Helen Phillips MSc, Dip. Phyt., MNIMH; Alison Denham MA, FNIMH.

Introduction

This review attempts to summarise current research and thinking around the risk associated with the use of unsaturated pyrrolizidine alkaloid-containing herbs by Western herbal practitioners. The topic has been brought back under the spotlight because of the discovery of contamination of herbal teas and tablets with unsaturated pyrrolizidine alkaloids (PA, henceforth refers to unsaturated PA unless stated).

This represents our personal views only. We worked together on this topic at the University of Central Lancashire, where Helen completed the MSc Herbal Medicine, and Alison taught on the course. Here is our current thinking and discussion since early 2016 – but this is open to change based on the ever-growing published research. The points are divided into two parts each with 8 sections: 8 summaries followed by 8 sections of background detail (see pdf Bookmarks). Of the enormous literature, we have referenced relevant reviews and papers but not attempted a complete reference list.

Why does this matter?

1. Acute toxicity and hepatic sinusoidal obstruction syndrome

Acute hepatic sinusoidal obstruction syndrome (SOS), also known as hepatic veno-occlusive disease (HVOD), resulting from consumption of unsaturated pyrrolizidine-containing medicinal herbs has been recognized since the early 1950s with cases first documented in 1920 in South Africa (Selzer & Parker, 1951). Cases of acute toxicity are unlikely in Western herbal practice, as it is dose-dependent and mainly results from use of *Senecio* spp. which were banned in the UK in 2008 by SI 2008 no. 548 (MHRA, 2014) and *Crotalaria* spp. which are listed under Part 1 of Schedule 20 for sale only by registered pharmacists (Human Medicines Regulations, 2012). Reports of cases possibly associated with the use of *Symphytum* spp. Comfrey led to negotiation of a voluntary ban on the sale of Comfrey tablets and capsules made from either root or leaf, and on consumption of Comfrey root (Parliament, 1993; Couet et al., 1996). Around that time, internal usage of PA-containing herbs was restricted in many countries.

2. Chronic toxicity and cancer

- There is growing concern over the risk of chronic toxicity to humans from low-dose long-term consumption of herbs containing unsaturated pyrrolizidine alkaloids. This is because latent toxic metabolites may be formed, with delayed toxin release, and because active formation of pyrroles (the toxic agent) by the CYP450 pathway may lead to occult tissue damage. Therefore, chronic PA toxicity may be more widespread than previously thought as the associated diseases are usually of unknown aetiology thus unlikely to be linked with low-dose PA consumption (Edgar et al., 2011). Sub-acute and chronic hepatic SOS can develop slowly and silently, with gradual cumulative hepatocellular injury leading to overt veno-occlusive disease or cirrhosis of the liver (Livertox, 2016; Prakash et al., 1999).

- PAs have repeatedly been shown to be genotoxic and carcinogenic in animal and laboratory studies (Neuman & Steenkamp, 2009). There have been no reports of cancer in humans associated with PA consumption, but it would be impossible to prove an association with chronic exposure to PA or intermittent PA exposure (Edgar et al., 2011; Wiedenfeld, 2011). Even though the concentration of PA is low, there could be a cumulative carcinogenic effect.

- This risk is unpredictable due to variable individual susceptibility factors and probable dietary PA load. It has been shown that people inadvertently ingest low doses of PA through their diet in honey, bee pollen, meat, eggs and milk. Recently, contamination of herbal teas and food supplements by PA-containing weeds has been discovered (see Key point 7).

- This is not a new concern. For example, a paper published in 1950 found that liver tumours were induced in rats when fed *Senecio jacobaea* (Cook et al., 1950). This research was undertaken to investigate whether there was a link between the high incidence of primary liver cancer in South Africa and the widespread consumption of *Senecio* spp. teas. It is easy to dismiss experiments on animals by questioning the dosage and other factors, but *Tussilago farfara* contains the same PA as *Senecio* spp.

3. What are Pyrrolizidine alkaloids?

- PA can be: saturated which are NON-TOXIC, or
  unsaturated which are TOXIC .

- There are hundreds of PA, with numerous isomers. They have a common necic base, but the degree of toxicity of each PA is determined by the chemical complexity of the associated necic acids. There are three main categories: macrocyclic diesters, branched diesters, and monoesters. PA occur as free bases and as N-oxides.

4. Where are they?

- Unsaturated PA occur in Fabaceae, Boraginaceae eg. *Borago officinalis*, *Symphytum officinale* and Asteraceae plant families eg. *Tussilago farfara*.
- PA are part of the plant's defence mechanism and are found in roots and aerial parts.
- Unsaturated PA are water- and alcohol-soluble and so can be found in teas and tinctures.

