macrocyclic esters, exemplified by senecionine (**2**) (**Figure 1**), which are particularly widely distributed in the genus *Senecio*, and can attain exceptionally high levels of as much as 18% of the dry weight of the plant. Other major groups are the nonmacrocyclic diesters, such as echimidine (**3**), and the monoesters, esterified either at the primary 9-OH or less frequently at the secondary 7-OH, as in lycopsamine (**4**) or 7-angelyl heliotridine (**5**), respectively. These nonmacrocyclic esters appear to be particularly characteristic of the plant family Boraginaceae. The structural complexities of pyrrolizidine alkaloids are further enhanced by their natural occurrence as oxidized forms, either as *N*-oxides, such as riddelliine *N*-oxide (**6**), which on many occasions may predominate in the plant almost to the exclusion of the corresponding free base form, or as *seco*-alkaloids, exemplified by senkirkine (**7**), in which the bridgehead bond of the necine base moiety has been cleaved and *N*-methylation has occurred.

Irrespective of their structural particulars, pyrrolizidine alkaloids are not significantly toxic to mammals per se. Their hazard as constituents of food or forage arises when the normal oxidative detoxification mechanisms of the liver convert them into pyrrolic metabolites (dehydroalkaloids) (**Figure 2**) (*16*).

* Corresponding author. Tel: 61-3-5227-5739. Fax: 61-3-5227-5555. E-mail: john.edgar@csiro.au.
† CSIRO Livestock Industries.
‡ Pharmazeutisches Institut der Universitat Bonn.
§ Western Regional Research Center.

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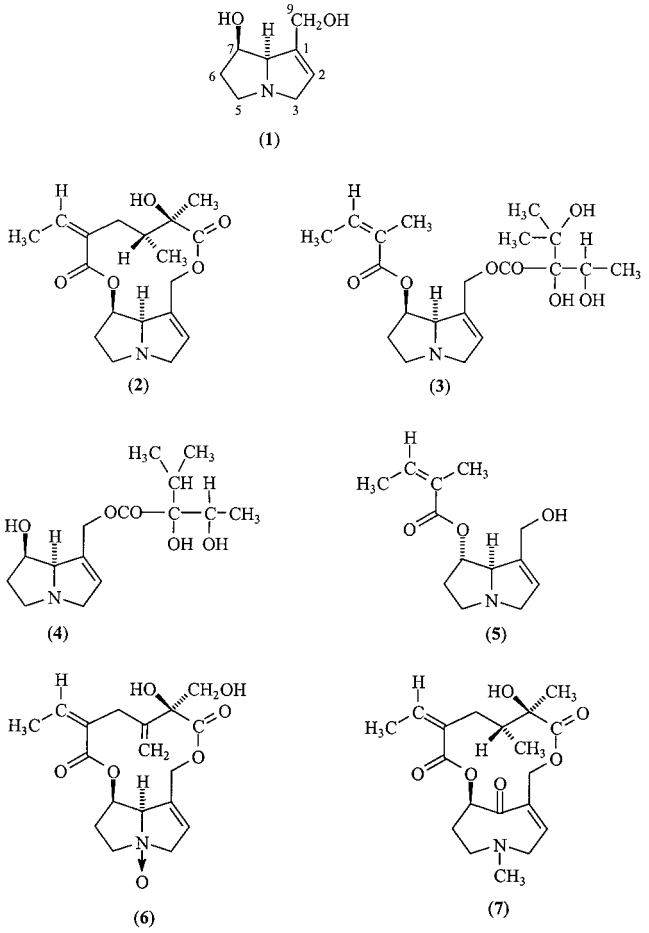

Figure 1. Representative structures of pyrrolizidine alkaloids: (**1**) retronecine (necine base); (**2**) senecionine; (**3**) echimidine; (**4**) lycopsamine; (**5**) 7-angelyl heliotridine; (**6**) riddelliine *N*-oxide ; (**7**) senkirkine.

These highly reactive electrophilic alkylating agents are capable of binding strongly to nucleophilic centers in tissues or cross-linking DNA, leading to hepatotoxicity or carcinogenicity. The necic acid moieties play a role in affecting solubility and transport of the metabolites so that other organs such as the lungs may be affected, rather than the primary target of the liver. Pyrrolizidine alkaloids based upon saturated necine bases cannot undergo oxidation to pyrrolic metabolites, because of the absence of the double bond at the 1,2-position, and can therefore be excluded from consideration as potential hepatotoxins. In theory, the pyrrolizidine alkaloid *N*-oxides should also be nontoxic because they already exist at the same oxidation state as the pyrroles, but in fact they have been shown to be reduced to the corresponding free bases in the digestive tract and, consequently, can be reoxidized to toxic pyrrolic metabolites (*16*). Evaluation of any particular plant as a potential source of toxic pyrrolizidine alkaloids should, therefore, take into account the sum of both free base and *N*-oxide forms of the alkaloids present.

After conducting a scientific risk assessment, using available human and animal toxicity data, the German Federal Health Bureau established regulations in 1992 governing the sale of herbal products containing pyrrolizidine alkaloids (*17*). In herbal products with proven health benefits the dose is restricted to 1 μg of pyrrolizidine alkaloids per day for oral administration or 100 μg of pyrrolizidine alkaloids per day for external use. Use of such products is, however, restricted to a maximum of six weeks per year. The allowed level for pyrrolizidine alkaloids is reduced to 0.1 μg per daily oral dose or 10 μg for external use if the product is to be used for greater than six weeks per year. Herbal products containing pyrrolizidine alkaloids that have no demonstrated health benefits are prohibited by the German regulations.

