COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

STATEMENT ON PYRROLIZIDINE ALKALOIDS IN FOOD

Introduction

1. Pyrrolizidine alkaloids (PAs) are a large group of natural toxins produced by plants, several of which are known to be highly hepatotoxic and have been shown to be carcinogenic in rats. They have been associated with a number of livestock diseases and cases of human poisoning following consumption of herbal remedies or after contamination of staple foods. There is also potential for PAs to be transferred to other food products such as honey, milk, eggs and offal.

2. In 2007, the report of a Food Standards Agency funded project on PAs in honey from borage and ragwort was published. This report was provided to the Committee along with a number of risk assessments of PAs from other countries.

3. The Committee was asked for its view on the risk assessment of PAs in food and whether it considered potential human exposure, particularly via honey and milk, to be of concern.

Background

4. PAs are found in a large number of plants around the world including the families *Boraginaceae* particularly *Heliotropium* and *Trichodesma* species, *Compositae* (*Asteraceae*) in the tribe *Senecioneae* and *Leguminosae* (*Fabaceae*) in *Crotalaria* species. It is estimated that approximately 3% of the world's flowering plants contain one or more toxic PAs[1].

5. Cases of human toxicity have been shown to occur following contamination of staple foods, generally grain crops, and after consumption of some herbal remedies. Other possible food sources of exposure include milk, honey, offal and eggs which have all been found to contain PAs in some instances[2], although cases of human poisoning resulting from exposure through these sources have not been reported. It is unknown whether PA residues are present in meat but the potential for exposure is thought to be slight due to the fast clearance of PAs from the body of the animal[3].

6. In humans, veno-occlusive disease is the most prominent hepatic lesion resulting from PA poisoning. Classical signs and symptoms are abdominal pain and rapidly developing ascites. The effects of PAs can take time to develop and could be due to long term low level exposure, although known cases of poisoning have usually presented as acute disease similar to Budd-Chiari syndrome[2].

7. Livestock poisonings have been reported worldwide, especially in cattle and horses, but also in some instances in sheep. One of the plants often associated with this is common or tansy ragwort (*Senecio jacobaea*)[2].

8. In animals, PA toxicosis is usually characterised by clinical signs relating to hepatic insufficiency including weakness, loss of appetite and wasting, jaundice and behavioural abnormalities. Extensive haemorrhagic necrosis of the liver is usually recorded in acute toxicity. Chronic disease, either resulting from a single sublethal dose or from repeated low level intake, shows a variety of lesions such as parenchymal megalocytosis, extensive fibrosis, obliteration of central and sub-lobular veins characteristic of veno-occlusive disease, bile duct proliferation and nodular regeneration[2].

9. Instances of poisoning in humans and livestock, combined with the results of studies in experimental animals indicate that there is variation between species in susceptibility to PAs. In general, cattle, horses, pigs, poultry, humans, rats and mice are considered to be sensitive while sheep, goats, rabbits and guinea pigs are less so[4,5].

Previous COT recommendations

Comfrey

10. The Committee last reviewed PAs in 1992[6], focussing on comfrey, a herb which at the time was available in tablet and capsule form as well as for tea and infusions. The recommendations of this review of comfrey were as follows:

- the public should be warned of the potential dangers associated with the consumption of comfrey and products containing comfrey. This advice applies equally to commercial and home-grown comfrey and preparations made from it.

- concentrated forms of comfrey such as tablets and capsules should no longer be available.

- the public should be advised against the ingestion of comfrey root and leaves, and of teas and infusions made from comfrey root.

- comfrey teas and tinctures may continue to be available to the public. However, this recommendation should not be construed as an endorsement of these products.

The COT's advice was subsequently endorsed by the Food Advisory Committee. Department of Health (DH) and MAFF Ministers accepted the committees' advice and action was taken to implement it.

Chemistry

11. PAs are a group of more than 350 natural toxins sharing a basic structure derived from esters of 4 necine bases: platynecine, retronecine, heliotridine and otonecine. The acid moieties of the esters are termed necic acids. A number of structural features determine the potency of the PAs.

12. PAs associated with adverse effects are esters of 1-hydroxymethyl 1,2-dehydropyrrolizidine (figure 1). There may be a second hydroxyl group at the C7 position. At least one of these hydroxyl groups must be esterified to exert toxicity and the acid moiety of the ester linkage must contain a branched chain. PAs can therefore exist as mono or open diesters or as a closed macrocyclic diester[2]. Figure 1 shows the basic structure of these PAs.

