

# **PYRROLIZIDINE ALKALOIDS IN FOOD**

## **A Toxicological Review and Risk Assessment**

TECHNICAL REPORT SERIES NO. 2

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## SUMMARY

Pyrrolizidine alkaloids (PAs) which may find their way into human and animal food in Australia are derived mainly from the plants *Heliotropium europaeum*, *Echium plantagineum*, *Symphytum* spp. and *Crotalaria retusa*. The *Symphytum* spp. (comfrey) are deliberately ingested while the remaining species are weeds in various grain crops. There is a long history of toxicity in livestock caused by grazing on PA-containing plants. There have also been a number of outbreaks of human poisoning as a result of ingestion of contaminated grain as well as case reports of poisoning caused by intentional ingestion of herbal medicines containing PAs.

### *Hazard assessment*

The PAs of relevance to human health are the hepatotoxic PAs which are esters of 1-hydroxymethyl dehydropyrrolizidine. Such compounds are metabolised in the liver to electrophilic derivatives referred to as pyrroles. These pyrroles cause damage in the hepatocytes in which they are generated, but depending on their persistence in aqueous media, can pass from the hepatocytes into the adjacent sinusoids and damage endothelial lining cells of the sinusoids and smallest hepatic veins. These effects give rise in man to hepatocellular injury, cirrhosis and veno-occlusive disease.

The pyrroles react with macromolecules in the cells in which they are either formed or gain access leading to the formation of S-bound protein adducts and DNA cross-linking. The pyrones have been shown to have mutagenic activity, mainly in *Drosophila* and many have been shown to be carcinogenic, mainly in the rat. There is no evidence of pyrrolizidine alkaloid-induced cancer in humans.

In laboratory and domestic animals, marked anti-mitotic activity due to the pyrones has been demonstrated but this is not a prominent feature of their toxicity in humans. The main pathological feature of this effect in animals is in the liver, and less so in other tissues. In humans, the major toxicological effect of chronic exposure to PAs is veno-occlusive disease. The available data on cases of veno-occlusive disease in humans indicates a tentative no-observed-effect level (NOEL) of 10 µg/kg bw/day can be established. If an uncertainty factor of 10 to account for human variability is applied to this NOEL, the provisional tolerable daily intake (PTDI) for PAs in humans is 1 µg/kg bw/day.

### *Dietary intake assessment*

Apart from the deliberate use of herbal remedies and nutritional supplements containing PAs, humans can become inadvertently exposed through consumption of contaminated food. The foods which have been found to contain PAs include grains, honey, milk, offal and eggs. It is still unknown whether there are residues of PAs in meat.

In Australian honey, levels of alkaloid up to 1 mg/kg have been recorded from hives where bees foraged exclusively on *Echium* spp., however, blending and bulking of honey from different sources would substantially reduce this level. In the liver and kidney of domestic animals, PA levels have ranged from <10 to 73 µg/kg while in eggs, the levels ranged from 5 to 168 µg/kg. In relation to milk from domestic animals, it is likely that no

more than about 0.1% of the ingested alkaloid base will be excreted in milk. PAs and PA N-oxides are known to be excreted in cows milk, but due to milk bulking, it is unlikely that significant exposures would come from this source. In relation to human milk, PAs have been found in human milk during PA poisoning epidemics and cases of veno-occlusive disease have occurred in both neonates and other infants by this means.

Substantial contamination of grain commodities has been recorded in various countries due to both contamination by seeds of PA-containing weeds growing in the crop as well as plant dust fragments from the same plants. The levels of PAs found in various grain commodities in Australia have ranged from <50 to >6000 µg/kg, but there has been no systematic analysis of the levels in grains entering the food supply. There is currently no data to indicate whether PAs occur in oilseed crops.

On the basis of the very limited data available, the major source of dietary exposure to PAs is grains, with eggs, offal and honey minor dietary contributors. However, on the basis of the currently available data, it is not possible to estimate the potential dietary exposure to PA from these food sources.