Table 1. Some Pyrrolizidine Alkaloid-Containing Plants Reported to Contribute to Honey Production in Various Countries

country	plant genus	reference
Albania	*Senecio*	*29*
Argentina	*Echium*	*30*
Austria	*Myosotis*	*31, 32*
Australia	*Echium, Ageratum, Heliotropium*	*27, 33–35*
Brazil	*Senecio, Eupatorium*	*36–39*
Burma	*Chromolaena*	*40*
Canada	*Borago*	*41*
Denmark	*Borago*	*42*
Egypt	*Borago*	*43*
Finland	*Borago*	*44*
Germany	*Borago, Petasites, Myosotis*	*45–47*
India	*Crotalaria, Senecio, Ageratum*	*48–53*
Italy	*Echium, Senecio, Borago, Myosotis,Cynoglossum, Petasites, Tussilago*	*54–61*
Lithuania	*Symphytum*	*62*
Mexico	*Eupatorium, Senecio*	*63*
Morocco	*Echium*	*64*
Netherlands	*Tussilago*	*65*
New Zealand	*Echium*	*66*
Nigeria	*Ageratum, Chromolaena*	*67, 68*
Poland	*Echium, Tussilago*	*69*
Portugal	*Echium*	*70–72*
Senegal	*Crotalaria*	*73*
Somalia	*Eupatorium*	*74*
South Africa	*Echium, Ageratum*	*75, 76*
Spain	*Echium*	*77–97*
Switzerland	*Myosotis, Senecio*	*11, 98–100*
Thailand	*Chromolaena (Eupatorium)*	*101*
Taiwan	*Ageratum*	*102*
Turkey	*Myosotis*	*103*
Ukraine	*Symphytum*	*104*
United Kingdom	*Senecio, Borago, Myosotis*	*28, 105–107*
Uruguay	*Echium*	*108*
USA	*Senecio, Borago*	*26, 109*
USSR (Former)	*Echium, Symphytum, Borago, Cynoglossum*	*110–112*
Venezuela	*Crotalaria*	*113*
Yugoslavia	*Echium*	*114*
Zimbabwe	*Senecio*	*115*

The use of pyrrolizidine alkaloid-containing herbal products by pregnant and lactating women is specifically prohibited by the regulations because fetuses and infants are particularly susceptible to poisoning by pyrrolizidine alkaloids (*1, 9*). The German regulations require all herbal products containing pyrrolizidine alkaloids within the prescribed level to be labeled with the following: "Not to be used in pregnancy and during the lactation period" (*17*). Austria and Switzerland have similar restrictions on exposure to pyrrolizidine alkaloids in herbal products, and comparable regulations may apply to the whole of Europe and elsewhere in due course (*12*). For example, in The Netherlands a maximum level of 0.1 μg of pyrrolizidine alkaloids per 100 g of food (1 ppb) has been suggested (*18*).

Other dietary sources of pyrrolizidine alkaloids include milk (*19−24*), eggs (*25*), and honey (*11, 26−28*). The potential for honey containing pyrrolizidine alkaloids to present a risk to the health of consumers is examined in this review.

DISCUSSION

Plants Containing Pyrrolizidine Alkaloids Used in Honey Production. Table 1 shows some of the pyrrolizidine alkaloid-