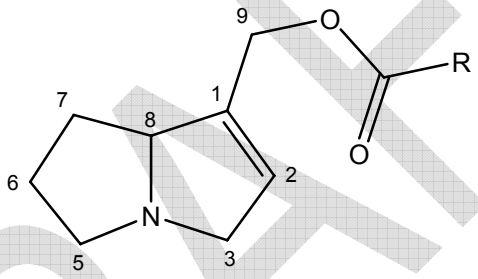

Figure 1: The generic structure required for PAs to cause toxicity.

13. PAs are fairly stable chemically and require metabolic activation to exert toxicity [5].

Metabolism

14. On ingestion of PAs, the majority of the dose is eliminated unchanged . Studies using a limited number of representative PAs have shown that three main pathways of metabolism occur[7].

Activation pathway

15. The activation pathway is oxidation of the PA to form the dehydropyrrolizidine derivative, which is biologically and chemically reactive (figure 2). Cytochromes P450 have been shown to be involved in this bioactivation of the PAs[8].

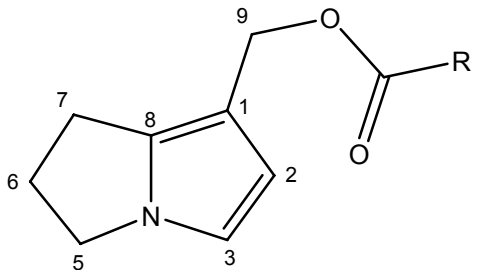

Figure 2: The generic structure of the dehydropyrrolizidine derivative of PAs.

16. Dehydropyrrolizidine derivatives can undergo further biotransformation by enzymic or non-enzymic glutathione conjugation[1]. Alternatively, the dehydropyrrolizidine derivative can be hydrolysed further at the ester bond to form the dehydronecine, often referred to as dehydropyrrolizine (DHP)[8].

Detoxification pathways

17. Esterase cleavage of the PA releases the necine base and necic acid(s). No further metabolism occurs and this is seen as a detoxification pathway[7].

18. *N*-oxidation of retronecine- and heliotridine-type PAs is generally catalysed by a variety of enzymes including cytochromes P450 and flavin-containing monoxygenases. The *N*-oxides are highly water soluble and are rapidly excreted in the urine[5].

19. *N*-oxides are also often found in plant materials. While metabolism to PA *N*-oxide is usually seen as a detoxification pathway, upon ingestion these can be converted to the alkaloid form in the gut[3].

20. The activity of the metabolic enzymes towards individual PAs plays an important role in determining toxicity and varies between species, sexes and at different developmental stages[7,9,10,11,12].

21. Following metabolism, rapid elimination occurs mainly via urine but some goes in to the bile. It is considered unlikely that large amounts of the PAs and their metabolites remain in the liver in the long term[3]. PAs and their metabolites can be excreted in the milk and possibly, in the case of poultry, into eggs[1].

Toxicity

22. Much of the data on PA toxicity is derived from studies on plant constituents or extracts and often arises from studies or reports of single dose administration. Information on the mechanism of toxicity has been taken from reviews, which have compiled data from a number of studies and do not quote the doses at which the individual effects occur. Where doses are given these are examples, generally of the lowest dose causing effect, and illustrate the range in the data available.

Human case reports

23. A 49 year-old woman, who had been consuming a herbal tea and comfrey-pepsin pills on a daily basis for 6 and 4 months respectively, was admitted to hospital with progressive swelling of the abdomen and extremities. This was diagnosed as veno-occlusive disease, a form of Budd-Chiari syndrome. A liver biopsy showed centrilobular necrosis and congestion. The hepatic venograms were consistent with moderate portal hypertension recording wedge pressures of 23 mmHg with corrected sinusoidal pressure of 17 mmHg. No demonstrable obstruction of outflow was observed but balloon distention of an intrahepatic venous tributary showed near obliteration of the smaller hepatic venules and extravasation of the dye into the hepatic parenchyma. There was no history of tumour or trauma, her last pregnancy was 22 years previously and no common causes of Budd-Chiari syndrome were evident. The herbal tea and comfrey-pepsin pills were analysed for PAs, based on monocrotaline as a standard and looking for alkaloids of the same molecular weight and with the same extinction coefficient for spectrophotometric detection. The subject's minimum daily PA intake was estimated as 15 µg/kg b.w. The researchers stated that it is possible she had other sources of exposure in this time[13]. This report is one of the few where an attempt has been made to evaluate the exposure level of a person with PA poisoning.

