

TOX/2008/20

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

FIRST DRAFT COT STATEMENT ON THE HAZARD TO CONSUMERS OF EATING FOODS DERIVED FROM ANIMALS THAT HAD EATEN BRACKEN

1. At the 1st April 2008 meeting, the Committee was presented with a paper (TOX/2008/12) reviewing the toxicological data on bracken and some of its constituent chemicals in relation to consumer exposure to bracken and residues of substances in foods derived from animals that had eaten bracken. The review had been instigated to address an enquiry from an interdepartmental Quarterly Review of Incidents about the consumer safety of foods derived from animals that had been poisoned with bracken. Members commented on the data presented in the review and made suggestions for the structure and content of a proposed statement on bracken.
2. Annex A contains the first draft COT statement which incorporates comments and suggestions made at the April 2008 meeting of the Committee and suggests some conclusions and recommendations.

Questions asked of the Committee

- I. Members are invited to comment on the overall structure and content of the draft statement (Annex A).
- II. If members are content with the overall structure, they may wish to make more detailed comments and to consider the proposed conclusions and recommendations.

COT Secretariat
May 2008

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

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Background

1. Several cases of bracken poisoning in farm animals have been reported. The Committee was asked to consider the hazard to the health of consumers eating foods derived from bracken poisoned animals and to consider whether there were sufficient data to establish how long poisoned animals should be left before they may be safely milked or slaughtered for human consumption.
2. Bracken is an invasive fern that is common throughout the world with several different sub-species and varieties. The varieties that are found in the UK are all of the *Pteridium aquilinum* subspecies *aquilinum*. The UK varieties of this subspecies are *aquilinum* (the most common), *latiusculum* and *atlanticum*¹. Bracken is found in all parts of the country and dense growths cover large amounts of land in Wales, Scotland and northern England².
3. Eating bracken can be harmful to farm animals and there is some evidence that it might also be harmful to humans. The varieties found in the UK are toxic and potentially fatal to farm animals if eaten, but there is great variability in the amount of the bracken toxin ptaquiloside, and possibly of other bracken toxins, in different varieties of bracken that are found throughout the world³. There is also variation between strains within particular varieties of bracken⁴ and at different times of the year⁵. All parts of the bracken plant contain potentially harmful chemicals, some of which can be excreted in milk and may leave residues in meat and offal derived from animals that ate the plant. Thus there is a potential hazard to consumers.
4. The COT and its sister committees COM and COC last advised on the safety of foods derived from animals reared on bracken infested land in the Annual Reports of COM in 1993⁶ and of COT in 1996⁷. The Committees had considered the available information on carcinogenicity and mutagenicity of bracken, along with the results of a government-sponsored study of the transfer of bracken mutagens into milk from goats fed on bracken. They advised that the risk to consumers was very low and that further research need not be undertaken on bracken mutagens. This advice was based partly on the observation that goats

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in the study were reluctant to eat bracken and it was expected that cattle and goats would not eat bracken if other food was available.

5. Several food producing species, including pigs and sheep, readily eat bracken and are used to clear bracken from pastures⁸. Furthermore, cattle have been observed to eat hay containing up to 30% bracken⁹. It has been reported that some horses and sheep eat bracken in preference to their normal pasture¹⁰.
6. Since 1996, the Veterinary Laboratory Agency (VLA) has identified several cases of suspected bracken poisoning in farm animals. There were 22 cases of bracken poisoning in cattle reported to the Veterinary Laboratories Agency between 1999 and 2007⁸. In addition, there was a report¹¹ in 2007 of two pigs that were suspected of having been poisoned by bracken. It is likely that many more cases of bracken poisoning would have gone unreported, as there is no requirement to report bracken poisoning. It is also likely that many other animals would have eaten bracken without showing clinical signs of poisoning.

Constituents of Bracken

7. Bracken contains a large number of potentially harmful substances, including illudane and protoilludane glycosides (such as ptaquiloside^{12, 13}, ptaquiloside Z¹⁴, isoptaquiloside¹⁵, pteroside A2¹⁶, pteridoside¹⁶ and caudatoside¹⁵, terpenic indanones (pterosins)^{15, 16}, *p*-hydroxystyrene glycosides (ptelatoides A and B)¹³, the cyanogenic glycoside prunacin¹⁷, braxin glycosides¹⁸, the flavinoid quercetin and its glycoside rutin^{19, 20}, kaempferol^{19, 20}, shikimic acid²¹, thiaminases²², ecdysteroids²² and tannins²². Other substances detected in bracken include dihydrocinnamic acids, phloretic acid, dihydroferulic acid, 2,3-butanediol, 3-methylbutan-2-ol, monomethylsuccinate, methyl-5-oxoproline, 2(3H)-dihydrofuranone and *t*-2-methylcyclohexanol^{13, 23, 24, 25, 26}. Little is known of the toxicology of many of these substances and information on the amounts in bracken is often lacking.

Toxicity of Bracken

Experimental Studies in Laboratory Animals and *In Vitro*

8. Most of the available studies were investigations of the carcinogenicity and mutagenicity of bracken. There is no available carcinogenicity bioassay of bracken that has been performed to modern standards, but the carcinogenicity of bracken has been investigated in numerous more limited studies in a variety of species. Feeding of bracken to various strains of mice at 25% or more in the diet for 6 weeks or longer^{27, 28, 29, 30} produced a variety of neoplasms, including leukaemia, lung adenomas, intestinal tumours, bladder tumours and liver nodules. In rats^{19, 31, 32, 33, 34}, 5% or more dietary bracken caused various tumours including gastrointestinal tumours, including adenocarcinomas and sarcomas, mainly in the ileum; transitional cell carcinomas of the urinary bladder; and pre-neoplastic nodules in the liver. In guinea-pigs^{35, 36, 37}, 30% dietary bracken caused intestinal adenomas and adenocarcinomas and

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transitional cell carcinomas of the bladder, and it also caused panmyelopathy of the bone marrow and haematuria. In toads³⁸, dietary bracken caused ileal adenocarcinomas and malignant liver tumours.

