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Review

Bracken-associated human and animal health hazards: Chemical, biological and pathological evidence

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ABSTRACT

Bracken (*Pteridium aquilinum*) is a widely distributed carcinogenic fern, to whose toxins human populations are exposed through multiple routes. Animals are also affected by bracken toxins, leading to serious production losses yearly. Accordingly, several governmental reports regarding the safeguard of public health against bracken carcinogens have been recently issued. This review describes the main bioactive compounds identified in bracken and their biological effects at the molecular, cellular, pathological and populational levels, with particular emphasis on ptaquiloside, the main bracken carcinogen. Recent biopathological studies shedding further light on the genotoxicity immunotoxicity and carcinogenicity of ptaquiloside are discussed. Key steps on the long effort to understand bracken toxicology are also reviewed, along with the latest findings on new bracken toxins and human exposures routes. The presence of ptaquiloside and related terpene glycosides in milk, meat and water are of particular concern from the viewpoints of both human and animal health.

Contents

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1. Bracken: taxonomy and geographical distribution

An increasing number of plants are recognised to be toxic to man, to animals, or both [1]. Not many plants, however, are as well-known as bracken (*Pteridium aquilinum* (L.) Kuhn), which is one of the few which can naturally cause cancer in animals. [2]. Bracken is a fern belonging to the Dennstaedtiaceae family and consists of two subspecies: *P. aquilinum* ssp. *aquilinum* and ssp. *caudatum* [3–5]. Eight varieties can be considered within ssp. *aquilinum*, which includes European bracken, and 4 within ssp. *caudatum*, which is mainly found in South and Central America, South-East Asia and Oceania, each defined by morphological and/or chemical criteria (Fig. 1). However, although the *Pteridium* genus forms a readily delimited taxonomic entity, infrageneric bracken taxonomy is still in dispute, with some authors proposing that varieties such as *revolutum* or *esculentum* be considered as independent species [6–12]. These disputes, based on chromosomal ploidy and on DNA and isoenzyme homology are largely due to the fern's phenotypic plasticity in response to environmental factors and to the occurrence, in the field, of numerous morphological intermediates between definable morphotypes [7]. Further research is needed before the taxonomy of this actively evolving fern can be firmly established. Considering this taxonomical controversy, the long-used classification system [3,4] was adopted as the basis for this review, for the sake of clarity. Alternative names for bracken varieties are occasionally referred in connection with works by authors who adopted other classifications. Bracken is one of the five most abundant plants on Earth, being present in all continents except Antarctica. The only limitations to its distribution are extreme temperatures and lack of humidity [13]. Bracken behaves in an opportunistic way, taking advantage of its extensive rhizomes [14], often invading abandoned, newly cut or burned areas, and can become quite a difficult weed to manage [3,15–17]. Bracken is actually occupying or invading 1.7 million hectares in the United Kingdom, which accounts for 7% of the land surface in that country [18], and the average annual increase in bracken-covered land has been estimated at 1%. Losses due to bracken invasion of heather moorland, used for grouse shooting, were pointed to amount to £15.5 million every year [18]. Bracken also causes other important economic losses due to its toxicity towards farm animals, as described further onwards.

2. Bracken bioactive compounds

Bracken contains a large number of chemically heterogeneous compounds with varied and, often, poorly understood biological activity. While some compounds are recognised as major determinants of bracken toxicity or as defences against insect or herbivore attack [19], the impact of many others is unknown or merely guessed. The whole plant is toxic [20] but young shoots (called crosiers) and fronds, which are preferred by grazing animals, accumulate some of bracken's most important toxic principles. [21].

2.1. Illudane and illudalane sesquiterpenes and their glycosides

Illudane and illudalane sesquiterpenes and *nor*-sesquiterpenes are found in bracken as well as in other members of the Dennstaedtiaceae and other ferns [22]. These compounds include the numerous and long-known family of pterosins and their