5. What is the toxic action?

- The pyrrole is the toxic compound.
- Unsaturated PA can be broken down and safely excreted via two 2 enzyme pathways in the liver: carboxylesterases and mono-oxygenases.
- If these two routes are saturated eg high dose of PA, high concentration of macrocyclic PA, compromised liver function or genetic tendency, then PA are metabolised by CYP450 enzymes. This results in formation of pyrroles.
- Pyrroles are highly reactive and 'lock' onto tissues in the hepatocyte leading to cellular destruction and damage to DNA, leading to hepatic sinusoidal injury.
- Pyrroles can be broken down and safely excreted by glutathione and glutathione S-transferase in the liver. There is substantial inter-individual genetic variation in levels of these compounds, and low levels are linked to poor diet and alcohol dependency.
- More rarely, toxic PA metabolites can travel via the bloodstream and have been suggested as a cause of pulmonary arterial hypertension in animal studies (Edgar et al., 2011).
- Unfortunately, research has not investigated what protection is given by the three detoxification mechanisms in humans, and thus potential rate of pyrrole formation.

6. People most at risk of PA-associated tissue damage

- Children aged between 1-14 years old are more at risk of PA toxicity than adults, even at low dose and when their lower body weight is taken into account.
- Foetuses and babies are greatly at risk of PA toxicity, even at low dose because of normal high levels of copper in their livers.
- Pregnant and lactating mothers are also at risk as PA appear to cross the placental barrier and are excreted in milk.
- Using honey as a therapeutic medium should be considered a potential source of PA. Children and the elderly tend to consume higher-than-average quantities of honey which could provide a chronic low-dose level of PA.
- Individuals whose drinking pipes are copper-lined are at more risk of PA-toxicity at low levels.
- Certain herbs eg. *Hypericum perforatum* and medications eg. drugs used in epilepsy, induce the CYP450 metabolic route and this could increase risk of toxicity, if taken concurrently.
- Some individuals may have increased susceptibility to PA-associated damage whereas mathematical calculations of risk only take into account individual's body weight. Factors include:
  - genetic factors (liver enzymes, CYP450 pathway, glutathione and glutathione S-transferase levels);
  - liver health (past or present viral and bacterial infection, alcohol intake, history of liver disease or alcohol dependency);
  - CYP450 pathway inducers (drugs, herbs, underlying conditions);
  - glutathione status (alcohol intake, low dietary sulphur, protein and selenium intake, erratic eating habits);
  - Individuals with a family history of HVOD/SOS, liver cirrhosis, pulmonary hypertension and right heart failure, particularly of unknown aetiology may be more at risk of low-dose PA toxicity due to genetic susceptibility (Edgar et al., 2011).

7. PA in food and herbal teas

In 2015, the European Food Standard Authority published a review of PA found as contaminants of food, food supplements and herbal teas (Mulder et al., 2015). The cause is contamination by low levels of weeds. The UK Food Standards Authority undertook further tests and discovered contamination of some batches of St Johns wort tablets which led to a precautionary recall (MHRA, 2016). Alongside this, the levels of PA in PA-containing plants was investigated (Mulder et al., 2015, Table 38). Concerns over PA-containing medicinal plants were recently reviewed by the European Medicines Agency (2014).

8. Where can we go?

There are many frustrating issues around PA, particularly with regard to:

- Dosage: if the dose is high enough, the PA will be metabolized by CYP450 into pyrroles which are carcinogenic and may cause occult tissue damage. But what is this dose? Substantial expertise and expanding technical skills have led to an enormous literature on PA. However, we have not found any experimental investigation of the activity of human esterases.
- Botanical studies: More field research could help to identify strains with low PA which could be used in cultivation.
- We have used three herbs: *Tussilago farfara, Borago officinalis*, and *Symphytum* spp. to start to discuss some of these issues. This may be theoretical if there is a permanent decision to ban all PA-containing herbs!

Background to key points

1. Acute toxicity and sinusoidal obstruction syndrome (SOS)

Hepatic SOS was originally identified as a discrete clinical and pathological picture linked to consumption of PA-containing herb teas in Jamaica (Stuart & Bras, 1957, Fan & Crawford, 2014). Young children are the most vulnerable as shown by a summary of reports from South Africa (Steenkamp, Stewart, & Zuckerman, 2000). For example, a report from South Africa firmly links the death of a girl aged 3 ½ months from HVOD with a herbal tea which contained PA (Zuckerman, Steenkamp, & Stewart, 2002). Cases linked to consumption of herbal teas, in particular *Senecio* and *Crotalaria* spp., have been summarised by Wiedenfeld (2011).

Worldwide, many cases of SOS have been caused by contamination of grain supplies, in particular in the context of low protein intake (Kakar, Akbarian, Lesliet et al., 2010). Animal species vary in susceptibility, and poisonings in both humans and animals have been summarized by Wiedenfeld and Edgar (2011).

Acute poisoning from PA is more likely if

- there has been ingestion of large quantities of PA-containing plants
- plants consumed contain macrocyclic diester PA which are more toxic (see below)
- there is increased individual susceptibility eg. young age group, malnourished.