### ***Risk characterisation***

The target organ for PA toxicity in both experimental animals and humans is the liver. In animals, this toxicity is manifested as anti-mitotic activity leading to extensive fibrosis, nodular regeneration, parenchyma and cancer, while in humans, the major effects are hepatocellular injury, cirrhosis and veno-occlusive disease. There is no evidence from the significant human epidemics which have occurred that PAs cause liver cancer in humans. Further research on the mechanisms of PA-induced hepatotoxicity may clarify the apparent differences in species specificity. At this time, the major toxicological endpoint for humans is considered to be veno-occlusive disease.

While there is survey data to suggest that significant levels of PAs can be found in some foods, and particularly in grains, there is virtually no data on the levels of PAs in foods as consumed. The effectiveness of measures taken to control *Heliotrope* seed contamination of grains is unknown. A realistic dietary exposure assessment for PAs, therefore, is not possible at this time.

On the basis of current knowledge regarding the toxicity of PAs, the PTWI in humans is 1µg/kg/day.

In order to further characterise the public health risk associated with PAs, further research is required on: (i) the levels of PAs in all foods, but particularly grains and foods derived from grains; (ii) the mechanisms of PA-induced hepatotoxicity, in order to clarify the apparent differences in species specificity.

## PYRROLIZIDINE ALKALOIDS IN FOOD

### A Toxicological Review and Risk Assessment

#### OCCURRENCE

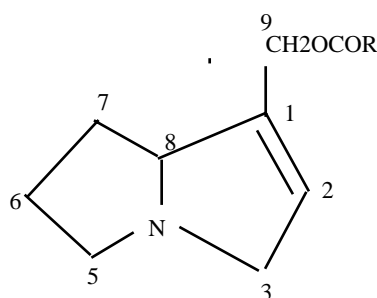
Some 13 families of the flowering plants contain PAs (Furuya et al, 1987). Only 6 of these families contain hepatotoxic PAs (Anon, 1988) but they represent some 3% of all the species of flowering plants (Culvenor, 1980). The principal families involved are the *Asteraceae* (*Compositae*), *Boraginaceae* and *Leguminaceae* (*Fabaceae*), while the main genera are *Senecio* (*Asteraceae*), *Crotalaria* (*Leguminaceae*) *Heliotropium*, *Trichodesma* and *Symphytum* (*Boraginaceae*). In Australia *Echium plantagineum* (*Boraginaceae*) is also an important PA-containing species. All three families are well represented in Australia and are causes of poisoning in grazing domestic livestock in all parts of the country (Seawright, 1989). Several *Crotalaria* spp. cause poisoning in cattle and horses in northern Australia while *Heliotropium* spp, *Echium plantaginium* and various *Senecio* spp. are responsible for toxicity in sheep, cattle and horses in southern Australia (Seaman & Walker 1985). Pigs (Hooper & Scanlan, 1977; Jones et al, 1981) and poultry (Ross & Tucker, 1977; Pass et al, 1979) have also become poisoned due to consumption of prepared feeds contaminated with the seeds of *Heliotropium europaeum* and *Crotalaria retusa* respectively.

#### CHEMISTRY OF PYRROLIZIDINE ALKALOIDS

The earliest studies of the alkaloids began with the isolation of two compounds from *Senecio latifolius* by Watt in 1909. These were subsequently tested for toxicity in frogs, cats, rats and rabbits by Cushny in 1911, who concluded correctly that they were responsible for the diseases in domestic animals caused by the consumption of *Senecio* spp in South Africa and elsewhere (Bull et al, 1968). Since that time these findings have been confirmed on numerous occasions and in many species.

