

# Toxicity of pyrrolizidine alkaloids to humans and ruminants

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**Abstract** 1,2-dehydro pyrrolizidine ester alkaloids (PA) are toxic for human and livestock. The PAs undergo a metabolic toxification process in the liver which is the first target organ for PA poisoning. World-wide many episodes of PA intoxications have been reported involving humans as well as ruminants. This intoxication is not only related to the amount and duration of the exposure to PAs but also to species, age and gender. Besides the metabolic toxification, detoxication processes are also important. The paper discusses the toxification and detoxication processes and gives an overview about PA poisoning cases in humans and ruminants.

**Keywords** Metabolic toxification · Detoxication · Poisoning in humans · Poisoning in ruminants

Thirteen plant families are reported to contain pyrrolizidine alkaloid (PA)-producing species. PAs possessing a 1,2 double-bond in their base moiety

(necine) are hepatotoxic, carcinogenic, genotoxic, teratogenic and sometimes pneumotoxic (IPCS 1988). PAs have been estimated to be present in about 3% of all flowering plants (Smith and Culvenor 1981). The toxicity of PA-containing plants from many plant genera, mainly of the families Asteraceae, Fabaceae and Boraginaceae, is well known (Bull et al. 1968).

The fact that *Senecio* and other genera containing toxic PAs are hazardous for livestock has been known for a long time: In 1903/1904 Gilruth established that tansy ragwort (*Jacobaea vulgaris* syn. *Senecio jacobaea* L.) produces chronic liver disease in livestock (Gilruth 1903, 1904; Bull et al. 1968). This was extended by investigations which showed that PAs were responsible and that ingestion of all PA-producing plants led to similar diseases (Bull et al. 1968). It is also well established that PAs are not only hazardous for livestock but also for humans: in the 1920s a widespread liver disease in South Africa was shown to be caused by the consumption of bread contaminated with seeds from *Senecio* species (Willmott and Robertson 1920; Steyn 1933). About 60 years ago it was also established that in the former USSR different endemic liver diseases also resulted from the consumption of PA-contaminated bread; the plant source for this PA contamination was found to be *Heliotropium lasiocarpum* (Boraginaceae; Bourkser 1947; Milenkov and Kizhaikin 1952). Many intoxications have been reported in Central Asia, all derived from PA contaminated cereals:

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1975/1976 in Afghanistan 8,000 people were affected by a contamination from *Heliotropium popovii* subsp. *gillianum*, 3,000 of them were seriously poisoned and many died (Tandon and Tandon 1975; Mohabbat et al. 1976). Similarly 4,000 people were hospitalized in Tajikistan by *Heliotropium lasiocarpum* contamination of grain in 1992 (Chauvin et al. 1994; Mayer and Lüthy 1993).

In 1989 the International Program on Chemical Safety (IPCS), a joint agency of WHO, FAO and ILO, summarized then current knowledge with the statement that “consumption of contaminated grain or the use of PA-containing plants as herbal medicine, beverages, or food by man, or grazing on contaminated pastures by animals, may cause acute or chronic disease” (IPCS 1989). In the last three decades different episodes of human intoxications by PA-containing medicinal plants have also been reported (Roeder 1995, 2000) and, in these cases, children in particular were effected due to their higher susceptibility to PA intoxication (IPCS 1988, 1989).

Awareness that herbal medicinal preparations can be hazardous for humans has led to extensive investigations on herbal teas or other extracts from medicinal plants which are suspected to contain toxic PAs (IPCS 1988). This awareness increased especially after it was shown that liver diseases in Jamaica and the West Indies, that were reported in the 1950s, were caused by so-called “bush-teas” containing PAs (Bras et al. 1961; Brooks et al. 1970). Also in Africa and parts of some other tropical or subtropical countries the use of herbal preparations containing PA-producing plants for the treatment of several diseases is common and this can be considered as an important reason for the liver diseases reported in those areas (Druckrey 1965; Schoental 1972).

While PA poisoning is mainly a problem in developing countries because there the use of traditional medicine is common, within the last 25 years, especially in industrialized countries, the use of herbal medicine has become more and more common due to an increased interest of people in alternative medicine, hand in hand with a greater influence of the “green wave”.

In Western countries like the EU, UK and USA many alternative medicine practitioners claimed that traditional medicines show only benefits without undesired side-effects. This has led to increasing fatal

intoxications being reported caused by the use of herbal products, e.g. comfrey, that contain toxic PAs.

Other potential sources of human exposure to PAs have been observed: Milk, for example, has been shown to contain PAs when milk-producing animals consume PA-containing plant material (Schoental 1959; Dickinson et al. 1976; Dickinson 1980; Johnson et al. 1978; Goeger et al. 1982; Lüthy et al. 1983; Candrian et al. 1984; Molyneux and James 1990). Human milk has also caused liver diseases in neonates and infants (Roulet et al. 1988).

Honey was shown to be another source of PA exposure; here it seems that the contamination may be due to the plant pollen, which is rich in PAs, being transferred by bees into the honey (Deinzer et al. 1977; Culvenor et al. 1981; Roeder 1995, 2000; Edgar et al. 2002; Beales et al. 2004; Boppré et al. 2005; Betteridge et al. 2005). Eggs from poultry exposed to PAs in PA-contaminated grain were also shown to be a possible source of PA exposure for humans (Edgar and Smith 1999).

Recently it was shown in Germany that salads can sometimes be contaminated with PA-containing plants (BfR 2007a). It was found that, especially in supermarkets, ready-packed rucola salads and also salad mixtures were contaminated by *Senecio vulgaris*, a typical weed of field-crops.

The use of medicinal plants or preparations of them are controlled by regulations in a number of countries: The IPCS confirmed in 1988 a possible health risk by PA-containing medicinal plants (IPCS 1989). For Australia and New-Zealand the ANZFA/FSANZ recommends that, on account of a PA-derived veno-occlusive disease (VOD), a daily dosage of 1 µg/kg bw toxic PA is tolerable (ANZFA 2001). According to ANZFA/FSANZ, the carcinogenic potential of PA demonstrated in rodents was not considered as established in humans. In contrast, from the results of an investigation with the PA ridelliine, which causes tumors in rodents, it was concluded that, based on the identical metabolism in human liver microsomes, this tumor-induction should also occur equally in humans (Xia et al. 2003). The USA FDA required all PA-containing Comfrey/*Symphytum*-preparations to be withdrawn from the market; based on the available scientific data, the FDA saw no possibility for a level of PAs where a possible human risk could be excluded (FDA 2001). In the EU the EFSA determined that

uptake of toxic PA produces VOD and that the carcinogenic potential of PAs is demonstrated in rodents but not yet proven in humans. For food and other sources of a PA-contamination and especially for milk, with respect to its use in high amounts in the food of neonates and children, more scientific data are required (EFSA 2007). In Germany the German Federal Department of Health established regulations in 1992, that allows specified herbs and preparations from them that may contain toxic PA, provided that the total daily dose of PA is less than 1 µg, the duration of treatment is not longer than 6 weeks and that those products are not used during pregnancy and breast-feeding; a use longer than 6 weeks reduces the daily dosage to 0.1 µg; these regulations are also applied for homeopathic descriptions up to D6 (Bundesanzeiger 1992). Austria has banned all PA-containing products from the market (Bundesgesetzblatt 1994). In The Netherlands it has been decided that all herbal preparations and extracts of PA-containing plants are limited to 1 µg of PAs/kg or 1 µg of PAs/l in the final product and this limit also applies to food (Staatsblaad 2001).

While several countries specify maximum levels of PA-containing seeds in grain for human and livestock consumption, most countries do not specify a maximum level of PAs in either food or fodder. In the EU the so-called “zero-tolerance principle” can be applied; this principle is used in cases where no safe or tolerable level can be determined based on available, valid scientific data, or if insufficient toxicological data are available. Due to their genotoxic and carcinogenic potential this principle can be applied for PA in food and fodder (BfR 2007b).

As already mentioned the presence of toxic PAs in herbal medicinal products and in food and fodder has taken on increased importance in industrialised countries. For example, in Middle Europe common groundsel (*Senecio vulgaris*, L.) and tansy ragwort (*Jacobaea vulgaris* syn. *Senecio jacobaea*, L.) are of particular concern. Common groundsel, a common field-weed, has been found as a contaminant in salads and tansy ragwort has been discussed recently on account of its extensive expansion into pastures and meadows which has led to a great number of intoxications in livestock (mainly horses), especially in Germany (<http://www.jacobskreuzkraut.de>; <http://www.izn.niedersachsen.de/servlets/download?C=39412784&L=20>).