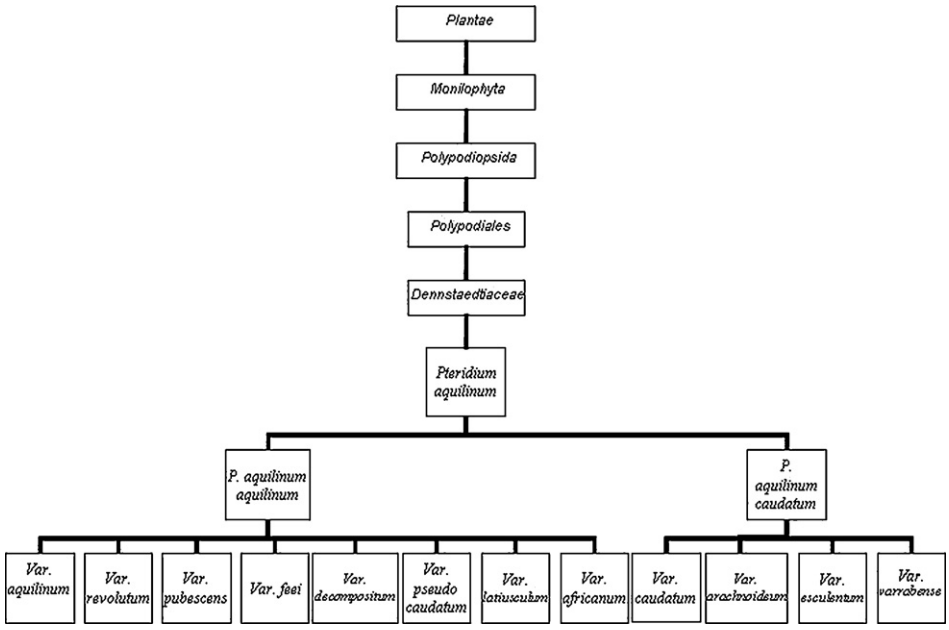
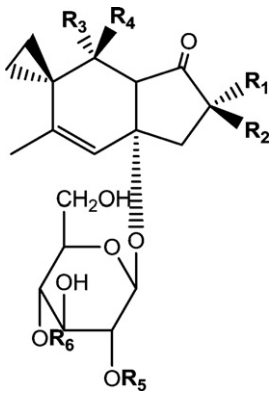
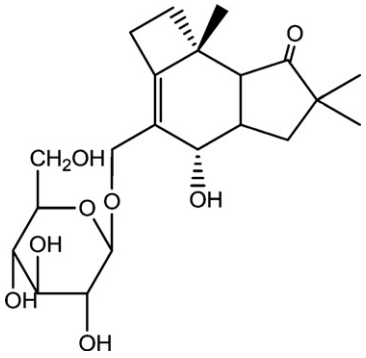
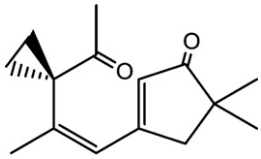


Fig. 1. The taxonomy of *P. aquilinum*, including subspecies and varieties [3,4].

Table 1
Some ptaquiloside-related illudanes, *nor*-illudane, proto-illudanes and seco-illudanes [33–35,47–54].







Compound	R_1	R_2	R_3	R_4	R_5	R_6
1	CH_3	H	CH_3	OH	H	H
2	CH_3	H	OH	CH_3	H	H
3	CH_3	CH_3	CH_3	OH	H	H
4	CH_2OH	CH_3	CH_3	OH	H	H
5	CH_2OH	H	CH_3	OH	H	H
6	CH_2OH	CH_3	OH	CH_3	$COCH_3$	H
7	CH_2OH	CH_3	OH	CH_3	$COCH_3$	*trans*-*p*-Coumaroyl
8	CH_3	CH_3	OH	CH_3	$COCH_3$	H
9	CH_3	CH_3	OH	CH_3	$COCH_3$	*cis*-*p*-Coumaroyl
10	CH_3	CH_3	OH	CH_3	$COCH_3$	*trans*-*p*-Coumaroyl

glycosides, called pterosides [23–30]. Although some pterosins and pterosides do show some *in vitro* cytotoxicity against neoplastic cell lines, they are not genotoxic and none of them was considered to be a determinant of bracken toxicity towards animals [31,32].