Several hundred PAs have now been described (Bull et al, 1968; Mattocks, 1986) and the list continues to grow. Not all PAs however are hepatotoxic. The PA molecule is made up of two five membered rings, inclined towards each other and which share a common nitrogen at position 4. Most naturally occurring PAs are derivatives of 1-methyl pyrrolizidine while hepatotoxic PAs are esters of 1-hydroxymethyl pyrrolizidine unsaturated in the 1,2 bond, namely esters of 1-hydroxymethyl 1,2-dehydropyrrolizidine. The basic structure of hepatotoxic pyrrolizidine alkaloids is shown in Figure 1. The minimum structural requirements for toxicity are:

- (1) an unsaturated 3-pyrroline ring;
- (2) one or preferably two hydroxyl groups, each attached to the pyrroline ring via one carbon atom;
- (3) at least one of the hydroxyls is esterified;
- (4) the acid moiety has a branched chain (Mattocks 1986).



**Figure 1. The basis structure of the hepatotoxic pyrrolizidine alkaloids**

Ester linkages are found at positions C9 and C7. The amino alcohol base is referred to as a necine and the branched chain esterifying acids are necic acids. Necic acids esterify at the C9 only or at both C9 and C7 positions. The alkaloids may then exist as mono or open diesters or as a closed macrocyclic diester. N-oxides of these alkaloids are often found together with the alkaloid base in plant material. N-oxides are themselves intrinsically of low or negligible toxicity but upon ingestion are converted in the alimentary tract to the base alkaloid, which is then able to cause typical PA toxicity (Mattocks 1986).

Examples of hepatotoxic pyrrolizidine alkaloids, which occur in a variety of plants, are:

- (i) Supinine (monoester with only active centre at C9. (Necine-supinidine);
- (ii) Indicine (monester with main active centre at C9 but C7 also active). Rat LD<sub>50</sub> >1000mg/kg (necine - retronecine);
- (iii) Echimidine (open diester - active centres at C9 and C7 - LD<sub>50</sub> 200mg/kg;
- (iv) Retrorsine (macrocyclic diester - active centres at C9 and C7 - rat LD<sub>50</sub> 34mg/kg. (From Mattocks 1986. p192).

## TOXICITY IN LIVESTOCK

A detailed account of the early investigations of the toxicity of the PA plants for livestock is given by Bull et al, (1968). Seneciosis (Winton Disease) was first investigated by Gilruth in 1903 in New Zealand. Similar diseases caused by *Senecio* spp. were simultaneously being investigated in South Africa (Molteno Disease) and Canada (Pictou Disease) respectively, and later in the USA, 'Walking Disease' of horses in Nebraska. In the latter part of the nineteenth century *Crotalaria sagittalis* came under suspicion as the cause of 'Missouri River Bottom Disease' of horses in the USA and *Crotalaria dura* was shown to be the cause of jaagseikte in South Africa. The basic hepatic pathology in these diseases of cattle and horses and that described later in sheep and cattle in Australia grazing *Heliotropium europaeum* and *Echium plantagineum* was similar. The syndromes in domestic animals caused by PA containing plants are recorded by Peterson & Culvenor (1983). Essentially, acute death from a large intake of the plants over a short period may occur under conditions of natural grazing but a longer time course is more usual, resulting from a number of sublethal episodes that may extend over a period of weeks or several years. Most commonly, all the clinical signs relate to hepatic insufficiency. Sluggishness,

weakness, loss of appetite, wasting, ascites, jaundice, photosensitisation, and behavioural abnormalities have been observed, although their frequency tends to vary in different species.

In acute toxicity, extensive haemorrhagic necrosis of the liver may be the only abnormality recorded. In the chronic disease, especially if it has developed intermittently over 2-3 years, lesions of various ages may be discerned in the same animal, with terminal acute damage superimposed on a background of extensive fibrosis, bile ductule proliferation, nodular regeneration, and parenchymal megalocytosis. There may be obliteration of central and sub-lobular veins characteristic of veno-occlusive disease, but this is normally obscured by the extensive fibrosis and nodular regeneration which may mainly characterise the hepatic pathology. Reports of new PA plant toxicities from various parts of the world continue to be recorded in the veterinary and scientific literature up to the present time.