24. A 5 day old female infant was referred to intensive care with jaundice, massive hepatomegaly and ascites. When the infant was 27 days old, a biopsy was taken. This showed centrilobular fibrosis, neovascularisation and iron deposition associated with widespread circumferential connective tissue occlusion of the small and medium size hepatic veins suggesting a diagnosis of hepatic veno-occlusive disease. The mother had had daily consumption of a herbal tea containing senecionine (including its *N*-oxide) at 0.60 mg/kg dry weight but no estimate of the dose to the mother or the fetus was given. A liver biopsy section of the mother was not obtained but physical appearance and blood tests showed no abnormalities[14].

25. A pregnant woman was admitted to hospital in the 27th week of gestation with fetal ascites. The male infant was delivered by emergency caesarean section during week 32 but died 12 hours later. Autopsy found no internal or external malformations. Liver histology showed veno-occlusive disease. Tea used by the family was found to be free of PAs. However, a herbal mixture of which 2 g/day was used for cooking contained 6 mg/kg lycopsamine, 3.5 mg/kg interrimine and 3 mg/kg of their acetyl derivatives. Neither the maternal or the fetal dose resulting from the use of this mixture was estimated. The dehydro-derivatives of these PAs were found in the fetal liver tissue[15].

26. Based on information provided by two paediatric liver centres to the Committee, it was noted that incidence of paediatric veno-occlusive disease was likely to be rare in the UK.

Acute Studies

27. LD50 data obtained following intraperitoneal administration to male rats are available for some PAs and are given in Table 1[16,5].

Table 1: Reported intraperitoneal LD50 values obtained for the male rat unless otherwise stated (Source: Cheeke and Shull, 1985 and WHO, 1988).

Alkaloid	**LD50 (mg/kg b.w.)**
Retrorsine	34
Senecionine	50 – also quoted as 85
Heliosupine	60
Lasiocarpine	72
Seneciphylline	77
Jacobine	77 (mouse)
Riddelliine	105 (mouse)
Symphytine	130 also quoted as 300
Heleurine	140
Jaconine	168 (female rat)
Monocrotaline	175
Echimidine	200
Spectabiline	220
Senkirkine	220
Retrorsine *N*-oxide	250
Heliotrine	300
Echinatine	350
Supinine	450
Lasiocarpine *N*-oxide	547
Europine	>1000
Heliotridine	1200
Intermedine	1500
Lycopsamine	1500
Heliotrine *N*-oxide	5000

28. Lesions in the lung following acute dosing include alveolar oedema and effects on the alveolar wall seen after a single dose of 60 mg/kg b.w. in rats given monocrotaline subcutaneously and at the same dose in dogs given monocrotaline intravenously. In the long term extensive pleural effusion occurs following intraperitoneal administration of a single dose of 50 mg fulvine/kg b.w. to female rats[5].

29. Necrotising pulmonary arteritis is observed following subcutaneous administration of monocrotaline at 120 mg/kg b.w. to male rats as a single dose or 30 mg/kg b.w. as 4 doses each 2 months apart in monkeys[5].

30. A study investigated the early pulmonary changes following PA exposure using monocrotaline pyrrole (the dehydropyrrolizidine derivative of monocrotaline) injected into the tail vein. A single dose of 3.5 mg/kg b.w. injected in the tail vein to male Sprague Dawley rats caused changes in lung from 4 hours after administration[17].

Repeated dose toxicity

31. Monkeys given monocrotaline at 60 mg/kg b.w. by subcutaneous injection at monthly intervals for 3 months showed varying degrees of occlusion

of the centrilobular, sublobular and larger veins in the liver Centrilobular haemorrhagic necrosis and megalocytosis were also observed[5].

32. Rats given 8 mg/kg b.w./day by gavage 5 days per week for 114 weeks of an alkaloidal extract of *Senecio nemorensis* ssp. fuchsii containing 50% fuchsisenecionine and 1% senecionine. Fatty changes, single cell and focal necrosis, fibroses and granulomatous reactions were observed in the livers[18].

33. In a study of riddelliine administered by gavage 5 days per week for 105 weeks to rats and mice, 0.033 mg/kg b.w./day in rats caused hepatocyte cytomegaly (NOAEL 0.01 mg/kg b.w./day) and at 0.33 mg/kg b.w./day regenerative hepatocyte hyperplasia was observed. In mice, focal necrosis was observed at 0.1 mg/kg b.w./day (lowest dose given) and hepatocyte cytomegaly and karyomegaly was observed at 0.3 mg/kg b.w./day (NOAEL 0.1 mg/kg b.w./day)[19].

Mechanism of action

Hepatotoxicity

34. Animal studies have demonstrated that hepatic parenchymal cell and sinusoidal endothelial cell injury occur early in the process of PA induced disease. The start of the process is thought to be disruption of protein synthesis and some of the mitochondrial processes. Once cell injury has occurred, zonal necrosis ensues where the zone affected depends on the species and the pathway of metabolism for the PA in question[5].

