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

COT Statement on the risk to consumers of eating foods derived from animals that have eaten Bracken

Background

1. Several cases of bracken poisoning in farm animals have been reported. The Committee was asked to consider the hazards to the health of consumers eating foods derived from bracken-poisoned animals, and whether there were sufficient data to establish how long poisoned animals should be left before they may safely be milked or slaughtered for human consumption. It was noted that bracken is eaten as a vegetable in some parts of the world, but the Committee was not aware of it being eaten in the UK.

2. Bracken (*Pteridium aquilinum*) is an invasive fern that is common throughout the world with several different sub-species (formerly referred to as varieties). The sub-species that are found in the UK are *aquilinum* (the common form), *latiusculum* (found in Scottish pine forests) and *atlanticum* (found mostly in limestone areas of Wales and Scotland)[1]. Bracken is found in all parts of the country and dense growths cover large areas of land in Wales, Scotland and northern England[2].

3. Eating bracken can be harmful to farm animals and there is some evidence that it might also be harmful to humans. The sub-species found in the UK are toxic and potentially fatal to farm animals if eaten. However, there is great variation in the amount of the bracken toxin ptaquiloside, and possibly of other bracken toxins, in the different sub-species of bracken that are found throughout the world[3]. There is also variation between strains within particular sub-species of bracken[4] and at different times of the year[5]. 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 have eaten the plant. Thus there is a potential hazard to consumers.

4. The COT and its sister committees the COM and the COC last advised on the safety of foods derived from animals reared on bracken-infested land in the Annual Reports of the COM in 1993[6] and of the COT in 1996[7]. 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. The COT Annual Report for 1996[7] reported that the COC concluded in 1988 that "*There were…few data available at that time about the extent to which farm animals grazed on bracken and the occurrence of bracken constituents in dairy products.*"

5. It was also stated in the COT Annual Report[7] that "*Human epidemiology data* [were] *limited and the COC concluded that evidence of carcinogenicity was inconclusive. Carcinogenicity studies in laboratory animals in which whole bracken had been administered in the diet, in some cases accounting for up to one third of the total diet, were flawed in their experimental design, execution and interpretation. However, despite these limitations, the COC concluded that these studies had demonstrated a clear trend for increased benign and malignant tumours of the small and/or large intestine and/or urinary bladder and that there was a need for properly conducted carcinogenicity bioassays in rats and mice. The COC also considered the active constituents of bracken and concluded that ptaquiloside, an inherent constituent of bracken, had been shown to be capable* [of] *reproducing some* [of] *the carcinogenic effects of whole bracken."* In addition, the International Agency for Research on Cancer (IARC) classified bracken in Group 2B: possibly carcinogenic to humans[125].

6. It was reported in the COM Annual Report for 1993[6] that:

- "*Solvent extracts of bracken fern showed mutagenic activity in bacterial assays. There was evidence that most of the mutagenic activity appeared to be due to the compound ptaquiloside, but other potentially mutagenic compounds might be present. There were some data suggesting that this activity was expressed in mammalian cells in vitro. There were limited data available which suggest this mutagenic potential might be expressed in vivo."*
- *"Work carried out by MAFF[50, 156]* [the Ministry of Agriculture, Fisheries and Food] *on the possibility of bracken mutagens being transmitted to the milk of bracken-fed goats suggested that very little, if any, mutagenic activity is present in the milk of the goats which were exposed to UK bracken for short time periods of time (1 month)."*
- *In view of the fact that cattle and goats would not eat bracken if other food is available, the Committee did not recommend that any further work should be carried out at present on the risk of transmission of mutagenic compounds from bracken into milk for human consumption, provided that the milk was bulked and processed centrally."*

7. It was reported in the COT Annual Report for 1996[7] that the COT "*accepted the advice of the COC and COM, and agreed that the risk to the population was very low and that further research need not be undertaken on bracken fern mutagens*".

8. Since 1996, there have been several reports of farm animals eating bracken. As a consequence, the expectation that farm animals would not readily eat bracken needs to be reconsidered. 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 VLA between 1999 and 2007[8]. In addition, there was a report[11] 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. Several food-producing species, including pigs and sheep, readily eat bracken and are used to clear bracken from pastures[8]. Furthermore, cattle

have been observed to eat hay containing up to 30% bracken[9]. It has been reported that some horses and sheep eat bracken in preference to their normal pasture[10].

9. In addition, several reports of new studies of the safety of bracken have been published since the last consideration by the COT, the COM and the COC. The areas of interest covered by the new studies include human epidemiology and possible modes of action for the carcinogenicity of ptaquiloside. Both new and old studies are summarised in this Statement. The criteria by which relevant research was identified are set out in the Appendix to the Statement.

Constituents of Bracken

10. Bracken contains a large number of potentially harmful substances, including illudane and protoilludane glycosides (such as ptaquiloside[12, 13], ptaquiloside Z[14], isoptaquiloside[15], pteroside A2[16], pteridanoside[16] and caudatoside[15], terpenic indanones (pterosins)[15, 16], *p*-hydroxystyrene glycosides (ptelatosides A and B)[13], the cyanogenic glycoside prunacin[17], braxin glycosides[18], the flavinoid quercetin and its glycoside rutin[19, 20], kaempferol[19, 20], shikimic acid[21], thiaminases[22], ecdysteroids[22] and tannins[22]. 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*

11. Most of the available studies are 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 of several species of bracken. Feeding of bracken to several strains of mice at 25% or more in the diet for 6 weeks or longer[27, 28, 29, 30] produced neoplasms, including leukaemia, lung adenomas, intestinal tumours, bladder tumours and liver nodules. In rats[19, 31, 32, 33, 34, 149], 1% or more dietary bracken caused tumours including gastrointestinal adenocarcinomas and sarcomas, mainly in the ileum; transitional cell carcinomas of the urinary bladder; and pre-neoplastic nodules in the liver. In female Sprague Dawley rats, 1% or 2% dietary bracken caused mammary adenomas and fibroadenomas, but these were not seen in female F344 rats given the same treatment[149]. In guinea-pigs[35, 36, 37], 30% dietary bracken caused intestinal adenomas and adenocarcinomas and transitional cell carcinomas of the bladder, and it also caused panmyelopathy of the bone marrow and haematuria. In toads[38], dietary bracken caused ileal adenocarcinomas and malignant liver tumours. In 1988, the COC concluded[7] that the carcinogenicity studies of bracken in laboratory animals had demonstrated a clear trend towards increased incidence of benign and malignant tumours of the small and/or large intestine and/or urinary bladder but that there was a need for properly-conducted carcinogenicity studies in rats and mice.

