

## **COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT**

### **Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

#### **Referral to COC**

1. The Committee is asked to evaluate the carcinogenicity of pyrrolizidine alkaloids (PAs), particularly those found in foods such as honey and milk.
2. In December 2007, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) 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. The topic has been discussed subsequently at COT meetings in February and April. During these discussions, the COT requested a view from the COC on the carcinogenicity of PAs to aid their risk assessment.

#### **Mutagenicity and carcinogenicity - Introduction**

3. PAs are a large group of natural toxins produced by plants, several of which are known to be highly hepatotoxic and a few which 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.
4. The mutagenic and carcinogenic effects of those individual PAs and their suggested metabolites for which there are *in vivo* data indicating tumour formation are summarised below. All the available data mutagenicity and carcinogenicity data on these compounds are presented as an overview in Annex A, Sections 1-9.
5. The COC is asked to review the carcinogenicity data. The mutagenicity data has been reviewed by the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) Secretariat. Essentially, riddelliine was the only compound for which there were adequate data to draw definite conclusion on potential for *in vivo* mutagenicity. However, conclusions on *in vitro* mutagenic activity could be drawn for some of the other compounds described in this paper. The COM Secretariat note that the assessment of monocrotaline was particularly complex and suggest a COM view could be sought on this compound only.

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6. An overview of mutagenicity data on PAs which have not been tested for *in vivo* tumour formation and a table with these data will be presented as Annex B for the meeting.
7. Riddelliine and lasiocarpine have both been studied by the NTP in a 2 year rodent bioassay. The results from these reports have been used to carry out benchmark dose modelling. A brief description is provided in this paper and a more detailed discussion of the methodology and the results are provided in Annex C.
8. The COC is asked whether it is possible to consider that the concept of a Cumulative Assessment Group might apply to PAs. The Cumulative Assessment Group has recently been described by the European Food Safety Authority (EFSA) Scientific Panel on Plant Protection products and their Residues in one of their opinions. The summary of this opinion and section 2.1 where the Cumulative Assessment Group is described is provided in Annex D. A more limited database is needed for Cumulative Assessment Group compared to making decisions on common mode of action for carcinogenicity. An assessment of mode of action or human relevance framework has not been undertaken in this preliminary paper.
9. The process by which papers have been identified for this review is presented in Annex E. The individual papers describing the evidence of *in vivo* tumour formation for the individual PAs and the papers discussed in the section on the Cumulative Assessment Group are provided in Annex F.

## **Background**

10. 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 (EFSA, 2007).
11. 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 (ANZFA, 2001), 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 (Mattocks, 1986).
12. 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 might result from long term low level exposure, although known cases of poisoning have usually presented as acute disease similar to

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Budd-Chiari syndrome, a condition associated with obstruction of the major hepatic veins or the inferior vena cava (ANZFA, 2001).

13. 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*) (ANZFA, 2001).

14. 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 (ANZFA, 2001).

15. 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 (McLean, 1970; WHO, 1988).

16. There are no substantial long term follow up data on people with known exposure but the World Health Organization (WHO) suggest that available clinical and experimental data indicate that single exposure or low level long term exposure may lead to cirrhosis in humans. In addition, instances of known human exposure are close to levels which have been shown to cause tumours in laboratory animals (WHO, 1988).

### ***Chemistry and Kinetics***

17. 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.

18. 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 (ANZFA, 2001).

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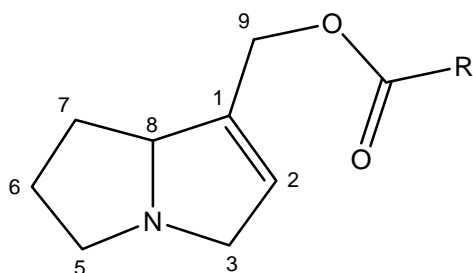


Figure 1: The generic structure required for PAs to cause toxicity.

19. PAs are fairly stable chemically and require metabolic activation to exert toxicity (WHO, 1988). Studies using a limited number of representative PAs have shown that three main pathways of metabolism occur (Prakash *et al.*, 1999).

20. 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 (Fu *et al.*, 2004).