35. Veno-occlusion is thought to occur because of damage to the sinusoidal and central vein endothelial cells leading to thickening and then collagenisation. Occlusion of the central vein occurs which is preceded by a functional blocking of the blood flow. Fibrosis occurs from the central vein through the sinusoids and into the space of Disse.

36. One of the typical features of PA toxicosis in animals is megalocytosis of hepatic parenchymal cells, though this has not generally been observed in humans. This is believed to occur where cells are stimulated to go through the cell cycle but do not divide. This anti-mitotic effect has been suggested to be a result of cross-linking of actin which plays a major role in cell division[20].

37. The WHO stated that adverse long term effects are similar whether resulting from one relatively high dose, which is not acutely lethal, or multiple low level doses[5].

Pulmonary toxicity

38. Pulmonary toxicity is sometimes, but not always, seen with hepatotoxic PAs though in some instances higher doses are required to elicit pulmonary toxicity than cause hepatotoxicity. The structural requirements for toxicity in the lung are the same as those for toxicity in the liver and metabolites produced in the hepatocytes cause toxicity in the lung. In general, the more stable or

persistent the dehydropyrrolizidine derivative is, the greater the possibility that this can be transported away from the liver to cause toxicity in other organs[5]. Some metabolism similar to that in the liver can occur in the pulmonary endothelial cells and type II pneumocytes of the lung[20].

39. Pulmonary toxicity manifests as pulmonary hypertension and can lead to cardiac right ventricular hypertrophy[5]. There may also be abnormal macrophages and a proliferation of mast cells. Initial damage is reported to be to the endothelial cells of the small blood vessels, this is followed by changes in the alveolar wall and then a reduction in the lumen of the small vessels[3].

Developmental studies

40. Studies in pregnant rats given heliotrine by intraperitoneal injection showed fetal malformations at doses above 100 mg/kg maternal b.w. along with subnormal maternal gestational weight gain. These included retarded development, musculoskeletal defects, cleft palate and at high dose (300 mg/kg maternal b.w.) cessation of growth, immature fetuses and intrauterine deaths and resorptions. Litters exposed to 50 mg/kg maternal b.w. showed decreased weight and length following a temporary reduction in maternal weight after injection. However, little liver damage was observed in the fetuses suggesting that the fetal liver may be relatively more resistant to these toxic effects[21].

41. Oral or intraperitoneal administration of two PAs to lactating rats did not result in maternal toxicity. The total doses, given as single or multiple doses, was 21 mg/kg or greater for retrorsine or 83 mg/kg or greater for lasiocarpine. However, liver biopsy samples from the pups showed marked changes. In pups that died aged 18 to 30 days, liver cells showed hydropic or fatty vacuolation. Pups dying after postnatal day 30 showed haemorrhagic necrosis and thickening of centrilobular veins upon examination of the liver. Susceptibility of suckling rats was shown to be greater than that of their mothers in this study[22].

Mutagenicity, Genotoxicity and Carcinogenicity

42. In 2004, a review of genotoxicity of PAs and the mechanisms involved was published[8]. Various PAs and PA containing plant extracts have been shown to be mutagenic in *Salmonella typhimurium* TA100 strain with S9 activated enzyme system. Seneciphylline, senkirkine, petasitene, monocrotaline, clivorine and ligularidine have been shown to be mutagenic in either *Drosophila melanogaster* or bacteria[23,24].

43. Male Drosophila flies were fed milk from lactating rats receiving 25 mg/kg b.w. at 0.5 ml per 10 male flies. The resulting number of sex-linked recessive lethals was compared to flies given seneciphylline directly and control flies receiving milk taken from the same rats before they were given seneciphylline. There was an increase in sex-linked recessive lethals compared to controls but not to as great an extent as flies receiving 10^{-5} M seneciphylline directly. The results indicated the presence of an indirect mutagen in the milk which the authors suggest to be unchanged seneciphylline[23].

44. PAs have been shown to have DNA binding and DNA to DNA or DNA to protein cross-linking abilities and cause sister chromatid exchange and chromosomal aberrations[8].

45. Mechanistic studies have shown that riddelliine induces liver tumours mediated by dehydronecine-derived DNA adducts. It has been proposed that these could be used as biomarkers of tumourigenicity and that they could be responsible for mutagenicity and teratogenicity of PAs[12]. It was subsequently suggested that monocrotaline formed dehydronecine-derived DNA adducts either by hydrolysis of dehydromonocrotaline and then reaction with DNA or by dehydromonocrotaline interacting with DNA and then being hydrolysed to dehydronecine[25]. The cross linking potency of a sample of PAs (dehydrosenecionine, dehydromonocrotaline, dehydroseneciphylline and dehydroriddelliine) was shown to be positively correlated with differences in the toxicity[26].