12. Mice given Welsh bracken spores by stomach tube were found to have DNA-adducts in their stomach and small intestines, but not in the liver[39]. In contrast, rats fed Brazilian bracken (a sub-species not found in the UK) did not have DNA-adducts in their stomach or ileum[160]. Cytogenetic analysis of blood taken from cows[40, 41, 42] or people[43, 44] who had eaten bracken showed increased numbers of chromosomal aberrations.

13. Processed bracken and various extracts from bracken have been tested for carcinogenicity[13] or mutagenicity[12]. All parts of the bracken plant were carcinogenic in rats but the tips of young fronds (parts of the plant that are eaten by humans in some parts of the world) were the most potent[45]. 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 rats[28]. However, drying or freezing preserved the carcinogenic/mutagenic potency of bracken and the carcinogenic/mutagenic component(s) was extractable in aqueous media and in several organic solvents[12, 13]. Various extracts of bracken (including boiling water, acetone, methanol and ethanol extracts) were mutagenic to Ames strains of *Salmonella typhimurium*[152, 153, 154, 155]. There is some evidence that bracken and/or its extracts may be mutagenic *in vivo*, as indicated by the results of cytogenetics assays on tissues from intraperitoneally-dosed mice[157], bracken-fed cattle[40, 41, 42] and bracken-consuming humans[43, 44]; a dominant lethal assay in *Drosophila*[46, 47]; an unspecified test in mice[46] and ^{32}P-postlabelling to detect DNA adducts in tissues of exposed mice[39] and rats[160]. Milk from bracken-fed cows[48, 49] was mutagenic to Ames strains of *Salmonella typhimurium,* and milk from cows fed on substantial amounts of bracken was carcinogenic in mice[51] and rats[48]. In 1993, when the COM considered[7] the available mutagenicity studies on bracken extracts, it concluded that there was evidence that mutagenic activity was expressed in mammalian cells *in vitro* and there was limited evidence (from observations of effects in exposed farm animals) that this activity might also be expressed *in vivo*. Positive results in several *in vivo* studies[41, 42, 43, 44, 157, 160] that have been performed since 1993 strengthen the evidence that bracken is an *in vivo* mutagen.

14. 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 rats[52] and rabbits[53], 25% dietary incorporation of dried bracken, containing 4.6 to 20.7 ppm 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-pigs[143] 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 treatment[54]. All of the cats suffered hepatotoxicity.

15. The results of a developmental toxicity study in mice[55] showed that bracken in the diet at a maternally toxic dose caused low fetal weights and skeletal abnormalities (extra cervical or lumbar ribs, incomplete fusion of sternebrae, retarded ossification) in the offspring. No studies have been performed in laboratory animals to investigate reproductive toxicity over several generations.

Clinical and Epidemiological Findings in Farm Animals

16. Acute poisoning of farm animals fed on bracken can be fatal[56, 57]. Prolonged ingestion of sub-lethal amounts of bracken can lead to toxic effects that differ between species. Consumption of bracken is also associated with the development of tumours. The sites where tumours develop vary between species.

17. Sudden death with signs of toxicity similar to cyanide poisoning has been reported in animals fed on young fronds of bracken[58]. This is thought to be related to the presence in bracken of the cyanogenic glycoside, prunacin.

18. In non-ruminant species, the principal adverse effect of dietary bracken is to cause a deficiency of thiamine (vitamin B_1) through the action of thiaminases in the bracken[56]. Bracken has not been seen to cause thiamine deficiency in ruminants, whose gut microflora can synthesise this vitamin, or in humans, perhaps because their level of exposure to bracken is lower and they have a more varied diet.

19. In adult cattle, dietary exposure to bracken can cause a severe panmyeloid depression of bone marrow activity, which is expressed clinically as an acute haemorrhagic syndrome[57]. Calves show a different acute clinical syndrome involving bradycardia, laryngeal oedema and death from heart failure[57]. Prolonged dietary exposure of cattle can result in chronic depression of bone marrow activity, which can cause leucopaenia and thrombocytopaenia, which in turn leads to widespread petechial haemorrhages[59]. It has been proposed[121] that immunosuppression resulting from the effects of bracken on the bone marrow might make animals more prone to infections. Chromosomal instability has been reported in lymphocytes taken from cattle that had been fed bracken[145]. 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 tumours[60]. Syndromes similar to BEH have also been described in buffalo, sheep and deer[61]. Bracken feeding of cattle has also been associated with a slow-developing epidermoid carcinoma of the upper digestive tract and a progressive retinal degeneration[56]. Sheep are more prone to progressive retinal degeneration (called bright blindness or PRD in sheep) than cattle[58].

20. 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 ileum[35].

Clinical and Epidemiological Findings in Humans

21. When COC reviewed bracken in 1988[7], it concluded that human epidemiological data were limited and that evidence of carcinogenicity was inconclusive. Since then, several new observations have been reported[54, 62, 67, 68, 70, 71], but the totality of evidence remains sparse. The human populations that have been investigated include people in Japan and Brazil who had been directly exposed by eating bracken and people in Wales and Costa Rica who may have been indirectly exposed as a result of living in bracken-infested areas (eg. by consuming residues of chemicals from bracken in drinking water or animal-derived foods such as milk).