9. Mice fed Welsh bracken spores were found to have DNA-adducts in their stomach and small intestines, but not in the liver³⁹. In contrast, rats fed Brazilian bracken (a sub-species not found in the UK) did not have DNA-adducts in their stomach or ileum³¹. Cytogenetic analysis of peripheral blood taken from cows^{40, 41, 42} or people^{43, 44} who had eaten bracken showed increased numbers of chromosomal aberrations.
10. Processed bracken and various extracts from bracken have been tested for carcinogenicity¹³ or mutagenicity¹². All parts of the bracken plant were carcinogenic in rats but the tips of young fronds (the parts eaten by humans) were the most potent⁴⁵. Traditional methods of preparation of bracken for human consumption (boiled, treated with wood ash or sodium bicarbonate, pickled in salt) reduced its carcinogenic potency in rats²⁸. However, drying or freezing preserved the carcinogenic/mutagenic potency of bracken and the carcinogenic/mutagenic component(s) was extractable in aqueous media and in various organic solvents^{12, 13}. Various extracts of bracken (including boiling water, acetone, methanol and ethanol extracts) were mutagenic to Ames strains of *Salmonella typhimurium*. It was very briefly reported that a fraction that had been isolated chromatographically from bracken was mutagenic to *Drosophila* and mice^{46, 47}, but no details of the test were given. Milk from bracken-fed cows^{48, 49} and goats⁵⁰ was mutagenic to Ames strains of *Salmonella typhimurium* and milk from bracken-fed cows was carcinogenic to mice⁵¹ and rats⁴⁸.
11. Repeat-dose toxicity studies have been performed in rats, guinea-pigs, rabbits and cats but a NOAEL was not identified in any of the studies. In rats⁵² and rabbits⁵³, 25% dietary incorporation of dried bracken, containing 4.6 to 20.7 mg/kg ptaquiloside, for 30-90 days caused various adverse effects, including reduced bodyweight gain, leucopaenia, oedema of the brain and degenerative changes in the liver and testes. In the rats there were also sub-epicardial haemorrhages in the heart and hypersecretion into the intestines. In the rabbit study, there was also haemorrhaging in various organs and depletion of lymphoid follicles in the spleen and mesenteric lymph nodes. In guinea-pigs¹⁴³ given 30% dried bracken in the diet, there was decreased feed intake and decreased bodyweight gain. Cats given 10 g of dried bracken every 48 hrs died at 9-10 days after the start of treatment⁵⁴. All of the cats suffered hepatotoxicity.
12. The results of a developmental toxicity study in mice⁵⁵ showed that bracken in the diet caused low fetal weights and skeletal abnormalities (extra cervical or lumbar ribs, incomplete fusion of sternbrae, retarded ossification) in the offspring of pregnant. These results demonstrate that a toxic component of bracken can cross the placental barrier. No studies have been performed in laboratory animals to investigate reproductive toxicity over several generations.

Clinical and Epidemiological Findings in Farm Animals

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13. Acute poisoning of farm animals feeding on bracken can be fatal^{56, 57}. Prolonged ingestion of sub-lethal amounts of bracken can lead to various ailments that can differ between species. Exposure to bracken is also associated with the development of various tumours. The site where the tumour develops can vary between species.
14. Sudden death with signs of toxicity consistent with cyanide poisoning has been reported in animals fed on young fronds of bracken⁵⁸. This is thought to be related to the presence in bracken of the cyanogenic glycoside, prunacin.
15. In non-ruminant species, the principal adverse effect of dietary bracken is to cause a deficiency of thiamine (vitamin B₁) as a result of the action of thiaminases in the bracken⁵⁶. Bracken has not been seen to cause vitamin B₁ deficiency in ruminants, as their gut microflora can synthesise this vitamin, or in humans, presumably because their level of exposure to bracken is lower and they have a more varied diet.
16. In cattle, dietary exposure to bracken can cause a severe pancytopenia depression of bone marrow activity which is expressed clinically as an acute haemorrhagic syndrome⁵⁷. Calves show a different acute clinical syndrome involving bradycardia, laryngeal oedema and death from heart failure⁵⁷. Continued exposure results in chronic depression of bone marrow activity, which causes leucopaenia and thrombocytopaenia, which in turn leads to widespread petechial haemorrhages⁵⁹. Prolonged exposure of adult cattle can result in a chronic disease called bovine enzootic haematuria (BEH) that involves changes to the blood vessels of the urinary bladder and the later development of benign and malignant bladder tumours⁶⁰. Syndromes similar to BEH have also been described in buffalo, sheep and deer⁶¹. Bracken feeding of cattle has also been associated with a slow-developing epidermoid carcinoma of the upper digestive tract and a progressive retinal degeneration⁵⁶. Sheep are more prone to progressive retinal degeneration (called bright blindness or PRD in sheep) than cattle⁵⁸.
17. In quail, the feeding of bracken caused reduced testis weight and reduced male fertility and feeding of a solvent extract of bracken caused adenocarcinomas of the caecum, colon and distal ileum³⁵.

Clinical and Epidemiological Findings in Humans

18. There are several epidemiological studies of human populations in Japan^{62, 63, 64, 65, 66} Brazil⁶⁷, Venezuela⁶⁸, Costa Rica⁵⁴ and Wales^{69, 70, 71, 72}, the results of which consistently showed a moderate association (mostly with relative risks of approximately 3) between eating bracken and the development of cancer of the stomach and some also showed an association with cancer of the oesophagus. Furthermore, a recent Japanese ecological study⁶³ showed an association between eating wild plants (mainly bracken) and pancreatic cancer in men. The associations between eating bracken and cancer were less strong in the human studies than was seen in cattle. Most of the studies were ecological studies, and as such were prone to confounding. For instance it is possible that the increased prevalence of gastric cancer in bracken infested areas of Wales,

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instead of being due to exposure to bracken carcinogens, might have been due to increased numbers of infections by *Helicobacter pylori* due to cramped living conditions in this area.

19. Many of the human studies were too small to show a dose-response relationship, but one Japanese study⁶⁶ showed a higher risk of oesophageal cancer in people who ate bracken regularly than in those who ate it only rarely.

