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Plants containing Pyrrolizidine Alkaloids - Toxicity and Problems

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Abstract:

Pyrrolizidine alkaloids (PA) are toxic for human and livestock. The PA undergo a metabolic toxication process in the liver which is the first target organ for PA poisoning. World-wide many episodes of human PA intoxications are well reported. In many cases the reason for these intoxications have been PA-contamination in food. The main tools for analyzing food and fodder on PA content are based on GC- and HPLC-separation, followed by MS(-MS) detection. Actual incidents with toxic PA are the "*Jacobaea vulgaris* (syn. *Senecio jacobaea*)-problem" in Europe and the "*Ageratum conyzoides*-problem" in Ethiopia.

Key Words: Pyrrolizidine alkaloids; poisoning in humans; PA-analysis; PA in food

Pyrrolizidine alkaloids (PAs) possessing a 1,2 double-bond in their base moiety (necine) are hepatotoxic, carcinogenic, genotoxic, teratogenic and sometimes pneumotoxic. PAs have been estimated to be present in about 3% of all flowering plants [Smith, Culvenor, 1981]. PAs mainly occur in species of the plant families Asteraceae, Fabaceae and Boraginaceae.

Several reports in literature proof the fact that PA containing plants are hazardous for livestock [Gilruth, 1903, 1904; Bull, Dick, 1959]. Since a long time ago, it is also well established that humans can be affected by toxic PA, too [Willmott, Robertson, 1920; Steyn, 1933; Bourkser, 1947; Milenkhov, Kizhaikin, 1952; Tandon, Tandon, 1975; Mohabbat, Srivasta, et al., 1976; Chauvin, Dillon, et al., 1994; Mayer, Lüthy, 1993]. Here, the main source for the intoxication was found to be PA contaminated grain and bread.

The International Program on Chemical Safety (IPCS), a joint agency of WHO, FAO and ILO, stated that "consumption of contaminated grain or the use of PA-containing

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3 plants as herbal medicine, beverages, or food by man, or grazing on contaminated
4 pastures by animals, may cause acute or chronic disease" [IPCS, 1989].

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6 Besides this, also different medicinal plants were found to show a hazardous
7 potential for humans [Roeder, 1995, 2000]. Many investigations on herbal
8 preparations or extracts from medicinal plants were done and prove the toxic risk by
9 the consumption of phytomedicines which contain PA plants. It can be assumed that
10 so-called "bush-teas" are the reason for several liver diseases reported in Jamaica
11 and several developing countries from Africa and West Indies [Bras et al., 1954,
12 1957, 1961]. While PA poisoning is mainly a problem in developing countries
13 because there the use of traditional medicine is common, within the last 25 years
14 especially in industrialised countries the use of herbal medicine has become more
15 and more common due to an increased interest of people in alternative medicine,
16 hand in hand with a greater influence of the "green wave".
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19 In addition, also several food products of animal origin can be a source for PA
20 ingestion: Milk has been shown to contain PA when milk-producing animals
21 consume PA-containing plant material [Schoental, 1959; Dickinson, Cooke, et al.,
22 1976; Dickinson, 1980; Johnson, Robertson, et al., 1978; Goeger, Cheeke, et al.,
23 1982; Lüthy, Heim, et al., 1983; Candrian, Lüthy, et al., 1984; Molyneux, James,
24 1990]. Human milk has also caused liver diseases in neonates and infants [Roulet,
25 Laurini, et al., 1988].
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28 Honey was shown to be another source of PA exposure; here it seems that the
29 contamination may be due to the plant pollen, which is rich in PAs, being transferred
30 by bees into the honey [Deinzer, Thomson, et al., 1977; Culvenor, Edgar, et al.,
31 1981; Roeder, 1995, 2000; Edgar, Roeder, et al., 2002; Beales, Betteridge, et al.,
32 2004; Boppré, Colgate, et al., 2005; Betteridge, Cao, et al., 2005]. Eggs from poultry
33 exposed to PAs in PA-contaminated grain were also shown to be a possible source
34 of PA exposure for humans [Edgar, Smith, 1999].
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37 Recently it was shown in Germany that salads can sometimes be contaminated with
38 PA-containing plants [BfR, 2007a]. It was found that, especially in supermarkets,
39 ready-packed rocket salad and also salad mixtures have been contaminated by
40 *Senecio vulgaris*, a typical weed of field-crops.
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43 While in several countries the use of PA containing plants or preparations from them
44 are controlled by state regulations, nothing exists in case of regulating contaminated
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3 food and fodder. In the EU the EFSA recommended in its opinion [EFSA, 2007] that
4 feed material which can be contaminated with PA should be monitored. More data
5 are needed to assess human PA exposure from resulting from feed and carry-over
6 into animal products. Meanwhile the so-called "zero-tolerance principle" can be
7 applied; this principle is used in cases where no safe or tolerable level can be
8 determined based on available, valid scientific data, or if insufficient toxicological
9 data are available. Due to their genotoxic and carcinogenic potential this principle
10 can be applied for PA in food and fodder [BfR, 2007b].