The outcome for people afflicted with hepatic SOS is unpredictable, particularly as cases mainly occur in countries where poverty leads to inadequate nutrition. Infants and the over-50s age group are at most risk of death. Some people recover and some go on to develop cirrhosis (Prakash et al., 1999). A recent prospective study in China of 23 cases associated with PA-containing herbs has provided more detail on prognosis (Gao et al., 2015). Most patients had consumed *Gynetum segetum* and the pathological changes in 16 further cases are discussed by Zhou et al. (2014).

Initial damage from PA toxicity occurs in the centrilobular area of the liver acinus, closest to the central vein. The diagnostic, characteristic primary injury is to the endothelial cells lining the sinusoids where toxic pyrroles bind to proteins in the cell membrane, causing blebs which rupture and obliterate the cell. Leakage into the Space of Disse leads to subendothelial oedema. Stellate cells begin to release fibrin in response to this damage and thrombotic factors increase within the venule wall. Haemorrhages occur in the sinusoids as hepatocytes are destroyed, with fragments of damaged cells obstructing blood flow through the sinusoids and terminal hepatic venules. Portal hypertension is an early feature of disease whilst fibrosis may be mild or even absent. Liver fibrosis occurs later, in response to extensive damage, haemorrhage and necrosis with subsequent cirrhosis of the liver. In severe cases, acute hepatic veno-occlusive disease/sinusoidal obstruction syndrome causes multi-organ failure with tissue hypoxia, renal dysfunction, encephalopathy and death (Chen & Huo, 2010; MSD Manuals, 2013; Medscape, 2014).

2. Chronic toxicity and cancer

In 1988 the World Health Organisation summarized what was known about PA toxicity and since then, the challenge of demonstrating the mechanism of tissue damage has been taken up by numerous researchers. The ongoing research has led to growing recognition of the possibility that that low level exposure to PA may be linked to HVOD and cirrhosis as well as pulmonary arterial hypertension and cancer. It has been argued that constant low-level ingestion of PA, through diet or medicinal herb consumption, may cause a chronic low-grade toxicity, whilst intermittent exposure to PA may result in the formation of latent pyrrole adducts with delayed toxin release causing damage weeks, months or even years later (Edgar et al., 2011; Prakash et al., 1999; Wiedenfeld, 2011).

Sub-acute veno-occlusive disease can develop slowly and insidiously with gradual hepatocellular injury leading to cirrhosis or HVOD/SOS (Prakash et al., 1999). Symptoms of chronic hepatoxicity may include insidious fatigue, abdominal distension, ascites, peripheral oedema and other signs of portal hypertension with persistent tender hepatomegaly upon clinical examination (Livertox, 2016, Prakash et al., 1999).

Pulmonary arterial hypertension may be idiopathic or caused by a number of conditions such as connective tissue disorders, drugs and toxins. Toxic PA pyrroles have been found to cause pulmonary micro-thrombi, acute inflammation and thickening of the vessel wall with subsequent fibrosis and occlusion. Some research authors suggest that intermittent exposure to PA may allow the liver to repair but could, over time, cause damage to lung tissue resulting in pulmonary arterial hypertension of unknown aetiology (Edgar et al., 2011). Pulmonary arterial hypertension caused by PA-toxicity has been demonstrated in non-human primate experiments (Edgar et al., 2011; Prakash et al., 1999).

It has also been argued that some copper-associated liver diseases in children may also be linked to chronic PA toxicity in that high copper levels in the liver are typical of PA poisoning (Edgar et al., 2011).

The Committee on Herbal Medicine Products of the European Medicines Agency has published an overview of the risk management issues surrounding contamination of herbal medicinal products by PA (HMPC, 2016). This summarises the risk of carcinogenicity, and states that the risk is low but definite. The tumour induced in animal studies is haemangiosarcoma. This is an extremely rare soft tissue sarcoma, a malignant neoplasm of the endothelial cells of blood vessels or lymphatic vessels. The paper cites a review of cases of liver haemangiosarcoma in Sweden, UK, USA and Norway which estimated a frequency of 0.5-2.5 cases per 10 million person per year.

In rodent experiments, PA metabolites have been shown to lead to cancer of the liver, pancreas, bladder, pituitary, bone, retroperitoneal tissue, leukaemia and rhabdomyosarcoma. There are, however, no reported cases of human carcinoma associated with PA ingestion but it is argued that if the cancer, just as in the case of pulmonary arterial hypertension is caused by a chronic low-dose exposure to toxic PAs, it would be very difficult to identify and attribute (Edgar et al., 2011; Prakash et al., 1999).