22. Ecological studies have indicated higher rates of stomach cancer (both sexes) and oesophageal cancer (men) in rural districts of Gwynedd, North Wales where a larger proportion of land area was covered by bracken[70], of stomach and oesophageal cancer in a bracken-infested as compared with a bracken-free region of Costa Rica[54], and of stomach cancer in highland areas of Venezuela where pastures were infested by bracken[68]. A large cohort study found elevated risk of oesophageal cancer in relation to combined intake of bracken fern and hot tea gruel among inhabitants of mountainous districts of Japan[66], and a case-control investigation, also in Japan, showed a similar association[65], although it is unclear from the brief descriptions available whether this was an independent investigation or part of the same study. A case-control study of stomach cancer in Gwynedd found a statistically significant association ($p < 0.001$) with bracken in the vicinity of the childhood home with an estimated relative risk (RR) of 2.34 (confidence intervals not reported)[71]. A small case-control study of upper gastrointestinal (stomach and oesophageal) cancer in Brazil gave an odds ratio of 3.63 (95%CI 1.24-10.63) for bracken consumption, but the choice of controls was not ideal, and the statistical analysis failed to account for the matching in the study design[67]. More recently, a large, prospective cohort study in Japan found a significantly increased risk of oesophageal cancer in men who reported frequent consumption of wild edible plants (mainly bracken) in a questionnaire at baseline (RR 2.98, 95%CI 1.46-6.07)[62]. However, the corresponding risk estimate in women was unremarkable (RR 1.39, 95%CI 0.56-3.47) [62].

23. Few conclusions can be drawn from these epidemiological findings. Many of the studies have been inadequately reported or were methodologically unsatisfactory. The three ecological investigations were limited by their inability to adjust for potential confounding factors, and the case-control study in Gwynedd[71] did not take into account the possibility of confounding by *Helicobacter pylori* infection, which has been linked to domestic crowding in childhood[150]. In the past, domestic crowding was common in rural North Wales.[151] In the strongest and best reported investigation[62], the association of oesophageal cancer with bracken consumption was found only in men and not in women.

Toxicity of the Main Constituents of Bracken

Ptaquiloside and activated ptaquiloside (APT)

24. In 1983, two separate groups of workers in Japan[13] and the Netherlands[12] independently identified ptaquiloside (a norsesquiterpene glycoside of the illudane type) as the principal substance responsible for the carcinogenicity and mutagenicity of bracken. They had used extraction and separation techniques to obtain various fractions, which they had then tested with mutagenicity assays[12] or short-term carcinogenicity assays in rats[13] to find those with the highest mutagenic/carcinogenic potency. The chemical structure of the principal compound (ptaquiloside) in the most potent fraction was then determined.

25. Ptaquiloside is a water-soluble substance that has been detected in all parts of the bracken plant[18]. It is stable in dried bracken[18]. 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 B[18], which is not carcinogenic. Composting bracken destroys the ptaquiloside[5].

26. Some of the observed clinical effects of dietary bracken in farm animals have been reproduced in experiments in which animals were dosed with ptaquiloside. Progressive retinal degeneration (PRD or bright blindness) has been reproduced in sheep given ptaquiloside intravenously[73]. Haemorrhagic cystitis and haematuria have been produced in guinea-pigs (but not rats or mice) given subcutaneous ptaquiloside[74].

27. It has been suggested that ptaquiloside is responsible for more than half of the mutagenic potency of bracken[12], and the COM agreed[7] that most of the mutagenic activity of bracken appeared to be due to ptaquiloside. No carcinogenicity bioassay of ptaquiloside 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, after which the rats received no further treatment for the rest of their lives[75]. 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). "Conspicuous preneoplastic hyperplasia of the mucous membrane of the urinary bladder" was seen in all of the treated rats that survived 40 days or more. In another study, rats given a diet containing 0.027 to 0.080% ptaquiloside in their diet (equivalent to 27 - 80 mg/kg bw/day) developed cancers of the ileum and/or bladder within 15 to 60 days[76]. 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 necrosis[77]. The COC noted[7] that ptaquiloside had been shown to be capable of reproducing some of the carcinogenic effects of bracken.

28. Oral dosing of rats with APT at 10 weekly doses of 20.7 mg/kg bw (equivalent to an average dose of 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 detectable when the animals were killed 30 weeks later[78]. Nor did administration of APT as 10 weekly intravenous injections of 20.7 mg/kg bw. However, other adverse effects were seen in all groups: tubular necrosis of the kidneys, monocytosis and elevated plasma tumour necrosis factor TNFα. In addition, the orally-treated rats showed necrosis of blood cell precursors in the bone marrow and had apoptotic bodies in their livers.

29. 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 pHs[82]. Ptaquiloside also produced DNA-adducts *in vitro*[83] and caused *in vitro* unscheduled DNA synthesis in a rat hepatocyte culture at pH 7.2[84]. Ptaquiloside and APT were shown to be alkylating agents, with APT being the more potent[60]. No *in vivo* mutagenicity assays of ptaquiloside were found.

30. It seems likely that ptaquiloside is responsible for at least some of the carcinogenicity and toxicity of bracken. The carcinogenicity appears to involve a genotoxic mode of action.

Illudane Substances Other than Ptaquiloside & APT

31. 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 ptaquiloside raises the concern that some of them might be similarly carcinogenic by a genotoxic mode of action.