## **TOXICITY IN HUMANS**

The first recorded example of human disease caused by PA-containing plants was that reported in 1920 in South Africa where multiple cases of cirrhosis occurred following consumption of bread made from flour contaminated mainly with the plant *Senecio burchellii* (Willmot & Robertson, 1920). Since that time, many further reports of human PA poisoning have occurred, including at least nine major epidemics mainly in Africa, Central and South Asia and the Caribbean, the last one occurring in the winter of 1992-93 in Tadjikistan in which some 3906 cases or 4% of the local population were affected (Chauvin et al, 1994). Huxtable (1989) provides a comprehensive account of the circumstances in which human PA poisoning occurs and makes the point that many more cases almost certainly occur than are recorded because physicians generally do not take into account the possibility of plant-induced poisoning when examining patients with symptoms of liver disease.

The classical symptoms and signs of human PA toxicosis are abdominal pain and rapidly developing ascites. Lassitude, anorexia, nausea, vomiting, diarrhoea, oedema, emaciation, hepatomegaly, splenomegaly and mild jaundice also occur. The condition may present as an acute toxicity but is more often the late manifestation of hepatic failure or circulatory obstruction resulting from chronic pathological changes which have been developing in the liver over previous weeks or months due to a low level intake of the alkaloids (Peterson & Culvenor, 1983).

Although haemorrhagic periportal necrosis occurs in acute poisoning, occlusion of the central and sub-lobular veins, the so-called veno-occlusive disease is the histologically and functionally most prominent hepatic lesion. Occluded vessels may become cannulated and the perivenular fibrosis may progress to a non-portal cirrhosis. Bile ductule proliferation and nodular hyperplasia are not prominent and hepatic megalocytosis is not a feature of the pathology. The liver failure and hepatic veno-occlusion are the causes of the rapidly developing ascites. (Peterson & Culvenor, 1983).

## METABOLISM OF PYRROLIZIDINE ALKALOIDS

Upon ingestion, PAs are absorbed mainly from the small intestine and carried to the liver. Highly hydrophilic PAs and PA N-oxides are substantially excreted unchanged in the urine within 24 hours. PAs may be hydrolysed by tissue esterases and converted to non-toxic necines and necic acids. The branched chain necic acid on the PA molecule, by steric hindrance tends to inhibit this hydrolysis and allows the PA ester to reach the hepatic microsomal oxygenases, where in the case of the more common retronecine-heliotridine based alkaloids either N-oxidation or C3 and C8 hydroxylation may occur. N-oxides are not readily metabolised further and are mainly excreted in the urine. The proposed C3 and C8 hydroxyalkaloids are extremely unstable and with loss of water and subsequent intramolecular rearrangement are converted to the corresponding didehydropyrrolizidine alkaloid or pyrrole. These pyrrolic alkaloids possess an allylic structure which promotes an increase in their reactivity. Alkaloids of the otonecine type undergo N-demethylation followed by the formation of an hydroxyl group at C8. This transient intermediate then also loses water and rearranges internally with the formation of a didehydropyrrolizidine alkaloid as above. These are known as the primary toxic metabolites. Didehydro PAs may still undergo hydrolysis within the liver cells with the formation of the corresponding pyrrolic alcohol. These are much less reactive than the pyrrolic esters but are far more persistent and are referred to as the secondary toxic metabolites.

The pyrrolic esters contain active centres at C9 and C7 with the latter the more reactive site. Upon formation by the microsomal enzymes, the pyrrolic metabolites react with cellular macromolecules at or near the site of formation. They bind most strongly with sulphydryl groups but also with amino groups of proteins and nucleic acid bases. The possibility for double alkylation gives rise to cross-linking between DNA molecules and between DNA and protein molecules. These reactions are considered to be the cause of the initial hepatocellular damage caused by PAs.