As well as the hazard for grazing animals (i.e. direct toxicity), the possibility of contaminated hay and silage and transfer of PAs into foods such as milk and milk products is under investigation and considered to be a severe problem.

## Toxicity of pyrrolizidine alkaloids

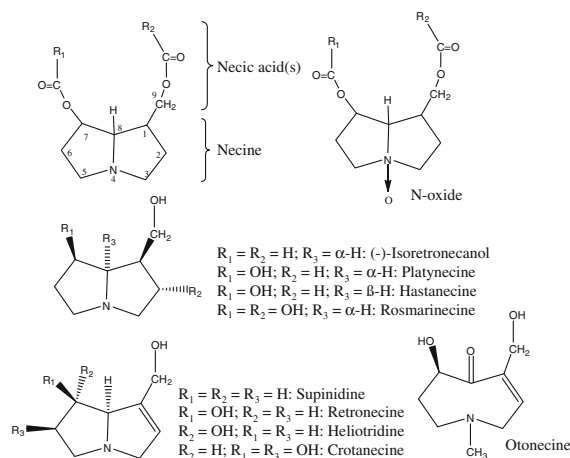
### Metabolic toxification of PA

PAs produced by *Senecio*-species are ester alkaloids derived mainly from the necines retronecine and otonecine (Fig. 1). They are carcinogenic, mutagenic, genotoxic, fetotoxic and teratogenic.

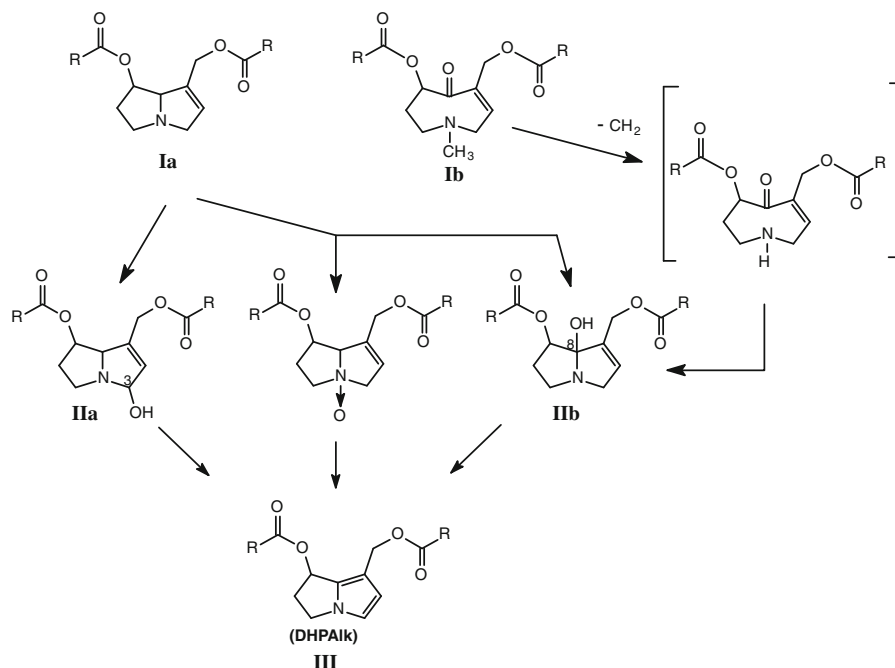
The relative toxicity of individual PAs is determined by their liver metabolites and also physical properties such as lipophilicity, hydrophilicity and pharmacokinetics. PAs prior to metabolic activation show a more or less low acute toxicity but in vivo they undergo a metabolic toxification process in the liver, which is, as a result, the first target organ for the toxicity.

This toxification process is well investigated (Mattocks 1968; Culvenor et al. 1969, 1971; Jago et al. 1970; Mattocks and White 1971a, b; Mattocks 1972; IPCS 1989).

After oral uptake and absorption of the PA (Fig. 2Ia, Ib), an hydroxyl-group is introduced adjacent to the nitrogen-atom in the necine (position 3 or 8) by the cytochrome P-450 monooxygenase enzyme complex in the liver (Fig. 2IIa, IIb).



**Fig. 1** Structures of necines occurring in PA



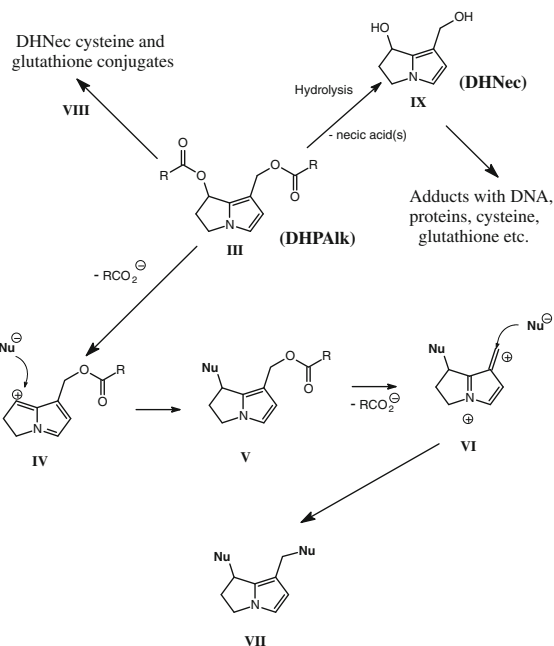
**Fig. 2** Enzymatic hydroxylation and didehydropyrrolizidine products

These Hydroxy-PAs (OHPAs) are unstable and undergo a rapid dehydration to the didehydropyrrolizidine alkaloids (DHPAlk; Fig. 2**III**). This dehydration results in a second double-bond in the necine followed by spontaneous rearrangement to an aromatic pyrrole-system **III**.

PAs occur mainly as their N-oxides in the plants and these cannot be directly converted to the OHPA, but on oral ingestion they are reduced by the gut enzymes or the liver microsomes and NADH or NADPH to the free bases and therefore they show equal toxicity to that of the free bases (Mattocks and White 1971a, b; Powis et al. 1979; Chou et al. 2003; Wang et al. 2005a, b, c).

Otonecine-type PAs (Fig. 2**Ib**) are metabolised to the OHPAs (Culvenor et al. 1971; Lin et al. 1998, 2000). These otonecine-PAs possess a methyl-function at the nitrogen and a quasi keto-function at the bridge-carbon 8. After hydroxylation of the N-methyl-group it is lost as formaldehyde leaving an NH-function which undergoes condensation with the C8 keto group to produce product **IIb** (Fig. 2) which spontaneously dehydrate to the DHPAlk **III**.

The metabolites **III** are able to generate stabilized carbonium ions (Fig. 3**IV**, **VI**) by loss of hydroxy groups or ester functions as hydroxyl or acid anions.



**Fig. 3** DHPAlk and carbonium ion building

These carbonium ions can react rapidly with nucleophiles (Fig. 3**VII**).

In the case of necine-diester (as shown in **Ia** and **Ib**, Fig. 2), typical of *Senecio* species, the formation

of the reactive carbonium ions is facilitated because the necic acid groups provide good leaving groups that facilitate rapid formation of the carbonium ions **III** in high yield. Where one of the hydroxy groups at C7 or C1 of the necine is not esterified formation of the carbonium ions is not as spontaneous. In this case the carbonium ions are most readily formed after protonation of the hydroxyls and loss of H<sub>2</sub>O (Culvenor et al. 1970).

In vivo the metabolites **IV** and **VI** react rapidly with nucleophilic mercapto, hydroxyl and amino groups on proteins and the amino groups of purine and pyrimidine bases in nucleosides like DNA and RNA. The resulting alkylated products show abnormal functions and in the case of DNA, mutations are possible. As this metabolic toxification takes place in the liver it is obvious that this organ is the first target for the intoxication leading to the veno-occlusive disease (VOD) in which the veins of the liver are narrowed (Bull et al. 1968; Prakash et al. 1999). Typical macrocyclic diester PAs (like senecionine, seneciophylline, retrorsine and senkirine which are PAs commonly found in *Senecio*-species) have been shown to produce liver damage due to cross-linking of DNA (Curtain and Edgar 1976; Hincks et al. 1991; Kim et al. 1995, 1999; Pereira et al. 1998; Coulombe et al. 1999; Yan et al., 2002; Fu et al., 2002, 2004; Xia et al. 2006). In the case of PA-monoesters (e.g. derived from the necine supinidine, which lacks a C7 hydroxyl (Fig. 1), cross-linking is not possible and they show a lower toxic potential. It has also been shown that the nucleophilic activity at C7 is higher than at C9 resulting in the primary nucleophilic attack at C7 followed by attack at C9 (Fig. 3; Mattocks 1986).