Terpenic Indanones

32. A large number of terpenic indanones have been isolated from bracken, including pterosins A, A2, B, C, D, E, F, G, J, K , l, N, O V and Z and pterosides A, B, C and M[16, 56]. Indanones are found in high concentrations (up to approximately 24 ppm) in young fronds[22], but they do not act as alkylating agents[22]. Pterosin B is much less electrophilic than ptaquiloside[84]. 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, 86]. 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 activation[87] (not tested in the presence of metabolic activation). No studies of the mutagenicity or carcinogenicity of pteroside A2 were available. Extracts of fronds and of rhizomes of Welsh bracken, containing high levels[a] of pterosins, pterosides and other non-specified indanones, did not cause leucopaenia or thrombocytopaenia in calves[88].

33. There is insufficient evidence to establish whether or not terpenic indanones are likely to be responsible for any of the toxicity of bracken.

p-Hydrostyrene Glycosides

34. The *p*-hydrostyrene glycoside, ptelatoside A (ρ-β-primerverosyloxystyrene) was tested in rats at a concentration of 1.3 ppm in the diet (equivalent to 0.065 mg/kg bw/day) for 109 or 125 days[13]. At this dose, there was no evidence of any carcinogenicity when the animals were killed at 520 days after the start of the experiment. There was insufficient ptelatoside A available to test higher concentrations.

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[a] Pterosins A, B, C and others were isolated from the fronds with yields of 40, 190, 40 and 20 ppm, respectively, and pterosins A, B and others and pterosides A, B, C and others were isolated from rhizomes with yields of 20, 20, 20, 320, 570, 370 and 90 ppm, respectively, and the concentrated extracts that were tested contained the equivalent of 3.6 and 1.8 kg of the dried frond and rhizome, respectively, in each litre. Each morning for 30 days, a calf was fed an amount of extract of frond or rhizome that was equivalent to 1 kg of dried bracken material. Thus, one calf received daily doses of frond extract supplying pterosins A, B, C and others at respective concentrations of 11, 52, 11 and 5.6 ppm; whereas another calf received daily doses of rhizome extract supplying pterosins A, B and others and pterosides A, B, C and others at respective concentrations of 11, 11, 11, 178, 317, 206 and 50 ppm.

35. Another *p*-hydrostyrene glycoside, ptelatoside B (ρ-β-neohesperidosyl oxystyrene), has also been isolated from bracken, but it has not been toxicologically tested. There is no evidence to indicate whether or not any of the toxicity of bracken is due to the presence of *p*-hydrostyrene glycosides.

Prunacin

36. Prunacin is a cyanogenic glycoside that is present in some sub-species 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 sub-species. 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 bracken[22, 56].

37. Up to 61 mg/g of prunacin has been detected in fresh plant material from a Venezuelan tropical sub-species of bracken (*Pteridium aquilinum* var. *arachnoideum*), with the highest concentrations being found in young fronds[17]. It was noted in Venezuela that the *arachnoideum* sub-species of bracken contained more prunacin than the *caudatum* sub-species[22]. No quantitative information is available on the amount of prunacin in British sub-species of bracken, but it has been reported that the highest concentrations occur in early to mid spring[22]. One gram of prunacin has the potential to release up to 96 µg of HCN[17]. Thus up to 5.9 µg of HCN might be released from 1 gram of the fresh Venezuelan bracken.

38. In its 2006 Statement on Cyanogenic Glycosides in Bitter Apricot Kernals[89], the COT concluded that the limited chronic toxicity data available were not sufficient to propose a tolerable daily intake (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 proposed 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 125 g per day of this bracken to exceed the TDI that was set by WHO. It is conceivable that an extreme consumer of bracken might experience acute cyanide toxicity as a result of eating a large portion of a sub-species of bracken that is high in prunacin. However, consumption of bracken by humans is not known to occur in the UK. No data are available on residues of prunacin or cyanide in foods derived from animals that have eaten bracken, so it is unclear whether 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. As the level of exposure of humans to prunacin and cyanide from foods derived from animals that have consumed bracken is likely to be less than that of the directly-exposed farm animals and as humans are not markedly more susceptible to cyanide than other species[158], it is considered likely that animals would show clear signs of toxicity if they had consumed sufficient bracken to leave toxic levels of cyanide or prunacin in foods derived from them. Furthermore, the Scientific Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) has advised[159] that the carry-over of cyanide and cyanogenic

residues into milk, meat and eggs derived from animals intoxicated with cyanogenic glycosides is likely to be “very low” in all food-producing species.

Braxin Glycosides

39. Braxins A1, A2 and B have been detected in rhizomes of bracken[90]. Braxins A1 and A2 were present in rhizomes at a combined concentration of up to 600 ppm, but were not detected in fronds[90]. Braxins A1 and A2 are aromatic β-glucopyranosides[90], but their precise chemical structure has not been established. The chemical structure of braxin B is not known. (“Braxin C” is ptaquiloside.)

40. 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,* with swelling of the cells[90]. The *in vitro* histamine-releasing activity of glycosides extracted from rhizomes (which include braxins A1 and A2) was about ten times greater than that of glycosides from the fronds (braxins A1 and A2 not present) [90].

41. It is possible that braxin glycosides play a part in the aetiology of the haemorrhagic cystitis that is seen in cattle and some other species.

Quercetin

42. Quercetin was found in bracken at concentrations of up to 860 ppm (dry weight)[20], but is also found in many fruits and vegetables, often at higher concentrations (eg. up to 65,000 ppm in onions)[24]. It has been estimated[146] from national dietary records in Australia, the Netherlands, Finland, Italy, Croatia, Japan, and the USA that the habitual diets of consumers gave them mean intakes of quercetin from <5 to approximately 40 mg/person/day, although intakes as high as 200-500 mg/person/day could be achieved by high consumers of fruit and vegetables.

43. Orally administered quercetin was not very well absorbed[92]. It is either converted to phenolic acids by the gut flora or voided unchanged in the faeces.

44. Quercetin was of low cytotoxicity when tested *in vitro* using CHO cells, 3T3 mouse fibroblasts and normal rat kidney (NRK) cells[93].