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12 Actually, in Middle Europe common groundsel (*Senecio vulgaris*, L.) and tansy
13 ragwort (*Jacobaea vulgaris*, syn. *Senecio jacobaea*, L.) are of particular concern.
14 Common groundsel, a common field-weed, has been found as a contaminant in
15 salads and tansy ragwort has been discussed recently on account of its extensive
16 expansion into pastures and meadows which has led to a great number of
17 intoxications in livestock (mainly horses), especially in Germany
18 [<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),
19 the possibility of contaminated hay and silage and transfer of PAs into foods such as
20 milk and milk products is under investigation and considered to become problematic.
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37 **Toxification process of pyrrolizidine alkaloids**

38 PA produced by *Senecio*-species are ester alkaloids derived mainly from the necines
39 retronecine and otonecine. They are carcinogenic, mutagenic, genotoxic, fetotoxic
40 and teratogenic.
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43 PAs themselves show a more or less low acute toxicity but *in vivo* they undergo a 3-
44 step metabolic toxication process in the liver, which is, as a result, the first target
45 organ for the toxicity.
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49 This toxication process is well investigated [Mattocks, 1968; Culvenor, Downing, et
50 al., 1969; Jago, Edgar, et al., 1970; Mattocks, White, 1971a, 1971b; Culvenor, Edgar,
51 et al., 1971; Mattocks, 1972; IPCS, 1989].
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54 After absorption an hydroxyl-group is introduced in the necine at position 3 or 8 by
55 the cytochrome P-450 monooxygenase enzyme complex in the liver (Figure 1: IIa and
56 IIb). These hydroxy-PAs (OHPAs) are unstable and undergo a rapid dehydration to
57 the didehydropyrrolizidine alkaloids (DHPAlk; Figure 1: III). This dehydration results
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3 in a second double-bond in the necine followed by spontaneous rearrangement to an
4 aromatic pyrrole-system III.
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9 Figure 1: Enzymatic hydroxylation and didehydropyrrolizidine products
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12 PAs occur mainly as their N-oxides in the plants and these cannot be directly
13 converted to the OHPA, but on oral ingestion they are reduced by the gut enzymes or
14 the liver microsomes and NADH or NADPH to the free bases and therefore they
15 show equal toxicity to that of the free bases [Mattocks, White, 1971a, 1971b; Powis,
16 Ames, et al., 1979; Chou, Wang, et al., 2003; Wang, Yan, et al., 2005a; Wan, Yan, et
17 al., 2005b; Wang, Yan, et al., 2005c].
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23 Otonecine-type PAs (Figure 1: Ib) are metabolised to the OHPAs [Culvenor, Edgar,
24 et al., 1971; Lin, Cui, et al., 1998; Lin, Cui, et al., 2000]. These otonecine-PAs
25 possess a methyl-function at the nitrogen and a quasi keto-function at the bridge-
26 carbon 8. After hydroxylation of the N-methyl-group it is lost as formaldehyde leaving
27 a NH-function which undergoes condensation with the C8 keto group to produce
28 product IIb (Figure 1) which spontaneously dehydrates to the DHPAlk III.
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34 The metabolites III are able to generate stabilized carbonium ions (Figure 2: IV and
35 VI) by loss of hydroxy groups or ester functions as hydroxyl or acid anions. These
36 carbonium ions can react rapidly with nucleophiles (Figure 2: VII).
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41 Figure 2: DHPAlk and carbonium ion building
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44 In *in vivo* the metabolites IV and VI react rapidly with nucleophilic mercapto, hydroxyl
45 and amino groups on proteins and the amino groups of purine and pyrimidine bases
46 in nucleosides like DNA and RNA. These alkylated products show abnormal
47 functions and in the case of DNA, mutations are possible. As this metabolic
48 toxification takes place in the liver it is obvious that this organ is the first target for the
49 intoxication and results in the liver damage leading to the veno-occlusive disease
50 (VOD) in which the veins are narrowed.
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58 **Detoxication of PA**

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3 As well as the metabolic activation, detoxification of PA also occurs *in vivo*: hydrolysis
4 of the ester bonds in PA from type Ia or Ib by esterases leads to necic acids and to
5 the free necines. Both are non-toxic products and - on account of their higher water-
6 solubility - can be renally excreted. The rate of hydrolysis is dependent on the level of
7 steric hinderance of the ester linkages (see before); and it has been shown that the
8 more highly branched the necic acids are the more resistance to hydrolysis
9 [Culvenor, Edgar, et al., 1976; Mattocks, 1986]. This means, that macrocyclic
10 diesters (Ia and Ib) with more complex acid moieties are more hazardous on account
11 of their lower rate of hydrolytic detoxication.
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14 The N-oxides of PAs (the form occurring most commonly in plant sources) are highly
15 water soluble and can therefore be excreted renally. Besides their natural
16 occurrence, N-oxidation of PAs also takes place in the liver and can be seen as a
17 detoxification process (Figure 2) [Mattocks, 1968; Jago, Edgar, et al., 1970;
18 Mattocks, White 1971b; Williams, Reed, et al., 1989; Miranda, Chung, et al., 1991].
19 However it has been shown that the N-oxides - besides excretion - can be converted
20 by dehydration or by acetylation followed by elimination of acetic acid to the DHPAik
21 (Figure 2: III) [Mattocks, 1986; Culvenor, Edgar, et al., 1970].
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34 35 **PA toxicity in humans**