On the other hand, there is an argument that human hepatocytes do not react to the genotoxic effects of PAs in the same way as animals do, and that low-dose PA exposure does not pose a carcinogenic threat to humans. This conclusion is reflected in the Food Standards Australia New Zealand safe exposure guidelines as listed by the European Medicines Agency (2014). This may have been influenced by the paper written by Prakash et al. (1999) whose conclusion was that there is no proven cancer risk. Their research was funded by the National Health and Medical Research Council of Australia, The University of Queensland and the Queensland Health.

3. What are pyrrolizidine alkaloids?

PA are compounds found in plant species Fabaceae (Leguminoseae), Boraginaceae and Asteraceae (Compositae). They are made up of a necine base and necic acid(s), bound together by ester bond(s).

There are two types of PA: saturated which are NON-TOXIC (eg. thesinine in the flowers of borage) and unsaturated PA which are TOXIC. It is the group of unsaturated PA that are of concern to herbalists.

There are three types of unsaturated PA, defined by their chemical complexity:

- monoester PA have a single necic acid branch
- open diester PA have two necic acid branches
- macrocyclic diester PA have two branches of necic acid joined together to form a chemical ring. (Figures 1 and 7 in Roeder (1995) give examples to understand this point.)

The ester bonds of macrocyclic diesters are harder for liver enzymes to break due to steric hindrance, so they are more toxic than monoesters. Plants containing macrocyclic diesters eg *Tussilago farfara* are considered more toxic than those containing mainly monoesters eg. *Borago officinalis* (Kim et al., 1995).

In plant material, PA occur in a 'free-base' form and as N-oxides (with an attached oxygen). The N-oxides are non-toxic and water-soluble but once ingested, N-oxides are converted into free-base PA, and are now therefore considered to contribute to total toxicity (Cao et al., 2008).

4. Where are they?

PA are thought to act as part of the plant's defence mechanism as their taste is extremely bitter thus deterring predators from eating young leaves. In some but not all plants, biosynthesis of PA occurs in the root system and they are transported as water-soluble N-oxides to aerial parts and stored in vacuoles (Roeder, 1995). Levels of PA vary between different parts of plants, and according to growing conditions and maturity of the plant. There could be exceptionally high or low levels in any one sample. Unfortunately, apart from Comfrey (Mutterlein & Arnold, 1993; Denham, 1996), there is little research which compares PA levels in different samples.

Table 1: Medicinal herbs containing unsaturated pyrrolizidine alkaloids

Medicinal herbs	
Boraginaceae	*Borago officinalis,* Borage *Symphytum officinale* Comfrey, *S. asperum*, *S. caucasicum*, *S. tuberosum*, *S. peregrinum,* *S.* x *uplandicum* Other herbs: *Alkanna* spp*., Cynoglossum* spp*., Lithospermum* spp*.,* *Pulmonaria officinalis* (see below).
Asteraceae	*Eupatorium purpureum* Gravelroot, *E. cannabinum*, *E. fortunii*, *E. japonicum* used in traditional Chinese medicine *Eupatorium perfoliatum* Boneset (see below) *Tussilago farfara* Coltsfoot *Other herbs: Farfugium japonicum* Leopard plant, *Ligularia dentata* Summer Ragwort, *Petasites spp.* including *P. hybridus* Butterbur.
Fabaceae	*Crotalaria* spp.

List compiled from Denham, 1996; Dharmananda, 2001; Edgar, et al., 2011; El-Shazly & Wink, 2014; Mulder et al., 2015; Roeder, 1995; Roeder, 2000; Roeder et al., 2015.

The recent investigations carried out on behalf of EFSA have identified PA in *Eupatorium perfoliatum* Boneset powder, whereas other recent research found no PA in *Eupatorium perfoliatum* (Mulder et al., 2015, Table 38, page 77; Hensel et al., 2011). Mulder et al. (2015) identified PA in samples of *Pulmonaria officinalis,* whereas previously the status of *P. officinalis* was unclear as only *P. obscura* (roots), Unspotted lungwort had been investigated (Haberer et al., 2002).

5. What is the toxic action?

Numerous animal and laboratory experiments have shown that unsaturated PA can be metabolised into toxic pyrroles. These investigations are summarized by Fu et al. (2004). The role of the reactive metabolites derived from pyrroles has been explored extensively, and recently demonstrated in vitro by Yang et al. (2016). PA may be ingested as non-toxic N-oxides or potentially toxic free-base PAs. N-oxide PAs are broken down by bacterial microflora and gut enzymes to become free-base PAs which are then absorbed in the gut and travel via the bloodstream to the liver. PA are then metabolised in one of three ways:

- In the hepatocyte, they are hydrolysed by carboxylesterase enzymes. This process breaks the ester bonds of the PA, leaving necic acid and necine. These are water-soluble, non-toxic and excreted via the urine.
- The second pathway involves oxidation by other liver enzymes, the flavin-containing mono-oxygenases. An oxygen binds to the free-base PA, thus making it an N-oxide again which is water-soluble, non-toxic and excreted in the bile and urine.
- If the first two pathways are saturated or compromised, the free-base PA is metabolised by cytochrome P450 mono-oxygenases, specifically CYP3A4, CYP2B and CYP2D located in the hepatocyte. The free-base PA becomes

a 6,7-dihydropyrrolizine ester (also known as an ester pyrrole).