45. In calves, oral doses of up to 20 g/calf/day for several months had no effect on incidences of BEH or papillomavirus-induced cancer of the urinary bladder[94]. It has been claimed that exposure to quercetin is associated with bovine cancers of the upper alimentary tract[22], but no evidence has been found to support this claim.

46. 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 ppm quercetin in their diet (equal to approximately 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 rats[96], 40000 ppm 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 concentrations of 100 or 1000 ppm. It is noted that renal tumours were not produced when rats were fed bracken. In a third study in F344 rats[149], females were given dietary levels of 10000 or 20000 ppm of quercetin (500 and 1000 mg/kg bw/day) throughout their lives (approximately 750 days) and at both dose levels there were increased incidences of liver pre-neoplastic foci, hepatomas and bile duct tumours. In a study in Norwegian albino rats, administration of 10000 ppm quercetin in the diet (equivalent to 400 mg/kg bw/day) for 406 days caused transitional cell carcinomas of the bladder[19]. 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 ppm (4000 mg/kg bw/day) for 850 days[97]; to F344 rats at 10000 ppm (400 mg/kg bw/day) for 540 days[98]; to F344 rats at up to 50000 ppm (2000 mg/kg bw/day) for 728 days[99]; to F344 rats at up to 2000 ppm (76 mg/kg bw/day for males and 58 mg/kg bw/day for females) for 448 days[147]; to ddY mice at 20000 ppm (equivalent to 3000 mg/kg bw/day) for a lifetime (about 842 days)[100]; to strain A mice at 50000 ppm (7500 mg/kg bw/day) for 161 days[148]; or to golden hamsters at up to 100 000 ppm (equivalent to 12000 mg/kg bw/day) for up to 735 days followed by treatment with 1% croton oil for a further 350 days[101].

47. 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 oncogenes[102]. Oral doses of quercetin given to rats caused 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 cancers[92].

48. There is some evidence that quercetin is genotoxic. It can bind to DNA and cause single-strand breaks[103, 146]. It gave positive results in several mutagenicity assays, including the *Salmonella*/microsome reverse mutation assay[95, 104, 105, 106], tests of SOS repair and reverse mutations in *Escherichia coli*[146], gene mutation (*tk* locus) assays in mammalian cells[24, 107, 108], cytogenetics tests in mammalian cells[24, 87, 95, 107, 109], and a sex-linked recessive lethal mutation test in *Drosophila*. On the other hand, quercetin gave negative results in a forward mutation assay in *Bacillus subtilis* and in mammalian cell gene mutation assays that used loci other than *tk* (*hprt, aprt*, ATPase)[146]. Quercetin gave inconsistent results in the mouse bone marrow micronucleus test: with one experiment finding it mutagenic[110] whilst others did not[111, 112]. Quercetin did not cause unscheduled DNA synthesis in gastric mucosal cells of rats that had been given oral doses[111]. Thus, although quercetin is mutagenic in several *in vitro* tests, the balance of evidence suggests that it does not express its mutagenicity in mammalian systems *in vivo*.

49. In a recent review[146] that covered the carcinogenicity and genotoxicity data on quercetin, it was concluded that quercetin at dietary levels of up to 50 mg/person/day "would not produce adverse health effects". Given that many commonly eaten fruits and vegetables contain higher concentrations of quercetin than are found in bracken, it is considered unlikely that quercetin is responsible for the adverse effects caused by eating bracken.

Kaempferol

50. Kaempferol is chemically similar to quercetin, from which it differs by lacking one hydroxyl group. It was found in bracken at a concentration of 1100 ppm (dry weight)[19]. It is also commonly found in other plants[24]. Tea can contain up to 10,000 ppm of quercetin plus kaempferol, combined[24].

51. A limited study in ACI rats (400 ppm feed given to 6 males and 6 females for 540 days) showed no evidence to suggest that kaempferol was carcinogenic[98]. 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 intraperitoneally[110]. It 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 (mutation at the *tk* locus of CHO cells[107] and development of resistance to 8-azoguanine in V79 cells[108]) and a cytogenetics assay in mammalian cells[107]. Negative results were obtained for induction of gene mutations at the *aprt, hgpt* and ATPase loci of CHO cells[107].

52. It is possible that the presence of kaempferol contributes to the overall carcinogenicity of bracken. However, given that many commonly eaten fruits and vegetables contain higher concentrations of kaempferol, it is considered unlikely that kaempferol is responsible for other features of the toxicity of bracken.

Shikimic Acid

53. Shikimic acid was found at 1440 ppm (dry weight) in Welsh bracken[21]. It is also present in several edible plants, including soybeans, star anise and green tea. Shikimic acid was destroyed in alkaline conditions[115].

54. 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 millimolar (139, 122 and 174 ppm)[93].

55. Intraperitoneal injection of 10 mg/mouse of shikimic acid caused death in mice within a few hours of dosing, with haemorrhaging and “denudation” of the intestinal mucosa[115].

56. Developmental toxicity was tested in pregnant CD-1 mice given 17 daily oral gavage doses of 250 or 1000 mg/kg bw shikimic acid[116]. There was a reduced number of implantations at both dose levels, as compared with untreated controls. The results showed no evidence of fetotoxicity or teratogenicity.

57. TF1 mice given single intraperitoneal doses of 1 to 30 mg of shikimic acid (equivalent to 50 to 1500 mg/kg bw) had increased incidences of cancer of the glandular stomach and of leukaemia when observed for up to 70 weeks[117]. The only mouse tested with a single oral gavage dose of 100 mg (5000 mg/kg bw/day) died after 34 weeks, having developed stomach cancer and leukaemia[117]. 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 ppm (equivalent to 150 mg/kg bw/day) for 142 days had no effect on tumour incidences, after an observation period of a further 70 days [21].