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37 PA poisoning of humans can progress from an acute to a sub-acute and finally to a
38 chronic state [McLean, 1970; Peterson, Culvenor, 1983; IPCS, 1989; Huxtable, 1989;
39 Prakash, Pereira, et al., 1999; Fu, Xia, et al., 2004; Stegelmeier, Edgar, et al., 1999].
40 Acute poisoning is characterised by haemorrhagic necrosis, hepatomegaly and
41 ascites; death is caused by liver failure due to necrosis and liver dysfunctions
42 [Peterson, Culvenor, 1983; IPCS, 1989; Huxtable, 1989; Prakash, Pereira, et al.,
43 1999].
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46 Sub-acute levels are characterised by hepatomegaly and recurrent ascites;
47 endothelial proliferation and medial hypertrophy leading to an occlusion of hepatic
48 veins, resulting in the VOD which can be seen as a characteristic histological sign for
49 PA poisoning [Peterson, Culvenor, 1983; IPCS, 1989; Huxtable, 1989; Prakash,
50 Pereira, et al., 1999; Fu, Xia, et al., 2004]. The VOD causes centrilobular congestion,
51 necrosis, fibrosis and liver cirrhosis, the end-stage of chronic PA intoxication.
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A very important fact is that PA toxicity not only correlates with the amount and duration of uptake. It is shown that the susceptibility for PA intoxication is also dependent on gender (males seem to be more sensitive than females) and on age: children and especially neonates and foetuses show a much higher sensitivity than adults: In 2003 it was shown that the daily uptake of ~ 7 µg 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 such a way that the newborn child died after 2 days [Rasenack, Müller, et al., 2003].

It has also been observed that cofactors can exacerbate the PA poisoning: liver damaging agents, bacterial or viral infections as well as medical drugs like barbiturates or metals like copper or mycotoxins like aflatoxins can increase the severity and likelihood of PA liver damage [Yee, Kinser, et al., 2000; Newberne, Rogers, 1973; White, Mattocks, et al., 1973; Tuchweber, Kovacs, et al., 1974; Lin, Liu, et al., 1974; Bull, Culvenor, et al., 1968].

There is a large number of reports in the literature about different liver diseases (mainly VOD) possibly connected with PA poisoning. But, on account of the fact that those reports mainly describe sub-chronic or chronic intoxications, in most cases the connection cannot be proven because there is often a long time period between the outbreak of the liver disease and a possible ingestion of PA-containing material.

The following table therefore lists only those cases where the source of PAs was identified and the liver disease was therefore undoubtedly caused by PA intoxication:

Table 1: human intoxications :

<i>Location and year</i>	<i>Affected people</i>	<i>Observed damage</i>	<i>Source of PA</i>	<i>Lit.</i>
South Africa, 1920	11 adult people	Abdominal pain, vomiting, cirrhosis	<i>Senecio illicifolius</i> , <i>S. burchelli</i>	Willmot, Robertson, 1920
Jamaica, 1954	23 adults	VOD	Bush-teas with <i>Crotalaria fulva</i>	Bras, Brooks, et al., 1961
South Africa, 1968	15 children; 10 died	VOD	Bush-teas; <i>Crotalaria</i> sp.?	Freiman, Schmaman, 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, Beker, 1972

Kuweit, 1970	Adults	Liver carcinoma	<i>Heliotropium ramosissimum</i> ("Ramram")?	Macksad, Schoental, et al., 1970
Jamaica, 1970	6 children	VOD	Bush-tea from <i>Crotalaria</i> and <i>Senecio</i> sp.	Brooks, Miller, et al., 1970
Iraq, 1970	9 children	VOD	food contaminated by a <i>Senecio</i> spec.	Al-Hasany, Mohamed, 1970
Afghanistan, 1970-72	7200 people	VOD	Wheat contaminated with <i>Heliotropium popovii</i> , ssp. <i>gillianum</i>	Mohabbat, Srivasta, et al., 1976
India, 1973	486 people	VOD	Cereals contaminated with <i>Crotalaria</i> spp.	Tandon, Tandon, et al., 1976
Ecuador, 1973	Woman	VOD	Herbal tea with <i>Crotalaria juncea</i>	Lyford, Vergara, et al., 1976
India, 1973, 1975	4 male people	Endemic ascites	Millet contaminated with <i>Crotalaria</i> spp.	Krishnamachari, Bhat, et al., 1977
China, 1973, 1978	2 adults	VOD	<i>Gynura segetum</i>	Hou, Xia, et al., 1980
India, 1974-1977	6 people	VOD	<i>Heliotropium eichwaldii</i>	Datta, Khuroo, et al., 1978
Martinique, 1975	2 children	VOD	Bush-teas with <i>Crotalaria retusa</i> and/or <i>Heliotropium</i> sp.	Saint-Aimé, Ponsar, et al., 1977
USA, 1976, 1977	4 children	Vein congestion and necrosis of liver	<i>Senecio longilobus</i>	Stillman, Huxtable, et al., 1977
UK, 1976	Woman	VOD	Maté (Paraguay tea) contaminated with PA of unknown origin	McGee, Patrick, et al., 1976
USA, 1984	49 year old woman	VOD	Food supplement containig <i>Symphytum</i> spp. root	Ridker, Okhuma, et al., 1985
China, 1985	4 women	VOD	Herbal tea containig <i>Heliotropium lasiocarpum</i>	Culvenor, Edgar, et al., 1986
Switzerland, 1985	59 years old man and 27 years old son	VOD	Herbal tea consisting of <i>Senecio</i> spp.	Margalith, Heraief, et al., 1985
Switzerland, 1986	5 days old baby	VOD	Herbal tea containing <i>Tussilago farfara</i> consumed during whole pregnancy	Roulet, Laurini, et al., 1988
UK, 1986	13 years old boy	VOD	Herbal tea containing <i>Symphytum</i> spp.	Weston, Cooper, et al., 1987
Tadjikistan, 1992, 1993	3906 people	Abdominal pain, hepatomegaly, ascites, alteration of consciousness	<i>Heliotropium lasiocarpum</i>	Chauvin, Dillon, et al., 1993
Peru, 1994	38 year old woman	VOD	Herbal tea from <i>Senecio tephrosioides</i>	Tomioka, Calvo, et al., 1995
Spain, 1995	73 years old man	VOD	<i>Senecio vulgaris</i>	Cansado, Valadés, et al., 1995
Austria, 1995	18 month old boy	VOD	Herbal tea with <i>Adenostyles alliariae</i>	Sperl, Stuppner, et al., 1995
Argentina;	23 old	VOD	Herbal tea containing	Vilar, Garcia, et al.,