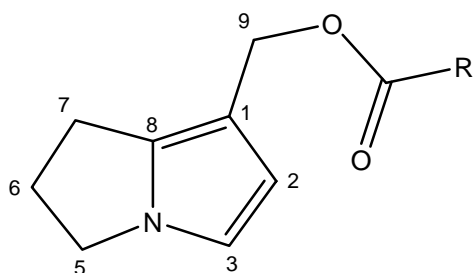


Figure 2: The generic structure of the dehydropyrrolizidine derivative of PAs.

21. Dehydropyrrolizidine derivatives can undergo further biotransformation by enzymic or non-enzymic glutathione conjugation (EFSA, 2007). Alternatively, the dehydropyrrolizidine derivative can be hydrolysed further at the ester bond to form the dehydronecine, often referred to as dehydropyrrolizine (DHP) (Fu *et al.*, 2004).

22. 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 (Prakash *et al.*, 1999).

23. *N*-oxidation of retronecine- and heliotridine-type PAs is generally catalysed by a variety of enzymes including cytochromes P450 and flavin-containing monooxygenases. The *N*-oxides are highly water soluble and are rapidly excreted in the urine (WHO, 1988). *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 (Mattocks, 1986).

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24. 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 (Cheeke and Pierson-Goeger, 1983; Fu *et al.*, 2002; Huan *et al.*, 1998a; Huan *et al.*, 1998b; Prakash *et al.*, 1999).

25. Following metabolism, rapid elimination occurs mainly via urine but some goes into the bile. It is considered unlikely that large amounts of the PAs and their metabolites remain in the liver in the long term (Mattocks, 1972). PAs and their metabolites can be excreted in the milk and possibly, in the case of poultry, into eggs (EFSA, 2007).

### **Summaries of data for PAs where *in vivo* studies have shown carcinogenic responses**

26. The descriptions provided below are a brief summary of the available data. The full collation for each PA can be found in Annex A and individual papers on *in vivo* tumour formation can be found in Annex F.

#### ***Riddelliine***

27. Riddelliine is a macrocyclic retronecine derived PA (figure 3) and is found in a number of species including *Senecio jacobaea* (tansy ragwort) and *Senecio vulgaris* (common groundsel or ragwort) as well as a number of other *Senecio* species (EFSA, 2007; WHO, 1988)

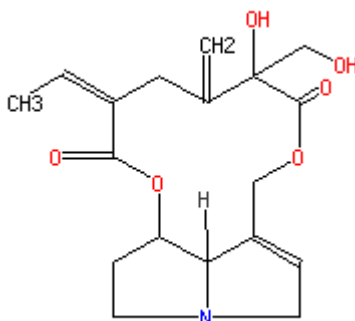


Figure 3: Riddelliine (obtained from the EPA website)

28. Riddelliine shows mutagenic activity *in vitro* in bacterial and mammalian cells in the presence of exogenous metabolic activation (Berry *et al.*, 1996; NTP, 2003). A positive response *in vitro* for sister chromatid exchange with and without S9 and for chromosomal aberrations with S9 have also been obtained (NTP, 2003). *In vivo* mutagenicity studies for gene mutation in liver endothelial cells and DNA adduct formation have given positive responses (Chou *et al.*, 2003; Chou *et al.*, 2004; Chou and Fu, 2006; Mei *et al.*, 2004a; NTP, 2003; Wang *et al.*, 2005b; Wang *et al.*, 2005c). Unscheduled DNA synthesis (UDS) assays *in vivo* have given positive responses in some reports and negative responses in other (Mirsalis *et al.*, 1993; NTP, 2003). Overall, the evidence suggests riddelliine is an *in vivo* mutagen in rats and mice. The data from DNA adduct studies and cll mutant

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spectra in liver endothelial cells (Mei *et al.*, 2004a; Mei *et al.*, 2004b) are consistent with the pattern of carcinogenicity reported and suggest a DNA reactive mechanism involving metabolism to DHP and DNA adducts and mutations are important elements in the carcinogenicity of riddelliine to endothelial cells in rats and mice.