58. Shikimic acid was not genotoxic *in vitro* in bacterial[118] or *in vivo* mammalian assays (mouse bone marrow assay and unscheduled DNA synthesis in rat gastric mucosa)[111]. However, the results of a dominant lethal assay[117] indicated that shikimic acid could cause mutations *in vivo*. Male TF1 mice were each treated either with a single intraperitoneal injection of 25 mg of shikimic acid or an oral dose of 80 mg of shikimic acid by stomach tube prior to mating with untreated virgin females. The proportions of embryos having dominant lethal mutations in the control, intraperitoneally-treated and orally-treated groups were 4.4, 22.1 and 13.6%, respectively.

59. It cannot be excluded that shikimic acid might make some contribution towards the overall carcinogenicity of bracken. There was limited evidence to suggest that it might be mutagenic and carcinogenic. Although a single intraperitoneal dose of 0.15 mg/kg bw/day or more produced tumours in mice, a dietary dose of 50 mg/kg bw/day did not appear to be carcinogenic in rats. Nevertheless, it is unlikely that shikimic acid makes a major contribution to the overall carcinogenicity of bracken as it was destroyed by alkaline conditions whereas the mutagenicity and carcinogenicity of bracken appears to be increased under such conditions.

Thiaminases

60. Anti-thiamine enzymes, thiaminases, seem to cause most of the short- to medium-term symptoms of bracken poisoning in monogastric animals. Thiaminase 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, respectively[119]. A third, more heat-stable, thiaminase (possibly caffeic acid, a substance that also has both pro- and anti-cancer properties) might also play a role[10].

61. Rats fed on bracken that had thiaminase activity developed lesions of the nervous system that were considered by the authors of the study[13] to be typical of antivitaminosis-B_1. The lesions could be cured by thiamine (vitamin B_1). Similar effects have been reported in monogastric farm animals, including horses and pigs[22].

62. In order to test whether the anti-thiamine activity of bracken contributed to its carcinogenicity, 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 subcutaneous injection of 2 mg/rat/week of thiamine[120]. No tumours were found in controls. All survivors in the two treatment 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 as thiamine supplements did not reduce the incidence of tumours (if anything, the incidence increased), it seemed unlikely that thiaminase caused the carcinogenicity of bracken.

63. 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 because they have a more varied diet. It is also likely that humans who eat bracken would eat much less bracken (per kg body weight) than animals fed on bracken.

Ecdysteroids

64. Bracken contains several ecdysteroids which can prevent insects from moulting and developing into adults[22]. This mode of action is not relevant to mammals.

65. 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.

66. There is no reliable evidence to suggest that ecdysteroids are a toxic hazard to humans.

Tannins

67. The tannins in bracken are mainly condensed tannins derived from procyanidin and prodelphinidin. Fronds of tropical bracken can contain more than 120,000 ppm of condensed tannins[22]. No information was available on the amount in sub-species 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 570 to 2.470 ppm[122], red kidney beans (*Phaseolus vulgaris*) contain 6300 to 9100 ppm raw and 3100 to 5500 ppm cooked[123], raw soyabeans[123] contain 500 ppm and "corn"[b] contains 100 ppm[123].

68. There is limited evidence suggesting that parenteral administration of some tannins might be carcinogenic. Subcutaneous injections caused liver tumours[124] and fibrous histiocytomas at the injection site[126] in rats, and caused local sarcomas and liver tumours in mice[124]. Although liver nodules were noted in one of the mouse carcinogenicity studies of bracken[33], the liver was not the major site for neoplasia in mice. More usual were leukaemias, lung tumours and gastrointestinal cancers[28, 29, 30, 33]. The feeding of bracken tannins at a dietary concentration of 4000 ppm (equivalent to 100 mg/kg bw/day) for up to 72 weeks did not cause any cancer in F344 or Sprague-Dawley rats[126]. 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 activation[126].

69. Although bracken can contain higher concentrations of tannins than commonly consumed foods, there is no evidence that ingestion of these tannins is harmful.

[b] It was not clear from the report whether "corn" referred to maize, wheat or some other cereal.

Summary of Toxicity

70. 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 to occur in humans. The more varied human diet and the lower dietary consumption of bracken are factors that make it less likely that bracken will cause thiamine deficiency in human consumers.

71. 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 ptaquiloside in bracken is at least partly responsible for causing these effects. Braxins might also play a role. These non-neoplastic effects of bracken have not been reported in humans, probably because levels of dietary exposure are lower than in animals.

72. 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 in bracken is almost certainly 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 have eaten bracken.

73. Bracken also seems to cause cancer in a wide variety of species. The site and type of cancer can differ between species. Evidence from epidemiological studies for a carcinogenic effect of bracken in humans is inconclusive. It is likely that the presence in bracken of the genotoxic carcinogen, ptaquiloside, contributes towards its carcinogenicity. Other components of bracken, such as kaempferol and shikimic acid, might make a more minor contribution towards the overall genotoxicity and carcinogenicity of bracken.

Modes of Action of Relevance to Humans

74. Carcinogenicity is the toxic effect of bracken that is of most relevance to humans exposed by eating bracken 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.

75. 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 can cause cancers by a genotoxic mode of action, although it is possible that non-genotoxic modes of action could also be involved.

Contributions of Constituent Chemicals to the Carcinogenicity of Bracken

76. 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.

77. 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 ppm were detected in sub-species that are found in the UK, but higher amounts have been detected in tropical sub-species.

78. Quercetin appears not to be genotoxic *in vivo,* but has been shown to cause tumours in experimental animals at sites 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.

79. The available mutagenicity test results indicate that kaempferol is genotoxic *in vivo*, but it has not been tested for carcinogenicity. It is possible that kaempferol contributes to the carcinogenicity of bracken.

80. Shikimic acid was not genotoxic in a limited range of *in vitro* tests, but *in vivo* it caused dominant lethal mutations in mice. 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 shikimic acid in bracken makes a major contribution to the overall carcinogenicity of bracken.