1999	woman		<i>Senecio vulgaris</i>	2000
Germany, 2002	Fetus	VOD	<i>Symphytum</i> spp.	Rasenack, Müller, et al., 2003
Afghanistan, 2008	270 people (50 fatalities)	VOD	Wheat contaminated with "charmac" (<i>Heliotropium popovii</i> , ssp. <i>gillianum</i>)	Kakar, et al., 2010

PA determination

Several methods can be used for the qualitative and quantitative determination of PAs in plant material or its preparations:

TLC: Thin-layer chromatography (using the detection method of Dann / Mattocks [Dann, 1960; Mattocks, 1967]) is a quick, sure and easy method for a qualitative detection of PAs. Using TLC in a densitometric way [Bartkowski, Wiedenfeld, et al., 1997], it is also possible to have quantitative results; the detection limit is about 1 - 10 µg.

LC-MS: Different HPLC methods are described. Obviously, this is the mostly used analytical method for the determination of PAs. A great benefit is the fact that PA-N-oxides can be analyzed as well as the free bases. Depending on the equipment, the detection limit is less than 0.1 µg per injection.

GC-MS: also in the case of GC a lot of methods are described. A GC problem is that N-oxides have to be reduced to the free bases before being analyzed. This reduction procedure is described intensively and doesn't seem to be a limiting factor. The detection limit is similar to that in LC.

All three methods can only be used accurately in case reference material is available. The reason is that the single PAs show different detector responses in the respective analyzing methods.

A further interesting - but unfortunately very specific - quantification method is the enzyme linked immuno sorbent assay (ELISA). Via a PA hemisuccinate and bovine serum albumine, enzyme linked antigens (AG-E) can be synthesized and used for the production of antibodies (polyclonal as well as monoclonal) in rats [Röder, Pflüger, 1986, 1995]. This method is very sensitive and needs no complicated cleanup procedure. Unfortunately, it is sensitive only for a concrete PA structure; that means, antibodies produced by a retrorsine-AG-E can only be used for the analysis

of retrorsine and senecionine, but are inactive for seneciphylline, jacobine or monocrotaline and, of course, also for all otonecine-type PAs.

Toxic PA in food and fodder

During the last three years an extensive increase of the spreading of *Senecio* species (especially *Jacobaea vulgaris*, syn. *Senecio jacobaea* L., Asteraceae) could be observed in central Europe. This led to severe problems as this spreading includes pastures and meadows: horses, which show a high sensitivity for PA intoxication, were affected in large numbers: To date more than 100 horses died on account of acute intoxication by *Jacobaea vulgaris* [<http://www.jacobskreuzkraut.de/wirkung.htm>; <http://www.jacobskreuzkraut.de>; <http://www.izn.niedersachsen.de/servlets/download?C=39412784&L=20>]. As for ruminants, an intoxication in grazing animals seems to be negligible because these animals show a very low susceptibility and the amount of *Senecio* species on meadows is too low for an intoxication; in this case fodder like hay or silage is responsible for the hazardous problems.

To evaluate a possible risk, plant material of *Jacobaea vulgaris* was analyzed for its PA content during the vegetation period. The PAs from the plant were isolated by column chromatography and their structures elucidated by spectroscopical methods. Figure 3 shows the structures of the isolated PAs. Figure 4 shows a GC from an alcoholic plant extract.

The plant parts were analyzed (Figure 5) and it could be shown that the PA content is ranging between 0.1 and 0.2%, except in flowers, where amounts up to 0.8% were found. These data show that the application of 2 - 4 kg of dried plant material reaches the lethal level in horses (~ 350 kg bw).

This is of great importance in case of hay or silage where a contamination with *J. vulgaris* of e.g. 1 - 5% is a possible scenario. In those cases the lethal dosage for horses will be reached in a few days of feeding such contaminated hay [http://www.jacobskreuzkraut.de/toedliche_dosis.htm]. In this context the question occurs whether there is a decrease during hay production and storage or during silaging.

In figures 6 and 7, PA contents are shown of hay and silage produced from *J. vulgaris*. The data show that in case of hay no reduction of the PA level (compared with dried plants) can be observed. Contrary to this, the results for silage show a decrease in the PA level down to 10%. It can be assumed that this is due to an

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3 enzymatic decomposition. Therefore, it can be concluded that in case of hay the
4 possible toxic risk is equal to original plant material, whereas feeding silage to
5 animals seems to be without any toxic risk.
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8 Feeding contaminated material is not only hazardous for the animals themselves but
9 can produce human risks, too, in case PAs are excreted in animal products like milk
10 and honey.
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12 A direct human exposure to PA was found in 2009 when salads contaminated with
13 *Senecio* plants (here, especially *Senecio vulgaris*, a common weed on field crops)
14 occurred in stores and supermarkets. Especially in the case of rucola and salad
15 mixtures containing rucola this problem became of high importance as it is not
16 possible for non-experts to distinguish between the salad leaves and *Senecio* leaves
17 (Figure: 8). Our own analysis of such a salad sample (45 g) resulted in an amount of
18 2606 μg PAs. Taking into consideration that for herbal medicinal preparations the
19 daily uptake limit (different in several countries) ranges from PA-free (Austria) over 1
20 $\mu\text{g}/\text{kg}$ or l (The Netherlands) to 1 μg (Germany) it becomes obvious that such
21 contaminated food is showing a severe health risk.
22
23

