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

COT 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 with 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 metabolism and elimination of PAs from the bodies of animals as determined experimentally[1,3].

6. In humans, veno-occlusive disease is the most prominent hepatic lesion resulting from PA poisoning. Classical symptoms and signs are abdominal pain and rapidly developing ascites. The effects of PAs can take time to develop and might

result from 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, resulting either from a single sublethal dose or from repeated low level intake, is characterised by various abnormalities including 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 that 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 advice was subsequently endorsed by the Food Advisory Committee. Department of Health (DH) and Ministry of Agriculture Fisheries and Food (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].

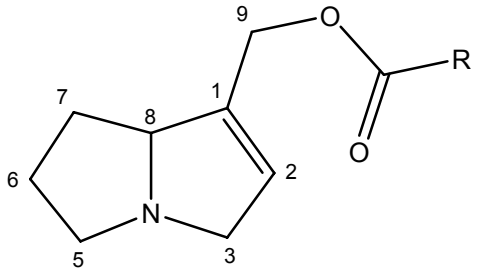

Figure 1: The generic structure required for PAs to cause toxicity.

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

Metabolism

14. On ingestion of PAs, parent alkaloid or metabolites can be found in the serum and only later in the urine and faeces indicating that absorption across the gastrointestinal tract occurs[7,8,9,10,11,12,13,14]. Studies using a limited number of representative PAs have shown that three main pathways of metabolism occur[15].

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[16].

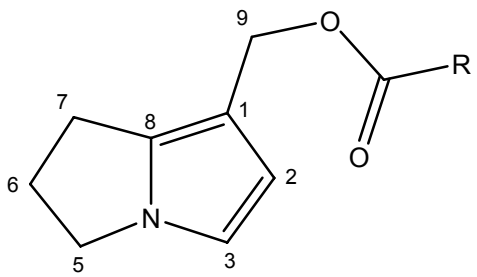

Figure 2: The generic structure of the dehydropyrrolizidine derivative of PAs.

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)[16].

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[15].

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*-oxides is usually seen as a detoxification pathway, upon ingestion these can be converted to the alkaloid forms 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[15,17,18,19,20].

21. Following metabolism, rapid elimination occurs mainly via urine but some through 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 is often derived 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.

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 pressure of 23 mmHg with corrected sinusoidal pressure of 17 mmHg. No demonstrable obstruction of outflow was observed but balloon distension 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 assuming 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 was possible she had other sources of exposure in the same period[21]. 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 was not obtained from the mother but her physical appearance and blood tests showed no abnormalities[22].

25. A pregnant woman was admitted to hospital in the 27th week of gestation with fetal ascites. A 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 nor 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[23].

26. Based on information provided by two paediatric liver centres to the Committee, it was noted that paediatric veno-occlusive disease was rare in the UK, and that cases were almost always attributable to other causes and therefore unlikely to be related to PA exposure.

Acute Studies

27. LD50 data obtained following intraperitoneal administration to male rats are available for some PAs and are given in Table 1[5,24]. These deaths, 3 to 7 days after administration, were associated with severe haemorrhagic liver necrosis[5].

Table 1: Reported intraperitoneal LD50 values obtained for the male rat unless otherwise stated (Source: Cheeke and Shull, 1985 and World Health Organization (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
Heliotrine	300
Echinatine	350
Supinine	450
Europine	>1000
Heliotridine	1200
Intermedine	1500
Lycopsamine	1500

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 was 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 of Sprague Dawley rats. A single dose of 3.5 mg/kg b.w. in male rats caused changes in the lung from 4 hours after administration[25].

Repeated dose toxicity

31. 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 of the liver 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[26].

32. 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].

33. Rats were given 8 mg/kg b.w./day of an alkaloidal extract of *Senecio nemorensis* ssp. fuchsii containing 50% fuchsisenecionine and 1% senecionine by gavage 5 days per week for 114 weeks. Fatty changes, single cell and focal necrosis, fibroses and granulomatous reactions were observed in the livers[27].

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 in the rat. 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 functional restriction of blood flow.

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[28].

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 the same metabolites as are 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 it 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[28].

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. Fetal effects 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[29]. This is in contrast to the effects seen in the human case report[22] where a 5 day old infant showed liver pathology following daily consumption of a herbal tea by the mother in the absence of maternal toxicity (para 24).

41. Oral or intraperitoneal administration of two PAs to lactating rats did not result in maternal toxicity. The total dose, given singly or in multiple fractions, was 21 mg/kg or greater for retrorsine and 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 in liver. Susceptibility of suckling rats was shown to be greater than that of their mothers in this study[30].