As shown in Fig. 3 the DHPAlks can also react with SH groups found in more soluble, less critical components like glutathione and cysteine (Fig. 3**VIII**). High levels of glutathione and cysteine therefore reduce the toxic potential of PAs (Cheeke and Gorman 1974; Nigra and Huxtable 1992; Reed et al. 1992; Lin et al. 1998).

Furthermore, hydrolysis can take place where the DHPAlks (Fig. 3) yield dehydronecines alcohols (DHNecs; Fig. 3**IX**) which are more water-soluble and less reactive like the DHPAlks but still display a moderate level of alkylating activity (Peterson and Jago 1980; Robertson 1982). This higher water-solubility and lower reactivity can lead to escape

from the liver tissue and subsequent reaction in other organs (Peterson et al. 1972; IPCS 1989; Prakash et al. 1999). It has been shown that a single exposure of DHPAlk can form macromolecular adducts that release DHNecs over a longer period which leads to on-going health problems (Peterson et al. 1972; Prakash et al. 1999). DHNecs like dehydroretronecine and dehydroheliotridine have also been shown to produce rhabdomyosarcoma, skin, liver and lung tumours (Allen et al. 1975; Shumaker et al. 1976; Johnson et al. 1978; Mattocks and Cabral 1982; Peterson and Culvenor 1983).

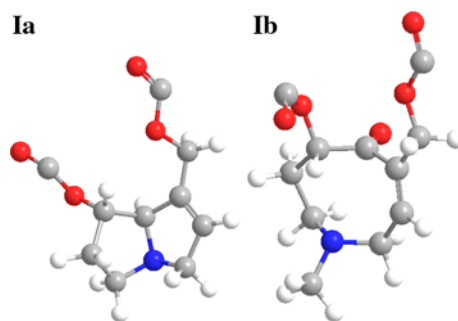
#### Toxicity and structural aspects

As mentioned before, the key didehydropyrrolizidine metabolites (e.g. **III**) are also generated from pyrrolizidine alkaloids of the otonecine type (Fig. 2**b**).

These otonecine derivatives do not show a C8–N bond but possess a keto function at C8 and a methyl group at the nitrogen atom. It is surprising therefore that these seco alkaloids are of identical toxicity to alkaloids of type **Ia** (1,2-dehydro-retronecine and heliotridine esters).

For energetic reasons, these seco compounds could be expected to occur in a different necine conformation than type **Ia** compounds: the missing C8–N bond should lead to a stable 8-membered macrocycle which may be expected to hinder the metabolism via an intermediate to **IIb** (Fig. 2).

Molecular modelling experiments support this assumption and show the energy minimised structure as depicted in Fig. 4 (energy minimisation: Chem 3D ultra; V. 10.0; Cambridge Soft).



**Fig. 4** Molecular modelling of PA from type **Ia** and **Ib**

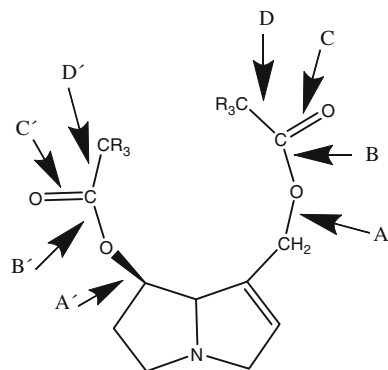
Interpretation of the X-ray structure analysis data helps to explain the identical toxicity of pyrrolizidine alkaloids of type **Ia** and **Ib**: In all nine otonecine-type alkaloids measured to date a necine conformation was found which is identical to those found in those having the C8–N bond (type **Ia**).

Both the distances between C8 and N are similar and equal values can be found for the plane angles built between plane C1–C3–N–C8 and plane C7–C5–N–C8 ( $\sim 125^\circ$ ). This indicates that the seco 1,2-dehydroesters do not exist in the optimal, energy minimised form (**Ib**, Fig. 4) but are of an equal conformation to those of type **Ia**, which finally enables the metabolism as shown in Fig. 2.

Furthermore, X-ray data show that the dedihydro metabolites (**III**, Fig. 2) derived from 1,2-dehydrodiester have a higher toxic potential compared with the low or missing toxicity of the monoesters of retronecine or heliotridine (Fig. 1). A possible detoxification mechanism is the hydrolysis of the ester bindings by esterases and the subsequent building of dehydronecine (IX, Fig. 3) which—due to their higher water solubility—can be easily excreted renally.

Disassociation to C7 as well as to C9 carbonium ions (especially the speed and therefore the rate of this cleavage) and the subsequent reaction with nucleophiles are seen as a key step determining the level of toxicity. The potential for alkylation and adduct formation of PA esters is indicated by analysing the X-ray data. Interpretation of the bond lengths shown in Fig. 5 leads to the following results:

In pyrrolizidine alkaloids of type **Ia** and **Ib** (1,2-dehydropyrrolizidine diesters) the C–O bonds A and A' occur in a normal range of 1.45 Å; the following



**Fig. 5** Bond characterisation of PA diesters

C–O bonds B and B' are considerably shortened, whereas the keto functions C and C' show a moderate shortening. The C–C bonds D and D' show—similar to A and A'—normal values of about 1.54 Å.

In contrast to these findings, the data for monoesters with retronecine or heliotridine give evidence of a different situation: here, the A and A' (C9 as well as C7) bonds are elongated and the corresponding keto bonds C or C' are of nearly ideal length (1.20 Å for keto-functions).

These results show that in 1,2-dehydro diesters the ester functions are a conjugated system leading to the conclusion that the bonds C9–O and C7–O (=A and A') are the target breaking points within the molecule, which makes possible a quick and easy carbonium ion formation and further reaction with nucleophiles as described before.

Contrary to that, in pyrrolizidine alkaloids of 1,2-dehydro monoesters more stable ester bonds are found (i.e. no conjugation) which leads to the fact that the building of the didehydro metabolites is more difficult and time-consuming. In this case, a hydrolysis by esterases can take place in higher amounts what leads to the detoxication via the free necines and necic acids, which explains the missing or generally lower toxicity of 1,2-dehydro monoester (see below).

These findings show that X-ray structure analysis data give evidence of the toxic potential of a single pyrrolizidine alkaloid and that furthermore, by interpreting these data, the extent of this toxic potential can be estimated.

#### Metabolic detoxication of PA

As well as the metabolic activation, detoxication of PA also occurs in vivo: hydrolysis of the ester bonds in PA from type **Ia** or **Ib** by esterases leads to necic acids and to the free necines. Both are non-toxic products and—on account of their higher water-solubility—can be renally excreted. The rate of hydrolysis is dependent on the level of steric hindrance of the ester linkages (see before); and it has been shown that the more highly branched, the necic acids are the more resistance to hydrolysis (Bull et al. 1968; Culvenor et al. 1976; Mattocks 1986). This means, that macrocyclic diesters (**Ia** and **Ib**) with more complex acid moieties are more hazardous on account of their lower rate of hydrolytic detoxication.

The N-oxides of PAs (the form occurring most commonly in plant sources) are highly water soluble and can therefore be excreted renally. Besides their natural occurrence, N-oxidation of PAs also takes place in the liver and can be seen as a detoxification process (Fig. 2; Mattocks 1968; Jago et al. 1970; Mattocks and White 1971b; Williams et al. 1989; Miranda et al. 1991). However, it has been shown that the N-oxides—besides excretion—can be converted by dehydration or by acetylation followed by elimination of acetic acid to the DHPAlk (Fig. 2III; Mattocks 1986; Culvenor et al. 1970) and, as previously mentioned, following oral ingestion they are converted in the gut and liver to the free base form.

Ruminants display another very effective detoxication process. Their rumens contain microorganisms that, by hydrogenolysis, cleave the C9 ester linkage of PAs to release the necic acids and produce 1-methylene pyrrolizidine metabolites, both of which are non-toxic (Lanigan and Smith 1970; Lanigan 1971). In some species (goats, sheep, cows) this rumen metabolism is very efficient and these species are, as a consequence, highly resistant to PA intoxication.

In conclusion it can be stated that the toxicity level of different PAs in non-ruminants is dependent on three aspects:

- The efficiency of metabolic activation to form the key-intermediate III (Fig. 2).
- The efficiency of ester hydrolysis to form non-toxic and water soluble necines and necic acids.
- The efficiency of N-oxidation and excretion via urine.