24 As already mentioned before, several human PA exposures have been observed due
25 to contaminated grain and/or bread. A similar problem was found in the Tigray region
26 of Ethiopia. In the last years many people (especially children) died there on account
27 of a severe liver failure. After excluding of all other risk potentials (aflatoxins, etc.), a
28 PA containing plant was suspected to be responsible for the problems: *Ageratum*
29 *conyzoides*. Several years ago we described this plant to contain toxic PAs
30 [Wiedenfeld, Röder, 1991]. The people in this region are mainly using millet for the
31 production of food but also for beverages like beer. An inspection of the grainfields in
32 this area resulted in the findings that indeed *A. conyzoides* is wide-spread there and
33 that seeds of this weed can contaminate the millet. We analyzed 6 millet samples
34 from different houses with respect to their PAs originated from *A. conyzoides*. Three
35 of them showed a PA contamination. The sample with the highest amount came from
36 a house where all family members already died ("Dagusha house 1"). We found 87.1
37 μg PAs in 182 g millet. As the normal daily consumption amounts to ~ 100 g millet/
38 person it becomes obvious that, consumed over a more or less longer period, such a
39 grain contamination can indeed produce the fatal problems observed in the Tigray
40 region.
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8 Figure 3: Structures of isolated PA from *Jacobaea vulgaris*, syn. *Senecio jacobaea*
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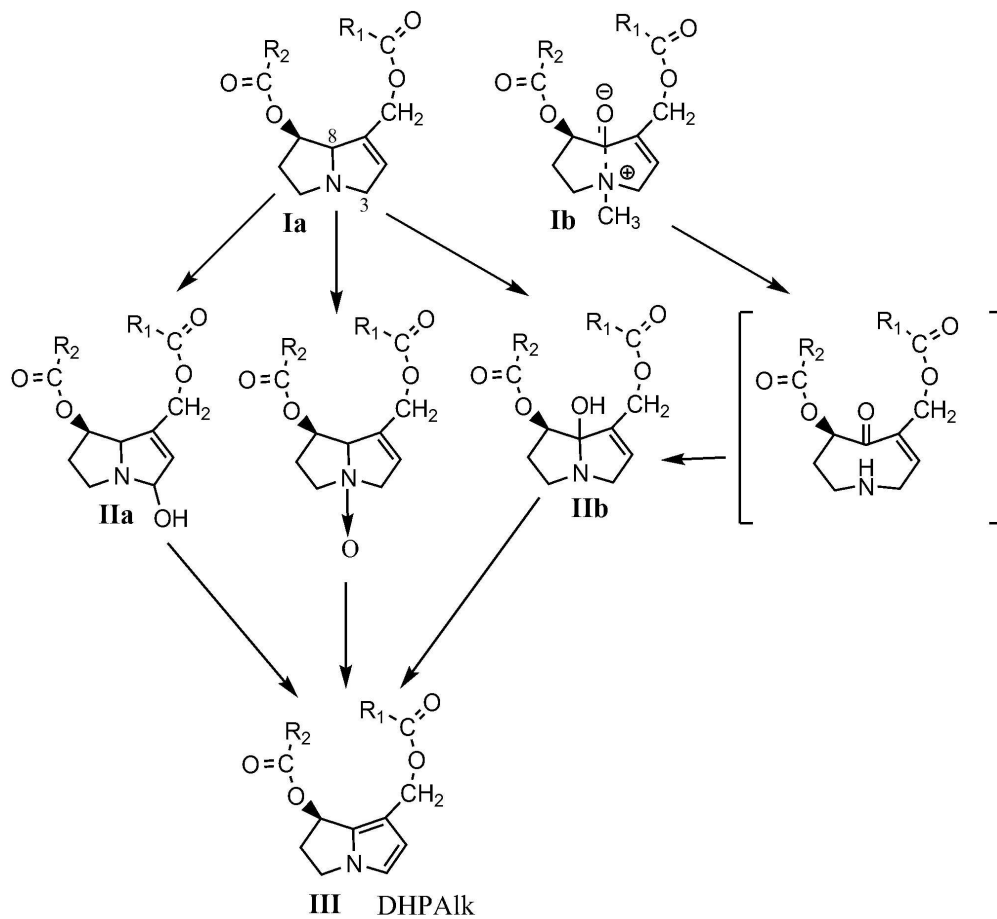
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11 Figure 4: Gas chromatogram of the PA from *Jacobaea vulgaris*, syn. *Senecio*
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16 Figure 5: PAs [$\mu\text{g} / \text{g}$ plant material] in *Jacobaea vulgaris*, syn. *Senecio jacobaea*
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20 Figure 6: PAs [$\mu\text{g} / \text{g}$] in hay from *Jacobaea vulgaris*, syn. *Senecio jacobaea*
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23 Figure 7: PAs [$\mu\text{g} / \text{g}$] in silage from *Jacobaea vulgaris*, syn. *Senecio jacobaea*
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27 Figure 8: Salad sample (left *Senecio vulgaris*; right rucola)
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Figure 1: Enzymatic hydroxylation and didehydropyrrolizidine products
171x157mm (300 x 300 DPI)