Toxicity of the Main Constituents of Bracken

Ptaquiloside and activated ptaquiloside (APT)

20. In 1983, two separate groups of workers in Japan¹³ and the Netherlands¹² independently identified ptaquiloside (a norsesquiterpene glycoside of the illudane type) as the principal substance responsible for the carcinogenicity of bracken. They had used various solvent extraction, resin adsorption and chromatographic techniques to separate various fractions which they had then tested with mutagenicity assays¹² or short-term carcinogenicity assays in rats¹³ to find those with the highest mutagenic/carcinogenic potency. The most purified fraction was then analysed by NMR spectroscopy to identify the chemical structure of the principal compound present, which is now named ptaquiloside.
21. Ptaquiloside is a water-soluble substance that is stable when present in plant tissues¹⁸. In mildly alkaline conditions, isolated ptaquiloside readily breaks down to its carcinogenically-active form, activated ptaquiloside (APT)²². Both ptaquiloside and APT slowly break down in acidic conditions to form pterosin B¹⁸, which is not carcinogenic. Composting bracken destroys the ptaquiloside in it⁵.
22. Ptaquiloside might be responsible for much of the toxicity of bracken that has been seen in ruminant farm animals. Progressive retinal degeneration (PRD or bright blindness) has been reproduced in sheep given ptaquiloside intravenously⁷³. Haemorrhagic cystitis and haematuria have been produced in guinea-pigs (but not rats or mice) given subcutaneous ptaquiloside⁷⁴.
23. It has been suggested that ptaquiloside is responsible for more than half of the mutagenic potency of bracken¹². There is no carcinogenicity bioassay of ptaquiloside that has been performed to modern standards, but its carcinogenicity has been investigated in more limited studies in rats. Ptaquiloside was administered as either an initial oral dose of 780 mg/kg bw followed by 8 weekly doses of 100-200 mg/kg bw or as twice weekly doses of 100-150 mg/kg bw for 8½ weeks and then the rats received no further treatment for the rest of their lives⁷⁵. The rats given the initial high dose developed haematuria and a loss of bodyweight and both treatments produced tumours of the mammary gland (adenocarcinomas, papillary carcinomas and anaplastic carcinomas) and ileum (adenocarcinomas). Hyperplasia was seen in the bladders of many of the treated rats. In another study, rats given a diet containing 0.027 to 0.080% ptaquiloside in their diet (equivalent to 29 - 80 mg/kg bw/day) developed cancers of the ileum and/or bladder within 15 to 60

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days⁷⁶. In a parenteral-dosing study, no tumours were seen in rats that had been given weekly intravenous doses of 20.7 mg/kg bw of ptaquiloside (equivalent to 3 mg/kg bw/day) for 10 weeks, followed by 30 weeks without further treatment, but these rats developed monocytosis and focal renal tubular necrosis⁷⁷.

24. Oral dosing with APT at 10 weekly doses of 20.7 mg/kg bw (equivalent to 3 mg/kg bw/day) or at 3 weekly doses of 41.4 mg/kg bw (equivalent to 6 mg/kg bw/day) did not produce any tumours when the animals were killed 30 weeks later⁷⁸. Similar results were obtained when APT was administered as 10 weekly intravenous injections of 20.7 mg/kg bw. Adverse effects were seen in all groups: tubular necrosis of the kidneys, monocytosis and elevated plasma tumour necrosis factor TNF α . In addition, the orally-treated rats showed necrosis of blood cell precursors in the bone marrow and had apoptotic bodies in their livers.
25. Ptaquiloside was tested for mutagenicity at different pHs. It was not mutagenic in either TA98 or TA100 strain of *Salmonella typhimurium* when tested at pH 7.4 in the absence of metabolic activation, but was mutagenic in both strains if it was pre-incubated at pH 8.5^{79, 80, 81}. It caused chromatid exchange type aberrations in CHL-cells in the presence and absence of S9 at pHs 5.3, 7.4 and 8.3, but the genotoxic potency was greater at the higher pHs⁸². Ptaquiloside also produced DNA-adducts *in vitro*⁸³ and caused *in vitro* unscheduled DNA synthesis in a rat hepatocyte culture at pH 7.2⁸⁴.
26. It seems likely that ptaquiloside is responsible for at least some of the carcinogenicity and toxicity of bracken. The carcinogenicity appears to have a genotoxic mode of action.

Illudane Substances Other than Ptaquiloside & APT

27. No toxicological data are available for the illudane and protoilludane glycosides other than ptaquiloside that have been identified in bracken: ptaquiloside Z, isoptaquiloside, pteroside A2, pteridanoside and caudatoside. However, their chemical similarity to the ptaquiloside raises the concern that some of them might be similarly carcinogenic by a genotoxic mode of action.

Terpenic Indanones

28. A large number of terpenic indanones have been isolated from bracken, including pterosins A, A2, B, C, D, E, F, G, J, K, I, N, O V and Z and pterosides A, B, C and M^{16, 56}. Indanones are found in high concentrations (up to approximately 24 mg/kg (w/w)) in young fronds²², but they do not act as alkylating agents²². Pterosin B is much less electrophilic than ptaquiloside⁸⁴. A range of different indanones (pterosins A, B, C, D, E, F, G, K, L, N and Z; acetyl pterosin C; benzoylpterosin B; and palmitylpterosins A and B) were shown to be non-mutagenic at pH 7.4, when tested in *Salmonella typhimurium* strains TA98 and TA100 in the presence and absence of S9^{79, 80, 85}. A selection of indanones (pterosins A, B, C, F AND L; and pterosides A, B and C) were also non-clastogenic when tested in CHL cells in the absence of metabolic activation⁸⁶.

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Extracts containing high levels of pterosins A, B & C, pterosides A, B & C, and other non-specified indanones did not cause leucopaenia or thrombopaenia in calves⁸⁷.

29. There is no evidence to suggest that terpenic indanones are responsible for any of the toxicity of bracken.

p-Hydrostyrene Glycosides

30. The p-hydrostyrene glycoside, ptelatocide A, was tested for carcinogenicity in rats at a concentration of 1.3 mg/kg in the diet (equivalent to 0.065 mg/kg bw/day) for 109 or 125 days¹³. At this dose, there was no evidence of any carcinogenicity. There was insufficient ptelatocide A available to test higher concentrations.
31. There is no evidence to indicate that any of the toxicity of bracken is due to the presence of p-hydrostyrene glycosides.