29. Riddelliine has been studied in a two year carcinogenicity study under the National Toxicology Program (NTP) in F344 rats and B6C3F<sub>1</sub> mice. Liver haemangiosarcomas were seen in 43 of 50 male rats and 38 of 50 female rats at 1 mg/kg b.w./day and in 3 of 50 female rats at 0.33 mg/kg b.w./day. In male mice, liver haemangiosarcomas were seen in 31 of 50 animals at 3 mg/kg b.w./day. Female mice showed alveolar and bronchiolar neoplasms in 13 of 50 animals at 3 mg/kg b.w./day. The authors of this study concluded that there was clear evidence of carcinogenic effects of riddelliine in F344 rats and B6C3F<sub>1</sub> mice (NTP, 2003). Details of these and other effects seen are summarised in Annex A, Section 1.

30. IARC have classified riddelliine as Group 2B “possibly carcinogenic to humans” (IARC, 2002).

Members are invited to consider whether:

i). the evidence is sufficient to consider riddelliine to be a genotoxic carcinogen?

### **Lasiocarpine**

31. Lasiocarpine is a diester heliotridine derived PA (figure 4) and is found in a number of *Heliotropium* and *Symphytum* species (WHO, 1988)

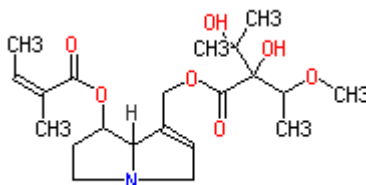


Figure 4: Lasiocarpine (obtained from the EPA website)

32. Lasiocarpine induces gene mutations in bacterial cells with exogenous metabolic activation (Yamanaka *et al.*, 1979) and in mammalian cells with or without exogenous metabolic activation (Takanashi *et al.*, 1980). It also showed DHP-derived DNA adducts with calf thymus DNA (Xia *et al.*, 2006). There are no *in vivo* studies which enable conclusions about *in vivo* mutagenic hazard to be drawn.

33. A two year carcinogenicity study has been carried out under the NTP testing lasiocarpine in F344 rats. Liver angiosarcoma was seen in 13 of 23 male and 2 of 23 female 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 and 7 of 24 females at 15 ppm in the diet and in 5 of 24 males and 8 of 22 females at 7 ppm in diet. The authors of this study concluded that

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this study had shown that lasiocarpine was carcinogenic in F344 rats (NTP, 1978). Details of these and other effects seen are summarised in Annex A, Section 2 as are the other studies.

34. Additional studies on lasiocarpine have been carried out using dietary and intraperitoneal administration. Following dietary administration of 50 ppm for 55 weeks, 9 of 20 had liver angiosarcoma and 7 of 20 had hepatocellular carcinoma (Rao and Reddy, 1978). Following intraperitoneal administration for 56 weeks, 11 of 18 animals surviving at termination had liver tumours of which 10 had hepatocellular carcinoma (Svoboda and Reddy, 1972).

35. IARC have classified lasiocarpine as Group 2B “possibly carcinogenic to humans” (IARC, 1976).

Members are invited to consider whether:

i). the evidence is sufficient to conclude that lasiocarpine has carcinogenic activity?

### **Clivorine**

36. Clivorine is a macrocyclic otonecine derived PA (figure 5) found in a number of *Ligularia* species (WHO, 1988).

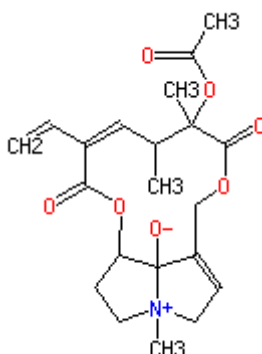


Figure 5: Clivorine (obtained from the EPA website)

37. Clivorine induces gene mutation in bacteria with exogenous metabolic activation (Yamanaka *et al.*, 1979). An *in vitro* UDS assay was positive in rat and hamster hepatocytes but negative in mouse hepatocytes (Mori *et al.*, 1985). It also showed DHP-derived DNA adducts with calf thymus DNA (Xia *et al.*, 2004). No data are available to draw conclusions on mutagenicity *in vivo*.