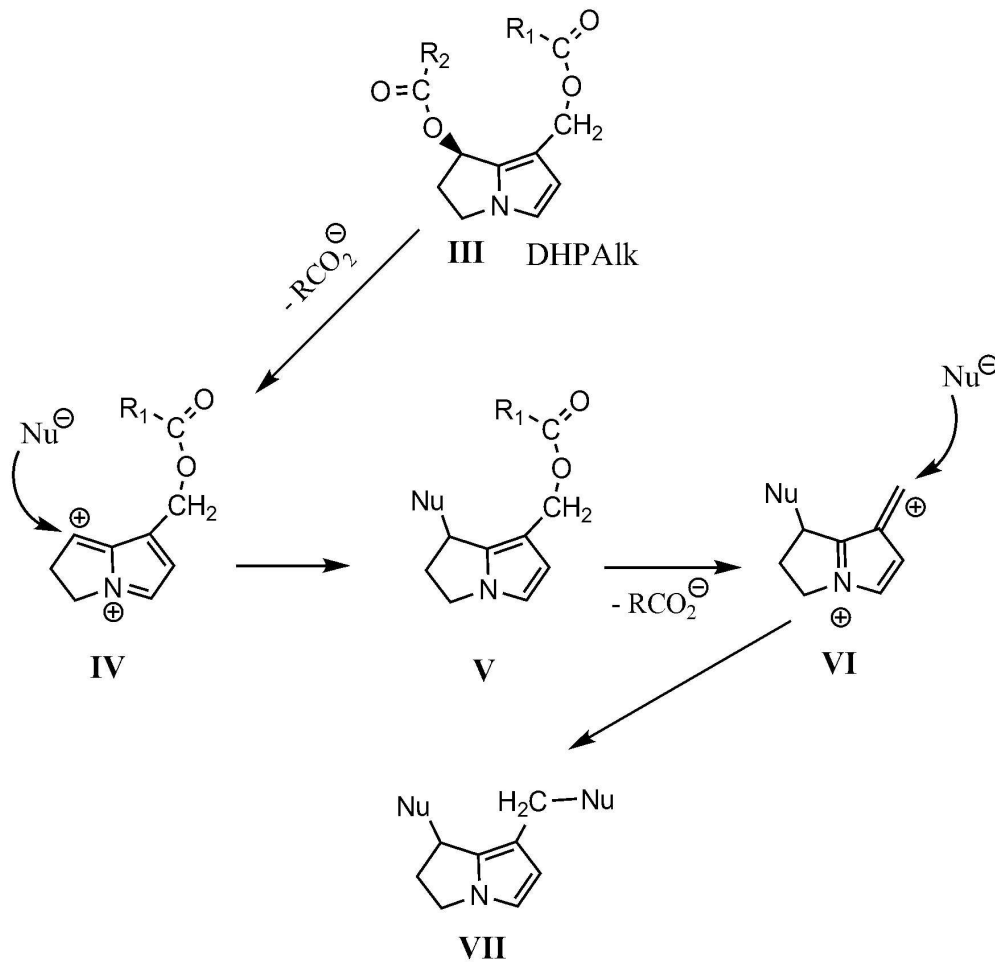


Figure 2: DHPAlk and carbonium ion building
148x144mm (300 x 300 DPI)