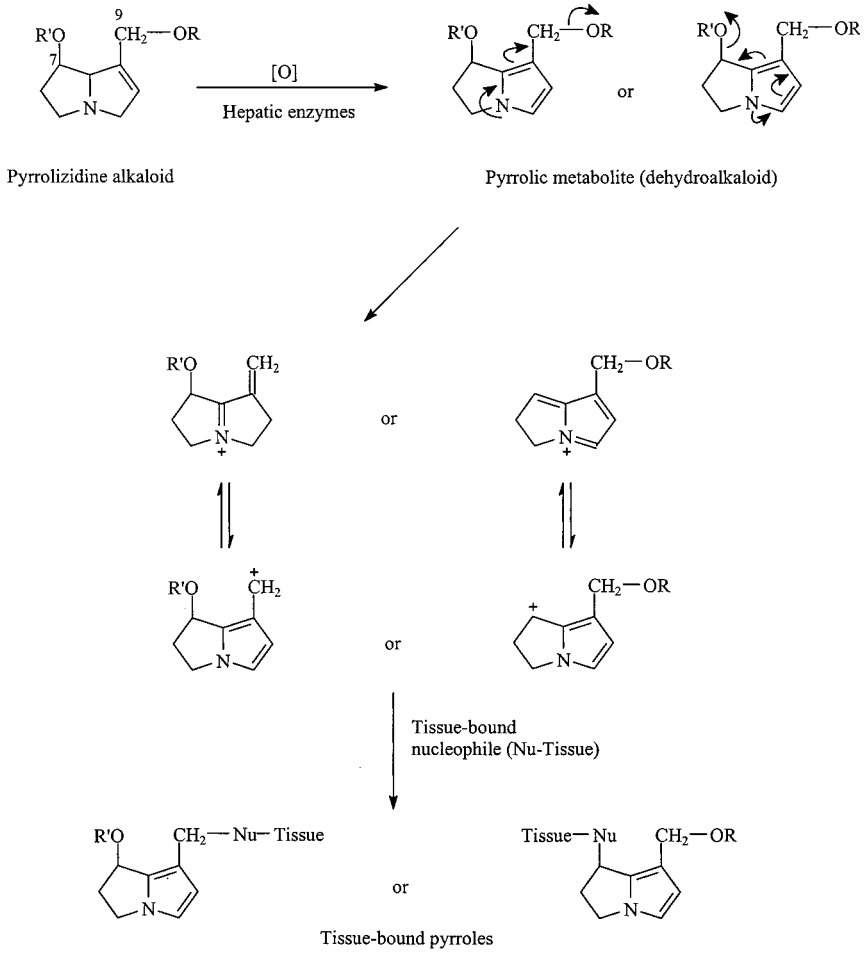

Figure 2. Pathway for conversion of pyrrolizidine alkaloids to reactive pyrrolic metabolites in the liver and subsequent binding to tissue nucleophiles.

containing plants that are recorded in the scientific literature as being sources of honey for human consumption. Generally, the studies cited in **Table 1** were largely undertaken to determine the origin of honeys rather than to determine the specific contribution of pyrrolizidine alkaloid-containing plants to honey production. The data therefore do not provide a comprehensive assessment of all pyrrolizidine alkaloid-containing plants contributing to honey production or the relative importance of such plants to honey production in different regions. The data are biased toward regions where the plant sources of honeys have been scientifically determined, for example by microscopic pollen analysis studies. The countries listed in **Table 1** are, therefore, not necessarily those most likely to produce honeys containing pyrrolizidine alkaloids.

A comprehensive worldwide survey of pyrrolizidine alkaloids in honey is necessary before regions producing honeys containing significant levels of pyrrolizidine alkaloids can be identified. However, careful consideration of the publications cited in **Table 1** leads to the conclusion that plant genera producing pyrrolizidine alkaloids are significant contributors to honey production in many countries. Many of these plants are not native to the countries under which they are listed but are inadvertently introduced weeds or plants deliberately naturalized for use as herbs and for their flowers. The latter may become widely distributed throughout the countryside as garden escapes. The frequent lack of natural predators in areas where such introductions have become established often results in a plant density far greater than that in their native areas and displacement of more desirable native species. Common examples of this phenomenon are the following: common borage (*Borago officinalis*) (**Figure 3**); Paterson's curse (*Echium plantagineum*) (**Figure 4**); tansy ragwort (*Senecio jacobaea*) (**Figure 5**); and, comfrey (*Symphytum officinale*) (**Figure 6**).

Figure 3. Common borage (*Borago officinalis*; Boraginaceae) cultivated in garden, Berkeley, California.

Levels of Pyrrolizidine Alkaloids in Honey. In all cases so far reported in the literature, honeys attributed to a single species of pyrrolizidine alkaloid-containing plants contain the pyrrolizidine alkaloids known to be present in that plant species (*11*, *26*−*28*). The highest level reported for a honey attributed to *Senecio jacobaea* is 3.9 μg of pyrrolizidine alkaloids per gram of honey, but this value was not corrected for extraction efficiency (*26*) so the amount present could have been higher. Crews et al. (*28*), for example, reported recoveries of pyrrolizi-

Figure 4. Paterson's curse (*Echium plantigineum*; Boraginaceae) infesting paddock in Victoria, Australia; the common name indicates the antipathy with which this plant is regarded by livestock producers.

Figure 5. Tansy ragwort (*Senecio jacobaea*; Compositae, tribe Senecioneae) growing in pastureland in New Zealand.

Figure 6. Comfrey (*Symphytum officinale*; Boraginaceae) cultivated in garden (Captain Cook's Cottage), Melbourne, Victoria, Australia.

dine alkaloids from *S. jacobaea* honeys of between 57 and 70%. In a study of *Echium plantagineum* honey, in which the highest recorded level of pyrrolizidine alkaloids was 0.950 μg per g of honey before correction for recovery, the extraction efficiency was considered to be 50% (*27*). Thus, "single species" honeys attributed to the pyrrolizidine alkaloid-containing genera listed in **Table 1** can be expected to contain several micrograms of pyrrolizidine alkaloids per gram of honey.

Honeys produced in nature rarely come from a single plant species even if they are attributed to a single species. In the scientific literature on the origins of honeys, including that cited in **Table 1**, the term "unifloral" is used to specify that a honey comes predominantly from a single species of plant. Unifloral honeys are generally defined as having more than 45% of the total pollen count in the honey confirmed microscopically to be from a single plant species; however, the definition of unifloral is subject to variation depending on the relative amount of pollen produced by different plant species. For example, some pyrrolizidine alkaloid-containing species, such as *Myosotis* spp. or *Cynoglossum officinale*, that produce honeys with high levels of pollens, are considered to be unifloral only when the predominant pollen is more than 90% of the total pollen (*116*).

In considering the literature cited in **Table 1**, we have assumed that honeys reported to be unifloral in respect to plants containing pyrrolizidine alkaloids could have levels of pyrrolizidine alkaloids approaching those in the single-species honeys examined in the references cited above, i.e., they are likely to contain several micrograms of pyrrolizidine alkaloids per gram of honey.