46. A number of rat studies on PAs and synthetically prepared pyrrolic metabolites have shown tumour development. A hemangiosarcomas at 0.33 mg/kg b.w./day in female rats (NOAEL 0.1 mg/kg b.w./day), 1 mg/kg b.w./day in male rats (lowest dose given) and 3 mg/kg b.w./day in male mice (NOAEL in males 1 mg/kg b.w./day). Other tumours observed in this study at or above the dose causing hemangiosarcomas include hepatocellular adenomas and carcinomas, mononuclear cell leukaemia, alveolar and bronchiolar adenoma or carcinomas[19].

47. Tumours have also been observed in the pancreas when weanling male rats were given a single dose by stomach tube of 500 to 1500 mg/kg b.w. of a mixture of lycopsamine and intermedine as alkaloids extracted from tarweed (*Amsinckia intermedia* Fisch and Mey) seeds. Three rats of 15 in the treated group were found to have pancreatic tumours; 1 receiving 600 mg/kg b.w. had an islet cell tumour, and of those receiving 1500 mg/kg b.w. 1 had an islet cell adenocarcinoma and 1 had an adenoma of the exocrine pancreas[27].

Transfer to food

48. The PA content of plants has been reported as varying from 100 mg/kg dry weight to 40,000 mg/kg, although the highest reported is 180,000 mg/kg in *Senecio riddelli*. The amount of PAs present in a plant depends on the season and locality[3]. In addition, various parts of the plants have different levels of PAs, some of which may be present in PA *N*-oxide form[5].

Feed

49. Although PA containing plants are present throughout the world, the plants are usually unpalatable to livestock. Most cases of poisoning with fresh plant material occur when pastures are overgrazed or if there is a limited supply of forage[1].

50. Where feed is preserved, contamination with PA-containing plant material is not readily recognised by animals. Experiments carried out on hay indicate that the concentration of PAs does not decrease with storage. The evidence for silage is more equivocal with some experiments suggesting that levels do decrease while others find no change. Where decomposition occurs, this is mainly enzymic and levels remain stable once the crop is dry[5].

51. In 2004 the Department for Environment, Food and Rural Affairs (Defra) published the Code of Practice on How to Prevent the Spread of Ragwort. The aim is to control the spread of ragwort where there is an identifiable risk to vulnerable animals including through the production of forage[28].

Food

52. Humans are thought to be exposed to PAs through plant products (either herbal products or contamination of grain crops), or animal derived products including honey, milk, eggs and offal[2].

Plant products

53. A number of reports of outbreaks of human PA poisoning exist from different parts of the world. These are generally as a result of contamination of cereal crops with PA containing plants or deliberate intake of herbal remedies which contain PAs.

54. Despite reports of acute symptoms and deaths in the human poisoning incidents, it is difficult to estimate exposure. As little or no follow up has been carried out on those recovering from the illness or others involved in the outbreaks, it is also unclear whether there are long term effects of these poisoning events[5].

55. The ANZFA have sampled various Australian grain commodities and found levels between <0.050 mg/kg to >6 mg/kg[2]. The use of Good Agricultural Practice in Europe has decreased the likelihood of grain contamination and therefore also the likelihood of human and livestock exposure via this route[1].

Milk

56. A number of studies have been carried out looking at transfer to milk as a possible route of excretion in lactating animals. The studies described above in paragraphs 41 and 43 show that lactational transfer of PAs occurs in rats.

57. Cows given ragwort containing 0.16% PAs by rumen cannula at 10 g/kg b.w./day showed weight loss, reduced milk output and persistent diarrhoea. Liver biopsy sections showed megalocytosis and portal fibroplasia. Their calves showed no gross or microscopical lesions and appeared normal throughout the study. While the ragwort contained jacobine, seneciphylline, jacoline, jaconine and jacozine, the milk was found to contain only jacoline and following correction for recovery, the highest mean concentration of PAs was 0.840 mg/l[29].

58. Studies in goats have shown that PAs are also transferred into their milk. In one instance, a goat was fed ragwort containing 0.18% PAs as 25% of the feed, which was at the upper limit of acceptance of the plant by the goats. A pooled milk sample collected from the goat twice daily for 236 days contained $7.5x10^6$ mg PAs/kg dried weight where the dry matter content of the milk was 12%[30].