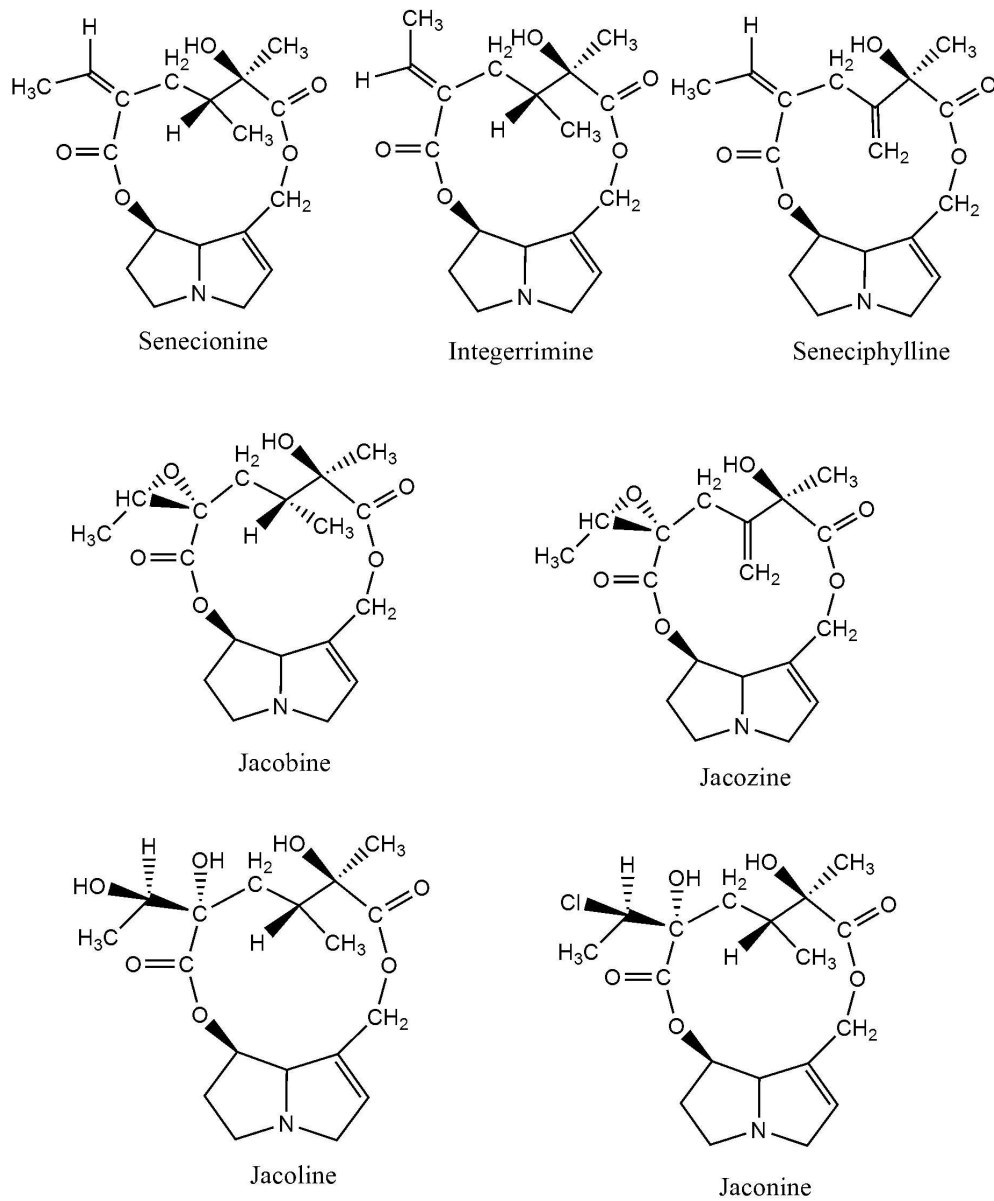


Figure 3: Structures of isolated PA from *Jacobaea vulgaris*, syn. *Senecio jacobaea*
156x188mm (300 x 300 DPI)

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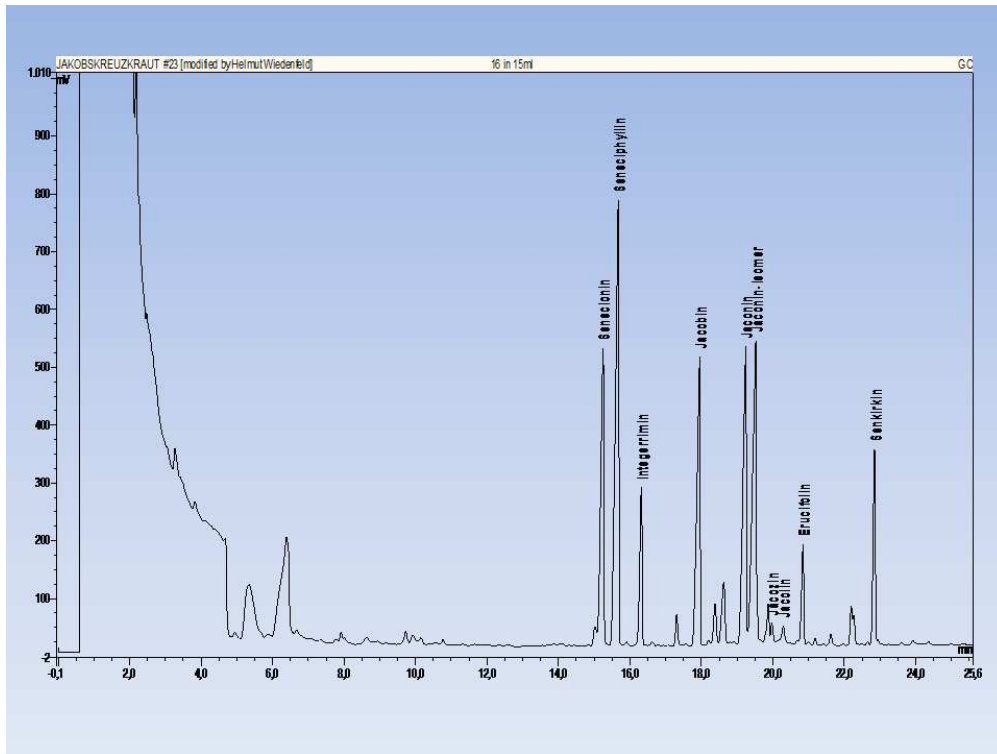


Figure 4: Gas chromatogram of the PA from *Jacobaea vulgaris*, syn. *Senecio jacobaea*
254x190mm (96 x 96 DPI)

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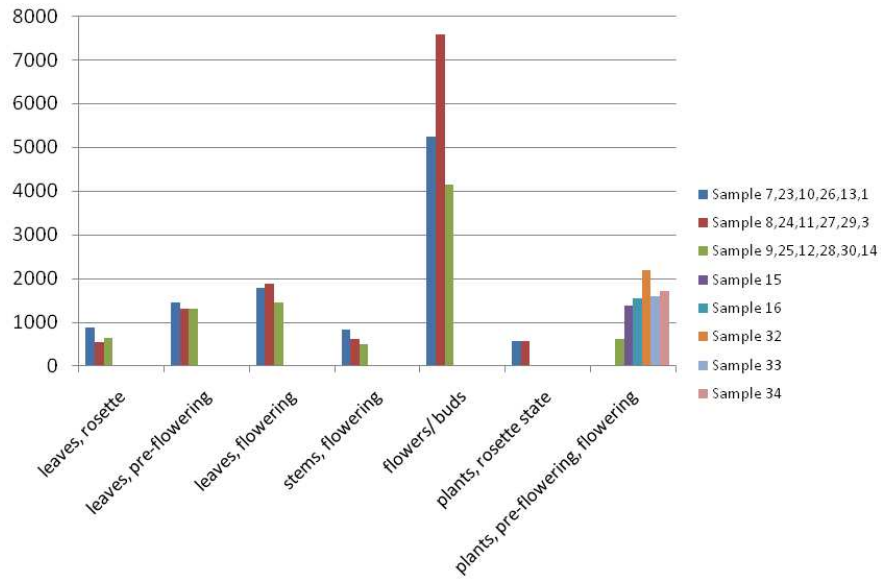