For ruminants the activity of PA-destroying rumen microbes makes them more resistant than monogastric species to PA poisoning. These considerations provide an explanation for the fact that the toxicity of PAs is different in different species and individuals: e.g. while horses are very sensitive to PA poisoning sheep, goats and cattle are much more resistant and tolerate much higher PA dosages. As has been shown in many experiments, the metabolic pathway of the PA intoxication and detoxication seems to be the same in all animals and also in humans (IPCS 1988) it can be assumed that relative rates of PA activation and PA detoxication in the different species and in different individuals determines the different susceptibilities to PA poisoning.

## PA toxicity in human

PA poisoning of humans can be characterised in three dose-related levels: acute, sub-acute and chronic. These levels can be progressive resulting ultimately in irreversible chronic toxic effects (McLean 1970; Peterson and Culvenor 1983; IPCS 1988; Huxtable 1989; Prakash et al. 1999; Fu et al. 2004; Stegelmeier et al. 1999).

Acute poisoning is characterised by haemorrhagic necrosis, hepatomegaly and ascites; death is caused by liver failure due to necrosis and liver dysfunctions (Peterson and Culvenor 1983; IPCS 1988; Huxtable 1989; Prakash et al. 1999).

Sub-acute levels are characterised by hepatomegaly and recurrent ascites; endothelial proliferation and medial hypertrophy leading to an occlusion of hepatic veins, resulting in the so-called veno-occlusive disease (VOD) which can be seen as a characteristic histological sign for PA poisoning (Peterson and Culvenor 1983; IPCS 1988; Huxtable 1989; Prakash et al. 1999; Fu et al. 2004). The VOD causes centrilobular congestion, necrosis, fibrosis and liver cirrhosis, the end-stage of chronic PA intoxication.

As well as the liver VOD, other organs can be affected by PAs. It has been shown that the pyrrolic metabolites (DHPAlks and DHNecs) can escape from the liver into pulmonary arterioles where they can produce damage similar to the VOD-changes in the liver (Huxtable 1989). It has been shown that from 62 tested PAs all can produce (dose-dependent) lung lesions (Culvenor et al. 1976) and it is speculated that pulmonary damages can result from long-term and low-level exposure to PAs (IPCS 1989; Huxtable 1989).

PA intoxication in humans is not only related to the amount and the duration of the exposure but also to age and gender: males react more sensitively than females and fetuses and children (especially neonates or infants) show the highest sensitivity for PA poisoning (IPCS 1988): in 2003 it was shown that the daily uptake of  $\sim 7 \mu\text{g}$  PA (from a herbal tea containing comfrey) during pregnancy did not show a toxic effect in the mother's liver but damaged the foetal liver in this way that the new born child died after 2 days (Rasenack et al. 2003).

It has also been observed that cofactors can exacerbate PA poisoning: liver damaging agents, bacterial or viral infections but also medical drugs

like barbiturates or metals like copper or mycotoxins like aflatoxins can increase the severity and likelihood of PA liver-damage (Yee et al. 2000; Newberne and Rogers 1973; White et al. 1973; Tuchweber et al. 1974; Lin et al. 1974; Bull et al. 1968).

There are a large number of reports in the literature about different liver diseases (mainly VOD) possibly connected with PA poisoning. But in most cases the connection cannot be proven because the outbreak of the liver disease and a possible ingestion of PA-containing material is often separated by a long time period.

The following table therefore lists only those cases where a source of PAs was identified and the liver disease was therefore undoubtedly caused by PA intoxication (Table 1).

#### PA toxicity in ruminants

The intoxications of cattle by PA poisoning is very well established. Over more than a hundred years numerous reports about intoxications of livestock can be found in the literature and—as the reports in literature show us—it is a problem world-wide (Bull et al. 1968).

Not surprisingly, given the ubiquity of cytochrome P450 enzymes involved in the activation of PAs and the high chemical reactivity of the DHPalks metabolites produced, more than 26 different animal species (including spiders and insects) have been shown to be adversely affected by toxic PAs. Within this series some animal species are of major importance because their products are used as food which can become contaminated by PAs and hazardous for human. Besides contamination of honey produced by bees visiting PA-plants (Deinzer et al. 1977; Culvenor et al. 1981; Roeder 1995; Edgar et al. 2002; Beales et al. 2004; Betteridge et al. 2005), the transfer of PAs into milk and into products such as cheese, yoghurt, etc., which are consumed in high amounts by humans, are of particular concern. Neonates and small children especially show a high toxic risk because milk is the main part of their food and furthermore they show an extreme susceptibility to PA intoxication.

Thus, the PA intoxications of cattle, sheep and goats should be considered as a particularly severe and problematic health risk. Farmers should be made aware of this hazardous aspect and should be obliged

to control and manage their pastures and meadows to ensure the absence of PA containing plant. As well as direct ingestion of PA-containing plants in rangeland and pasture, there is also the possibility of contamination of fodder: reports can be found where the intoxication was due to contaminated straw, hay or silage. It has been shown that PAs are not destroyed during production of straw and hay and that these products retain toxicity over a very long period. In the case of silage, however, a significant reduction of the PA level is observed. It can be assumed that during the fermentation process, some of the PAs are enzymatically decomposed. Our findings show that under normal conditions a reduction of the PA content down to 20% is possible during production of silage.

An important aspect in this context is that ruminants show a low susceptibility for PA intoxication. This means that calves, sheep and goats need very high doses of PA for an acute intoxication; leading to the observation that the reports about ruminant PA poisonings are mainly describing sub-acute or chronic toxicity because the PA level in contaminated fodder or the distribution of PA-containing plants on pastures and meadows does not often reach a level causing acute toxicity (Molyneux et al. 1988).

This leads to the conclusion that ruminants rarely show acute poisoning and generally progress from sub-acute to chronic toxic effects. The first symptoms observed seem to be loss of appetite, depression, wandering, uncoordinated walking and diarrhea; a disease pattern which over a long period and in different countries has been observed and variously named “Winton disease”, “Walking disease” or “Pictou Cattle Disease” (Bull et al. 1968). The behavioural symptoms have been attributed to poor liver function affecting the brain and the behavior of the animals.

Table 2 below gives a far from complete overview of reports of PA intoxications in ruminants (not feeding experiments) and indicates the range of plant species responsible. PA poisoning is the most common plant-associated poisoning disease of domestic livestock worldwide (Prakash et al. 1999).

Besides these field cases of PA poisoning, reports exist of many feeding experiments in cattle, sheep and goats (see Bull et al. 1968). The data produced can give information on dosage levels leading to intoxication, however, often the duration of the

**Table 1** Human intoxications

Location and year	Affected people	Observed damage	Source of PA	Lit.
South Africa, 1920	11 Adult people	Abdominal pain, vomiting, cirrhosis	<i>Senecio illicifolius</i> , <i>S. burchelli</i>	Willmott and Robertson (1920)
Jamaica, 1954	23 Adults	VOD	Bush-teas with <i>Crotalaria fabva</i>	Bras et al. (1961)
South Africa, 1968	15 Children; 10 died	VOD	Bush-teas; <i>Crotalaria</i> sp.?	Freiman et al. (1968)
Venezuela, 1969	5 Years old girl	VOD	<i>Crotalaria anagyroides</i> , <i>C. pumila</i> consumed as infusion and as vegetable soup	Grases and Beker (1972)
Kuwait, 1970	Adults	Liver carcinoma	<i>Heliotropium ramosissimum</i> ("Ramram")?	Macksad et al. (1970)
Jamaica, 1970	6 Children	VOD	Bush-tea from <i>Crotalaria</i> and <i>Senecio</i> sp.	Brooks et al. (1970)
Iraq, 1970	9 Children	VOD	Food contaminated by a <i>Senecio</i> spec.	Al-Hasany and Mohamed (1970)
Afghanistan, 1970–1972	7,200 People	VOD	Wheat contaminated with <i>Heliotropium popovii</i> , ssp. <i>gillianum</i>	Mohabbat et al. (1976)
India, 1973	486 People	VOD	Cereals contaminated with <i>Crotalaria</i> spp.	Tandon et al. (1976)
Ecuador, 1973	Woman	VOD	Herbal tea with <i>Crotalaria juncea</i>	Lyford et al. (1976)
India, 1973, 1975	4 Male people	Endemic ascites	Millet contaminated with <i>Crotalaria</i> spp.	Krishnamachari et al. (1977)
China, 1973, 1978	2 Adults	VOD	<i>Gynura segetum</i>	Hou et al. (1980)
India, 1974–1977	6 People	VOD	<i>Heliotropium eichwaldii</i>	Datta et al. (1978)
Martinique, 1975	2 Children	VOD	Bush-teas with <i>Crotalaria retusa</i> and/or <i>Heliotropium</i> sp.	Saint-Aimé et al. (1977)
USA, 1976, 1977	4 Children	Vein congestion and necrosis of liver	<i>Senecio longilobus</i>	Stillman et al. (1977)
UK, 1976	Woman	VOD	Maté (Paraguay tea) contaminated with PA of unknown origin	McGee et al. (1976)
USA, 1984	49 Year old woman	VOD	Food supplement containing <i>Symphytum</i> spp. root	Ridker et al. (1985)
China, 1985	4 Women	VOD	Herbal tea containing <i>Heliotropium lasiocarpum</i>	Culvenor et al. (1986)
Switzerland, 1985	59 Years old man and 27 years old son	VOD	Herbal tea consisting of <i>Senecio</i> spp.	Margalith et al. (1985)
Switzerland, 1986	5 Days old baby	VOD	Herbal tea containing <i>Tussilago farfara</i> consumed during whole pregnancy	Roulet et al. (1988)
UK, 1986	13 Years old boy	VOD	Herbal tea containing <i>Symphytum</i> spp.	Weston et al. (1987)
Tadjikistan, 1992, 1993	3,906 People	Abdominal pain, hepatomegaly, ascites, alteration of consciousness	<i>Heliotropium lasiocarpum</i>	Chauvin et al. (1993)
Peru, 1994	38 Year old woman	VOD	Herbal tea from <i>Senecio tephrosioides</i>	Tomioaka et al. (1995)