These ester pyrroles are highly reactive and can quickly bind to cell proteins in the liver. They can also bind to red blood cells and travel via the bloodstream to the lungs and other organs. Within the liver, toxic ester pyrroles may be metabolised in a number of ways.

- Some pyrroles re-bind to CYP3A4 and are metabolised into N-oxides which are then excreted via the bile and urine.
- Others are bound to the protein glutathione in hepatocytes by the enzyme glutathione S-transferase, and become non-toxic thiol pyrroles. These are then excreted via the bile and urine.
- Some pyrroles, however, are converted into 6,7-dihydropyrrolizine alcohol. These compounds cause cellular destruction by damaging DNA and the proteins responsible for cell integrity. They have been implicated in hepatic veno-occlusive disease and cirrhosis of the liver.
- These compounds also bind to the nucleotide proteins DNA and RNA where they form cross-linkages, interrupt replication and cause mutation. This appears to be the mechanism behind the genotoxicity and carcinogenicity of PA.
- Pyrroles may also become protein-bound as dihydropyrrolizine adducts which have been found in the liver, lung, kidneys, spleen and blood of animals. These adducts can lie tissue-bound for several days, months or even years after the initial ingestion of PAs until they are released and become toxic 6,7-dihydropyrrolizine alcohols. These latent adducts are steadily released until they are all used up; the trigger that releases them from tissue is unclear. This pathway is believed to underlie cases of delayed or insidious toxicity.

(Information compiled from Edgar et al., 2011; EMA, 2014; Prakash et al., 1999; Roeder, 1995; Wiedenfeld, 2011).

How is this relevant to humans?

Most research into PA has been conducted upon animals with some studies using human tissue samples or human tissue enzymes to establish biochemical reactions (Prakash et al., 1999). Some researchers argue that humans are more sensitive to PA toxicity at lower doses than animals (WHO, 1988).

Most research studies use isolated PA which does not reflect herbal usage of the whole plant. However, animal studies into the ingestion and metabolism of PA-containing plants, reveal that the PA are broken down by gut microflora (Roeder, 1995), gut enzymes, liver microsomes, NADP and NADPH (Wiedenfeld, 2011) into free-base PA (Mei et al., 2010). There is no evidence to suggest that PA-containing plants also contain hepato-protective mechanisms (eg. CYP450 inhibitors). Animal experiments often use very high doses of PA, and some involve the administration of PA intravenously or by intramuscular injection and are therefore not reflective of Western herbal practice. However, it is the process of natural digestion that converts the plant PA into a potentially toxic pyrrole, so these experiments cannot just be ignored.

6. People most at risk of PA-associated tissue damage

Dose dependent: High doses of toxic PA saturate the initial detoxification routes, thus initiating the CYP450 pathway and formation of pyrroles. If the dose is high enough (or the individual susceptible), acute poisoning occurs. Even in lower doses, if initial detoxification routes are saturated, cellular damage can occur and latent toxic metabolites bind to tissue. It is an accepted probability that most people regularly ingest a low dose of PA through their diet. It has been argued that a slight increase in PA consumption may push them over the threshold and into toxic PA metabolism. For example, if someone starts to take a therapeutic (ie. higher) dose of PA-containing herbal remedies, there is a risk of low-grade chronic toxicity even if taken short-term.

Complexity of PA in plant consumed: Plants containing a higher concentration of macrocyclic diesters are considered more toxic than those containing monoesters.

Copper, foetuses and newborns

- High copper levels in the liver causes increased susceptibility to low-level PA toxicity (Edgar et al., 2011).
- Foetuses (in third trimester) and newborns (until 4-6 months) naturally have higher levels of copper stored in their liver thus potentially making them more susceptible to PA poisoning (Edgar et al., 2011).
- It is believed that during pregnancy, lipophilic toxic pyrroles ingested by the mother can cross the placental barrier causing poisoning to the foetus (Prakash et al., 1999).
- Animal studies reveal that water-soluble PA are excreted in the mother's milk and ingested by the infant

(Prakash et al., 1999), and therefore pregnant women, infants and lactating mothers should avoid PA-containing medicinal herbs (EMA, 2014).

- Copper in drinking water (eg. from copper pipes) does not, in itself, cause liver problems but it is argued that this might potentiate the toxicity of PAs even at low dose (Edgar et al., 2011).

Age and prognosis: Reports of PA-associated disease have shown that children and young adolescents (age 1-14yrs) are most susceptible to PA toxicity, even at low dosage. Case series such as Stuart and Bras (1957) have attempted to estimate prognosis after diagnosis of HVOD, but the role of inadequate diet and poor health in these patients makes the figures unreliable.