Health Risk from Pyrrolizidine Alkaloids in Honey. Taking into account the maximum quantities of honeys eaten (up to 93 g per day for some adult consumers and 32 g per day for infants; see below), even at the highest level of pyrrolizidine alkaloids thus far found in honey, acute pyrrolizidine alkaloid poisoning is an unlikely outcome for most consumers of honey (*1*). However, levels of pyrrolizidine alkaloids present in honeys from pyrrolizidine alkaloid-containing plants are well above that considered by the German Federal Health Bureau (*17*) to be capable of causing progressive chronic toxicity, especially in infants and fetuses. Although no incidents of pyrrolizidine alkaloid poisoning have been attributed to consumption of honey, the evaluation of pyrrolizidine alkaloids conducted by IPCS concluded that the level of pyrrolizidine alkaloids found in honeys may contribute to chronic liver disease or liver tumors (*1*).

The German risk assessors considered pyrrolizidine alkaloids to be genotoxic carcinogens for which there is theoretically no threshold of toxicity, and any exposure to pyrrolizidine alkaloids could potentially induce a harmful, ultimately fatal, mutation (*17*). It was, however, argued that for herbal medicines proven to be efficacious, some level of exposure to pyrrolizidine alkaloids could be justified. This argument does not apply in the case of a nonessential food such as honey, and another justification for allowing consumers to be exposed to pyrrolizidine alkaloids is needed in this case.

If the herbal medicine regulations on pyrrolizidine alkaloids in Germany were applied to honeys, and there is no logical reason they should not be, then consumption of pyrrolizidine alkaloid-containing honey by pregnant or lactating women would not be allowed. Sale of single-species and unifloral honeys containing pyrrolizidine alkaloids would be severely curtailed because the amount that could be eaten before 1 μg of pyrrolizidine alkaloids per day was exceeded would not be sufficient to justify marketing the product (**Table 2**). If consumption of such honeys continued for more than 6 weeks, to comply with the allowed maximum daily exposure to pyrrolizidine alkaloids of 0.1 μg, the daily intake of honey would be very small indeed (**Table 2**).

Likelihood of Honey Containing Pyrrolizidine Alkaloids. It has been estimated that 3% of the world's flowering plants contain pyrrolizidine alkaloids (*117*). All habitable regions of the world have indigenous and/or introduced pyrrolizidine alkaloid-containing plants. It is, thus, not surprising that there are many records in the scientific literature showing pyrrolizidine alkaloid-containing plants as sources of commercial, single-

Table 2. Amount of Honey Containing Various Concentrations of Pyrrolizidine Alkaloids Typical of Those Reported in the Literature that Can Be Consumed Without Exceeding the German Standards of 1 μg of Pyrrolizidine Alkaloids per Day for a Maximum of Six Weeks, and 0.1 μg per Day for a Longer Period

concentration of pyrrolizidine alkaloids in honey (μg/g)	amount of honey containing 1 μg of pyrrolizidine alkaloids[a]	amount of honey containing 0.1 μg of pyrrolizidine alkaloids[a]
4	0.25 g	0.025 g
3	0.33 g	0.033 g
2	0.5 g	0.05 g
1	1.0 g	0.1 g

[a] Examples of daily honey consumption include 1.3 g per capita in the GEMS/Food regional European diet (*119*) and a maximum daily intake of 93 g by adults in the U.K. (*121*).

species, and unifloral honeys (e.g., *27, 42, 43, 56, 67, 71, 78−80, 82, 89, 90, 93, 101, 107, 108, 111*).

A number of pyrrolizidine alkaloid-containing species are widespread weeds. These include *Chromolaena odorata* (*Eupatorium odoratum*), *Ageratum conyzoides*, *Echium plantagineum*, *Cynoglossum officinale*, and several *Senecio* species. Some of these plants have been promoted and used for honey production without regard to the possibility that they may contain pyrrolizidine alkaloids (e.g., *51, 101, 108*).

Pyrrolizidine alkaloid-containing plants such as *Borago officinalis* and *Echium* spp. are being grown as sources of seed oils that are marketed as health products because they are high in alpha- and gamma-linolenic acids (*118*). Honeybees are used to pollinate the plants, and the honey produced is considered to be a valuable byproduct of this industry (e.g., *41, 107*). Such honeys are likely to contain pyrrolizidine alkaloids and contribute to exposure of consumers to pyrrolizidine alkaloids. It is apparent from this review of the literature that honey from many regions of the world will contain pyrrolizidine alkaloids and a considerable number of honeys may exceed the standards set for pyrrolizidine alkaloid levels in herbal medicines in Germany and other countries.

Potential for Dilution of Pyrrolizidine Alkaloids in Honey to be Below a Toxic Threshold. In many cases pyrrolizidine alkaloid-plants are only contributors to honeys classified as multifloral or being primarily from another floral source. It has been suggested, for example, that French lavender honey be allowed to have a maximum of 30% *Echium* spp. pollen (*88*). When this level of *Echium* pollen is present, the honey is likely to contain significant amounts of pyrrolizidine alkaloids (*27*). However, when pyrrolizidine alkaloid-containing plants are only a very minor source of nectar, it is conceivable that natural dilution, due to honeybees visiting sources of nectar or honeydew devoid of pyrrolizidine alkaloids, may be sufficient to reduce pyrrolizidine alkaloids to tolerable levels.