The simultaneous discovery of ptaquiloside (**1**) by two different groups in Japan and in the Netherlands [33–35] was a landmark in bracken research. This compound proved to be the long-awaited toxin responsible for both acute and chronic bracken toxicity, and especially for its carcinogenic affects [36]. Ptaquiloside's discovery took a very long time because, in fact, this compound is highly unstable towards water, heat, light and both acid and alkaline pH, so several attempts have been made to perfect the isolation methods [37–41]. Methods for quantifying ptaquiloside (**1**) in bracken have also been developed [42–44]. Maximum ptaquiloside (**1**) concentrations are found in young crosiers. Great variations in ptaquiloside (**1**) concentration occur during the year, between bracken strains and even between different bracken stands located near each other [2,45], with up to 12.9 mg/g in some Australian bracken samples [46]. Ptaquiloside (**1**) and several other ptaquiloside-like molecules such as isoptaquiloside (**2**), ptaquiloside Z (**3**), caudatoside (**4**), the recently identified ptesculentoside (**5**), the dennstosides A (**6**) and B (**7**) and hypolosides A (**8**), B (**9**) and C (**10**), the protoilludane pteridanoside (**11**) and the seco-illudane hypacrone (**12**) (Table 1) are still being discovered both in bracken and in other related ferns [47–54]. In general, they share the reactive cyclopropylidene ring, with the remarkable exception of pteridanoside (**11**) which presents a cyclobutane ring, and the C-1 carbonyl group. These structurally related molecules are thought to be pterosin precursors and, indeed, they originate their corresponding pterosins by hydrolysis in aqueous solution. Although little is known about the toxic properties of these compounds, they are expected to show much the same reactivity and toxic effects as ptaquiloside (**1**) [36,55], whose toxicity is discussed in Section 3.

2.2. *p*-Hydroxystyrene derivates

This group of substances includes five compounds named ptelatosides A, B and C, *p*-hydroxystyrene β-D-glycoside and glycoside A [37,56]. Not much is known about the toxicity of these substances, but ptelatoside A was fed to rats as part of their diet at a concentration of 0.065 mg/kg/day for 109 or 125 days [57] and no toxic effects were noted when euthanasia took place, 520 days after the experiment started. Accordingly, *p*-hydroxystyrene derivates are not thought to play a major role in bracken-induced toxicity.

2.3. Cyanogenic glycosides

Cyanogenic glycosides are enzymatically hydrolysed by β-glucosidases, thereby releasing hydrocyanic acid (HCN) and glucose. Not all bracken varieties are cyanogenic and the only such molecule reported in bracken is prunasin, which is thought to act as feeding deterrent against insects and also against herbivore mammals, due to its acrid taste [58]. Prunasin is most abundant in the young crosiers [59] and was present in amounts varying from 10 to 61 mg/g plant biomass [60] or 1.84 to 107.70 mg/g of dry weight [58] in samples from *P. aquilinum* var. *arachnoideum*, from Venezuela but in only 0.07 mg/g in *P. aquilinum aquilinum* crosiers samples from northern Portugal [61]. Cases of cyanide poisoning in animals caused by bracken ingestion have very seldom been reported [23,62].

2.4. Flavonoids and tannins

A number of flavonoids and their corresponding glycosides have been identified in bracken, including quercetin, kaempferol and many of their derivatives [63–65]. These compounds are present in up to 10–25 mg/g of plant material and are most abundant during spring and early autumn [66].

Toxicity due to ingestion of large amounts of tannins is sometimes observed in farm animals fed with oak (*Quercus* spp.) leaves or acorns. Tannins and their metabolites (gallic acid, digallic acid and pyrogallol) are responsible for the observed toxic effects, mainly renal tubular necrosis with interstitial oedema and haemorrhage and gastrointestinal ulcers [67]. Bracken tannins are mostly condensed tannins derived from procyanidin

and prodelphidin, and can reach up to 120 mg/g in plant material [62,68]. Although bracken toxicity is not commonly linked with tannins, it is conceivable that these compounds may contribute to irritate the gastrointestinal tract of bracken-fed animals. During the long search for the ultimate bracken carcinogen, tannins were also tested for carcinogenicity in rats and mice with conflicting results. Subcutaneous injection caused liver tumours and local sarcomas at the injection site, but rats fed a tannin-containing diet (4 mg/g) did not develop any tumours [69]. Considering this and bearing in mind their natural abundance, tannins are not currently considered one of brackens main toxins.