38. Clivorine has been studied in a non-standard test where 6 male and 6 female rats received a 0.005% solution in drinking water for 340 days and were kept until 480 days. Haemangioendothelial sarcoma in the liver was found in 2 of 12 treated rats following administration in drinking water compared to no rats with liver tumours in the control group (Kuhara *et al.*, 1980). Details of the effects seen are summarised in Annex A, Section 3.

39. No IARC classification has been found for clivorine.

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Members are invited to consider whether:

i). the evidence is sufficient to conclude that clivorine has carcinogenic activity?

### **Petasitenine**

40. Petasitenine is a macrocyclic otonecine derived PA (figure 6) found in *Farfugium japonicum* Kitam. and *Petasites japonicus* Maxim (WHO, 1988).

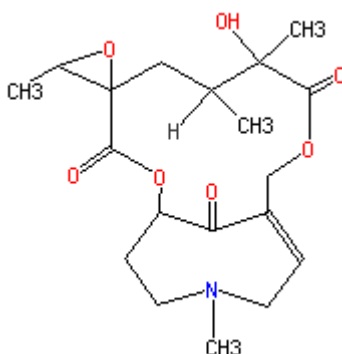


Figure 6: Petasitenine (obtained from the EPA website)

41. Petasitenine induces gene mutation in bacterial cells with exogenous metabolic activation (Yamanaka *et al.*, 1979). In addition, *in vitro* studies have shown that petasitenine can induce chromosomal aberrations and UDS in mammalian cells (Mori *et al.*, 1985; Takanashi *et al.*, 1980).

42. One *in vivo* study has been carried out where rats were given a 0.01% solution of petasitenine in drinking water. At termination after 480 days treatment, liver haemangioendothelial sarcomas were observed in 5 of 10 treated rats (Hirono *et al.*, 1977). Details of the effects seen are summarised in Annex A, Section 4.

43. IARC have classified petasitenine as Group 3 “not classifiable as to its carcinogenicity to humans” (IARC, 1983).

Members are invited to consider whether:

i). the evidence is sufficient to conclude that petasitenine has carcinogenic activity?

### **Senkirkine**

44. Senkirkine is a macrocyclic otonecine derived PA (figure 7). It is found in a number of plants including *Senecio* species and *Tussilago farfara* (coltsfoot) (EFSA, 2007; WHO, 1988).

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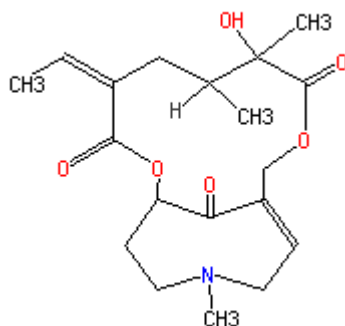


Figure 7: Senkirikine (obtained from the EPA website)

45. Senkirikine induces gene mutations in bacterial and mammalian cells with exogenous metabolic activation (Takanashi *et al.*, 1980; Yamanaka *et al.*, 1979). Senkirikine did not cause chromosomal aberrations in mammalian cells (Takanashi *et al.*, 1980) though an increase in sister chromatid exchange was described in another study (Bruggeman and van der Hoeven, 1985). However, the COM does not attach significant weight of evidence to sister chromatid exchange alone. Rat, mouse and hamster hepatocytes all showed UDS *in vitro* following treatment with senkirikine (Mori *et al.*, 1985). In addition, senkirikine gave a positive response in a sex-linked recessive lethal test in *Drosophila melanogaster* (Candrian *et al.*, 1984), though the COM does not attach significant weight of evidence to this type of study. No data are available to draw conclusions on mutagenicity *in vivo*.

46. Senkirikine has been studied in a non-standard test where groups of 20 male rats were given 22 mg/kg b.w. by intraperitoneal injection twice weekly for 4 weeks and then weekly for 52 weeks. 9 animals of 20 showed liver cell adenomas compared to no animals in the control group (Hirono *et al.*, 1979). Details of the effects seen are summarised in Annex A, Section 5.

47. IARC have classified senkirikine as Group 3 “not classifiable as to its carcinogenicity to humans” (IARC, 1983).

Members are invited to consider whether:

i). the evidence is sufficient to conclude that senkirikine has carcinogenic activity?