Honeybees collect nectar from all flowers and honeydew sources in the vicinity of their hives. There is no evidence in the literature to suggest that pyrrolizidine alkaloid-containing plants are not attractive to honeybees, or that the alkaloids adversely affect the bees themselves. To the contrary, honeybees are frequently observed gathering nectar from pyrrolizidine alkaloid-containing plants. It is therefore likely that the pyrrolizidine alkaloid content of a particular honey will reflect the percentage of pyrrolizidine alkaloid-containing plants in the forage area. Given that 3% of flowering plants contain pyrrolizidine alkaloids and that pyrrolizidine alkaloid-producing plants are widely distributed, pyrrolizidine alkaloid-containing honeys will, on average, constitute 3% of all honeys. In reality, the placement of hives could result in honey with a much higher percentage of pyrrolizidine alkaloid-containing honey or none at all. In light of this, it is relevant to determine the approximate percentage of a pyrrolizidine alkaloid-containing honey produced naturally or by post-production blending that would provide a honey meeting the German standards for pyrrolizidine alkaloids in herbal medicines.

Table 3. Effect of Natural or Artificial Dilution on the Amount of Honey Containing the Maximum Permitted Daily Intake of Pyrrolizidine Alkaloids Specified in Germany for Herbal Medicines, viz. 0.1 μg, Using, as an Example, a Typical Honey Containing 2 μg/g of Pyrrolizidine Alkaloids Blended with Different Percentages of Honey from Nonpyrrolizidine Alkaloid Sources

% honey from pyrrolizidine alkaloid source	level of pyrrolizidine alkaloids in blended honey (μg/g)	amount of honey containing 0.1 μg of pyrrolizidine alkaloids (g)[a]
100	2	0.05
50	1	0.1
20	0.4	0.25
10	0.20	0.50
1	0.02	5.0
0.05	0.001[b]	100.00

[a] Examples of daily honey consumption include 1.3 g per capita in the GEMS/Food regional European diet (*119*) and a maximum daily intake of 93 g by adults in the U.K. (*121*). [b] Standard for pyrrolizidine alkaloids under discussion in The Netherlands (*18*).

Table 3 shows the effect of natural or artificial blending of a honey containing a typical concentration of 2 μg pyrrolizidine alkaloids per g with honey from nonpyrrolizidine alkaloid sources, on the amount of honey containing 0.1 μg of pyrrolizidine alkaloids, the Germany standard for pyrrolizidine alkaloid exposure exceeding 6 weeks per year. Dilution to 1% allows 5 g of honey to be consumed whereas dilution to 0.05% is required to produce a concentration of pyrrolizidine alkaloids of 0.1 μg per 100 g being considered in The Netherlands (*18*) (**Table 3**).

This demonstrates that in regions where pyrrolizidine alkaloid-plants are present, even as minor sources of nectar (for example less than 1% of available nectar sources), natural dilution resulting from the contributions of other flora may not be sufficient to bring pyrrolizidine alkaloid concentrations down to levels allowed by herbal medicine regulations.

Exposure to Pyrrolizidine Alkaloids in Honey. Regional diets provided by the Global Environmental Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food) of the World Health Organization (WHO) (*119*) are used internationally in assessing the risk from lifetime exposure to harmful chemicals, particularly pesticides (*119*). For the five regional diets currently available, namely Middle Eastern, Far Eastern, African, Latin American, and European, the per capita daily consumption of honey is 0.8, 0.0, 0.5, 0.3, and 1.3 g per person, respectively (*119*). This indicates that, of these dietary groupings, Europeans are the highest consumers of honey. Assuming that pyrrolizidine alkaloids are distributed homogeneously in the honeys from the different dietary regions, the European diet is likely to result in the greatest risk from exposure to pyrrolizidine alkaloids in honey.

The GEMS/Food regional honey consumption figures are per capita and include both consumers and nonconsumers of honey. These figures therefore underestimate the actual consumption of particular individuals. This is especially true in the case of a food such as honey that is consumed by a minority of people.

Table 4. Percentage of Honey Containing Various Levels of Pyrrolizidine Alkaloids that Must Be Blended with Honey Containing No Pyrrolizidine Alkaloids to Give a Product that Allows Consumption of 1.3 g of Honey per Day (GEMS/Food regional diet per capita consumption figure for Europeans) or 3.9 g of Honey per Day (GEMS/Food regional diet per capita consumption figure for 95th percentile European consumers) without Exceeding a Maximum Tolerable Daily Intake of 0.1 μg of Pyrrolizidine Alkaloids Specified by the German Herbal Regulations

concentration of pyrrolizidine alkaloids in unblended honey (μg/g)	% of blended honey for 1.3 g to contain 0.1 μg of pyrrolizidine alkaloids	% of blended honey for 3.9 g to contain 0.1 μg of pyrrolizidine alkaloids
6	1.3	0.4
3	2.6	0.9
2	3.8	1.3
1	7.7	2.6

National consumption figures for the 95th percentile eaters of honey, when available, provide a more useful measure of likely exposure to pyrrolizidine alkaloids from the consumption of honey. National honey consumption figures for high consumers of honey in the United Kingdom (U.K.) and Australia are, therefore, also used below as examples of exposure of consumers at highest risk from pyrrolizidine alkaloids in honey.