Notwithstanding their reactivity, the pyrroles can persist for long enough in aqueous media to react at sites remote from the site of formation. This can occur within the cell of their formation itself but the primary metabolites can also pass from the hepatocyte into the adjacent sinusoids where they can react with the endothelial cells of the sinusoid and the associated hepatic vein, even reach the lungs and heart and also bind on red blood cells passing down the sinusoids. It has been shown in fact that PAs which produce persistent primary metabolites cause characteristic lung damage as well as liver damage, while with those from which short lived metabolites are produced, damage is restricted to the liver (Mattocks & Jukes 1990a). The more persistent secondary metabolites, the pyrrolic alcohols, are not acutely toxic but can cause extensive extrahepatic injury, characteristic of radiomimetic effect, and particularly in young animals, involving almost all rapidly developing tissues of the body (Peterson et al, 1972; Mattocks, 1986).

The main point of interaction of pyrroles with DNA is the reaction of the C7 or C9 of the pyrrole with the exocyclic nitrogen of guanosine (Robertson, 1982), preferentially at 5'-d(CG) sites (Woo et al 1993). Many PAs have been shown to be powerful dose-dependent mutagens in *Drosophila melanogaster* but PAs have not shown reliable mutagenicity in bacteria-based mutagenicity assays. In all such cases where mutagenicity has been demonstrated, incubation with liver microsomal preparations was necessary with all compounds. PAs have nevertheless been shown to be capable



of damaging chromosomes in a variety of biological forms including various tissue cell cultures. A suitable test *in vivo* is the transplacental micronucleus test in pregnant mice (Stoyel & Clark, 1980). Several of the alkaloids have been shown to induce sister chromatid exchange and also to induce DNA repair synthesis. Chromosome damage has been observed in blood cells of children affected with veno-occlusive disease and several alkaloids have been shown to induce chromosome aberrations in human lymphocytes *in vitro*. A summary of the results of various mutagenicity tests with PAs is reported in WHO Environmental Health Criteria Document 80 (Anon, 1988). On the data available to that time, the mutagenicity behaviour of the PAs parallels their carcinogenicity potential but appears to have little relation to their hepatotoxicity (Culvenor & Jago, 1979).

Capacity of the alkaloids through their primary and secondary metabolites to induce an anti-mitotic effect is also considered to be associated with mutation in one or more cell cycle genes and further comment will be made on this effect in a subsequent section.

## **THE MECHANISM OF CHRONIC TOXICITY**

A special feature of chronic PA-induced liver pathological changes is that after low level single or multiple exposure and well after the alkaloid and its soluble metabolites have been eliminated from the body, the disease in the liver is commonly progressive (Schoental & Magee 1957; Molyneux et al, 1988). Liver failure due to cirrhosis, veno-occlusive disease or hepatic atrophy may occur suddenly months to years after the last episode of PA exposure (Bras et al, 1961). It has been shown that the binding of pyrrolic metabolites to various nucleophiles in the liver is reversible (Mattocks, 1986) and it has been suggested that this constitutes a reservoir of electrophiles from which active molecules could be released in a continuing or intermittent manner, able to react at vital sites and maintain the progress of damage in the cell. This might in fact go on until all the reservoir of electrophile has been eliminated as soluble glutathione and other conjugates in the bile and urine. After this, if the damage to the liver is not too severe recovery may be expected to occur. Sulphur bound pyrrolic metabolites can be recovered as pyrrolic ethers from the proteins of liver and red cells for several weeks at least after exposure to the alkaloid (Mattocks & Jukes 1990b). These pyrrolic adducts may constitute such a source of electrophiles. The re-released electrophile would be the pyrrolic alcohol or secondary PA metabolite.