Mutagenicity, Genotoxicity and Carcinogenicity

42. Several PAs have been evaluated by the International Agency for Research on Cancer (IARC) and categorised either as Group 2B, possibly carcinogenic to humans, or Group 3, not classifiable as to its carcinogenicity to humans. Lasiocarpine, monocrotaline and riddelliine have been classified as Group 2B while hydroxysenkirkine, isatidine, jacobine, retrorsine, seneciphylline, senkirkine and symphytine have been classified as Group 3[31,32,33].

43. A review of the genotoxicity of PAs and the mechanisms involved was published in 2004[16]. Various PAs and PA-containing plant extracts have been shown to be mutagenic in *Salmonella typhimurium* TA100 strain with an S9 activated enzyme system. Seneciphylline, riddelliine, lasiocarpine, senecionine, retrorsine, heliotrine, senecivernine, senkirkine, petasitene, monocrotaline, clivorine, ligularidine, 7-acetyl intermedine, 7-acetyl lycopsamine, indicine, intermedine, jacoline and symlandine have been shown to be mutagenic in either *Drosophila melanogaster* or bacteria[34,35,36,37,38,39].

44. Male Drosophila flies were fed milk from lactating rats receiving 25 mg/kg b.w. seneciphylline 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 suggested to be unchanged seneciphylline[34].

45. 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[16]. The cross linking potency of a sample of PAs (dehydrosenecionine, dehydromonocrotaline, dehydroseneciphylline and dehydroriddelliine) was shown to be correlated positively with differences in toxicity[40].

46. Mechanistic studies have shown that riddelliine induces liver tumours mediated at least in part by DHP-derived DNA adducts. It has been proposed that these could be used as biomarkers of tumourigenicity and that they could be responsible for the mutagenicity and teratogenicity of PAs[20]. Subsequently it has been suggested that monocrotaline formed DHP-derived DNA adducts either by hydrolysis of dehydromonocrotaline and then reaction with DNA or by dehydromonocrotaline interacting with DNA and then being hydrolysed to DHP[41]. Other PAs, namely riddelliine, lasiocarpine, clivorine, heliotrine and retrorsine have also been shown to form DHP-derived DNA adducts either *in vivo* or *in vitro*[42,43,44,45,46].

47. A number of animal studies on PAs and synthetically prepared pyrrolic metabolites have shown tumour development. In one study under the National Toxicology Program (NTP), using riddelliine administered by gavage 5 days per week for 105 weeks, hemangiosarcomas were observed in 0/50 female control rats and 0/50 females given 0.01, 0.033 and 0.1 mg/kg b.w./day, 3/50 at 0.33 mg/kg b.w./day ($p=0.118$) and 38/50 at 1.0 mg/kg b.w./day ($p<0.001$). In male rats, hemangiosarcomas were observed in 0/50 controls but in 43/50 given 1 mg/kg b.w./day ($p<0.001$). In male mice, hemangiosarcomas were observed in 2/50 controls, 1/50 at 0.1 mg/kg b.w./day, 0/50 at 0.33 mg/kg b.w./day and 2/50 at 1 mg/kg b.w./day but in 31/50 mice at 3 mg/kg b.w./day ($p<0.001$). No female mice in the control group showed hemangiosarcomas and only 1/50 given 3 mg/kg b.w./day (only dose given). Other tumours observed in rats at or above the doses causing hemangiosarcomas included hepatocellular adenomas and carcinomas, mononuclear cell leukaemia, alveolar and bronchiolar adenoma and carcinomas[26]. Similarly, a two year carcinogenicity study has been carried out under the NTP testing lasiocarpine in F344 rats with dietary administration for 104 weeks. High mortality was seen in both sexes at the high dose with all females dead by week 69 and males by week 88. Liver angiosarcoma was seen in 13 of 23 male ($p<0.001$) and 2 of 23 female (not significant) rats (though the authors suggested that only female rats surviving beyond 52 weeks should be used for the analysis so they quote this as 2 of 9 in the main report) following dietary administration at 30 ppm, in 11 of 23 males ($p<0.001$) and 7 of 24 females ($p=0.005$) at 15 ppm in the diet and in 5 of 24 males ($p=0.025$) and 8 of 22 females ($p=0.002$) at 7 ppm in diet. The authors concluded that this study had shown that lasiocarpine was carcinogenic in F344 rats[47].

48. Other PAs have also been shown to cause tumours in animals. Clivorine, petasitenine and symphytine all produce angiosarcomas in liver in non-standard

carcinogenicity assays[48,49,50]. Senkirkine caused liver adenomas in a non-standard assay[50].