Potentially increased susceptibility to low-dose PA overload should be considered in the 1-14 yr age group.

Gender: Gender may influence an individual's susceptibility to PA toxicity but it is yet unclear as to which gender is more at risk as there are conflicting views among researchers.

Liver function and health: Some viruses, bacteria and fungi act as hepatoxic agents, working synergistically with PA and increasing the risk of hepatoxicity. Compromised liver function (low levels of liver enzymes and microsomal enzymes, viral and bacterial infections, alcohol dependancy) will encourage use of all pathways to deal with ingested PA, including the CYP450 route to toxic metabolite formation. It has also been shown in studies in China of people diagnosed with primary liver cancer that carcinogenic factors, in this case Hepatis B infection and consumption of aflatoxins in spoiled grains have a cumulative effect (Wogan, 2000).

Genetic variation: Levels of liver enzymes vary hugely between individuals, with a probable genetic pattern. If these are compromised or at inherently low levels, there is a greater chance of PA being pushed down the CYP450 pathway and converted into toxic metabolites.

Cytochrome P450 pathway: Rates of CYP450 enzyme activity can vary significantly between individuals resulting in considerable variability of response to PA exposure. Some drugs and herbs induce CYP450 enzymes and could increase the risk of toxicity if taken alongside PA-containing herbs.

Table 2: Factors that induce CYP450 enzyme pathway

Drugs	Griseofulvin (anti-fungal)
	Rifampcin (antimycobacterial)
	Carbamazepine, phenytoin, primidone, topiramate (anti-convulsants) Phenobarbital (however, also induces glutathione S-transferase, encouraging detoxification of pyrrole ester)
	Dexamethasone (corticosteroid): lower potency glucocorticoids, for example, prednisolone have minimal effect on CYP3A4
Herbs	*Hypericum perforatum* (St John's wort)
Other	DDT and edrin (organo-chlorine pesticides)
	Acute systemic hypoxia (chronic respiratory or cardiac insufficiency increases CYP3A4 activity)

Levels of glutathione and glutathione S-transferase: Individual variations in levels of hepatic glutathione and glutathione S-transferase are also likely to be genetically influenced. Low levels of glutathione have been found to increase toxic pyrrole formation in rodent experiments. Glutathione levels naturally drop by 50% after overnight fasting.
People with low intake of sulphur-containing foods (eg. cruciferous veg, shellfish, eggs, red meat, dried apricots, *Allium* spp.) and therefore lower levels of glutathione, are potentially more susceptible to PA poisoning.
Alcohol dependency can deplete glutathione levels and result in poor hepatic function, increasing risk of PA toxicity even at low dose.
Protein malnutrition (ie. poor diet, erratic eating, fasting) and selenium deficiency also deplete glutathione levels, potentially making the individual more susceptible to PA poisoning.

7. PA in herbal teas and food supplements

Recently, pyrrolizidine alkaloids have been discovered in herbal teas sold as food supplements or as registered medicinal teas in Europe. The teas surveyed are popular single herbs or mixtures.
Six studies have identified PA in food supplements and herbal teas in Europe, and four of these are included in a review carried out for the European Food Safety Authority (Mulder et al., 2015). They review studies from Germany and Ireland

(Bodi et al., 2014; Griffin et al., 2014; Mathon et al., 2014; Schulz et al., 2015). Studies have also been carried out in Switzerland and Belgium (Madge et al., 2015; Huybrechts & Callebaut, 2015). The results in each study vary depending on the number of PA tested for and the specific testing methods. For example, Bodi et al. (2014) purchased 282 teas and 43 mixed teas a retail outlets in Berlin in 2012-2013. They tested for 17 PA and found 14 of these PA in some samples. Taking Melissa teas, they identified some PA in all the samples (14/14). The range was 5-2579 µg kg^{-}. This is an example of the wide variation in levels. Rooibosch tea was the worst performer in most studies. For example, of 24 samples, 100% contained PA with the maximum 5647µg kg^{-l} (Bodi et al., 2014).

These teas should not contain any PA, and the main cause is contamination by weeds eg *Senecio* spp., *Heliotropium* spp. and *Myosotis* spp. Forget-me-not. The wide range of PA identified means other weeds must be involved, and there is substantial activity within Europe to confront the problem through testing and agricultural methods (HMPC, 2016).

Why does this matter as such low levels?

PAs have been shown to be carcinogenic in animal and laboratory studies (Neuman & Steenkamp, 2009). Even though the concentration of PA is low, there could be a cumulative carcinogenic effect as some people may consume the same brand of the same herb tea for years on end.