2.5. Shikimic acid derivates

A number of benzoic and cinnamic acid derivates have been identified in bracken [70,71]. Shikimic acid was found at 1.44 mg/g (dry weight) in bracken from Wales [72]. Conflicting evidence exists concerning its mutagenicity and carcinogenicity [72–74]. Nevertheless it is unlikely that this compound plays a major role in bracken-induced carcinogenesis [13], since bracken's mutagenicity and carcinogenicity are enhanced at high pH, while shikimate's is eliminated.

2.6. Thiaminases

Bracken contains two types of thiaminases, which cause thiamine (vitamin B1) deficiency [1,62], especially in horses, as described in Section 4.1. Bracken thiaminases 1 and 2 activities have been quantified at 3.1 and 3.5 μg of destroyed thiamine per gram of plant material per hour, respectively [75]. Thiaminase concentrations are higher in rhizomes and very young fronds, falling sharply in the fronds as they unfold.

2.7. Braxins

In 1986 Saito and Mochizuki [76] reported the isolation of two active glycosides from bracken rhizomes, called braxins A1 and A2. These compounds were shown to be aromatic β-glucopiranosides, but their structures were not fully elucidated. Braxins A1 and A2 were found to induce histamine release from rat mast cells [76] and to induce acute haemorrhagic cystitis in Guinea pigs [77]. The same lesions were induced by braxin C, which was found to be identical to ptaquiloside (**1**) [78,79]. The structure of braxin B has not been reported [80].

3. Bracken-associated toxicity

Bracken has long been known as a plant of pharmaceutical and toxicological interest [81]. During periods of reduced food availability, domestic animals (herbivores and pigs) readily consume bracken, especially its younger and tender parts [82]. The effects of bracken ingestion are various, depending on the animal species involved and in the ingested dose(s). Five different syndromes are described in farm animals: thiamine deficiency, acute bracken poisoning, progressive retinal degeneration, bovine enzootic haematuria and upper alimentary tract carcinomas [1,83].

3.1. Thiamine deficiency

Monogastric animals, mostly horses and, to a lesser extent, pigs [84], develop a thiamine (vitamin B1) deficiency syndrome, due to the action of bracken anti-thiamine factors described in Section 2.7. This syndrome, akin to human B1 avitaminosis (*beri beri*), is initially characterised by anorexia and ataxia, followed by convulsions and death. Polyoencephalomalatia is the main lesion found in affected animals [85]. Ruminants, whose ruminal flora produces enough thiamine, are generally resistant, although the syndrome has been reproduced experimentally in bracken-fed sheep [86,87]. A similar experiment in rats also resulted in thiamine deficiency [86].

3.2. Acute bracken poisoning

Bracken poisoning has been reported in animals since the late nineteenth century [88,89]. Acute bracken poisoning occurs mostly in ruminants, especially in cattle and, to a lesser extent, in sheep [1] due to bracken toxins that target the more rapidly dividing cells of animals [82]. Many of its main features are haematological changes resulting from bone marrow depression, with reduced production of platelets, erythrocytes and leukocytes. The resulting severe thrombocytopaenia leads to widespread haemorrages, while leukopaenia results in increased susceptibility to infections. Affected cattle often show severe neutropaenia [90], while sheep are reported to show lymphopaenia [91]. Necrosis of the laryngeal, pharyngeal and intestinal epithelial layer may also occur, leading to the so-called laryngeal and intestinal forms of the disease [1,92]. The intoxication was experimentally induced in bracken-fed cattle [93] and sheep [94]. The same effect was obtained when ptaquiloside (**1**), a bracken constituent (see Section 3.1), was administered to a calf [95], showing ptaquiloside (**1**) to be the etiological agent of acute bracken poisoning in cattle.

3.3. Progressive retinal degeneration

Progressive retinal degeneration is observed in sheep grazing on bracken-infested areas and results in a so-called "bright" blindness, and in a behaviour known as "star gazing" [96]. Administration of bracken [97,98] or ptaquiloside (**1**) isolated from bracken [99] reproduced the disease, showing this toxin to be its etiological agent.

3.4. Teratogenesis

When pregnant ICR-JCL mice were fed a diet containing 30% (w/w) bracken the embryos suffered several bone malformations including rib anomalies and incomplete fusion of sternebrae [100]. Newborn mice dosed with ptaquiloside (**1**) showed karyorrhexis in the granular layer of the cerebellum and rosette formation in the neuroblastic layer of the retina [101].