The reversibility of chronic damage in the liver following PA exposure is uncertain and unpredictable. In man, it is reported that following a poisoning outbreak in which significant acute toxicity is observed, some 50% of patients will recover completely and 20% will die rapidly. Of the survivors, about 20% will appear to recover clinically but may go on to develop cirrhosis and liver failure years later. Others may develop subacute liver pathological changes, which will either eventually resolve or go on to cirrhosis and liver failure (Stuart & Bras 1957).

In animals in which the antimitotic effect, megalocytosis is a feature of the liver pathology, Mattocks (1986) has suggested that this effect is not reversible. He suggests further that the mainly secondary metabolites target a specific area of the DNA with the result that a G2 + M cell cycle bypass occurs with the G1 and S phases unaffected. This then leads to nuclear polyploidy with cellular enlargement, so

characteristic of the megalocytosis of PA intoxication. An explanation of such a G2 + M bypass in the cell cycle in mammals is not so far available but in yeast this effect has been observed following an over expression of the p25<sup>rum + 1</sup> protein in that system (Moreno et al, 1994). This could be of relevance in megalocytosis due to PAs (Prakash et al, 1999).

The antimitotic effect of PAs has been used in an attempt to inhibit neoplasia in man. The PA indicine N-oxide given intravenously to humans has been shown to have some anticancer activity but causes chronic liver damage and some bone marrow aplasia. The activation of the N-oxide to pyrrolic metabolites appears to be sufficient in degree to produce this adverse effect in the liver over time but the mechanism in this instance of bone marrow toxicity is not fully understood.

A recent observation of some interest is that following exposure to the PA lasiocarpine followed 4 weeks later by partial hepatectomy in male mice in order to produce megalocytosis in the liver, an intraportal infusion of syngenic hepatocytes (hepatocytes produced from inbred animals of the same strain) prevented hepatic atrophy and caused a reversal of the megalocytosis already established (Laconi et al, 1995). It may well be that, as the mechanistic basis for the antimitotic effect is understood, a procedure for reversing this and possibly other chronic effects due to PA may be developed.

## **CARCINOGENICITY**

It has been pointed out in various reviews of PA toxicity, (Mattocks 1986; Furuya et al, 1987; Anon 1988) that several PAs, which are hepatotoxic in rats, are also carcinogenic in this species and that this property may be common to other PAs. While tumours of the liver are most commonly observed, neoplasms have been recorded in numerous other organs including lung, kidney, gastrointestinal tract, brain, spinal cord and pancreas. Leukaemia has also been recorded (Culvenor 1983). PA poisoning of domestic animals and human populations has been extensively observed since the discovery of the association of liver disease and ingestion of PA-containing plants in the first decade of this century but there has been no increased incidence of cancer linked to such exposures to date. There are also no known reports of cancer in domestic animals caused by exposure to PAs in their diet.

In an analysis of the estimated daily intakes of PAs in reported outbreaks of human poisonings up till 1983, Culvenor (1983) reported that the dose range of the alkaloids was 0.01 to 50 mg/kg/day. A review of the dose rates of PAs used to induce cancer in rats revealed that dose rates initially could be in the 2 to 6 mg/kg/day range for 4 weeks reducing to 0.2 to 3 mg/kg/day for the remainder of the 12 month period of the studies. In several of the human outbreaks of PA toxicosis the estimated daily exposure of PAs was clearly within these ranges. Follow-up observations on survivors in these outbreaks were advocated but, to date, there are no known reports of abnormal cancer incidence in these populations. Considering the high incidence of complete recovery from acute and chronic PA toxicosis in humans (>50%), it would appear that there are important differences in the response of the human liver and other tissues to PAs compared with those in laboratory and domestic animals. To date, there is no evidence that PAs are carcinogenic in humans. It has been proposed that human liver DNA repairs more efficiently after damage by PAs compared with lower animals (Prakash et al.1999).