Table 1 continued

Location and year	Affected people	Observed damage	Source of PA	Lit.
Spain, 1995	73 Years old man	VOD	<i>Senecio vulgaris</i>	Sansado et al. (1995)
Austria, 1995	18 Month old boy	VOD	Herbal tea with <i>Adenosyyles alliariae</i>	Sperl et al. (1995)
Argentina, 1999	23 Years old woman	VOD	Herbal tea containing <i>Senecio vulgaris</i>	Vilar et al. (2000)
Germany, 2002	Foetus	VOD	<i>Symphytum</i> spp.	Rasenack et al. (2003)

feeding experiments is too short to give clear evidence for absolute dosages and long-term (chronic) toxicity. Reports are cited below where, for a single experiment, toxic effects are connected with a specific dosage of a PA or PA mixture from a single plant species.

In 1975 sheep were individually fed with 100 g dried *Senecio jacobaea* plant material for 20 weeks. Mortality was observed after 11, 18 and 46 weeks (Mortimer and White 1975). As *S. jacobaea* normally contains about 0.5% PA it can be assumed that the daily uptake was about 500 mg of alkaloid. Goeger and coworkers fed 1.2–4.04 kg/kg bw of *Senecio jacobaea* to goats. They found 1.2 kg/kg bw of tansy ragwort produced chronic toxicity; that means the animals could take up about 100% of their initial body weight of this plant. It was shown that goats were much more resistant to PA intoxication than cattle (always 5–20% tansy ragwort of initial body weight were tolerated; Goeger et al. 1982). A similar experiment compared toxicity in sheep and goat: Dried plant material of *Heliotropium ovalifolium* was fed to the animals in a dosage of 10 and 5 g/kg bw daily. All goats died after a total uptake of 1–5 kg plant material while the sheep survived about 20 kg plant material (Abu Damir et al. 1982). Also goats were used and fed daily 10 g/kg bw of *Crotalaria saltiana* up to a total dose of 0.5 kg plants. The animals showed hepatocellular necrosis and fibrosis (Mes et al. 1984). An interesting result gave a sheep-feeding experiment where *Echium plantagineum* was added to the diet in 4 periods each 12 weeks long; the PA content in the diet was estimated between 0.03 and 0.1% and no effect on the liver function was observed (Culvenor et al. 1984). Similar results were found by feeding a total dose of 81.4 mg/kg bw to calves and 105.6 mg/kg bw to sheep, respectively. While the sheep showed no toxicity the calves developed hepatopathy (Craig et al. 1986). The lethal dose for *S. riddellii* in goats was found to be 400 g plant material over a period of 20 days what means 15 mg/kg bw of total PA (Johnson et al. 1985). Low toxic effects are found by feeding *Senecio vernalis* over a period of +100 days with an application of totally 12.1 kg (Hippchen et al. 1986). *Senecio jacobaea* was given to calves in an amount of 1.3 kg/day (~3 mg/kg bw PA) what resulted in megalocytosis of the animals after 182 days (Molyneux et al. 1988). Also to calves 45 mg/kg bw of PA

**Table 2** Overview of some PA intoxications in ruminants

Location and year	Affected animal	Source of PA
Albania		
1995	Cattle	<i>Senecio subalpinus</i>
Argentina		
1994	Cows	<i>Senecio selloi</i>
Australia		
1962	Sheep	<i>Echium plantagineum</i>
1968	Sheep	<i>Crotalaria mucronata</i>
1972	Heifers	<i>Heliotropium europaeum</i>
1985	Calves	<i>Heliotropium europaeum</i>
1987	Cattle	<i>Heliotropium amplexicaule</i>
1987	Sheep	<i>Echium plantagineum</i> , <i>Heliotropium europaeum</i>
1991	Heifers	<i>Senecio lautus</i>
1997	Cattle	<i>Heliotropium europaeum</i>
Bhutan		
1994	Yaks	<i>Senecio raphanifolius</i> , <i>S. biligulatus</i> , <i>Ligularia</i> spp.
Brazil		
1987	Cows, steers, heifers, calves	<i>Senecio brasiliensis</i> , <i>S. selloi</i> , <i>S. heterotrichius</i> , <i>S. crispatinus</i> , <i>S. leptilobus</i>
1987	Bovines	<i>Senecio brasiliensis</i> , <i>S. selloi</i>
1988	Bovines	<i>Senecio brasiliensis</i> , <i>S. selloi</i>
1993	Cows, heifers, steers	<i>Senecio tweediei</i>
2001	Sheep	<i>Crotalaria retusa</i>
2005	Sheep	<i>Senecio brasiliensis</i>
Canada		
1969	Heifers	<i>Senecio jacobaea</i>
Mexico		
1982	Sheep	<i>Senecio sanguisorbe</i>
Russia		
1979	Calves	<i>Cynoglossum officinale</i>
Sudan		
1981	Calves	<i>Crotalaria saltiana</i>
Switzerland		
1980	Cattle	<i>Senecio alpinus</i>
The Netherlands		
2002	Cattle	<i>Senecio jacobaea</i>
UK		
1917	Cattle	<i>Senecio jacobaea</i>
Uruguay		
1978	Cattle	<i>Senecio brasiliensis</i>
USA		
1962–1963	Heifers	<i>Amsinckia intermedia</i> , <i>Senecio vulgaris</i>
1989	Calves	<i>Cynoglossum officinale</i>

(from *Senecio riddellii*) were fed over 20 days what led to the typical VOD with a mortality of 100% (Molyneux et al. 1991). Another experiment with calves showed that a single dose of 60 mg/kg bw PA (from *Cynoglossum officinale*) killed the animals within 48 h; where a dosage of 15 mg/kg bw daily for 21 days was tolerated; the animals were killed after 35 days and a hepatocellular necrosis was found (Baker et al. 1991). *Senecio oxyphyllus* was used for an experiment where a dosage from 0.5 to 4 g/kg bw of plant material was fed to cattle; 1 g/kg bw for 60 days of plant material was estimated to be the toxic range (Driemeier and Barros 1992).

Based on these data it is not possible to establish a concrete lethal dose of particular PAs for each ruminant species, the dosages and especially the duration of administration are too variable. On the other hand these experiments give clear evidence for the fact that indeed the susceptibility for PA intoxication is different in individual species: in case of ruminants it is decreasing from cattle (which seem to show the highest sensitivity) to goats and sheep which are the most resistant animals discussed in this overview presumably due in large part to their different rumen microflora.