Per capita Exposure to Pyrrolizidine Alkaloids in Honey. Using the GEMS/Food per capita consumption figure for a European diet of 1.3 g of honey per day, consumption of a typical single-species honey or unifloral honey containing 2 μg of pyrrolizidine alkaloids per g, would result in a daily ingestion of 2.6 μg of pyrrolizidine alkaloids. This is well above the German herbal medicine standard of 0.1 μg of pyrrolizidine alkaloids per day.

Consumption of the 95th percentile consumer is considered to be approximately three times the estimated daily intake for a particular food (*120*). Based on the GEMS/Food per capita honey consumption figures, the 95th percentile European consumer would therefore eat 3.9 g of honey per day. Such a consumer would ingest 7.8 μg of pyrrolizidine alkaloids per day from eating honey containing 2 μg of pyrrolizidine alkaloids per g, compared to the maximum tolerable daily intake of 0.1 μg of pyrrolizidine alkaloids per day specified by the German regulations for herbal medicines. **Table 4** also shows the level of dilution needed to ensure that the 95th percentile European consumer does not exceed the German herbal medicine standard for pyrrolizidine alkaloid exposure. For example, honey containing 2 μg per g of pyrrolizidine alkaloids would need to be naturally or artificially diluted to 1.3% with honey from a nonpyrrolizidine alkaloid plant source for such a consumer to avoid exceeding the German herbal medicine standard of 0.1 μg pyrrolizidine alkaloids per day (**Table 4**).

Natural blending of pyrrolizidine alkaloid and nonpyrrolizidine alkaloid honeys occurs when bees from a particular hive visit both pyrrolizidine alkaloid and nonpyrrolizidine alkaloid-containing nectar sources. Therefore, honey from regions of the world where pyrrolizidine alkaloid-containing plants contribute more than 1.3% to honey production are theoretically likely to contain pyrrolizidine alkaloids exceeding levels determined to be tolerable for herbal products in Germany for 95th percentile European consumers eating 3.9 g of honey per day. This assumes honeys from these regions are naturally blended by bees to achieve a uniform 1.3% pyrrolizidine alkaloid-containing honey content, which is unlikely. Thus, some honeys from regions with 1.3% or less of bee forage plants containing pyrrolizidine alkaloids could still exceed the German herbal medicine standards. Given the estimate that 3% of plants produce pyrrolizidine alkaloids (*117*), many regions of the world are likely to produce honeys with levels of pyrrolizidine alkaloids that are hazardous according to the German herbal medicine regulations.

Table 5. Maximum Honey Consumption in the U.K. for Adults, Schoolchildren, and Infants (*121*) Showing the Level of Pyrrolizidine Alkaloids that Would Be Ingested from Honey Containing 2 μg of Pyrrolizidine Alkaloids per g of Honey and the Dilution Required to Meet the German Tolerable Daily Intake of 0.1 μg of Pyrrolizidine Alkaloids

group	honey consumption per day	amount of pyrrolizidine alkaloids ingested	dilution needed to meet German standard
adults	93 g	186 μg	0.05%
schoolchildren	60 g	120 μg	0.08%
infants	32 g	64 μg	0.16%

Exposure of the Highest Consumers of Honey to Pyrrolizidine Alkaloids. As previously mentioned, the amount of honey consumed by some individuals is very much higher than the average daily per capita consumption given in the GEMS/Food regional diets. For example, British infants who eat honey are reported to consume up to 32 g of honey per day, school children consume up to 60 g per day, and adults consume as much as 93 g per day (*121*).

Table 5 summarizes the daily exposure to pyrrolizidine alkaloids in these groups using honey containing 2 μg of pyrrolizidine alkaloids per g as an example. Also shown in **Table 5** is the dilution with honey containing no pyrrolizidine alkaloids that is required to meet the German tolerable daily intake of 0.1 μg of pyrrolizidine alkaloids per day. Similar maximum consumption levels of honey are found in other countries. For example, the 95th percentile consumers of honey in Australia eat 28.6 g per day as 2−4 year old infants, and 13−19 year old consumers in Australia in this category eat 64.2 g of honey per day (*122*).

It is clear that honey can be a significant source of pyrrolizidine alkaloids for consumers of honey and that natural dilution with honey from nonpyrrolizidine alkaloid-containing plants is unlikely to reduce levels of pyrrolizidine alkaloids in honey to tolerable levels according to the German regulations for herbal products. Postproduction blending, should such a process be deemed ethical, of pyrrolizidine alkaloid-containing honey with honey containing no pyrrolizidine alkaloids in order to achieve the German standard is also likely to be problematic. The wide occurrence of pyrrolizidine alkaloid-containing plants may make it difficult to find a sufficient volume of pyrrolizidine alkaloid-free honey in some regions. One aim of the German regulations on pyrrolizidine alkaloids in herbal products is to prevent exposure of infants to pyrrolizidine alkaloids, so that the potential for exposure of infants to pyrrolizidine alkaloids in honey demonstrated here is of particular concern.