There is convincing evidence that chronic PA exposure is carcinogenic for rats and some other laboratory animal species. In a study by Hirono et al (1976), it was demonstrated that when coltsfoot-containing diet was fed to rats for up to 600 days, there was a no-observable-effect level (NOEL) for the senkirkine in the plant of 300 µg/kg/day. Applying an uncertainty factor of 100, this would give a tolerable intake for humans based on carcinogenicity of 3 µg/kg /day. In the analysis of the Hirono study by Wolf (1983), it was concluded that the tolerable intake for humans should be about 2 mg PA/year or 0.08 µg/kg/day, but no rationale was provided by Wolfe for the use of a 5000 uncertainty factor for extrapolation to humans.

## DOSE RESPONSE RELATIONSHIPS FOR CHRONIC LIVER DISEASE

A study of human case reports of PA poisoning has been provided in IPCS Environmental Health Criteria Document No. 80 (Anon., 1988). It was acknowledged that estimates of PA intakes were approximate and that the acute toxicities of the different alkaloids and the mixtures of them which may be present in the plant source vary from highly toxic as in most *Crotalaria* macrocyclic alkaloids to PAs of comparatively low toxicity such as the monoester, indicine (Mattocks 1986). There may also be lifestyle and dietary factors that could impact on the toxicity of the PAs in any particular instance.

The main alkaloids involved in cases of human toxicity until 1988 were heliotrine from *Heliotropium*, echimidine from *Symphytum*, ridelliine and retrorsine from *Senecio longilobus* and crotananine and cronaburmine from *Crotalaria nana* (Anon., 1988). The approximate acute rat LD<sub>50</sub> for these toxins is 300, 500, 50, and 100 mg/kg respectively. These relativities need to be taken into consideration in a discussion of dose-response relationships for PAs. This was done by expressing the estimated PA LD<sub>50</sub> dose in terms of the mg/kg heliotrine-equivalent dose. The estimated daily intake of heliotrine in PA toxicity outbreaks caused by this PA varied from 0.033 mg/kg (6 mg/kg total over 180 days) to 3.3 mg/kg (67 mg/kg with death in 20 days and 167 mg/kg with death in 50 days in 2 patients, respectively). The collective data suggest that the daily PA intakes were cumulative in doses down to 0.033 mg/kg (heliotrine equivalent).

With *Symphytum* spp. (comfrey) toxicity (Ridker et al, 1985), the estimated daily intake by an affected individual of echimidine and related alkaloids was 0.015 mg/kg (total dose was 1.7 mg/kg over 4 months). The daily dose rate in terms of heliotrine equivalent was 0.009 mg/kg. It was suggested both by Mattocks (1986) and Huxtable (1989) on the basis of their extensive experience with PA toxicity that this level appeared too low to have accounted for PA poisoning and that alkaloids derived from other sources than the comfrey-pepsin capsules consumed, may well have contributed. The Environmental Health Criteria (Anon., 1988) concluded that daily PA intake as low as 0.01 mg/kg bw/day (10 µg/kg bw/day) may lead to veno-occlusive disease in humans. On the other hand, on the basis of the comments of Mattocks (1986) and Huxtable (1989), a daily intake 10 µg/kg bw may well be close to the no-observed-effect level (NOEL) for humans. If an uncertainty factor of 10 is applied to this figure to take into account individual variation, a daily intake of PAs of 1 µg/kg bw/day could be regarded as the provisional tolerable daily intake (PTDI) for humans.

## DIETARY EXPOSURE ASSESSMENT

Apart from the deliberate use of herbal remedies and nutritional supplements containing PAs (Huxtable 1989), humans can become inadvertently exposed through consumption of contaminated food. The foods that have been found to contain PAs include grains, honey, milk, offal and eggs. It is still unknown whether there are residues of PAs in meat.