Prunacin

32. Prunacin is a cyanogenic glycoside that is present in some varieties of bracken. Cyanogenic glycosides can become toxic by releasing hydrocyanic acid (HCN) when hydrolysed by enzymes that may be released when tissues are damaged. A polymorphism exists in bracken: not all plants are cyanogenic as some lack either prunacin or the enzymes needed to liberate hydrocyanic acid from it. Farm animals seem to avoid eating the cyanogenic varieties. Prunacin is usually present in bracken at harmless quantities, but there have been fatal cases of cyanide poisoning in animals that have been fed on young fronds of bracken²².
33. Up to 61 mg/g of prunacin has been detected in fresh plant material from a Venezuelan tropical variety of bracken (*Pteridium aquilinum* var. *arachnoideum*), with the highest concentrations being found in young fronds¹⁷. It was noted in Venezuela that the *arachnoideum* variety of bracken contained more prunacin than the *caudatum* variety²². No quantitative information was available on the amount of prunacin in British varieties of bracken, but it was reported that the highest concentration occur in early to mid Spring²². One gram of prunacin has the potential to release up to 96 µg of HCN¹⁷. Thus up to 5.9 µg of HCN might be released from 1 gram of fresh Venezuelan bracken.
34. In its the Statement on Cyanogenic Glycosides in Bitter Apricot Kernels⁸⁹, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) concluded that the limited chronic toxicity data available were not sufficient to it to propose a TDI for cyanide, but it noted that the World Health Organisation (WHO) and the Council of Europe (CoE) had established TDIs of 12 and 20 µg/kg bw, respectively. The COT identified a nominal acute reference dose (ARfD) of 5 µg/kg bw, by applying a 100-fold uncertainty factor to the lowest reliably observed acute lethal dose in humans of 0.5 mg/kg bw. A person of 60 kg bodyweight would need to eat more than 50 g of the Venezuelan bracken to exceed this ARfD and to regularly eat about 140 g per day of this bracken to exceed the TDI that was set by WHO. It is conceivable

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that an extreme consumer of bracken might experience acute signs of cyanide toxicity as a result of eating a large portion of a variety of bracken that is high in prunacin. However, consumption of bracken is not known in the UK. No data are available on residues of prunacin or cyanide in foods derived from animals that ate bracken. Nevertheless, it seems unlikely that UK foods derived from bracken-exposed animals would contain sufficient prunacin or cyanide to cause toxicity in human consumers without causing serious toxicity in the animals. This because:

- there is no evidence to suggest that UK bracken contains as much prunacin as the Venezuelan variety;
- there is no evidence that ingested prunacin or cyanide will leave residues in edible tissues and milk;
- we are not aware of any reports of cyanide poisoning in people eating foods from animals that have eaten bracken; and
- it is likely that the exposed animals will be more affected by the direct exposure to prunacin and cyanide than will be consumers of foods derived from these animals. Signs of toxicity in the animals will alert people that foods derived from poisoned animals should not be used for human consumption.

Braxin Glycosides

35. Braxins A1, A2 and B have been detected in rhizomes of bracken⁹⁰. Braxins A1 and A2 were present in rhizomes at a combined concentration of up to 600 mg/kg, but were not detected in fronds⁹⁰. Braxins A1 and A2 are β -glucopyranosides with an aromatic structure⁹⁰, but their precise chemical structure has not been identified. The chemical structure of braxin B is not known. ("Braxin C" is ptaquiloside.)
36. Subcutaneous injections of braxins A1, A2 and B induced haemorrhagic cystitis in guinea-pigs (as did ptaquiloside)⁹¹. Braxins A1 and A2 also caused a dose-related release of histamine from rat peritoneal cells *in vitro* and also caused the cells to swell⁹⁰. The *in vitro* histamine-releasing activity of glycosides extracted from rhizomes (includes braxins A1 and A2) was about ten times greater than that of glycosides from the fronds (braxins A1 and A2 not present)⁹⁰.
37. It is possible that braxin glycosides might play a part in the aetiology of the haemorrhagic cystitis that is seen in cattle and some other species.

Quercetin

38. Quercetin was found in bracken at concentrations of up to 860 mg/kg (dry weight)²⁰, but it is also found in many other fruits and vegetables, often at higher concentrations (eg. up to 65,000 mg/kg in onions)²⁴.
39. Orally administered quercetin was not very well absorbed⁹². It is either converted to phenolic acids by the gut flora or voided unchanged in the faeces.

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40. Quercetin was of low cytotoxicity when tested in vitro using CHO cells, 3T3 mouse fibroblasts and normal rat kidney (NRK) cells⁹³.
41. In calves, oral doses of up to 20 g/calf/day for several months had no effect on incidences of BEH or papilloma-induced lesions of the urinary bladder⁹⁴. It was claimed that exposure to quercetin is associated with bovine cancers of the upper alimentary tract²², but no evidence has been found to support this claim.
42. There is limited evidence to suggest that quercetin could be carcinogenic. In a two-year feeding study performed in F344 rats given 1000, 10000 or 40000 mg quercetin per kg diet (equivalent to 40, 400 and 1900 mg/kg bw/day) there were increased incidences of hyperplasia and adenomas of renal tubules at all doses in males with adenocarcinomas also being seen in the top-dose males (no adverse effects in females)⁹⁵. In another study in F344 rats⁹⁶, 40000 mg/kg in the diet (1900 mg/kg bw/day) caused benign tumours in the renal tubules of males, but not in females, although no adverse effects were seen at dietary levels of 100 or 1000 mg/kg. It is noted that renal tumours were not produced when rats were fed bracken. In a study in Norwegian albino rats, administration of 10000 mg quercetin per kg diet (equivalent to 400 mg/kg bw/day) caused transitional cell carcinomas of the bladder¹⁹. It is noted that bladder transitional cell carcinomas were also produced in rats fed bracken^{31, 34, 35, 51, 62, 73}. The results of other carcinogenicity studies gave no evidence to suggest that quercetin was carcinogenic when given in the diet to ACI rats at up to 100 000 mg/kg (4000 mg/kg bw/day) for 850 days⁹⁷, to F344 rats at 10000 mg/kg (400 mg/kg bw/day) for 540 days⁹⁸, to F344 rats at up to 50000 mg/kg (2000 mg/kg bw/day) for 104 weeks⁹⁹, to ddY mice at 20000 mg/kg (equivalent to 3000 mg/kg bw/day) for a lifetime¹⁰⁰, or to golden hamsters at up to 100 000 mg/kg (equivalent to 12000 mg/kg bw/day) for up to 735 days followed by treatment with 1% croton oil for a further 350 days¹⁰¹.
43. There is also some evidence that quercetin has anti-cancer properties. It has been suggested that it causes inhibition and induction of different phase I and phase II metabolism enzymes, *that* it has antioxidant effects, that it can induce apoptosis and that it can down-regulate oncogenes¹⁰². Oral doses of quercetin given to rats cause a decrease in the ability of benzo(a) pyrene metabolites to bind to DNA, and *in vitro* it inhibited the growth of cells from various human cancers⁹².
44. There is some evidence that quercetin might be genotoxic. It can bind to DNA and cause single-strand breaks¹⁰³. It gave positive results in a variety of mutagenicity assays, including the *Salmonella*/microsome reverse mutation assay^{95, 104, 105, 106}, gene mutation assays in mammalian cells^{24, 107, 108}, cytogenetics tests in mammalian cells^{24, 87, 95, 107, 109}, and sex-linked recessive lethal mutations in *Drosophila*. Quercetin gave inconsistent results in the mouse bone marrow micronucleus test: with some tests finding it mutagenic¹¹⁰ whilst others did not^{111, 112}. Quercetin did not cause unscheduled DNA synthesis in gastric mucosal cells of rats that had been given oral doses¹¹¹. Thus, although quercetin is an *in vitro* mutagen, the balance of evidence is that it does not express its mutagenicity in mammalian systems *in vivo*.