49. Plant extracts have also been tested for tumour formation, for example tumours have 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[51].

Transfer to food

50. The PA content of plants has been reported as generally 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 plants have different levels of PAs, some of which may be present in PA *N*-oxide form[5].

Feed

51. 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].

52. 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].

53. In 2004 the Department for Environment, Food and Rural Affairs (Defra) published a 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[52].

Food

54. 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

55. 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.

56. Where acute symptoms and deaths in human poisoning incidents have been reported, it is generally difficult to estimate the exposures responsible. 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].

57. The Australian New Zealand Food Authority (ANZFA) has sampled various Australian grain commodities and found levels from <0.050 mg/kg to >6 mg/kg[2]. In the EU, legislation specifies a maximum level for weed seeds and unground and uncrushed fruits containing alkaloids in animal feed[1].

Milk

58. 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 44 show that lactational transfer of PAs occurs in rats.

59. Cows given ragwort containing 0.16% PAs by rumen cannula for 2 weeks 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 microscopic 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 was 0.840 mg/L[53].

60. 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 7.5×10^{-3} mg PAs/kg dried weight where the dry matter content of the milk was 12%[54].

61. 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[55].

62. The European Food Safety Authority (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 if present.

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

Eggs

64. Free PAs were not detected in the eggs of laying hens fed up to 4% *Senecio vernalis*. 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, but they noted that reduced feed intake and egg production occurred at 2 and 4% of feed levels[56].

65. In contrast Edgar and Smith (2000) reported 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[57].

Meat

66. 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].

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

Honey

68. In a 1994 UK survey, honey samples were collected from hives placed close to ragwort, or obtained 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 data on 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[58].

69. 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 except ragwort as described by the Honey International Packers Association. 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[59].

70. The 2002 review considered exposure assessments which were carried out using a WHO database. The consumption data included non-consumers and therefore averages tended to underestimate consumption by consumers. In Europe average honey consumption is 1.3 g/day and high level (by the 95th percentile consumer) 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[59].

71. 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 provisional tolerable daily intake (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[60].

72. 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 levels of groundsel, another PA-containing plant. Of the retail samples, 28% contained PAs at levels between 0.001 and 0.365 mg/kg. Four of the eight 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[61].

73. 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[61].

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

74. 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[62].

75. 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 indicated 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 the honey tastes unpleasant and therefore would not be consumed[62].

76. Honey produced in areas with high levels of borage showed the presence of one PA which could have been either intermedine or lycopsamine. Honey produced from borage has a distinctive taste and is seen as a speciality product so attracts a premium price[62].

77. 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 (1988)

78. A WHO report provided a full account up to 1988 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, the WHO 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 was 9 µg/kg b.w./day. Therefore the WHO considered it prudent to conclude that a dose equivalent to 10 µg/kg b.w./day heliotrine may lead to disease in humans without providing further explanation[5].

79. The WHO considered that the dose estimates derived indicated that 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 might be liver cirrhosis or cancer but there had been a lack of long term follow up where exposure was known to have occurred[5].

Australia New Zealand Food Authority (2001)

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

81. 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].

82. 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]

83. 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 ”may well be close to the NOEL for humans”[2]. Both authors had highlighted the uncertainty stated in the original paper as to whether the woman had had exposure to PAs from other sources[3,63].

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

84. 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 tolerable daily intake (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 31 above, and using an uncertainty factor of 100 (10 for interspecies and 10 for intraspecies variation)[64]. 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”[61].

European Food Safety Authority (2007)

85. 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 up to 2006. 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].

UK Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (2008)

86. The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) reviewed the mutagenicity and carcinogenicity of 7 PAs and 2 proposed metabolites. The COC concluded that riddelliine was genotoxic and carcinogenic and it would be prudent to assume at least part of the carcinogenic effect was through a genotoxic mechanism. Similarly, lasiocarpine is possibly a genotoxic carcinogen. There were sufficient data to conclude that monocrotaline was carcinogenic. There was limited evidence of carcinogenicity for clivorine, petasitenine

and symphytine while for senkirkine there was insufficient evidence of carcinogenicity. A limited number of studies using non-oral routes of administration had been carried out on the two metabolites, dehydroretronecine and dehydroheliotridine. On balance, these did not indicate carcinogenic activity of the metabolites.