### **Symphytine**

48. Symphytine is a diester retronecine derived PA (figure 8) found in a number of *Symphytum* species including comfrey (WHO, 1988).

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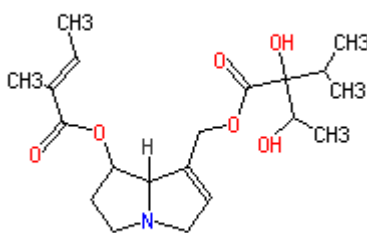


Figure 8 Symphytine (obtained from the EPA website)

49. No mutagenicity tests have been found on symphytine.
50. A non-standard test has been carried out where groups of 20 male rats were given 13 mg/kg b.w. symphytine by intraperitoneal injection twice weekly for 4 weeks and then weekly for 52 weeks. Of the 20 treated rats, one animal had liver cell adenoma and three showed liver haemangioendothelial sarcoma compared to no control animals showing liver lesions (Hirono *et al.*, 1979). Details of the effects seen are summarised in Annex A, Section 6.
51. IARC have classified symphytine as Group 3 “not classifiable as to its carcinogenicity to humans” (IARC, 1983).

Members are invited to consider whether:

- i). the evidence is sufficient to conclude that symphytine has carcinogenic activity?

### ***Monocrotaline***

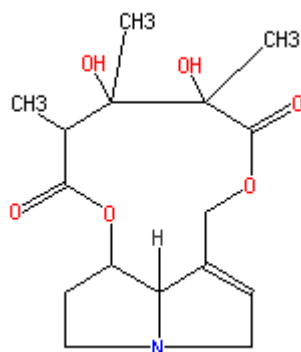


Figure 9: Monocrotaline (obtained from the EPA website)

52. Monocrotaline is a macrocyclic retronecine derived PA (figure 9) found in a number of *Crotalaria* species (WHO, 1988). In addition to hepatotoxicity, some PAs have also been shown to cause pulmonary toxicity. Monocrotaline and fulvine are known to be particularly active in this respect. The structural requirements of pulmonary toxicity are the same as for hepatotoxicity but the stability of the dehydropyrrolizidine derivative is believed to be key in

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determining whether the active metabolite can be transported away from the liver (WHO, 1988).

53. Three studies for gene mutation in bacterial cells with and without exogenous metabolic activation have showed negative responses for monocrotaline (Green and Muriel, 1975; White *et al.*, 1984; Yamanaka *et al.*, 1979). Chromosome aberrations have been observed in mammalian cells in the presence of exogenous metabolic activation (hepatocytes from rat, mouse or hamster) (Muller *et al.*, 1992). In addition, monocrotaline causes sister chromatid exchange in mammalian cells with exogenous metabolic activation (chick embryo hepatocytes) (Bruggeman and van der Hoeven, 1985). Two studies showed a positive UDS response rat hepatocytes *in vitro* (Berry *et al.*, 1996; Mori *et al.*, 1985). It also showed DHP-derived DNA adducts with calf thymus DNA (Wang *et al.*, 2005b). Micronucleus formation was observed in mice given 125 mg/kg b.w. (0.75 of the LD50) monocrotaline by intraperitoneal injection (Sanderson and Clark, 1993). Experiments in female F344 rats show the formation of DHP-derived DNA adducts (Wang *et al.*, 2005b).

54. Monocrotaline has been studied in an experiment administering 5 mg/kg b.w. biweekly for 12 months subcutaneously. 5 of 60 treated rats had hepatocellular carcinomas and 10 of 60 animals showed pulmonary adenocarcinomas (Shumaker *et al.*, 1976). Details of the effects seen are summarised in Annex A, Section 7.

55. IARC have classified monocrotaline as Group 2B “possibly carcinogenic to humans” (IARC, 1976).

Members are invited to consider whether:

- i) monocrotaline should be referred to the COM for assessment?
- ii). the evidence is sufficient to conclude that monocrotaline has carcinogenic activity?

### ***Dehydroretronecine and dehydroheliotridine***

56. Dehydroretronecine and dehydroheliotridine are the DHP metabolites of many PAs. They are enantiomers of each other at the C7 position.