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45. Given that many non-toxic plants contain higher concentration of quercetin than are found in bracken, it is considered unlikely that quercetin is responsible for the toxicity or carcinogenicity of bracken.

Kaempferol

46. Kaempferol is chemically similar to quercetin, from which it differs by lacking one hydroxyl group. It was found at 1100 mg/kg (dry weight) in bracken¹⁹. It is also commonly found in other plants²⁴. Tea can contain up to 10,000 mg/kg of quercetin plus kaempferol, combined²⁴.
47. A limited study in ACI rats (400 mg/kg feed given to 6 males and 6 females for 540 days) showed no evidence to suggest that kaempferol was carcinogenic⁹⁸.
48. The results of mutagenicity tests suggest that kaempferol is an *in vivo* mutagen. It gave a positive result for mutagenicity in a bone marrow micronucleus assay in which mice were dosed intraperitoneally¹¹⁰. Kaempferol also gave positive results in a sex-linked recessive lethal assay in *Drosophila*¹¹³ and in several *in vitro* mutagenicity tests: the *Salmonella*/microsome assay^{86, 104, 105, 106, 114}, gene mutation tests in mammalian cells^{107, 108} and a cytogenetics assay in mammalian cells¹⁰⁷.
49. It is possible that the presence of kaempferol might contribute to the overall carcinogenicity of bracken. Otherwise, given that many non-toxic plants also contain kaempferol, it is considered unlikely that kaempferol is responsible for the toxicity of bracken.

Shikimic Acid

50. Shikimic acid was found at 1440 mg/kg (dry weight) in Welsh bracken²¹. It is also present in several edible plants, including soybeans, star anise and green tea. Shikimic acid was destroyed in alkaline conditions¹¹⁵.
51. Shikimate was of low cytotoxicity when tested *in vitro* in CHO cells, 3T3 mouse fibroblasts and normal rat kidney (NRK) cells, with the concentrations inhibiting cell growth by 50% after 48 h of incubation being respectively 0.8, 0.7 and 1.0 millimolar⁹³.
52. Intraperitoneal injections of 10 mg on shikimic acid caused death in mice within a few hours of dosing, with haemorrhaging and “denudation” of the intestinal mucosa¹¹⁵.
53. Developmental toxicity was tested in CD-1 mice given oral gavage doses of 0.25 or 1 g shikimic acid¹¹⁶. There was a slightly reduced number of implantations at both dose levels, as compared with untreated controls. The results showed no evidence of fetotoxicity or teratogenicity.
54. TF1 mice given single intraperitoneal doses of 1 to 30 mg of shikimic acid had increased incidences of cancer of the glandular stomach and of leukaemia when observed for up to 70 weeks¹¹⁷. A single mouse given a single oral gavage dose

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of 100 mg died after 34 weeks, having developed stomach cancer and leukaemia. These lesions were consistent with the sites of tumours seen in mice fed bracken. In ACI rats, however, the feeding of shikimic acid at a dietary concentration of 1000 mg/kg for 142 days, followed by an observation period of a further 70 days had no effect on tumour incidences²¹.

55. Shikimic acid was not genotoxic in bacterial¹¹⁸ or *in vivo* mammalian assays (mouse bone marrow assay and UDS in rat gastric mucosa)¹¹¹.
56. It is unlikely that shikimic acid has any appreciable contribution to the toxicity of bracken. Although it was carcinogenic in mice, it was not carcinogenic in rats and appeared to be non-mutagenic. Furthermore, shikimic acid was destroyed by alkaline conditions whereas the mutagenicity and carcinogenicity of bracken was increased under such conditions.

Thiaminases

57. Anti-thiamine enzymes, thiaminases, seem to cause most of the short- to medium-term symptoms of bracken poisoning in monogastric animals. Thieminase activity is highest in rhizomes and very young fronds. Thiaminases types 1 and 2 have been found in bracken at activities of 3.1 and 3.5 µg thiamine destroyed/g plant material/hour, respectively¹¹⁹. A third more heat-stable thiaminase (possibly caffeic acid, a substance that also has both pro- and anti-cancer properties) might also play a role¹⁰.
58. Rats fed on bracken that had thiaminase activity developed lesions of the nervous system that were considered by the authors of the study¹³ to be typical of antivitaminosis-B₁. The lesions could be cured by thiamine (vitamin B₁). Similar effects have been reported in monogastric farm animals, including horses and pigs²².
59. In order to test if the anti-thiamine activity of bracken contributed to the carcinogenicity of bracken, a 52 week feeding study was performed in groups of rats fed either control diet, a diet containing bracken or a diet containing bracken supplemented with a sc injection of 2 mg/rat/week of thiamine¹²⁰. No tumours were found in controls. All survivors in the other two groups developed multiple intestinal tumours. Bladder tumours were found in 11% of males and 7% of females in the group given bracken alone and in 53% of males and 67% of females in the group given bracken plus thiamine. It was noted that thiamine supplements did not reduce the incidence of tumours, so it seemed unlikely that thiaminase caused the carcinogenicity of bracken.
60. No reports of thiamine deficiency in bracken-consuming human populations have been found. It is possible that humans are less prone to thiamine deficiency than farm animals as a result of having a more varied diet. It is also likely that humans who eat bracken would eat much less bracken than animals fed on bracken.

Ecdysteroids

61. Bracken contains several ecdysteroids which can prevent insects from moulting and developing into adults²². This mode of action is not relevant to mammals.
62. It has been claimed that α -ecdysone induced neoplastic lesions in Egyptian toads (*Bufo regularis*)¹²¹, but no details or evidence were presented to back-up this claim.
63. There is no reliable evidence to suggest that ecdysteroids are a toxic hazard to humans.