59. A survey carried out by MAFF in 1988 analysed 21 retail bulked samples of milk from an area which had the highest reported incidence of ragwort poisoning in cattle for the 2 years beforehand. No senecionine, seneciphylline or jacobine were detected in any sample and it was concluded that detectable levels were unlikely to be present elsewhere in the UK[31].

60. EFSA noted that milk can be a relevant source of PAs when obtained from a single animal which has ingested considerable amounts of PAs[1]. However, common commercial practice in the UK is to bulk milk samples from all the cows at one farm and then also at the dairy, which results in dilution of the PAs.

61. EFSA also suggested that a possible source of human infant exposure is via their mother's milk[1].

Eggs

62. In laying hens fed up to 4% *Senecio vernalis* free PAs were not detected in the eggs. The authors considered this may have been due to residues being below the level of detection, stated as 0.4 mg/ml dissolved residue, or the PAs being bound to egg protein[32].

63. In contrast Edgar and Smith (2000) report that in chickens fed contaminated wheat containing 26 mg/kg of PAs (heliotrine, europine and lasiocarpine), up to 0.168 mg/kg was detected in the eggs[33].

Meat

64. No published reports are available where PAs have been detected in meat from livestock which have ingested PA containing plants. Results from experimental animals suggest that levels in tissues would fall rapidly after ingestion. Mattocks suggested that unless animals are killed soon after a large dose, PAs are not expected to be at a high level in tissues[3].

65. The ANZFA reported PA levels of <0.010 to 0.073 mg/kg in livers and kidneys of domestic animals[2].

Honey

66. In a 1994 UK survey honey samples were collected from hives placed close to ragwort, or obtained them from farmgate producers and a small independent retailer. Eight of 23 honey samples contained ragwort pollen and

six of these had detectable levels of PAs. The two honey samples with the highest levels were dark, waxy samples, which were considered unpalatable and would not be used for blending with other honeys. Excluding these two samples, the highest detected level of PAs, was 0.06 mg/kg though the method used for this analysis was not reported. Using the data for maximum honey consumption at any one time for adults (93g), children (60g) and infants (32g), the authors concluded that PA consumption from locally produced honey was not a cause for concern[34].

67. A 2002 review of PAs in honey noted that the highest identified level of 3.9 mg PAs/kg was in honey reported to be from ragwort. This value was not corrected for extraction efficiency. The authors recognised that where bees are used to pollinate plants such as borage, the resulting honey is likely to contain PAs and data from the literature did not indicate that bees avoid PA containing plants. Therefore it was thought likely that the PA content of a particular honey will depend on the number of PA containing plants in the forage area[35].

68. Exposure assessments were carried out using a WHO database. The consumption data included non-consumers and is therefore likely to underestimate actual consumption by consumers. In Europe average honey consumption is 1.3 g/day and high level is 3.9 g/day. The estimated population average European dietary exposure resulting from honey containing 2 mg PAs/kg, which the review authors described as typical of a honey attributed to a single PA containing plant, was 2.6 µg PAs/day[35].

69. In 2004, Food Standards Australia New Zealand (FSANZ) reported that Australian honey samples had levels up to 2 mg/kg PAs though it was noted that blending could substantially reduce this level. The highest levels were found in honey from Paterson's Curse/Salvation Jane (*Echium plantagineum*). The FSANZ considered that 2-4 year old children of approximately 17kg with high levels of consumption at 28.6 g honey/day would be the most vulnerable subgroup of the population. To keep this subpopulation within the ANZFA PTDI of 1 µg/kg b.w./day, the honey consumed would need to contain no more than 0.594 mg PAs/kg. However as other food sources need to be considered, levels would need to be lower than this. As a result, the FSANZ advised that people consuming more than 2 tablespoons of honey every day (approximately 5% of the population) should not eat honey made exclusively from Paterson's Curse/Salvation Jane[36].

70. A recent Dutch study analysed honey samples for PA content of which 171 were retail samples of Dutch or imported origin and 8 were from hives deliberately placed in areas with high groundsel levels. Of the retail samples, 28% contained PAs at levels between 0.001 and 0.365 mg/kg. Four of the 8 non-retail samples had detectable levels of PAs with the highest at 0.010 mg/kg. Pollen counts indicated that the bees had foraged on many other plants not just the groundsel[37].

71. The authors stratified Dutch honey consumers into groups depending on whether they consumed honey from different sources or from the same manufacturer which could coincidentally contain high levels of PAs. Each group

was further subdivided into average (13 g honey/day) or high level consumers (30 g/day). The authors concluded that "only in cases of prolonged consumption of types of honey which contain high concentrations of PA is there any suggestion of a significantly increased risk of cancer and possibly acute liver damage." This was considered as rare so warning consumers of the risk was not felt to be useful[37].