In Australian honey, levels of alkaloid up to 1 mg/kg have been recorded (Culvenor, 1983) from hives where bees foraged exclusively on *Echium* spp., however, blending and bulking of honey from different sources would substantially reduce this level. In liver and kidney of domestic animals, PA levels have ranged from < 10 to 73 µg/kg while levels in eggs, the levels ranged from 5 to 168 µg/kg (Edgar, unpublished data).

In relation to milk from domestic animals, Molyneux & James (1990) have reviewed the available data and concluded that no more than about 0.1% of the ingested alkaloid base will be excreted in milk. PAs and PA N-oxides are known to be excreted in cows milk (Dickinson et al, 1976), but due to milk bulking, it is unlikely that significant exposures would come from this source.

In relation to human milk, PAs have been found in human milk during PA poisoning epidemics and cases of veno-occlusive disease have occurred in both neonates and other infants by this means (Roulet et al, 1988). The water-soluble N-oxides would be more readily excreted in milk, and when ingested by nursing infants would be converted to the base alkaloid in their alimentary tract. It was speculated that as much as 1% of ingested N-oxide could be excreted in human milk and oxidation of ingested base alkaloid in the mothers liver could also contribute to the available N-oxide for excretion in the milk. Therefore, foodstuffs contaminated by PA N-oxides and ingested by nursing mothers are potentially greater risk to babies than if the contaminating PAs were in the base form. Many PA containing plants contain up to 90% of the alkaloid in the N-oxide form (Molyneux et al, 1979).

Substantial contamination of grain commodities has been recorded in various countries due to both contamination by seeds of PA-containing weeds growing in the crop as well as plant dust fragments from the same plants (Gaul et al, 1994). Sometimes this has been sufficient to cause toxicity in livestock when the grains are used for stockfeed. The levels of PAs found in various grain commodities in Australia have ranged from <50 to >6000 µg/kg, but there has been no systematic analysis of the levels in grains entering the food supply (Edgar, unpublished). Levels of PAs in barley in excess of 6000µg/g have also been recorded (Edgar, unpublished). There is currently no data to indicate whether PAs occur in oilseed crops.

On the basis of the very limited data available, the major source of dietary exposure to PAs is grains, with eggs, offal and honey minor dietary contributors. However, on the basis of the currently available data, it is not possible to estimate the potential dietary exposure to PA from these food sources.

## RISK CHARACTERISATION

The target organ for PA toxicity in both experimental animals and humans is the liver. In animals, this toxicity is manifested as anti-mitotic activity leading to extensive fibrosis, nodular regeneration, parenchymal megalocytosis and cancer, while in humans, the major effects are hepatocellular injury, cirrhosis and veno-occlusive disease. There is no evidence from the significant poisoning outbreaks that have occurred that PAs cause liver cancer in humans. Further research on the mechanisms of PA-induced hepatotoxicity may clarify the apparent differences in species specificity. At this time, the major toxicological endpoint for humans is considered to be veno-occlusive disease.

In relation to dietary exposure, while there is survey data to suggest that significant levels of PAs can be found in some foods, and particularly in grains, there is virtually no data on the levels of PAs in foods as consumed. The effectiveness of measures taken to control *Heliotrope* seed contamination of grains is unknown. A realistic dietary exposure assessment for PAs, therefore, is not possible at this time.

On the basis of the limited human data on the incidence of veno-occlusive disease, a tentative NOEL of 10 µg/kg bw/day is suggested based on the human study reported by Ridker et al (1985). Applying an uncertainty factor of 10 to this figure to take into account individual variation, the provisional tolerable daily intake (PTDI) for PAs is 1 µg/kg bw/day.

Further characterisation of the potential human health risk from exposure to PAs in food is not possible because there is currently inadequate dietary exposure information.

### ***Further work***

In order to further characterise the public health risk associated with PAs, further research is required on: (i) the levels of PAs in all foods, but particularly grains and foods derived from grains; (ii) the mechanisms of PA-induced hepatotoxicity is needed to clarify the apparent differences in species specificity.

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