Tannins

64. The tannins in bracken are mainly condensed tannins derived from procyanidin and prodelfinidin. Fronds of tropical bracken can have more than 120 mg/g of condensed tannins²². No information was available on the amount in varieties of bracken that are found in the UK. Tannins are present in many foods (including legumes, chocolate, fruits and smoked foods) and drinks (including tea, wine and beer). For instance, wine contains 0.57 to 2.47 mg/mL¹²², red kidney beans (*Phaseolus vulgaris*) contain 6.3 to 9.1 mg/g raw and 3.1 to 5.5 mg/g cooked¹²³, raw soybeans¹²³ contain 0.5 mg/g and “corn”^a contains 0.1 mg/g¹²³.
65. There is limited evidence to suggest that parenteral administration of some tannins might be carcinogenic. Subcutaneous injections caused liver tumours¹²⁴ and fibrous histiocytomas at the injection site¹²⁶ in rats, and caused local sarcomas and liver tumours in mice¹²⁴. Although liver nodules were noted in one of the mouse carcinogenicity studies of bracken³³, the liver was not the major site for neoplasia in mice, more usual were leukaemias, lung tumours and gastrointestinal cancers^{28, 29, 30, 33}. However, the feeding of bracken tannins at a dietary concentration of 4000 mg/kg for up to 72 weeks did not cause any cancer in F344 or Sprague-Dawley rats¹²⁶. IARC has classified tannic acid and tannins in Group 3: “The agent (mixture or exposure circumstance) is not classifiable as to its carcinogenicity to humans”¹²⁵. Bracken tannins were not mutagenic to strains TA98 and TA100 of *Salmonella typhimurium* when tested in the absence of metabolic activation¹²⁶.
66. Although bracken can contain higher concentrations of tannins than commonly consumed foods, there is no evidence that ingestion of these tannins is harmful.

Summary of Toxicity

67. In monogastric animals the main effect of eating bracken is often a deficiency of thiamine as a result of the action of thiaminases in the bracken. However, this effect is not known in humans, possibly as a result of the more varied human diet and lower level of consumption of bracken in the diet.

^a It was not clear from the report whether “corn” referred to maize, wheat or some other cereal.

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68. Ruminants do not experience thiamine deficiency as a result of eating bracken as their gut flora can make thiamine from other substances in the diet. Instead they develop several other non-neoplastic diseases as a result of eating bracken, including panmyelopathy of the bone marrow, haemorrhagic cystitis and progressive retinal degeneration. It is likely that the illudanes glycoside, ptaquiloside, in bracken is at least partly responsible for causing these effects. Braxins might also play a role. Again these non-neoplastic effects are not seen in humans, probably as a result of lower levels of dietary exposure.
69. Animals have died, showing signs of toxicity consistent with cyanide poisoning, following ingestion of large amounts of bracken containing the cyanogenic glycoside, prunacin. The amount of prunacin found to be present in bracken is too low to cause cyanide poisoning in humans exposed either by direct consumption of bracken as a vegetable or by eating foods derived from animals that ate bracken.
70. Bracken also seems to cause cancer in a wide variety of species. The site and type of cancer can differ between species. In humans, there is some evidence from epidemiological studies to suggest an association between exposure to bracken and the development of cancers of the stomach and oesophagus.

Modes of Action of Relevance to Humans

71. Carcinogenicity is thought to be the only toxic effect of bracken that is of relevance to humans exposed by eating the plant or foods derived from animals that have eaten bracken. The non-neoplastic effects that have been seen in heavily exposed laboratory animals and farm animals have not been reported as occurring in exposed humans.
72. Bracken and various extracts of bracken were mutagenic in a range of *in vitro* and *in vivo* tests. As bracken is both carcinogenic and mutagenic, it is reasonable to assume that it causes cancers by a genotoxic mode of action.

Contributions of Constituent Chemicals to the Carcinogenicity of Bracken

73. Bracken contains a large number of component chemicals, some of which have been shown to be mutagenic and/or carcinogenic: ptaquiloside, quercetin, kaempferol, shikimic acid and tannins. Illudanes other than ptaquiloside have not been tested, but their chemical similarity to ptaquiloside raises suspicions about their possible carcinogenicity and mutagenicity.
74. There is evidence that ptaquiloside is responsible for at least some of the carcinogenicity of bracken. In addition it produced DNA adducts in rat ileum that gave a spot in thin-layer chromatography in an identical position to the adducts that had been found when rats were treated with bracken^{127, 128}. Ptaquiloside has been shown to be an *in vivo* mutagen and it produces similar types of tumours to bracken in the various species that have been tested. It can be present in bracken in appreciable amounts: between 447 and 1211 mg/kg were

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detected in varieties that are found in the UK, but higher amounts have been detected in tropical varieties.

75. Quercetin appears not to be genotoxic *in vivo*, but has been shown to cause tumours in experimental animals that are consistent with the sites of tumours formed when these animals were fed bracken. However, it seems unlikely that quercetin contributes to the carcinogenicity of bracken, as the concentration in bracken is considerably lower than in other innocuous foods, such as red onions.
76. The available mutagenicity test results indicate that kaempferol is genotoxic *in vivo*, but has not been tested for carcinogenicity. It is possible that kaempferol might contribute to the carcinogenicity of bracken.
77. Shikimic acid was not genotoxic in a limited range of tests. Although subcutaneous doses caused tumours in mice, a large oral dose was not carcinogenic in rats. Furthermore, it is destroyed in alkaline conditions, whereas the genotoxicity of bracken increased in such conditions. It seems unlikely that the presence of shikimic acid contributes to the carcinogenicity of bracken.
78. The evidence for bracken tannins being the cause of the carcinogenicity of bracken is weak because the only bioassay of the carcinogenicity of oral doses of bracken tannins gave a negative result, and there was no evidence that they were genotoxic.