87. The COC agreed that the $BMDL_{10}$ derived from the NTP studies on riddelliine and lasiocarpine would be an appropriate basis for a Margin of Exposure (MOE) approach to the risk assessment of PAs. The lowest $BMDL_{10}$ of 0.073 mg/kg b.w/day was derived from the study on lasiocarpine in male rats and included adjustment of the high dose to take into account cessation of dosing and termination of all animals by 88 weeks. Previously the COC have agreed that an MOE of less than 10,000 may be a concern, an MOE between 10,000 and 1,000,000 is unlikely to be a concern and above 1,000,000 is highly unlikely to be a concern[65]. This is similar to the 2005 EFSA Scientific Committee view that "an MOE of 10,000 or higher, if it is based on the $BMDL_{10}$ from an animal study, would be of low concern from a public health point of view and might be considered as a low priority for risk management"[66].

88. With evidence that the same rare tumour type, angiosarcoma, is produced by a number of PAs and that formation of the DHP metabolite has been demonstrated with various PAs, the COC felt that a cumulative assessment approach based on the most potent PA, lasiocarpine, as proposed by EFSA[67] would be appropriate for all PAs.

COT evaluation

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

90. The Committee discussed the differing ANZFA and WHO assessments of the case report by Ridker *et al.*, 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.

91. The Committee concluded that a single human case report, with considerable uncertainties in the exposure assessment did not provide a reliable basis for deriving a TDI. Therefore use of the 2 year rat and mouse study with riddelliine administered by oral gavage was considered to be the most appropriate basis for assessing risks of the non-cancer effects of PAs. A NOAEL of 0.01 mg/kg b.w./day for hepatocyte cytomegaly in rats was observed in this study. Applying an uncertainty factor of 100, 10 for interspecies and 10 for intraspecies variation, indicated that non-cancer effects would not be expected at doses of riddelliine up to 0.1 µg/kg b.w./day. It was noted that this dose was 100 fold lower than the dose in heliotrine equivalents as defined by the WHO which the woman received in the report by Ridker *et al*.

92. In their review, the WHO used the ratio of LD50s to compare doses of different PAs. The LD50 data are based on intraperitoneal administration rather than oral dosing. However, as severe haemorrhagic necrosis of the liver caused death in the animals studied, the data are relevant to the impairment of hepatic function, which is the basis of the risk assessment. Therefore the Committee considered that using the ratio of LD50 values is acceptable to extrapolate between PAs. It was noted that where they occur, *N*-oxides should be considered as equivalent to their parent alkaloid, because of possible conversion to this form in the gut following oral administration. This does not occur with intraperitoneal administration, by which *N*-oxides are much less toxic than their parent alkaloid.

93. The Committee accepted the COC advice that PAs should be considered as a cumulative assessment group where it is prudent to assume that PAs are genotoxic carcinogens. As a result, the Committee used the $BMDL_{10}$ of 0.073 mg/kg b.w./day to assess margins of exposure (MOE) for any PA assuming an equivalent potency to lasiocarpine. In line with COC[65] and EFSA[66] opinions, the Committee considered that MOEs of 10,000 and above, corresponding to doses of up to 0.007 µg /kg b.w./day, would be unlikely to be of concern. Such doses are below the 0.1 µg/kg b.w./day identified as not expected to be associated with non-cancer effects of PAs. The Committee noted the COC's acknowledgement that a cumulative assessment group approach assuming equal potency for all PAs would be likely to be over-precautionary where little was known about the PAs in question.

94. Using the limit of detection for honey of 1 µg/kg honey reported by Betteridge *et al.* in 2005[68] and for milk of 10 µg/L milk reported by MAFF in 1994[55], hypothetical exposure assessments were carried out for all age groups based on the UK National Diet and Nutrition Surveys. The age group with highest PA exposure on a body weight basis in both instances would be infants.

95. High level (97.5^{th} percentile) infant consumers have an intake of 6.97 g honey per day (equivalent to 1.14 g/kg b.w./day), which includes honey in other foodstuffs. This is despite FSA advice for infants not to consume honey due to the very small possibility of bacterial contamination that could cause infant botulism. Following consumption of honey with a PA concentration at the limit of detection (1µg/kg honey[68]), these high level infant consumers would receive 0.0011 µg PAs/kg b.w./day. This is 66,000 fold below the $BMDL_{10}$ and 90 fold below the dose of 0.1 µg/kg b.w./day, below which non-cancer effect would not be expected (this assumes that all the PAs present have equivalent potency to riddelliine, as no quantitative data on individual PAs present in honey are available). The maximum PA concentration in honey, which would still maintain an MOE of 10,000 compared to the $BMDL_{10,}$ for high level infant consumers, would be 6.4 µg/kg honey.