Food Standards Agency funded project T01037 "Collection and Analysis of Honey Samples Potentially Contaminated with Pyrrolizidine Alkaloids from Ragwort and Borage"

72. This project aimed to investigate the potential for PA contamination of honey if bees forage on PA containing flowers. Borage (*Borago officinalis*) and ragwort were the two flowers of interest and honey was produced in areas where either borage or ragwort was growing in abundance. While the PA concentrations in honey could not be quantified due to a lack of analytical standards, they could be compared from one honey sample to another and relative to the amount of PAs in a fixed weight of plant material[38].

73. Honey produced in areas with high levels of ragwort showed little difference in the PA profile compared to control sites except in honey from one site, which showed increased seneciphylline N-oxide levels. However conditions were very different at this site compared to sites for commercial honey production. Honey produced from ragwort is seen by beekeepers as a contaminant and is unlikely to be used for consumption. The authors concluded that the results indicate that even where there appears to be little else to forage on, the honey produced showed no conclusive evidence of ragwort contamination in terms of PA profile and pollen contained in the honey. The Honey International Packers Association has suggested that bees do not like foraging on ragwort or producing honey from it. They also state that where it is found, it tastes unpleasant and would therefore not be consumed[38].

74. Honey produced in areas with high levels of borage showed the presence of one PA which could either be intermedine or lycopsamine. Honey produced from borage has a distinctive taste and is seen as a speciality product so attracts a premium price[38].

75. This was a preliminary project to determine whether further quantitative analysis would be required for risk assessment. A standard for lycopsamine is now commercially available and the Food Standards Agency plans to fund further work to assess the levels of PAs in borage honey.

Previous risk assessments

World Health Organization (WHO)

76. The WHO report provided a full account of experimental animal studies in addition to cases of livestock and human poisoning events. Using data from outbreaks of human disease, the authors estimated total intake and length of

exposure. Total doses in known outbreaks or cases of veno-occlusive disease were estimated to be 1 to 167 mg/kg b.w. Data from the Ridker *et al.* (1985) report on ingestion of comfrey indicated that ingestion of 15 µg PA/kg b.w./day, may lead to acute or subacute liver disease in humans. As comfrey contains echimidine and related alkaloids, they used rat LD50 data to derive the equivalent heliotrine dose so exposures from different case reports of human disease could be compared. The heliotrine equivalent dose for this report is 9 µg/kg b.w./day. Therefore the WHO concluded that a dose of 10 µg/kg b.w./day heliotrine an equivalent PA dose may lead to disease in humans[5].

77. The WHO considered that the dose estimates carried out indicate that the effects are cumulative at very low intakes and that chronic exposure even at low levels may present a health risk. It therefore recommended that exposure should be minimised if possible. Long term effects in humans may be liver cirrhosis or cancer but there is no evidence of causative effects as there has been a lack of long term follow up in cases where exposure has occurred[5].

Australia New Zealand Food Authority (ANZFA)

78. This report provides a brief summary of the occurrence, chemistry, toxicity in livestock and humans, metabolism, mechanism, carcinogenicity and dose-response for chronic liver disease of PAs.

79. The ANZFA concluded that the major human dietary source of exposure is contaminated grains, with eggs, offal and honey being minor contributors. However the authors noted that the data available were very limited and it was not possible to estimate the potential dietary exposure to PAs from these food sources[2].

80. The conclusion of the ANZFA risk characterisation was: “On the basis of the limited human data on the incidence of veno-occlusive disease, a tentative NOEL for all PAs of 10 µg/kg b.w./day is suggested based on the human data reported by Ridker et al. (1985). Applying an uncertainty factor of 10 to this figure to take into account individual variation, the PTDI for PAs is 1 µg/kg b.w./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.”[2]

81. Despite 10 µg/kg b.w./day being quoted as causing disease by the WHO, the ANZFA cited comments by Mattocks (1986) and Huxtable (1989) as suggesting that this dose was probably close to the real NOEL[2]. Both authors had highlighted the uncertainty in the original paper as to whether the woman had had exposure to PAs from other sources[3,39].

Dutch National Institute for Public Health and the Environment (2005)

82. The Dutch National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) established a virtually

safe dose (VSD) for PAs of 0.00043 µg/kg/day leading to an increased risk of at most one person in a million developing cancer. For non-cancer effects, a TDI of 0.1 µg/kg b.w./day was derived from the rat NOAEL of 0.01 mg/kg b.w./day for non-neoplastic changes (hepatocyte cytomegaly) in the 105 week study cited in paragraph 32 above, and using an uncertainty factor of 100 (10 for interspecies and 10 for intraspecies variation[40]. A 2007 report of the Dutch Food and Consumer Product Safety Authority (Voedsel en Waren Autoriteit, VWA) concluded that "only in cases of prolonged consumption of types of honey which contain high concentrations of PA is there any suggestion of a significantly increased risk of cancer and possibly acute liver damage"[37].