57. Dehydroretronecine induces mutations in *S. typhimurium* strain TA92 in the absence of exogenous metabolic activation (Ord *et al.*, 1985). No test was undertaken with metabolic activation. A positive response was obtained in an *in vitro* sister chromatid exchange assay (Ord *et al.*, 1985) though the COM does not attach significant weight of evidence to such studies. Madin Darby bovine kidney epithelial cells were studied for DNA cross-links and the authors concluded that dehydroretronecine is not a potent DNA cross-linker (Kim *et al.*, 1995).

58. Studies where dehydroretronecine was administered by subcutaneous injection or topical application for between 4 weeks and 12 months have shown skin tumour formation and injection site rhabdomyosarcomas (Allen *et*

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*al.*, 1975; Johnson *et al.*, 1978; Mattocks and Cabral, 1982; Shumaker *et al.*, 1976). Details of the effects seen are summarised in Annex A, Section 8.

59. No IARC classification has been found for dehydroretronecine.

Members are invited to consider whether:

i). the evidence is sufficient to conclude that dehydroretronecine has carcinogenic activity?

60. No studies on the mutagenicity of dehydroheliotridine have been carried out.

61. Dehydroheliotridine has been studied in rats given intraperitoneal injections every 4 weeks for 32 weeks. The authors reported a significantly higher tumour incidence in treated animals compared to control (Peterson *et al.*, 1983). Details of the effects seen are summarised in Annex A, Section 9.

62. No IARC classification has been found for dehydroheliotridine.

Members are invited to consider whether:

i). the evidence is sufficient to conclude that dehydroheliotridine has carcinogenic activity?

### **Summaries of data for PAs where only mutagenicity studies have been carried out**

63. While relatively few PAs have been studied *in vivo* for long enough to make any assessment of carcinogenicity, there is data on the mutagenicity of a number of other PAs. A summary and a table of the collated data which forms Annex B will be available for the meeting.

### **Benchmark dose modelling of riddelliine and lasiocarpine**

64. The US EPA BMD software, version 1.4.1 was used for modelling the dose response for liver hemangiosarcoma incidence in rats and mice in 2-year studies, with riddelliine and lasiocarpine conducted under the NTP. The BMD<sub>10</sub> and BMDL<sub>10</sub> values for a 10% increase in incidence of haemangiosarcomas compared with the background incidence in controls were estimated using 6 dose response models for carcinogenicity.

65. For the current assessment the approach adopted by JECFA (Joint FAO/WHO Expert Committee on Food Additives) in its recent evaluation of Ochratoxin A (2007) and by EFSA in its assessment of PAHs (2008) was used to determine the acceptability of the models. This approach involves assessment of statistics on the goodness of fit calculated by the BMD software. The lower the chi-square value the better the fit, and the calculated *P*-value should be greater than 0.1. This *P*-value was therefore applied as a rejection level.

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66. For riddelliine in female rats, the range of BMD<sub>10s</sub> in accepted models was 0.377-0.508 mg/kg b.w./day and the range of BMDL<sub>10s</sub> in accepted models was 0.301-0.418 mg/kg b.w./day. In male mice, the range of BMD<sub>10s</sub> in accepted models was 1.252-1.615 mg/kg b.w./day and the range of BMDL<sub>10s</sub> in accepted models was 1.040-1.175 mg/kg b.w./day.

67. For lasiocarpine in male rats, the range of BMD<sub>10s</sub> in accepted models was 0.134-0.260 mg/kg b.w./day and the range of BMDL<sub>10s</sub> in accepted models was 0.078-0.200 mg/kg b.w./day. In female rats, none of the models was accepted.

68. In their assessments, JECFA and EFSA have felt it prudent to use the lowest BMDL<sub>10</sub> derived from an acceptable model to derive a margin of exposure. If the same approach were to be used here, lasiocarpine studied in male rats gives the lowest BMDL<sub>10</sub> of 0.078 mg/kg b.w./day.

69. A full description of the approach used and the results of the benchmark dose modelling is available in Annex C.

### **Discussion of a Cumulative Assessment Group**

70. The EFSA Scientific Panel on Plant Protection products and their Residues recently published a review of approaches to toxicological assessment of mixtures. "Cumulative Assessment Groups" were suggested to aid risk assessors in situations where there is insufficient information or time to undertake an assessment and reach conclusions on a common mechanism group (EFSA, 2008). The summary of this paper and the criteria for defining a Cumulative Assessment Group are available in Annex D.