Palatability of Honeys containing Pyrrolizidine Alkaloids. It has been said that honey from *Senecio jacobaea* is distasteful, and pure *S. jacobaea* honey is therefore not sold commercially (*26*, *28*). However, honeys containing pyrrolizidine alkaloids are not generally distinguished by unpleasant taste. This characteristic may not be associated with pyrrolizidine alkaloids. For example, honey from *Echium plantagineum* is considered to have no adverse taste qualities (*27*) and *Borago officinale* honey is said to have a bland taste (*107*) and to produce an

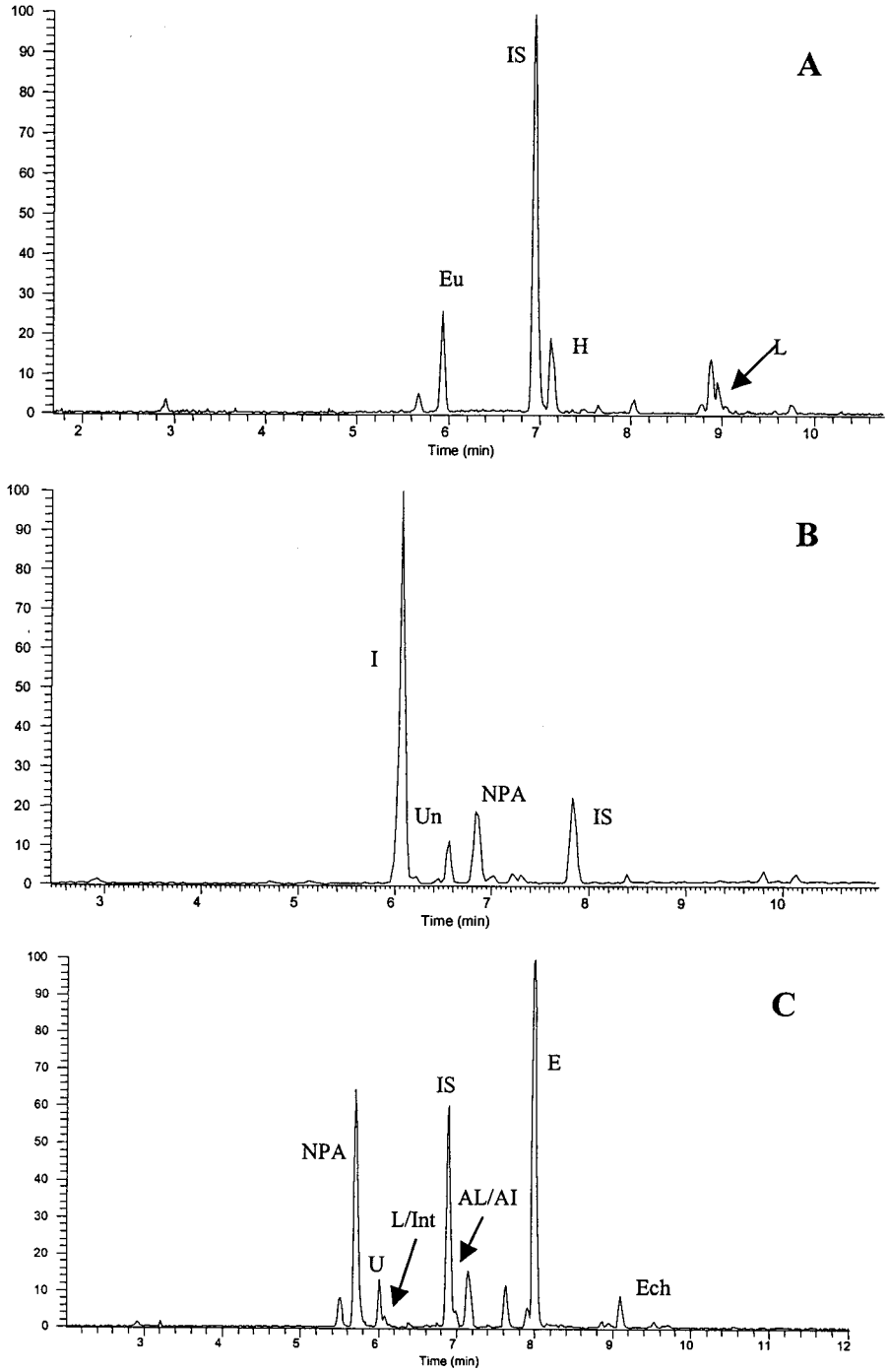

Figure 7. C8 reversed-phase HPLC/APCI-MS of extracts of honey using acetonitrile–water gradients showing pyrrolizidine alkaloids characteristic of **A**, *Heliotropium europaeum*; **B**, *H. amplexicaule*; and **C**, *Echium plantagineum*. Pyrrolizidine alkaloids identified by retention time and mass spectra are echimidine (**3**)(E), echiumine (Ech), europine (Eu), heliotrine (H), lasiocarpine (L), indicine (I), lycopsamine (**4**)/intermedine (L/Int), acetyllycopsamine/acetylintermedine (AL/AI), and uplandicine (U). Unidentified pyrrolizidine alkaloids (Un) and nonpyrrolizidine alkaloid material (NPA) were also observed; internal standard (IS).

excellent honey (*41*). *Eupatorium odoratum* honey is said to have a pleasant aroma and fine flavor (*101*). Even species that produce distasteful honeys, such as *S. jacobaea*, when naturally or deliberately blended with other honeys to less than 1% (as appears necessary to achieve tolerable levels of pyrrolizidine alkaloids) are likely to produce a honey with acceptable organoleptic characteristics.