81. The evidence for bracken tannins being a 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 is no evidence that they are genotoxic.

Mode of Action of Ptaquiloside

82. 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 8.5[80]. It was also discovered that ptaquiloside was less potent at causing chromosomal aberrations when tested at pH 5.3[82]. The higher genotoxic potency of ptaquiloside at higher pH is probably because, under mildly alkaline conditions, ptaquiloside is converted by β-elimination into the illudane-dienone compound, APT (activated ptaquiloside)[82]. APT possesses a highly reactive cyclopropyl ring. It is electrophilic with a greater capacity to alkylate DNA than ptaquiloside[60]. 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].

83. Compounds that are chemically similar to APT but lack an activated cyclopropane moiety (hypoloside B, hypoloside C, illudin M and illudin S) were not mutagenic to *Salmonella typhimurium* TA98 or TA100 strains[82]. It is not known whether the other illudanes that have been found in bracken form an activated cyclopropane moiety in a similar way to that in which ptaquiloside forms APT.

84. APT binds covalently to purine bases on DNA[130], opening the cyclopropyl ring of the molecule and forming adducts with the N-7 of guanine and the N-3 of 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 molecule[131]. The abasic sites were unstable and breakage of the phosphodiesterpentose backbone of the DNA molecule occurred. Investigations were made[132] of the H-*ras* and *p53* genes in cells 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* gene[78]. Mutations of the H-*ras* proto-oncogene also occurred in cells from the ileums of cattle fed bracken in their diet[132, 134, 135].

85. It has been shown that infections of cattle by papillomaviruses increase 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 proposed[121] 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

86. It is 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 as a vegetable. Analysis of fronds and rhizomes of the bracken sub-species that is most common in the UK (*Pteridium aquilinum* var. *aquilinum*) found them to contain ptaquiloside concentrations of 213 to 2145 ppm and 11 to 902 ppm, respectively.

87. The Committee is not aware of any UK commercial sources of bracken for human consumption. In August 2007, the FSA received 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. As bracken does not appear to have been marketed as a food in the EU, it is likely that it would require a pre-market safety assessment in accordance with the Novel Foods Regulation (EC) 258/97 before it could be sold for human consumption. The Food Standards Agency obtains expert advice on the risk assessment of all novel foods from the Advisory Committee on Novel Foods and Processes (ACNFP).

88. The most likely food-related route by which UK consumers could 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 bracken[8], 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 and to reduce invasion of pastures. Exposure might occur at a lower level as a result of animals eating bracken that is growing as a weed in their enclosures. There might also be some exposure to bracken as a result of the traditional use of bracken as bedding for animals.

89. 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 in meat and offal of residues of any toxic substances derived from bracken or on their rates of depletion from edible tissues.

90. Substances from bracken can be excreted into the milk. The milk from bracken-fed cows caused leucopaenia in calves, produced bladder cancer in mice, and produced cancers of the intestines, bladder and kidneys in rats. Various solvent extracts from 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.

91. Ptaquiloside has been detected in milk from bracken-fed cows[140]. 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 250 ± 50 ppm 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 study[143], two cows transferred into their milk 8.6 ± 1.2% of the ptaquiloside they ingested from 6 kg/cow/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. The limit of quantitation of the analytical method was approximately 0.5 ppm of pterosin, which is equivalent to about 1 mg/L of ptaquiloside. It may be concluded that concentrations of ptaquiloside fell off rapidly following withdrawal of oral exposure and minimal amounts would be present in milk at 4 days after ingestion of bracken ceased.

92. Data from the most recent UK food surveys[c] 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[143] described in the preceding paragraph that a UK infant having the upper 97.5th percentile chronic intake of milk (851 g/person/day for milk excluding infant formulae and breast milk) from a cow that had been fed a sub-clinical dose of 5000 mg/cow/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 an estimate of the maximum consumer intake of ptaquiloside from milk from bracken-exposed cows that show no clinical signs.

93. The estimated intake by 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).

94. 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 information[142] on possible amounts of ptaquiloside in cows' milk as used to estimate the intake of infants, the intakes of ptaquiloside by high (97.5th percentile) consumers of milk have been estimated to be between 2.9 and 22.1 mg/person/day (0.047 to 0.36 mg/kg bw/day) for the institutional elderly and between 2.8 and 21.6 mg/person/day (0.19 to 1.49 mg/kg bw/day) for toddlers.

95. 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 sub-clinical dose of ptaquiloside. It is possible that milk or edible tissues from bracken-poisoned cows could contain higher amounts of ptaquiloside. No quantitative data are available on the levels of exposure from sources other than milk.

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[c] 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).

Summary of Exposure Data

96. Bracken is not eaten by humans in the UK, but it is uncertain whether this situation will remain unchanged. With the continuing interest in international cuisine, it is conceivable that in future there may be moves to introduce bracken as an exotic vegetable. If this were to be done, bracken would be regarded as a novel food and it is likely that it would need to be assessed for safety before authorisation could be given for its sale in the UK as a food.

97. 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.

98. It has been estimated that, in the UK, the sub-population with the highest milk intake, infants, might be exposed to up to 2.62 mg/kg bw/day of ptaquiloside as a result of consuming milk from cows that had eaten 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 any high concentrations in individual samples will be diluted.

99. 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.

100. 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 have eaten bracken.

101. No information was available on the rate at which residues of ptaquiloside or other components of bracken can be cleared from edible tissues. However, information on the rate of decrease of ptaquiloside residues in milk from bracken-exposed cows indicated that minimal amounts of residues would be present in the milk at 4 days or more after exposure ended.

Conclusions

102. The Committee agreed the following conclusions:

I. Bracken is sometimes eaten by food-producing animals.

II. Although no modern carcinogenicity bioassays have been performed on bracken, observations from farm animals, laboratory animals and

mutagenicity studies suggested that it is carcinogenic and genotoxic. Few conclusions can be drawn from the small number of epidemiological studies of humans. It is prudent to regard bracken and at least one of its constituents (ptaquiloside) as being potentially carcinogenic to humans at all levels of ingestion.