Figure 5: PAs [$\mu\text{g} / \text{g}$ plant material] in *Jacobaea vulgaris*, syn. *Senecio jacobaea*
254x190mm (96 x 96 DPI)

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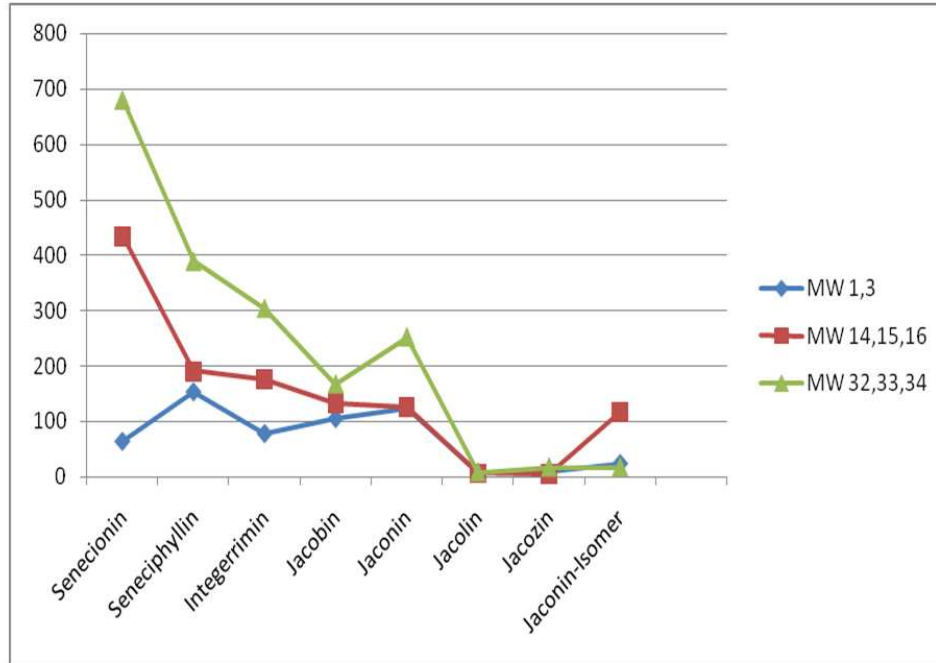


Figure 6: PAs [$\mu\text{g} / \text{g}$] in hay from *Jacobaea vulgaris*, syn. *Senecio jacobaea*
254x190mm (96 x 96 DPI)

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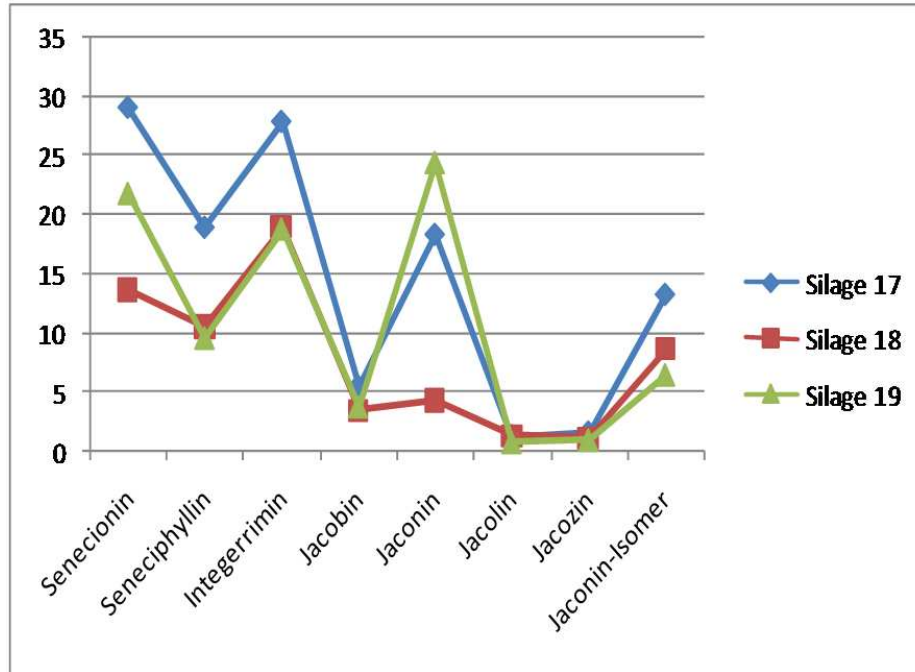


Figure 7: PAs [$\mu\text{g} / \text{g}$] in silage from *Jacobaea vulgaris*, syn. *Senecio jacobaea*
254x190mm (96 x 96 DPI)

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Figure 8: Salad sample (left *Senecio vulgaris*; right rucola)
254x142mm (96 x 96 DPI)

Review Only