96. There were limitations in the methods used in the Food Standards Agency funded study assessing honey samples potentially contaminated with PAs from ragwort and borage. The lack of analytical standards at the time of commissioning raised the possibility that where PAs were judged to be not present in the samples, this resulted from an inability to detect them. There was also concern that the PAs sought were the most prevalent in the plants but were not necessarily the most toxic PAs present. Overall, however, it was considered 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. The Committee noted that PAs had been found in honeys sampled around the world. However, in the absence of quantitative data on individual PAs present in UK commercial samples, it is difficult to assess the risk to the UK consumer.

97. For milk, high level (97.5th percentile) infant consumers receive 1054 g milk per day (equivalent to 187.6 g/kg b.w./day), where the specific gravity of milk is 1.03 kg/L. In milk with a PA concentration at the limit of detection (10µg/L milk[55]), infants would be exposed to 1.8 µg/kg b.w./day, which is only 40 fold below the $BMDL_{10}$. Hence the analytical method lacks sensitivity to detect levels in milk, which result in high level infant consumer exposure with a sufficient MOE to be of low concern.

98. Data on toxicity to young rats and calves following transfer of PAs to milk raised concern that human children and infants may be vulnerable following exposure to cows' milk and breast milk. Given the relatively low intake of PAs, the Committee doubted that levels in breast milk would be sufficiently high to cause significant effects in the neonate. In addition, the overall incidence of paediatric veno-occlusive disease in the UK appears to be extremely low with the majority of cases accounted for by other known causes such as cytotoxic drugs. Consumption of milk with PAs at the limit of detection would lead to exposure only 40 fold below the $BMDL_{10}$. Therefore the analytical method is insufficiently sensitive to identify concentrations of PAs in milk that would be of low concern following human exposure, particularly in high level infant consumers. However, the practice of bulking dairy milk supplies in the UK provides some reassurance that PA exposure through milk is unlikely to be a human health concern.

99. In addition to the precautionary nature of the assessment of carcinogenic potential, the Committee noted that the exposure assessments are precautionary as they assume that all foodstuffs consumed will be contaminated, which, while possible in the short term, is unlikely in the long term.

100. Data were not available on concentrations of PAs in grain, eggs or meat on the UK market and, therefore, an assessment of UK consumer exposure from these foodstuffs could not be carried out. The Committee noted that, if grain in the UK were contaminated to the same extent as the upper level identified in Australia, human exposure from this source could be significant. However, the absence of data prevented any further assessment of possible risk to the consumer.

101. It was noted that the Food Standards Agency research on PAs in honey is continuing while other organisations are looking at PAs in milk and other products. The Committee will monitor this research and other assessments made on PAs.

COT conclusions

102. PAs are known to cause veno-occlusive disease in humans. The available reports of human cases of poisoning do not provide sufficiently reliable exposure data to be used in establishing a health-based guidance value.

103. We conclude that the two-year study in rats administered riddelliine by oral gavage is the most robust basis for assessing the non-cancer effects of PAs. Applying uncertainty factors of 10 for interspecies and 10 for within species variability to the NOAEL of 0.01 mg/kg b.w./day for hepatocyte cytomegaly, indicates that 0.1 µg riddelliine/kg b.w./day would not be expected to result in non-cancer effects. The ratio of LD50 values can be used to convert other PAs to riddelliine equivalents for comparison with this dose.

104. We endorse the COC recommendation to assess all PAs as a cumulative assessment group using the $BMDL_{10}$ with an adequate MOE, while acknowledging the precautionary nature of this approach. A $BMDL_{10}$ of 0.073 mg/kg b.w./day was derived from a 2 year carcinogenicity study of lasiocarpine and should be used to assess exposure for any PA. Allowing an MOE of at least 10,000 indicates that PA doses of up to 0.007 µg /kg b.w./day are unlikely to be of concern for cancer risk. Such doses would also not be expected to result in non-cancer effects.

105. The maximum PA concentration in honey, to maintain an MOE of 10,000 for high level infant consumers, would be 6.4 µg/kg honey.

106. We note that consumption of milk with PAs at the limit of detection by high level (97.5^{th} percentile) infant consumers leads to exposure only 40 fold below the $BMDL_{10}$. We conclude that there is a need for more sensitive analytical methods to detect PAs in milk to enable assessment of exposure with an adequate MOE compared to the $BMDL_{10}$. However, 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. There is a need for more information on the levels of PAs in grain in the UK to enable assessment of exposure and risk to consumers from this source.

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