## References

- Abu Damir H, Adam SEI, Tartour G (1982) The effects of *Heliotropium ovalifolium* on goats and sheep. *Br Vet J* 138:463–472
- Al-Hasany M, Mohamed AS (1970) Venous-occlusive of the liver in Iraq. *Arch Dis Childhood* 45:722–724
- Allen JR, Hsu I-C, Carstens LA (1975) Dehydroretronecine induced rhabdomyosarcomas in rats. *Cancer Res* 35:997–1002
- ANZFA (2001) Pyrrolizidine alkaloids in food. A toxicological review and risk assessment. Technical report series no. 2. ANZFA, Canberra
- Baker DC, Pfister JA, Molyneux RJ, Kechele P (1991) *Cynoglossum officinale* toxicity in calves. *J Comp Path* 104:403–409
- Beales KA, Betteridge K, Colegate SM, Edgar JA (2004) Solid phase extraction and LCMS analysis of pyrrolizidine alkaloids in honeys. *J Agric Food Chem* 52:6664–6672
- Betteridge K, Cao Y, Colegate SM (2005) An improved method for extraction and LCMS analysis of pyrrolizidine alkaloids and their N-oxides in honey: application to *Echium vulgare* honeys. *J Agric Food Chem* 53:1894–1902
- Boppré M, Colegate SM, Edgar JA (2005) Pyrrolizidine alkaloids of *Echium vulgare* honey found in pure pollen. *J Agric Food Chem* 53:594–600
- Bourkser GV (1947) On the question of the aetiology and pathogenesis of toxic hepatitis with ascites (heliotrope toxicosis). *Hyg Sanit* 6:24–26
- Bras G, Brooks SEH, Watler DC (1961) Cirrhosis of liver in Jamaica. *J Pathol Bacteriol* 82:503–511
- Brooks SEH, Miller CG, McKenzie K, Audretsch JJ, Bras G (1970) Acute veno-occlusive disease of the liver. *Arch Path* 89:507–520
- Bull LB, Culvenor CCJ, Dick AT (1968) The pyrrolizidine alkaloids. North Holland Publishing Co., Amsterdam
- Bundesamt für Risiokobewertung (2007a) Salatmischung mit Pyrrolizidinalkaloid-haltigem Greiskraut verunreinigt; Stellungnahme Nr.028/2007 des BfR vom 10. Januar 2007
- Bundesamt für Risiokobewertung (2007b) Nulltoleranzen in Lebens- und Futtermitteln—Positionspapier des BfR vom 12.März 2007, Berlin, Germany
- Bundesanzeiger (1992) June 17, 4805 (cited by: Dtsch. Apoth. Ztg., 132, 1406–1408)
- Bundesgesetzblatt (1994) Verordnung des Bundesministers für Gesundheit und öffentlicher Dienst vom 5. Mai 1989 betreffend Arzneimittel, die nicht in Verkehr gebracht werden dürfen. 555/1994 Wien, Österreich
- Candrian U, Lüthy J, Graf U, Schlatter CH (1984) Mutagenic activity of the pyrrolizidine alkaloids seneciphylline and senkirikine in *Drosophila* and their transfer into rat milk. *Food Chem Toxicol* 22:223–225
- Chauvin P, Dillon J-C, Moreu A, Talbak S, Barakaev S (1993) Heliotrope poisoning in Tadjikistan. *Lancet* 341:1633
- Chauvin P, Dillon J-C, Moren A (1994) Épidémie d' intoxication alimentaire à l' héliotrope, Tadjikistan, Novembre 1992-Mars 1993. *Cahiers Santé* 4:263–268
- Cheeke PR, Gorman GR (1974) Influence of dietary protein and sulphur amino-acid levels on the toxicity of *Senecio jacobaea* (tansy ragwort) to rats. *Nutr Rep Int* 9:197–207
- Chou MW, Wang YP, Yan J, Yang YC, Berger RD, Williams LD, Doerge DR, Fu PP (2003) Riddelliine N-oxide is a phytochemical and mammalian metabolite with genotoxic activity that is comparable to the parent pyrrolizidine alkaloid riddelliine. *Toxicol Lett* 145:239–247
- Coulombe RA, Drew GL, Stermitz FR (1999) Pyrrolizidine alkaloids crosslink DNA with actin. *Toxicol Appl Pharmacol* 154:198–202
- Craig AM, Blythe LL, Lassen ED, Slizeski ML (1986) Resistance of sheep to pyrrolizidine alkaloids. *Isr J Vet Med* 42:376–384
- Culvenor CCJ, Downing DT, Edgar JA, Jago MV (1969) Pyrrolizidine alkaloids as alkylating and antimetabolic agents. *NY Acad Sci* 163:837–847
- Culvenor CCJ, Edgar JA, Smith LW, Tweeddale HJ (1970) Dihydropyrrolizines. III. Preparation and reactions of derivatives related to pyrrolizidine alkaloids. *Aust J Chem* 23:1853–1867
- Culvenor CCJ, Edgar JA, Smith LW, Jago MV, Peterson JE (1971) Active metabolites in the chronic hepatotoxicity of pyrrolizidine alkaloids, including otonecine esters. *Nature New Biol* 229:255–256
- Culvenor CCJ, Edgar JA, Jago MV, Outteridge A, Peterson JE, Smith LW (1976) Hepato- and pneumotoxicity of pyrrolizidine alkaloids and derivatives in relation to molecular structure. *Chem Biol Interact* 12:299–324