71. There is evidence that PA carcinogenicity in rodents encompasses both endothelial and parenchymal cell tumours of the liver. In addition, a number of investigators have reported evidence for either *in vitro* or *in vivo* metabolism to DHP and formation of DHP adducts for 4 of the PAs described in this paper and 2 other PAs, retrorsine and heliotrine (Wang *et al.*, 2005b; Wang *et al.*, 2005a; Xia *et al.*, 2003; Xia *et al.*, 2004; Xia *et al.*, 2006; Xia *et al.*, 2008). Xia *et al.* (2008) reported on *in vitro* evidence for common formation of DHP across PAs from different structural groups (retronecine, heliotridine, and otonecine derived PAs). Table 1 provides an overview of the liver tumour profile and the evidence for DHP-derived DNA adducts for the PAs described in this paper. The original papers on which this evidence is based are provided in Annex F.

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Table 1: Evidence for DHP-derived DNA adduct formation by PAs inducing liver tumours in rodents.

<b>PA</b>	<b>Liver tumour profile in rodents</b>	<b>Evidence for DHP-derived DNA adducts <i>in vitro</i> or <i>in vivo</i>.</b>
Riddelliine	Haemangiosarcoma (rat male and female, mouse male) Adenoma (rat male and female)	<i>In vitro</i> using male and female rat and human liver microsomes <i>In vivo</i> using female rats
Lasiocarpine	Angiosarcoma (rat male and female) Adenoma (rat male and female)	<i>In vitro</i> using male and female rat liver microsomes
Clivorine	Haemangioendothelial sarcoma (rat gender not specified)	<i>In vitro</i> using male rat liver microsomes
Petasitenine	Haemangioendothelial sarcoma (rat gender not specified) Adenoma (rat gender not specified)	No data obtained
Senkirkine	Adenoma (rat male)	No data obtained
Symphytine	Haemangioendothelial sarcoma (rat male) Adenoma – equivocal response (rat male)	No data obtained
Monocrotaline	Hepatocellular carcinoma (rat male)	<i>In vitro</i> using female rat liver microsomes <i>In vivo</i> using female rats
Dehydro-retronecine	Inadequate data	Data on riddelliine, retrorsine and monocrotaline – all retronecine-type PAs
Dehydro-heliotridine	Inadequate data	Data on lasiocarpine and heliotrine – both heliotridine-type PAs

This is a background paper for discussion.  
It does not reflect the views of the Committee and should not be cited.

**Questions on which the views of the Committee are sought**

72. Members are invited to consider the questions in each section of this discussion paper, repeated below for completeness, and the additional questions and to raise any other matters that arise.

- i). Is the evidence sufficient to conclude that each PA described in this paper has carcinogenic activity? In the case of riddelliine, is the evidence sufficient to conclude that it is a genotoxic carcinogen?
- ii) Should monocrotaline be referred to the COM for assessment?
- iii). Is there evidence of a common tumour pattern? Would a Cumulative Assessment Group approach be appropriate for PAs?
- iv). Could the BMDL<sub>10</sub> of 0.078 mg/kg b.w./day for lasiocarpine be used as a basis to consider a Margin of Exposure approach to the risk assessment of PAs?

**Secretariat  
July 2008**

This is a background paper for discussion.  
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It does not reflect the views of the Committee and should not be cited.

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**CC/08/13 ANNEX A**  
**Section 1**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**Riddelliine overview**

This Annex provides a summary of the carcinogenicity and mutagenicity data for riddelliine. The original papers which provide evidence for *in vivo* tumour formation are available in Annex F.

**Secretariat**  
**July 2008**

This is a background paper for discussion.  
It does not reflect the views of the Committee and should not be cited.

**CC/08/13 ANNEX A**  
**Section 2**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**Lasiocarpine overview**

This Annex provides a summary of the carcinogenicity and mutagenicity data for lasiocarpine. The original papers which provide evidence for *in vivo* tumour formation are available in Annex F.