Analysis for Pyrrolizidine Alkaloids in Honey. To monitor levels of pyrrolizidine alkaloids in honey it is essential that extremely sensitive, accurate analytical methods, capable of handling large numbers of samples if regulations are imposed, be generally available. The exceptional structural diversity of this large group of alkaloids renders this a challenging task relative to the situation pertaining with mycotoxins such as the aflatoxins, where only a few structurally similar compounds need to be quantitated. Numerous methods have been developed for qualitative and quantitative analysis of pyrrolizidine alkaloids (*123*) but these have been applied primarily to detection of the alkaloids in plant samples (in which they generally occur at relatively high levels) and herbal remedies; reports of honey analysis are very limited (*11, 26*−*28*). Although plant analysis may provide a first line of defense against the entry of

pyrrolizidine alkaloids into honey through identification of plant sources that should be eliminated from nectar collection, it cannot control the alkaloids once they have been incorporated into the honey. The analytical problem is compounded by the presence of large amounts of potentially interfering substances such as organic acids, amino acids, and sugars. Organic acids may present a particular problem because they could form salts with the basic alkaloids, necessitating careful pH adjustment prior to extraction to liberate all of the alkaloids. Solid-phase extraction techniques have the potential to further cleanup the honey matrix prior to analysis (*28*).

Gas chromatography, particularly in association with mass spectrometric detection (GC/MS), has been successfully applied to analysis of a wide range of pyrrolizidine alkaloids. Witte et al. (*124*) have demonstrated that ca. 100 underivatized pyrrolizidine alkaloids encompassing diverse structural types can be identified by retention indices (RIs) on two different capillary columns in combination with the molecular ion and group-specific fragmentation patterns. An interlaboratory collaboration showed that such data were sufficient to unequivocally identify the individual alkaloids without the need for standards of each individual alkaloid to be available. Further refinement of this approach should be possible through the use of selected-ion monitoring (SIM). Although GC methods have very high sensitivity for analysis of trace amounts of pyrrolizidine alkaloids, a major defect is the inability to analyze for the *N*-oxides without prior reduction to the free bases, because of their salt-like nature and susceptibility to on-column thermal deoxygenation.

The need to assess both free base and *N*-oxide pyrrolizidine alkaloids, preferably simultaneously, is a crucial problem. High-performance liquid chromatography offers the greatest potential to surmount this, but the significantly different solubility properties of the two alkaloid forms, one being lipophilic and the other being hydrophilic, creates significant difficulties. HPLC separation of a number of macrocyclic pyrrolizidine alkaloid free bases and their corresponding *N*-oxides has been achieved by using an ion-pairing technique that converts all of the alkaloids into ionized forms (*125*). Nevertheless, conventional HPLC methods are restricted by detection problems, with their sensitivity being severely limited by the lack of a significant chromophore in the UV spectrum. The increasing availability of lower-cost LC/MS systems may enable such problems to be circumvented, and in concert with tandem mass spectrometry (LC/MS/MS) could provide the advantages of high sensitivity and analysis of the alkaloids within a complex matrix without prior cleanup. **Figure 7** shows the HPLC/MS analysis of extracts of honey from *Heliotropium europaeum*, *H. amplexicaule*, and *Echium plantagineum*. The chromatograms illustrate the excellent resolution that can be achieved between structurally similar pyrrolizidine alkaloids, and comparison of retention time with standards and characteristic mass spectra, in combination, allows most of the alkaloids to be unequivocally identified.

Immunoassays have a particular advantage in that they are extremely sensitive, capable of detecting most natural products in the ppb range, do not require the use of dedicated, fixed-location equipment, and can ultimately be developed to the point that they can be used by personnel who do not have to be highly skilled. A number of pyrrolizidine alkaloid immunoassays have been described, most of which are specific for a particular alkaloid or show cross-reactivity to a small group of alkaloids having similar structure (*126*−*130*). A class-specific immunoassay utilizing one of the necine bases, retronecine, has also been reported (*131*). More recently, it has been demonstrated that the problem of detection of both free base and *N*-oxide forms of the same alkaloid can be overcome (*132*). The successful application of immunoassays to honey analysis will depend on the development of a single method, or at least a very limited number, capable of detecting all pyrrolizidine alkaloids likely to be found in honey samples from diverse, worldwide sources.

IMPLICATIONS

The data presented in this review suggest that all honeys, regardless of their origin, need to be assessed for their pyrrolizidine alkaloid content to ensure they are not contributing to dietary pyrrolizidine alkaloid exposure and consequent genetic damage or other pyrrolizidine alkaloid-related adverse health effects in consumers. Where possible, sources of pyrrolizidine alkaloids should also be avoided in the production of honey.

If pyrrolizidine alkaloid ingestion from honey is shown to be substantially above the German standards for herbal products, but there is no evidence that honeys containing pyrrolizidine alkaloids cause adverse health outcomes then this should be considered in future risk analysis and in the determination of tolerable daily intakes for pyrrolizidine alkaloids. It may be necessary, in the case of foods such as honey, to set a level for pyrrolizidine alkaloids that is "as low as reasonably achievable", as is done with aflatoxins and some other genotoxic mycotoxins (*133*).

Considering that grains, milk, eggs, and meat can also be sources of pyrrolizidine alkaloids exceeding the level allowed in herbal products in Germany and other countries (*25*), it would be of value to determine total dietary pyrrolizidine alkaloid exposure in different regions of the world to see if this correlates with observed health effects associated with pyrrolizidine alkaloid ingestion, including childhood cirrhosis, veno-occlusive disease, and liver cancer.

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