- Culvenor CCJ, Edgar JA, Smith LW (1981) Pyrrolizidine alkaloids in honey from *Echium plantagineum* L. J Agric Food Chem 29:958–960
- Culvenor CCJ, Jago MV, Peterson JE, Smith LW, Payne AL, Campbell DG, Edgar LA, Frahn JL (1984) Toxicity of *Echium plantagineum* (Paterson's Curse). I marginal toxic effects in merini wethers from long-term feeding. Aust J Agric Res 35:293–304
- Culvenor CCJ, Edgar JA, Smith LW, Kumana CR, Lin HJ (1986) Heliotropium lasiocarpum Fisch and Mey identified as cause of veno-occlusive disease due to a herbal tea. Lancet 1:978
- Curtain CC, Edgar JA (1976) The binding of dehydroheliotridine to DNA and the effect of it and other compounds on repair synthesis in main and satellite band DNA. Chem Biol Interact 13:243–256
- Datta DV, Khuroo MS, Mattocks AR, Aikat BK, Chhuttani PN (1978) Herbal medicines and veno-occlusive disease in India. Postgrad Med J 54:511–515
- Deinzer ML, Thomson PA, Burgett DM, Isaacson DL (1977) Pyrrolizidine alkaloids: their occurrence in honey from tansy ragwort (*Senecio jacobaea* L.). Science 195:497–499
- Dickinson JO (1980) Release of pyrrolizidine alkaloids into milk. Proc West Pharmacol Soc 23:377–379
- Dickinson JO, Cooke MP, King RR, Mohamed PA (1976) Milk transfer of pyrrolizidine alkaloids in cattle. J Am Vet Med Assoc 169:1192–1196
- Driemeier D, Barros CSL (1992) Intoxicação Experimental por *Senecio oxyphyllus* (Compositae) em Bovinos. Pesq Vet Bras 12:33–42
- Druckrey H (1965) Ein karzinogenes Lebergift in der Nahrung der Bantus. Med Klin 60:1558
- Edgar JA, Smith LW (1999) Transfer of pyrrolizidine alkaloids into eggs: food safety implications. In: Tu AT, Gaffield W (eds) Natural and selected synthetic toxins biological implications, chap. 8. ACS symposium series 745. American Chemical Society, Washington, DC, pp 118–128
- Edgar JA, Roeder E, Molyneux RJ (2002) Honey from plants containing pyrrolizidine alkaloids: a potential threat to health. J Agric Food Chem 50:2719–2730
- EFSA (2007) Opinion of the scientific panel on contaminants in the food chain of the European Food Safety Authority (EFSA) on a request from the European Commission related to pyrrolizidine alkaloids as undesirable substances in animal feed. EFSA J 447:1–51
- FDA (2001) FDA advises dietary supplement manufacturers to remove comfrey products from the market
- Freiman I, Schmaman A, Zamit R, Appleberg M (1968) Veno-occlusive disease of the liver—some new aspects. S Afr Med J 42:126–129
- Fu PP, Xia Q, Lin G, Chou MW (2002) Genotoxic pyrrolizidine alkaloids mechanisms leading to DNA adduct formation and tumorigenicity. Int J Mol Sci 3:948–964
- Fu PP, Xia Q, Lin G (2004) Pyrrolizidine alkaloids—genotoxicity, metabolism enzymes, metabolic activation, and mechanism. Drug Metabol Revs 36:1–55
- Gilruth JA (1903) Hepatic cirrhosis affecting horses and cattle (so-called “Winton disease”). New Zealand Department of Agriculture, 11th annual report. pp 228–279
- Gilruth JA (1904) Hepatic cirrhosis due to ragwort (*Senecio jacobaea*). Div Vet Sci New Zealand Dept Agric Bull 9:252–254
- Goeger DE, Cheeke PR, Schmitz JA, Buhler DR (1982) Toxicity of tansy ragwort (*Senecio jacobaea*) to goats. Am J Vet Res 43:252–254
- Grases PJ, Beker SG (1972) Veno-occlusive disease of the liver—a case from Venezuela. Am J Med 53:511–516
- Hincks JR, Kim HY, Segal HJ, Molyneux RJ, Stermitz FR, Coulombe RA (1991) DNA cross-linking in mammalian cells by pyrrolizidine alkaloids: structure-activity relationships. Toxicol Appl Pharmacol 111:90–98
- Hippchen C, Entzeroth R, Roeder E, Greuel E (1986) Experimentelle Untersuchungen zur Lebertoxizität von Senecioalkaloiden aus *Senecio vernalis* an Ziegen. Der Praktische Tierarzt 67:322–324
- Hou JG, Xia YT, Yu CS, An Y, Tang YH (1980) Veno-occlusive disease of the liver—with two fatal cases. Zhonghua Neike Zazhi 19:187–191
- Huxtable RJ (1989) Human health implications of pyrrolizidine alkaloids and herbs containing them. In: Cheeke PR (ed) Toxicants of plant origin, vol 1. CRC Press, Boca Raton, pp 42–86
- International Programme on Chemical Safety (IPCS) (1988) Pyrrolizidine alkaloids. Environmental health criteria 80. WHO, Geneva
- International Programme on Chemical Safety (IPCS) (1989) Pyrrolizidine alkaloids health and safety guide. Health and safety guide no. 26. WHO, Geneva
- Jago MV, Edgar JA, Smith LW, Culvenor CCJ (1970) Metabolic conversion of heliotrine based pyrrolizidine alkaloids to dehydroheliotridine. Mol Pharmacol 6:402–406
- Johnson WD, Robertson KA, Pounds JG, Allen JR (1978) Dehydroretrotronecine-induced skin tumours in mice. J Natl Cancer Inst 61:85–89
- Johnson AE, Molyneux RJ, Stuart LD (1985) Toxicity of Riddell's groundsel (*Senecio riddellii*) to cattle. Am J Vet Res 46:577–582
- Kim H-Y, Stermitz FR, Coulombe RA (1995) Pyrrolizidine alkaloid-induced DNA-protein cross-links. Carcinogenesis 16:2691–2697
- Kim H-Y, Stermitz FR, Li JK, Coulombe RA (1999) Comparative DNA cross-linking by activated pyrrolizidine alkaloids. Food Chem Toxicol 37:619–625
- Krishnamachari KAVR, Bhat RV, Krishnamurthi D, Krishnaswamy K, Nagarajan V (1977) Aethiopathogenesis of endemic ascites in Surguja district of Madhya Pradesh. Indian J Med Res 65:672–678
- Lanigan GW (1971) Metabolism of pyrrolizidine alkaloids in the ovine rumen. III The competitive relationship between heliotrine metabolism and methanogenesis in rumen fluid in vitro. Aust J Agric Res 22:123–130
- Lanigan GW, Smith LW (1970) Metabolism of pyrrolizidine alkaloids in the ovine rumen. Aust J Agric Res 21:493–500
- Lin JJ, Liu C, Svoboda DJ (1974) Long-term effects of aflatoxin B<sub>1</sub> and viral hepatitis on marmoset liver: a preliminary report. Lab Invest 30:267–278
- Lin G, Cui Y, Hawes EM (1998) Microsomal formation of a pyrrolic alcohol glutathione conjugate of Clivorine firm evidence for the formation of a pyrrolic metabolite of an

- otonecine-type pyrrolizidine alkaloids. *Drug Metabol Disposit* 26:181–184
- Lin G, Cui Y, Hawes EM (2000) Characterization of rat liver microsomal metabolites of clivorine, an hepatotoxic otonecine-type pyrrolizidine alkaloid. *Drug Metabol Disposit* 28:1475–1483
- Lüthy J, Heim TH, Schlatter CH (1983) Transfer of [<sup>3</sup>H] pyrrolizidine alkaloids from *Senecio vulgaris* L. and metabolites into rat milk and tissues. *Toxicol Lett* 17:283–288
- Lyford CL, Vergara GG, Moeller DD (1976) Hepatic Venooclusive disease originating in Ecuador. *Gastroenterology* 70:105–108
- Macksad A, Schoental R, Coady A (1970) The hepatotoxic action of a traditional bedu plant remedy “Ramram”. *J Kwt Med Assoc* 4:297–299
- Margalith D, Heraief C, Schindler AM, Birchler R, Mosimann F, Aladjem D, Gonvers JJ (1985) Venooclusive disease of the liver due to the use of tea made from *Senecio* plants. *J Hepatol* 1(Suppl):280
- Mattocks AR (1968) Toxicity of pyrrolizidine alkaloids. *Nature (London)* 217:723–728
- Mattocks AR (1972) Acute hepatotoxicity and pyrrolic metabolites in rats dosed with pyrrolizidine alkaloids. *Chem Biol Interact* 5:227–242
- Mattocks AR (1986) Chemistry and toxicology of pyrrolizidine alkaloids. Academic Express, London
- Mattocks AR, Cabral JRP (1982) Carcinogenicity of some pyrrolizidine alkaloid metabolites and analogues. *Cancer Lett* 17:61–66
- Mattocks AR, White INH (1971a) Pyrrolic metabolites from nontoxic pyrrolizidine alkaloids. *Nat New Biol* 231:114–115
- Mattocks AR, White INH (1971b) The conversion of pyrrolizidine alkaloids to dihydropyrrolizine derivatives by rat-liver microsomes in vitro. *Chem Biol Interact* 3:383–396
- Mayer F, Lüthy J (1993) Heliotrope poisoning in Tadjikistan. *Lancet* 342:246–247
- McGee JOD, Patrick RS, Wood CB, Blumgart LH (1976) A case of venooclusive disease of the liver in Britain associated with herbal tea consumption. *J Clin Path* 29:788–794
- McLean EK (1970) The toxic actions of pyrrolizidine (*Senecio*) alkaloids. *Pharmacol Rev* 22:429–483
- Mes B, Sei A, OH O (1984) Effects of *Crotalaria saltiana* on Nubian goats. *Vet Hum Toxicol* 26:476–480
- Milenkov SM, Kizhaikin Y (1952) Toxic hepatitis with ascites. In: Proceedings of symposium on V. M. Molotov medical institute, Tashkent
- Miranda CL, Chung W, Reed RE, Zhao X, Henderson MC, Wang JL, Williams DE, Buhler DR (1991) Flavin-containing monooxygenase: a major detoxifying enzyme for the pyrrolizidine alkaloid senecionine in guinea pig tissues. *Biochem Biophys Res Commun* 178:546–552
- Mohabbat O, Srivastava RN, Younos MS, Sediq GG, Menzad AA, Aram GN (1976) An outbreak of hepatic venooclusive disease in north-western Afghanistan. *Lancet* 308:269–271
- Molyneux RJ, James LF (1990) Pyrrolizidine alkaloids in milk: thresholds of intoxication. *Vet Hum Toxicol* 32:S94–S103
- Molyneux RJ, Johnson AE, Stuart LD (1988) Delayed manifestation of *Senecio*-induced pyrrolizidine-alkaloidosis in cattle: case reports. *Vet Hum Toxicol* 30:201–205
- Molyneux RJ, Johnson AE, Olsen JD, Baker DC (1991) Toxicity of pyrrolizidine alkaloids from *Ridell* groundsel (*Senecio riddellii*) to cattle. *Am J Vet Res* 52:146–151
- Mortimer PH, White EP (1975) Toxicity of some composite (*Senecio*) weeds. In: Proceedings of 28th N.Z. weed and pest control, pp 88–91
- Newberne PM, Rogers AE (1973) Nutrition, monocrotaline and aflatoxin B<sub>1</sub> in liver carcinogenesis. *Plant Food Man* 1:23–31
- Nigra L, Huxtable RJ (1992) Hepatic glutathione concentrations and the release of pyrrolic metabolites of the pyrrolizidine alkaloid, monocrotaline, from the isolated perfused liver. *Toxicol* 30:1195–1202
- Ortiz Cansado A, Crespo Valadés E, Morales Blanco P, Sáenz de Santamaria J, Gonzales Campillejo JM, Ruiz Téllez T (1995) Enfermedad venooclusiva hepática por ingestión de infusiones de *Senecio vulgaris*. *Gastroenterol Hepat (Barcelona)* 18:413–416
- Pereira TN, Webb RL, Reilly PE, Seawright AA, Prakash AS (1998) Dehydromonocrotaline generates sequence-selective N-7 guanine alkylation and heat and alkali stable multiple fragment DNA crosslinks. *Nucleic Acids Res* 26:5441–5447
- Peterson JE, Culvenor CCJ (1983) Plant and fungal toxins. In: Keeler RF, Tu AT (ed) Handbook of natural toxins, vol 1. Marcel Dekker, New York, pp 637–681
- Peterson JE, Jago MV (1980) Comparison of the toxic effects of dehydroheliotridine and heliotrine in pregnant rats and their embryos. *J Pathol* 131:339–355
- Peterson JE, Samuel A, Jago MV (1972) Pathological effects of dehydroheliotridine, a metabolite of heliotridine-based pyrrolizidine alkaloids in the young rat. *J Pathol* 107:107–189
- Powis G, Ames MM, Kovach JS (1979) Metabolic conversion of indicine-N-oxide to indicine in rabbits and humans. *Cancer Res* 39:3564–3570
- Prakash AR, Pereira TN, Reilly PEB, Seawright AA (1999) Pyrrolizidine alkaloids in human diet. *Mutation Res* 443:53–67
- Rasenack R, Müller C, Kleinschmidt M, Rasenack J, Wiedenfeld H (2003) Venooclusive disease in a foetus caused by pyrrolizidine alkaloids of food origin. *Fetal Diagn Ther* 18:223–225
- Reed RL, Miranda CL, Kedzierski B, Henderson MC, Buhler DR (1992) Microsomal formation of a pyrrolic alcohol glutathione conjugate of the pyrrolizidine alkaloid senecionine. *Xenobiotica* 1:1321–1327
- Ridker PM, Ohkuma S, McDermott WV, Trey C, Huxtable RJ (1985) Hepatic venooclusive disease associated with consumption of pyrrolizidine alkaloid containing dietary supplements. *Gastroenterology* 88:1050–1054
- Robertson KA (1982) Alkylation of N<sup>2</sup> in deoxyguanosine by dehydroretronecine, a carcinogenic metabolite of the pyrrolizidine alkaloid monocrotaline. *Cancer Res* 42:8–14
- Roeder E (1995) Medicinal plants in Europe containing pyrrolizidine alkaloids. *Pharmazie* 50:83–98
- Roeder E (2000) Medicinal plants in China containing pyrrolizidine alkaloids. *Pharmazie* 55:711–726
- Roulet M, Laurini R, Rivier L, Calarme A (1988) Hepatic venooclusive disease in newborn infant of a woman drinking herbal tea. *J Pediatr* 112:433–436