**Secretariat**  
**July 2008**

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It does not reflect the views of the Committee and should not be cited.

**CC/08/13 ANNEX A**  
**Section 3**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**Clivorine overview**

This Annex provides a summary of the carcinogenicity and mutagenicity data for clivorine. The original papers which provide evidence for *in vivo* tumour formation are available in Annex F.

**Secretariat**  
**July 2008**

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It does not reflect the views of the Committee and should not be cited.

**CC/08/13 ANNEX A**  
**Section 4**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**Petasitenine overview**

This Annex provides a summary of the carcinogenicity and mutagenicity data for petasitenine. The original papers which provide evidence for *in vivo* tumour formation are available in Annex F.

**Secretariat**  
**July 2008**

This is a background paper for discussion.  
It does not reflect the views of the Committee and should not be cited.

**CC/08/13 ANNEX A**  
**Section 5**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**Senkirkine overview**

This Annex provides a summary of the carcinogenicity and mutagenicity data for senkirkine. The original papers which provide evidence for *in vivo* tumour formation are available in Annex F.

**Secretariat**  
**July 2008**

This is a background paper for discussion.  
It does not reflect the views of the Committee and should not be cited.

**CC/08/13 ANNEX A**  
**Section 6**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**Symphytine overview**

This Annex provides a summary of the carcinogenicity and mutagenicity data for symphytine. The original papers which provide evidence for *in vivo* tumour formation are available in Annex F.

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This is a background paper for discussion.  
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**CC/08/13 ANNEX A**  
**Section 7**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**Monocrotaline overview**

This Annex provides a summary of the carcinogenicity and mutagenicity data for monocrotaline. The original papers which provide evidence for *in vivo* tumour formation are available in Annex F.

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**July 2008**

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It does not reflect the views of the Committee and should not be cited.

**CC/08/13 ANNEX A**  
**Section 8**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**Dehydroretronecine overview**

This Annex provides a summary of the carcinogenicity and mutagenicity data for dehydroretronecine. The original papers which provide evidence for *in vivo* tumour formation are available in Annex F.

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It does not reflect the views of the Committee and should not be cited.

**CC/08/13 ANNEX A**  
**Section 9**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**Dehydroheliotridine overview**

This Annex provides a summary of the carcinogenicity and mutagenicity data for dehydroheliotridine. The original papers which provide evidence for *in vivo* tumour formation are available in Annex F.

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**CC/08/13 ANNEX B**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**Overview of mutagenicity data on PAs which have not been tested *in vivo* for tumour formation**

This Annex is currently being prepared and the contents will be available for the COC meeting on 17<sup>th</sup> July 2008.

**Secretariat  
July 2008**

This is a background paper for discussion.  
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**CC/08/13 ANNEX C**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**Benchmark dose modelling of riddelliine and lasiocarpine**

This Annex provides details of the approach used and results obtained from the benchmark dose modelling using the data from the National Toxicology Program studies on riddelliine and lasiocarpine.

**Secretariat  
July 2008**

This is a background paper for discussion.  
It does not reflect the views of the Committee and should not be cited.

**CC/08/13 ANNEX D**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**EFSA Report**

The summary and section 2.1 of the report are provided here.

Opinion of the Scientific Panel on Plant Protection products and their Residues to evaluate the suitability of existing methodologies and, if appropriate, the identification of new approaches to assess cumulative and synergistic risks from pesticides to human health with a view to set MRLs for those pesticides in the frame of Regulation (EC) 396/2005

The EFSA Journal (2008) 704: 1-84.

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**CC/08/13 ANNEX E**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**Search Strategy**

This Annex contains the search strategy by which the papers on mutagenicity and carcinogenicity of pyrrolizidine alkaloids and the papers on DHP-derived DNA adducts were found.

**Secretariat  
July 2008**

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

**Pyrrrolizidine Alkaloids in Food – Initial Assessment of Carcinogenicity**

**Literature**

This Annex contains all the studies identified reporting *in vivo* tumour formation as described in the main paper and Annex A. Papers reporting the formation of DHP-derived DNA adducts as discussed in the main papers are also available here. The following papers are provided in alphabetical order:

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