European Food Safety Authority (EFSA)

83. This is a report on the opinion of the Scientific Panel on Contaminants in the Food Chain on pyrrolizidine alkaloids as undesirable substances in animal feed. The report provides a summary of available data. In relation to human exposure, EFSA recommended obtaining more data on carry over of PAs into milk as infants may have high exposure via this pathway. Also the need for quantitative assessment of the contribution of honey to human exposure was highlighted[1].

COT evaluation

84. The Committee noted that PAs are a large class of compounds with differing toxicities and the variability in potency is an important consideration in the risk assessment of these toxins.

85. It was noted that extracts of PAs have shown evidence of genotoxicity, mainly in *in* vitro models, but that the in vivo relevance of these studies to plant constituents, or their residues, was unclear. The Committee concluded that protecting against veno-occlusive disease, which is the predominant lesion in human case reports, provided the most appropriate basis for the risk assessment.

86. The Committee discussed the differing ANZFA and WHO assessments of the Ridker *et al.* case report, together with the additional comments made by Mattocks and Huxtable. The ANZFA supported the WHO approach of extrapolation of the subject's dietary exposure to 10µg/kg b.w./day heliotrine equivalent. The authors of the case report recognised that the affected subject may have had further sources of PA exposure in addition to those identified. This source of uncertainty was further highlighted by Mattocks and Huxtable in their commentaries. While the ANZFA judged that this was sufficient to deem 10 µg/kg b.w./day a tentative NOAEL, the WHO concluded that this exposure level may cause disease.

87.
(a) The additional comments by Mattocks and Huxtable, provided reassurance that 10µg/kg b.w./day was likely to be close to a NOAEL for human illness. Taking into account that the single subject reported to experience adverse

effects at this exposure level is likely to be more sensitive than average, and applying the default uncertainty factor of 10 to account for human variability, the Committee agreed that the ANZFA PTDI of 1 µg/kg b.w./day is appropriate.

or

(b) The Committee concluded that the possible additional sources of PA exposure did not provide a basis for assuming that the NOAEL was 10 µg/kg b.w./day. An additional uncertainty factor of 3 [or 10] was required to take into account that this was a LOAEL, as well as a factor of 10 to allow for variation between humans. The Committee therefore established a TDI of 0.3 [or 0.1] µg/kg b.w./day. It was noted that this TDI based on the human illness reflects an margin of exposure of 30 [or 100] compared to the NOAEL for non-neoplastic effects in a chronic study in rats, which was considered to be adequate.

88. There were limitations in the methods used in the Food Standards Agency funded study. The lack of analytical standards at the time of commissioning raised the possibility that where PAs were judged to be not present, this resulted from an inability to detect them. There was concern that the PAs sought during the project were the most prevalent in the plants but were not necessarily the most toxic PAs present. Overall, it was felt that the data from the project supported the hypothesis that honey produced in areas with a high concentration of ragwort is unlikely to be a concern for human health.

89. Data from rats and cows on toxicity to young following transfer of PAs to milk raised concern that the human children and infants may be vulnerable following exposure to cows' milk and breast milk. It was doubted that levels in breast milk would be sufficiently high to cause transfer to the neonate. In addition the incidence of paediatric veno-occlusive disease is low. The data showing that no PAs were detected in retail milk samples and the practice of bulking dairy milk supplies led to agreement that PA exposure through milk is unlikely to be a human health concern.

90. [*further paragraphs may be added if relevant following discussion of TOX/2008/04*]

COT conclusions

91.
*(a) We **agree** with the conclusions of ANZFA that a daily intake of 1 µg/kg b.w./day can be regarded as a Provisional Tolerable Daily Intake..*

*(b) We **note** that 10 µg/kg b.w./day is a LOAEL for veno-occlusive disease in humans. Applying uncertainty factors of 3 [or 10] to extrapolate to a NOAEL and 10 for human variability we **conclude** that a Tolerable Daily Intake of 0.3 [or 0.1] µg/kg b.w./day can be established for exposure to PAs in food.*

92. While noting the limitations of the methods used for the Food Standards Agency research project, exposure to ragwort PAs through honey is unlikely to be a cause for concern.

93. Based on the available data on PA content in milk and the practice of bulking dairy milk supplies, which is likely to lead to dilution of any contamination present, we conclude that PAs in milk are unlikely to be a human health concern.

COT Statement 2008/XX
XXX 2008

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