- Saint-Aimé MM, Ponsar C, Lacombe C, Lacombe W (1977) Maladie veino-occlusive du Foie chez L'Enfant martiniquais. *Bordeaux Médical* 10:665–670
- Schoental R (1959) The chemical aspects of seneciosis. *Proc Roy Soc Med* 53:284–288
- Schoental R (1972) The hepatotoxic and carcinogenic effects of some East African plants. *Bull Epizoot Dis Afr* 20:301–302
- Shumaker RC, Robertson KA, Hsu IC, Allen JR (1976) Neoplastic transformation in tissues of rats exposed to monocrotaline or dehydroretronecine. *J Natl Cancer Inst* 56:787–789
- Smith LW, Culvenor CCJ (1981) Plant sources of hepatotoxic pyrrolizidine alkaloids. *J Nat Prod* 44:129–152
- Sperl W, Stuppner H, Gassner I, Judmaier W, Dietze O, Vogel W (1995) Reversible hepatic veno-occlusive disease in an infant after consumption of pyrrolizidine-containing herbal tea. *Eur J Pediatr* 154:112–116
- Staatsblad (2001) Besluit van 19 januari 2001, houdende vaststelling van het Warenwetbesluit Kruidenpreparaten. *Staatsblad van het Koninkrijk der Nederlanden* 56:1–12
- Stegelmeier BL, Edgar JA, Steven M, Colegate SM, Gardner DR, Schoch TK, Coulombe RA Jr (1999) Pyrrolizidine alkaloids plants, metabolism, toxicity. *J Nat Toxins* 8:5–116
- Steyn DG (1933) Poisoning of human beings by weeds contained in cereals (bread poisoning). *Onderstepoort. J Vet Sci Anim Ind* 1:219–266
- Stillman AE, Huxtable RJ, Fox DW, Hart MC, Bergeson PS, Counts JM (1977) Poisoning associated with herbal teas—Arizona, Washington. *Morb Mortal Wkly Rep* 26:257–259
- Tandon HD, Tandon BN (1975) Epidemic of liver disease—Gulran District, Herat Province, Afghanistan, Alexandria, World Health Organization, Regional Office for the Eastern Mediterranean (Assignment report No. EM/AFG/OCD/001/RB)
- Tandon BN, Tandon RK, Tandon HD, Narndranathan M, Joshi JK (1976) An epidemic veno-occlusive disease of liver in central India. *Lancet* 271–272
- Tomioka M, Calvo F, Siguas A, Sanchez L, Nava E, Garcia U, Valdivia M, Reategui E (1995) Enfermedad hepática veno-oclusiva asociada a l ingestión de humanripa (*Senecio tephrosioides*). *Rev Gastroenterol Peru* 15:299–302
- Tuchweber B, Kovacs K, Jago MV, Beau lieu T (1974) Effect of steroidal and nonsteroidal microsomal enzyme inducers on the hepatotoxicity of pyrrolizidine alkaloids in rats. *Res Commun Chem Pathol Pharmacol* 7:459–480
- Vilar JH, Garcia M, Cabrera P (2000) Enfermedad veno-oclusiva hepática de causa Tóxica por *Senecio vulgaris*. *Gastroenterol Hepatol* 23:285–286
- Wang X, Yan J, Fu PP, Chou MW (2005a) Metabolic activation of the tumorigenic pyrrolizidine alkaloid, retrorsine, leading to DNA adduct formation in vivo. *Int J Environ Res Public Health* 2:74–79
- Wang Y-P, Yan J, Beger RD, Fu PP, Chou MW (2005b) Metabolic activation of the tumorigenic pyrrolizidine alkaloid, monocrotaline, leading to DNA adduct formation in vivo. *Cancer Lett* 226:27–35
- Wang Y-P, Yan J, Fu PP, Chou MW (2005c) Human liver microsomal reduction of pyrrolizidine alkaloid N-oxides to form the corresponding carcinogenic parent alkaloid. *Toxicol Lett* 155:411–420
- Weston CFM, Cooper BT, Davies JD, Levine DF (1987) Veno-occlusive disease of the liver secondary to ingestion of comfrey. *Brit Med J* 295:183
- White INH, Mattocks AR, Butler WH (1973) The conversion of the pyrrolizidine alkaloid retrorsine to pyrrolic derivatives in vivo and in vitro and its acute toxicity to various animal species. *Chem Biol Interact* 6:207–218
- Williams DE, Reed RL, Kedziarsk B, Dannan GA, Guengerich FP, Buhler DR (1989) Bioactivation and detoxication of the pyrrolizidine alkaloid senecionine by cytochrome P-450 enzymes in rat liver. *Drug Metab Dispos* 17:387–392
- Willmott FC, Robertson GW (1920) Senecio disease or cirrhosis of the liver due to *Senecio* poisoning. *Lancet* 196:848–849
- Xia Q, Chou MW, Kadlubar FF, Chan P-C, Fu PP (2003) Human liver microsomal metabolism and DNA adduct formation of the tumorigenic pyrrolizidine alkaloid, riddelliine. *Chem Res Toxicol* 16:66–73
- Xia Q, Chou MW, Edgar JA, Doerge DR, Fu PP (2006) Formation of DHP-derived DNA adducts from metabolic activation of the prototype heliotridine-type pyrrolizidine alkaloid, lasiocarpine. *Cancer Lett* 231:138–145
- Yan J, Nichols J, Yang Y-C, Fu PP (2002) Detection of riddelliine-derived DNA adducts in blood of rats fed riddelliine. *Int J Mol Sci* 3:1019–1026
- Yee SB, Kinser S, Hill DA, Barton CC, Hotchkiss JA, Harkema JR, Ganey PE, Roth RA (2000) Synergistic hepatotoxicity from coexposure to bacterial endotoxin and the pyrrolizidine alkaloid monocrotaline. *Toxicol Appl Pharmacol* 166:173–185