III. Bracken contains some genotoxic or possibly genotoxic substances, including ptaquiloside, kaempferol and shikimic acid.

IV. Ptaquiloside from bracken ingested by food-producing animals (eg. dairy cows) can be passed into milk that might be consumed by humans.

V. Ptaquiloside from ingested bracken is likely to be present in meat and offal derived from animals that have recently eaten bracken.

VI. 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 discarding milk or not slaughtering bracken-exposed animals for a length of time consistent with the clearance of residues of toxic substances.

VII. The available data suggest a withdrawal period of at least 4 days for ptaquiloside in milk. Current evidence does not provide a basis for specifying an adequate withdrawal period prior to slaughter for human consumption of meat and offal.

Recommendations

104. 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 meat and offal derived from animals that have been poisoned by bracken.
- Identify the amount of ptaquiloside and other harmful bracken constituents that can occur in meat and offal 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 (eg. those that are actively used to clear bracken).

105. It is recommended that priority should be given to identifying the rate of depletion of ptaquiloside from edible tissues of animals that had high exposure to bracken. Such information could be used by risk managers to help them decide with more confidence how long bracken-poisoned animals should be left before slaughter for human consumption.

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Appendix to COT statement on the Risk to Consumers of Eating Foods Derived from Animals that had Eaten Bracken

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

LITERATURE SEARCH

Articles were identified and selected for review as follows.

Computerised literature search

Several computerised searches of the scientific literature published over the last 10-38 years were conducted by the Information Centre of the Food Standard Agency. The computerised searches performed were:

- Search on DART Developmental Toxicology Literature, NewsQuest, Food Science and Technology Abstracts 1969-2007, Foodlineweb 1972-2007, and National Library for Health (incorporating Biomed Central, Dialog Datastar, MyLibrary, NHL Evidence, NHL Guidance, NHL Specialist Libraries, Proquest and PubMed) for
  "bracken".

- Searches on Campden and Chorleywood Food Research Association (CCFRA) Site, Barbour Index, British Library Inside, Current Contents 1998-2007, FoodlineWeb (Leatherhead Food International Ltd.), Nature – journal, and New Scientist - journal for:
  (bracken OR bracken fern OR Pteridium aquilinum OR Pteridium)
  AND
  (ptaquiloside OR bracken toxin OR sesquiterpene glycoside)
  AND
  (toxicology OR OR toxicological OR cancer OR carcinogenic OR mutagenic OR genotoxic OR epidemiology)

- Searches on TOXLINE and FoodlineWeb (Leatherhead Food International Ltd.), Current Contents 1998-2007 and Food Science and Technology Abstracts (FSTA) for:
  pterosin OR pterosin-B OR isoptaquiloside OR iso-ptaquiloside OR caudatoside OR thiaminase
  AND
  pharmacokinetics OR toxicology OR toxicological OR cancer OR carcinogenic OR mutagenic OR genotoxic OR epidemiology

- Searches on TOXLINE 1900-2007, British Library Inside, Ingenta.com, Food Science and Technology Abstracts (FSTA), TOXLINE, Foodlineweb 1972-2007, and Current Contents 1998-2007 for:
  quercetin OR prunacin
  AND
  bracken

The computerised literature searches identified a large number of articles, many appearing on several of the databases. Printouts of details of articles including abstracts were read and articles that looked like they would be relevant to the consumer safety of bracken were ordered. The main criterion for ordering was that the article should deal with some aspect of the exposure to or toxicology of bracken and/or its component chemicals.

Secondary search of the literature

Review articles on bracken were read and from these further key articles were identified. This was particularly helpful for finding older articles.

The relevant articles were read and summarised for this COT paper. During this process, several further important articles that had been missed earlier were identified and ordered.

Selection of articles for review in TOX/2008/12

A large number of articles on the safety of bracken and on related issues were obtained by the Secretariat. As a result of time constraints, not all of the articles were read in detail. Some selection had to be made. It was quickly apparent that certain articles were too general or only dealt peripherally with the issues of interest. Such articles were left aside.

Sometimes several articles referred to the same piece of work. Wherever possible the primary source of information was the source that was cited. In some instances, information was pieced together from several sources and it was necessary to cite several articles for a single piece of information.

Criteria for selection of articles cited in TOX/2008/12

Articles were selected for inclusion in the review on the basis that they dealt with some aspect of the safety of bracken and its constituent chemicals to human consumers. This could be a direct reference to human safety by dealing with exposure, toxicological or epidemiological aspects or less direct by looking at effects in exposed animals or of exposure by routes other than the diet. Related issues of interest included the taxonomy and worldwide distribution of different sub-species of bracken ferns.

Some articles were excluded as they covered areas of work already dealt with in other articles. This was particularly the case concerning the large number of studies from the 1980s that reported the testing various fractions of bracken in standard short-term assays for mutagenicity or carcinogenicity in order to isolate the carcinogenic component of bracken.

There were numerous review articles available that added no new information. Most of these were not cited.

Articles Cited in the Statement

Not all of the articles cited in the review paper TOX/2008/12 were cited in the Statement on the Risk to Consumers of Eating Foods Derived from Animals that had Eaten Bracken. Some aspects covered in the review paper were not considered to be directly relevant to the Statement and thus articles cited in these parts of the review paper were not cited in the Statement. Also, when several articles dealt with the same issue, some of the articles that contributed no unique information were omitted from the Statement.

Articles Added to the Statement

After the COT had discussed the first draft of the statement, Members identified a few additional articles that had not been identified in the Secretariat's search of the scientific literature. These articles and some of the articles giving primary data that they had cited were added to later drafts of the Statement.