Mode of Action of Ptaquiloside

79. The genotoxic potency of ptaquiloside was found to be dependent upon the pH of the medium. It was not mutagenic to *Salmonella* strains TA98 or TA100 when tested at pH 7.4 but was mutagenic without metabolic activation when it was first preincubated at pH 8.5⁸⁰. It was also discovered that ptaquiloside was less potent at causing chromosomal aberrations when tested at pH 5.3⁸². It is thought that the reason for the higher genotoxic potency of ptaquiloside at higher pH is because under mildly alkaline conditions, ptaquiloside is converted by β -elimination into an illudane-dienone compound, which is referred to as APT (activated ptaquiloside)⁸². APT possesses a highly reactive cyclopropyl ring. It is electrophilic with a greater capacity to alkylate DNA than ptaquiloside⁶⁰. It is stable in mildly alkaline conditions, but under acidic conditions it is converted to a less reactive non-genotoxic substance, pterosin B. Pterosin B is also formed from the breakdown of ptaquiloside under acidic conditions^{12, 129}.
80. Compounds that were chemically similar to APT but lacked an activated cyclopropane moiety (hypoloside B, hypoloside C, illudin M and illudin S) were not mutagenic to *Salmonella typhimurium* TA98 or TA100 strains⁸². It is not known whether the other illudanes that have been found in bracken would form an activated cyclopropane moiety in similar way to which ptaquiloside forms APT.
81. APT binds covalently to purine bases on DNA¹³⁰, opening the cyclopropyl ring of the molecule and forming adducts with the N-7 of guanine and the N-3 of

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adenine. Alkylation of adenine (but not that of guanine) caused cleavage of the N-glycosidic linkage of the modified adenines to produce abasic sites on the DNA molecule¹³¹. The abasic sites were unstable and breakage of the phosphodiester-pentose backbone of the DNA molecule occurred. Investigations were made¹³² of the H-*ras* and *p53* genes from the mammary glands of rats that had received weekly intravenous doses of 20.7 mg/kg bw and had been killed immediately after dosing. No mutations were found in *p53*³¹, but there were double mutations at codons 58 and 59 of the H-*ras* gene⁷⁸. Mutations of the H-*ras* proto-oncogene also occurred in the ileums of cattle fed bracken in their diet^{132, 134, 135}.

82. It has been shown that infections of cattle by papillomaviruses increases the chances of them developing benign papillomas of the upper gastrointestinal tract (associated with BPV-4 infection) or the urinary bladder (associated with BPV-2 infection) and repeated dietary exposure to bracken can further increase the chances of developing these tumours and of them progressing to malignancy^{56, 94, 121, 133, 134, 135, 136, 137, 138, 139}. It has been proposed¹²¹ that immunosuppression caused by bracken's suppression of the bone marrow makes the animals more prone to viral infection and that this makes them more likely to develop cancers of the upper gut and bladder when exposed to bracken. Such a mode of action might be relevant to humans.

Exposure

83. It is thought to be rare for people in the UK to eat bracken. However, in some parts of the world including Japan, Brazil, New Zealand, Canada and the USA, the young curled bracken fronds (called crosiers or fiddleheads) are eaten. Analysis of fronds and rhizomes of the bracken variety that is most common in the UK (*Pteridium aquilinum* var. *aquilinum*) found them to contain ptaquiloside concentrations of 213-2145 mg/kg and 11-902 mg/kg, respectively.
84. The Committee is not aware of any UK commercial sources of bracken for human consumption. The FSA received in August 2007 a single hearsay report of vacuum-packed bracken shoots being on sale in the UK, but could find no evidence to substantiate this. Although it is possible that bracken could be imported from abroad or harvested on a small scale locally, the Committee is not aware of any instances of this happening. If bracken were to be introduced for human consumption in the UK, its safety would need to be evaluated and approved by the Advisory Committee on Novel Foods and Processes (ACNFP) even though it is already consumed in several parts of the world outside of the EU.
85. The most likely route by which UK consumers may be exposed to bracken-derived substances is by eating foods derived from animals that have eaten bracken. Some food producing animals eat bracken. There have been 22 recent (1999-2007) documented cases of UK cattle being poisoned by bracken⁸, although the total number of poisonings is likely to be greater than this. Intensive grazing, usually by sheep or pigs, is used in the UK to clear bracken

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and to reduce invasion of pastures. There may also be some exposure to bracken as a result of the traditional use of bracken as bedding for animals.

86. It is theoretically possible that animals that are exposed to bracken could have residues of harmful bracken-derived chemicals in their tissues, which could be eaten by human consumers. No information is available on the concentrations of residues of any toxic substances derived from bracken in meat and offal or their rates of depletion from edible tissues.
87. Substances from bracken can be excreted into the milk. The milk from bracken-fed cows caused leucopaenia in calves, produced bladder cancer in mice, produced cancers of the intestines, bladder and kidneys in rats. Various solvent extracts of the milk were mutagenic to *Salmonella typhimurium* strains TA98 and TA100, and caused pulmonary adenomas in the offspring of mice that had been exposed during pregnancy. Thus it seems that toxic agents in bracken can be passed into the milk and can cross the placental barrier. There is a potential hazard from toxic components of bracken being passed into milk intended for human consumption. People who consume local unbulked milk or dairy products from bracken infested areas would be expected to be at greater risk than those drinking only bulked milk from commercial dairies.
88. Ptaquiloside has been detected in milk from bracken-fed cows¹⁴⁰. A concentration of 0.11 mg/L of ptaquiloside was found in milk from a cow that had been fed for 7 days on 6 kg/day of fresh bracken fronds that contained 0.25 ± 0.05 mg/g of ptaquiloside^{141, 142}. The total amount of ptaquiloside that was excreted into the milk of this cow was equal to 1.2% of the amount of ptaquiloside that it ingested. In another study¹⁴², two cows transferred into their milk $8.6 \pm 1.2\%$ of the ptaquiloside they ingested from 6 kg/day of fresh fronds of bracken that provided doses of 2400 to 10000 mg/cow/day of ptaquiloside. Doses of 2400, 4500, 8100 and 10000 mg/cow/day of ptaquiloside in bracken produced peak concentrations of 10, 27, 38 and 55 mg/L of ptaquiloside in the milk. After a few days the cows refused to eat the feed containing the highest concentration of ptaquiloside. Ptaquiloside was first detected in milk at 38 h after the start of dosing and peaked at 86 h. After feeding of bracken was stopped, the concentrations gradually dropped off until none was detectable at 86 h after the end of the dosing period.
89. Data from the most recent UK food surveys^b indicate that the sector of the UK population with the highest chronic intake of milk is infants aged 6-12 months (1992-1993 survey). It has been estimated by extrapolation from the results of the study described in the preceding paragraph¹⁴² that a UK infant having the upper 97.5th percentile chronic intake of milk (851 g/person/day for milk

^b The information on UK intakes of milk was obtained using data on individual consumption that are compiled in the "Intake 2" programme. The data include intakes of cows', sheep's and goats' milk, milk in chocolate and milk used in recipes. Chocolate was assumed to be 25% milk.

The "Intake 2" programme used data from the following food intake surveys: Infants 86, Toddlers' Survey, Young Persons '98 Survey, Vegetarian 1994-95 Survey, Adults 2001 National Diet and Nutrition Survey and Free-Living and Institutional Elderly Surveys (Mills and Tyler, 1992; Gregory, *et al.*, 1995; Gregory, *et al.*, 2000; MAFF, 1996; Henderson, *et al.*, 2002 and Finch, *et al.*, 1998).