PA in diet

Unsaturated PA have also been found in foodstuffs including milk, eggs, meat, bee pollen and honey. This is because the animal has consumed PA-containing plants, and the bees have visited PA-containing plants. The topic is discussed in a report for the European Food Safety Authority by Mulder et al. (2015). Vegans, for example, have a much smaller risk of chronic low-level PA exposure.

Honey: Unsaturated PA have been found in honey collected by bees from PA-containing flowers. Daily consumption of 15 - 25g of honey is enough for chronic toxicity (Edgar et al., 2011). Now that the problem has been identified, then efforts are being made to reduce the level of PA in honey. Children and the elderly are of particular concern with regard to chronic overload of dietary PAs, as both tend to consume higher-than-average quantities of honey (Edgar et al., 2011). Using honey as a therapeutic medium should also be considered a potential source of PA.

Bee Pollen: A daily amount of 10mg of bee pollen gives on average, 15ug PA which is sufficient to give risk of chronic toxicity (Kempf et al., 2010, cited in Edgar et al., 2011).
Wheat and salad: There is some concern that there may be residual low levels of PA-containing plants in wheat that are not fully eradicated by the grain-filtering processes used by countries in the West (Edgar et al., 2011). PA-containing plants have been detected in salad boxes in Germany (Wiedenfeld, 2011).
Milk: Lipophilic toxic PA have been found in milk (EMA, 2014).
Meat: Meat, especially liver, may contain toxic tissue-bound PA ingested by the animal before its slaughter. PA are not destroyed by cooking (EMA. 2014).
Eggs: PA have been found in eggs, as a consequence of PA contamination in the hen's grain but were not found when hens were deliberately fed PA-containing plants. The risk or incidence rate of PA-contaminated eggs has not been fully evaluated (EMA, 2014).

8. Where can we go with this?

Attribution of causation in individual cases is replete with problems. Teschke and his team have investigated these problems in cases of hepatoxicity and drug-induced liver disease attributed to consumption of medicinal plants (Teschke et al., 2014). We have not discussed cases here as the issue we are highlighting is the potential significance of chronic tissue damage which is even less likely than acute cases to be able to be connected with a definitive diagnosis.

We have pointed out the lack of research relevant to herbal practice, on dosage and botanical studies of relevant plants and discuss these points a little further here.

***Tussilago farfara* Coltsfoot:** In 2011, *Tussilago farfara* Coltsfoot was included in a review of herbs with longstanding usage in Europe, and that led to a detailed discussion of the safety aspects (Tobyn et al., 2011). Coltsfoot is in the Asteraceae family and contains the same macrocyclic PA as in *Senecio* spp. albeit at lower levels. I do not use this herb as in 1996, I argued that the macrocyclic PA are more dangerous (Denham, 1996). However, it is interesting that the 1992 PA limits set in Germany (1 µg /day for 6 weeks) made an exception for Coltsfoot (10 µg /day for 6 weeks). A recent paper found that this level would be exceeded by a 400 mg sample of dried herb (Nedelcheva et al., 2015). The

recommendations derived from the review of the traditional literature were “In cough prescription for up to 6 week’s use; particularly for stubborn old cough; particularly for emphysema and silicosis; and external applications for lung problems, as a poultice.” Safety recommendations were: “1. Use caution in dosage and duration of usage and consider using other expectorants before using coltsfoot. 2. Coltsfoot should not be used in children under 18 or in pregnancy and lactation. 3. Coltsfoot should be used short term unless there is an overriding reasons for continuing its use in a particular patient. Coltsfoot should not be taken alongside *Hypericum perforatum* or any medications for the treatment of epilepsy.” (Tobyn et al., 2011, pp. 317-326).

These recommendations strike at the heart of some of the frustrating issues around PA. If the dose is high enough, the PA will be metabolized by CYP450 into pyrroles which are carcinogenic and may cause occult tissue damage. But what is this dose? It may be that in most people, the liver detoxification mechanisms are sufficiently effective and the threshold where they are overridden is not crossed - and there is no metabolism by CYP450. If glutathione and glutathione-S-transferase levels are sufficient then the pyrroles formed will be metabolized and excreted - but there is no way of guaranteeing this.

***Symphytum* spp. Comfrey:** The internal use of Comfrey leaf can only be contentious and excite conflicting views! PA in Comfrey have been shown to be genotoxic and carcinogenic (Mei, et al., 2010). I argued that there was less risk because the PA are monoesters or open diesters and thus more likely to be metabolised before the CYP route comes into play. The argument against this is that in the end a pyrrole is a pyrrole, and the risks associated with ingestion of PA override the value of the plants. But what if the comfrey was prescribed for a life-threatening disease, for example for bleeding associated with gastric or duodenal ulcers (BHMA, 1983), or where the patient had survived oesophageal varices? But would you then have direct absorption (possibly of free base PA), bypassing any initial intestinal detoxification? And, equally would sharing clinical experience within the profession lead us to discover that use of, for example, Rad. *Althaea officinalis*, *Calendula officinalis*, *Agrimonia eupatoria* or *Potentilla erecta* would be equivalent or better?