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excluding infant formulae and breast milk) from a cow that had been fed a sub-clinical dose of 5000 mg/day of ptaquiloside in bracken could receive a dose of up to 22.8 mg/person/day of ptaquiloside (or 2.62 mg/kg bw/day for an infant of the UK mean bodyweight of 8.7 kg). This gives a conservative estimate of the maximum consumer intake of ptaquiloside from milk from bracken-exposed cows that show no clinical signs.

90. The estimated intake of infants of 22.8 mg/person/day of ptaquiloside is regarded as an extreme intake as it takes the highest measured amount of ptaquiloside in milk from cows given the highest tolerable dose of bracken and compares it with a high estimate of the cows' milk intake of infants. Most infants would be expected to have a lower intake of cows' milk than this as the UK Government advises that cows' milk should not be directly fed to infants of one year of age or less. Furthermore, most consumers would consume bulked milk, where the milk from any cows receiving such high intakes would be diluted with milk from cows with low or zero intakes of ptaquiloside. It is also conceivable that pasteurisation and other processing of milk would further reduce the levels of ptaquiloside present (although there are no data to confirm this).
91. The results of UK food surveys indicate that, after infants, the subpopulation with the highest per capita chronic intake of milk is the institutional elderly and those with the highest intake in relation to bodyweight are toddlers aged 1½-4½ years. Using the same information¹⁴² on possible amounts of ptaquiloside in cows' milk as used to estimate the intake of infants, the 97.5th percentile intakes of ptaquiloside by the institutional elderly and toddlers have been estimated to be 2.9-22.1 mg/person/day (0.047-0.36 mg/kg bw/day) and 2.8-21.6 mg/person/day (0.19-1.49 mg/kg bw/day), respectively.
92. The above estimates of intake of ptaquiloside from milk represent the maximum intake that might be anticipated to occur as a result of drinking milk solely from cows receiving the maximum dose of ptaquiloside that will not cause clinical signs of toxicity in the animals. Milk from bracken poisoned cows might well contain higher amounts of ptaquiloside and the acute human exposure from occasionally consuming milk from poisoned cows would be expected to be higher. Exposure to ptaquiloside and other bracken toxins might also occur from other sources, including drinking water and consumption of foods derived from bracken-exposed animals. However, no quantitative data are available on the levels of exposure from sources other than milk.

Summary of Exposure Data

93. There appears to be no bracken eaten by humans in the UK, but it is uncertain whether this situation shall remain unchanged. With the continuing interest in international cuisine, it is plausible that in future there may be moves to introduce bracken as an exotic vegetable. It is noted that eating bracken could be hazardous. Bracken has been shown to be mutagenic and carcinogenic and the eating of it has been associated with the development of cancers of the stomach and oesophagus. It contains several genotoxic carcinogens.

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94. There is a potential for exposure to component chemicals of bracken as residues in foods derived from animals that have eaten bracken. There is evidence that some animals readily eat bracken and there have been cases of bracken poisoning in farm animals. As component chemicals in bracken (eg. ptaquiloside) can cause systemic toxicity in farm animals, it is reasonable to assume that they have the potential to leave residues in edible tissues and other foods derived from animals that have eaten bracken. Little is known about the amounts of the component chemicals that can occur as residues in foods, but it is clear that ptaquiloside can pass into the milk of cows.
95. It has been estimated that, in the UK, the sub-population with the highest milk intake might be exposed to up to 2.62 mg/kg bw/day of ptaquiloside as a result of an infant consuming milk from cows that ate bracken without showing clinical signs of poisoning. However, if the advice not to give cows' milk to infants below the age of one year is followed, the highest estimate of intake on a bodyweight basis is 1.49 mg/kg bw/day, for toddlers. Most consumers will be exposed to less than this as they drink less milk. Furthermore, most milk supplies will be bulked so the high concentrations in individual samples will be diluted.
96. No information was available on the amount of ptaquiloside or other components of bracken that can occur in milk derived from animals that have been poisoned by bracken.
97. No information was available on the amount of ptaquiloside or other components of bracken that can occur in meat and offal derived from animals that had eaten bracken.
98. No information was available on the rate at which residues of ptaquiloside or other components of bracken can be cleared from edible tissues and milk obtained from bracken exposed animals.

Conclusions

99. The Committee agreed the following conclusions:
 - I. It is prudent to regard bracken as being carcinogenic to consumers at all levels of ingestion.
 - II. It is unlikely that human consumers of bracken will experience toxic effects other than carcinogenicity.
 - III. Bracken contains some genotoxic substances, including ptaquiloside and kaempferol, that might be at least partly responsible for its carcinogenicity.
 - IV. Bracken is sometimes eaten by food-producing animals.
 - V. Ptaquiloside from bracken ingested by food-producing animals (eg. dairy cows) can be passed into milk that might be consumed by humans.

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- VI. Ptaquiloside from ingested bracken is likely that to be present in meat and offal derived from animals that ate bracken.
- VII. The level of consumer exposure to ptaquiloside and other bracken derived genotoxic substances such as kaempferol should be kept as low as reasonably practicable. Measures that could be considered to achieve this could include:
 - humans not eating bracken.
 - preventing food-producing animals from eating bracken, and
 - not milking or slaughtering bracken-exposed animals for a length of time consistent with the clearance of residues of most known substances (eg. an arbitrary withdrawal time of 28 days is sometimes used for residues of veterinary medicines when no residue depletion data are available).

Recommendations

100. The Committee recommended the following actions to reduce uncertainty about the risk to consumers from bracken:
 - Identify the amount of ptaquiloside and other harmful bracken constituents that can occur in foods derived from animals that have been poisoned by bracken.
 - Identify the amount of ptaquiloside and other harmful bracken constituents that can occur in foods derived from animals that have eaten bracken without showing any signs of toxicity.
 - Identify the rate at which residues of ptaquiloside and other harmful bracken constituents are cleared from edible tissues of food-producing animals.
101. It is recommended that priority should be given to identifying the rate of depletion of ptaquiloside from edible tissues and milk of animals that have been poisoned with bracken. Such information could be used by risk managers to help them decide how long bracken-poisoned animals should be left before slaughter for human consumption or how long should be left before milk taken from poisoned animals can be used for human consumption.

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This is a draft statement for discussion.
It does not reflect the final views of the Committee and should not be cited.

**Secretariat
May 2008**

Draft