Finally, my argument depended on dosage, and I argued that mature leaves should be used, and that *S. officinale* should be used not *S.* x *uplandicum* as the level of PA is lower (Denham, 1996). This highlights the need for further studies into wild and cultivated plant materials to investigate this point!

Borago officinalis* Borage*: There appears to be no evidence, as yet, to suggest that borage flowers contain toxic unsaturated PA, unlike the leaves. The only alkaloid found in the flowers of *Borago officinalis* is thesinine, a saturated and therefore non-toxic alkaloid but this study dates from 1986 (Dodson & Stermitz) and there have been no further biochemical studies on Borage flowers since.

Traditionally, Borage flowers were considered superior to the leaves as a warming remedy for the heart, seat of all emotions, to ease pensiveness and sorrow, and to increase joy (Culpeper, 1653, 1816; Evelyn, 1699; Gerard, 1597; Parkinson, 1640). The fresh leaves were used but with other pot herbs during convalescence, their high potassium nitrate level now known to be able to soften meat and beans. This is in contrast to the more popular current use of the leaf tincture as a long-term treatment for adrenal exhaustion, burn-out and fatigue (Bartram, 1995; Hoffman, 1983, 1988). This finding reveals a shift in medical thinking and herbal materia medica and, I believe, is worth further investigation as it may provide a possible alternative of using borage flowers, not the leaves, as a safe and valuable medicine.

Dosage using *Borago officinalis* as example
Six unsaturated toxic PA have been isolated from the leaves of *Borago officinalis.*
There have been no reports of human cases of acute toxicity through the ingestion of Borage. The PA found in borage leaves include diesters and monoesters only, indicating a relatively low risk of acute toxicity.
However, the presence of any unsaturated PA may pose the threat of insidious chronic or delayed toxicity.

Levels of PA found in the dried leaves of borage are seemingly very low (see Table 3). However, the research study which identified this PA level (Larson, Roby & Stermitz, 1984) has a number of limitations and may present a false conclusion regarding levels of PA in borage leaves. Experimental techniques are now much more sensitive (These et al., 2013).
For the purpose of the study, dried *Borago officinalis* had been purchased from a local store which raises the question of correct identification. The fresh plant material, which was also studied, was grown to maturity to ensure correct identification. This may present a false low reading of PA content, as mature leaves of many PA-containing plants contain lower levels of unsaturated PA than the young leaves.

Table 3: Percentage of alkaloid content of dried Borago officinalis

"Dried bulk plant fragments (496g) yielded 48mg of crude alkaloid mixture" (Larson, Roby & Stermitz, 1984).
therefore the percentage of alkaloid content of dried *Borago officinalis* is calculated as follows,
Therefore % = 48mg/496000mg x 100 = 0.00967%, ie. **less than 0.01% alkaloid content**

Later statements on borage are founded upon this one study in 1984, and further research has been considered unjustified as there is a lack of proof for the efficacy of borage as a medicine. This does not, however, reflect how it is still widely used in Western Herbal Medicine.

Worldwide health authorities have established safe dosage guidelines for PAs found in food which vary from country to country (EMA, 2014). In an attempt to establish a safe daily dose of a 1:2 25% specific tincture of *Borago officinalis*, calculations were made using the formula for the total daily intake recommended by the German Federal Health Bureau (Bundesanzeiger, 1992 cited by EMA, 2014), as it does not consider adult body weight when establishing dosage and, in this way, reflects herbal practice. Levels of PA present in borage leaf were taken as cited by Larson et al. (1984), and the formula proposes that:

- The recommended daily dosage of dried borage herb is 10.3 mg if taken for less than six weeks in a year.
- The recommended daily dosage of dried borage herb is 1.03 mg if taken for more than six weeks in the year.
- The recommended daily dosage of 1:2.5 25% specific borage tincture is 0.009ml if taken for less than six weeks in a year. This is practically impossible to administer.

Honey as a therapeutic medium: Honey is only contaminated by PA if the bees visit PA-containing plants so, in theory, the time of year and site of beehives will have an influence. For example, forest honey collected in the summer may be more likely to contain PA from plants such as *Senecio* spp. than heather honey or orchard honey collected in the Spring. However, Edgar et al. (2011) argue that the named flower source only represents the majority of plants flowering near the hives and that other nearby PA-containing flowering plants, such as *Myositis* spp. and *Cynoglossum* spp. could contribute to the honey produced. The huge variations in PA found in honey supports this and therefore no honey can be guaranteed free from PA.

Conclusion

Finally, we could say well, a qualified practitioner will judge whether the herb is necessary. This separates herbal practice from potential long-term usage of herbs by people who are innocent of any potential risks – for example who are buying herbs online. But what is necessary? There are big unknowns – and unknowns generate controversy and opposing points of view.

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