3.5. Carcinogenesis

The prevailing theory of carcinogenesis explains it as a complex multistep process, driven by the accumulation of somatic genetic mutations and epigenetic changes caused by chemical, physical and biological carcinogens. Such changes often target the so-called tumour-suppressor genes and proto-oncogenes, and contribute to deregulate essential cellular functions, such as cell cycling and proliferation, apoptosis and differentiation, as well as to induce a remodelling of the surrounding stroma. This process is commonly divided into three consecutive steps, known as initiation, promotion and progression, and often gives rise to a sequence of morphologically and clinically distinct entities, including preneoplastic, benign and malignant lesions [102–105].

3.5.1. The carcinogenic properties of bracken

Since the observations of Rosenberger and Heeschen [106] and Rosenberger [107], relating bracken feeding with bovine

haematuria and bladder tumours, bracken became a suspect carcinogen. The carcinogenic potential of bracken was experimentally demonstrated by Evans and Mason [108] who obtained ileal adenocarcinomas in rats fed bracken. Slightly later findings [109] have shown that rats would develop both ileal and bladder tumours if the exposure time was long enough. Apparently, in rats, the development of bracken-induced bladder tumours is slower than that of ileal tumour and requires a longer period of bracken exposure. Several phenotypically distinct bladder neoplasms were induced [110,111] by feeding bracken to cattle, thereby demonstrating the link between bracken and bovine enzootic hematuria. In the long search for the ultimate bracken carcinogen, Evans [112] reflected on the radiomimetic nature of bracken's carcinogen, while Pamucku et al. [113] wondered if the anti-thiamine effects of this plant had anything to do with its carcinogenicity. Another strategy used by the Pamucku group, at the University of Ankara, was to study the effects in yet another species [114]. The authors reported that, in mice, leukaemia and pulmonary adenomas and adenocarcinomas occurred instead of bladder or intestinal tumours, thus stressing the importance of inter-species variation in response to bracken toxicity. Bracken was also found to induce tumours in the terminal jejunum of C57Bl/6 mice [115], although these findings could not be reproduced in dd mice or Swiss mice [112]. In both these mice strains the lung, rather than the ileum, was the target organ. Pamucku et al. [116] demonstrated that milk from bracken-fed cows induced ileal, bladder and kidney carcinomas in rats. When a diethyl ether fraction was prepared from milk and implanted unto the bladder of mice, these developed urothelial carcinomas in high incidence. These experiments clearly showed that the carcinogenic principle of bracken could be transmitted through milk.

A Japanese group from the University of Nagoya adopted a different strategy, by systematically fractionating bracken in search of its ultimate carcinogen [117]. The same group also demonstrated that rats fed bracken could develop mammary carcinomas, besides ileal and bladder tumours [118], and showed bracken to promote carcinogenesis initiated by *N*-propyl-*N*-nitrosourethan [119]. Other species, such as guinea pigs, were shown to develop mostly bladder tumours when fed a bracken diet [112,120,121]. The fractionation strategy proved successful, though laborious, and led to the discovery of ptaquiloside (**1**) [33,34] (see Section 3.1), which proved to be the main bracken carcinogen [117,122]. Other compounds, such as the flavonoid quercetin [123] and shikimic acid [73] were proposed to be important bracken carcinogens. However, it has not been possible to reproduce the findings of Pamucku et al. [123] and evidence to support a possible role for shikimic acid is still lacking [72,124].

3.5.2. Reactivity and mutagenicity of ptaquiloside

In aqueous solution ptaquiloside (**1**) is gradually converted into pterosin B with D-glucose liberation, conversion rates depending on temperature and pH. Under weakly alkaline conditions a highly reactive intermediate compound, referred to as bracken dienone or activated ptaquiloside, is rapidly generated [125], which is thought to be ptaquiloside's active form [33]. This is remarkable because while most chemical carcinogens are either directly active or dependent on metabolic activation [104], ptaquiloside (**1**) is unique in that its activation is not enzyme-mediated. Bracken dienone is stable in alkaline solutions for at least 1 h, but is extremely unstable under acidic conditions being immediately converted into pterosin B [33]. The reaction of ptaquiloside (**1**) and its derivatives with biomolecules was extensively studied by Professor Yamada's group at the University of Nagoya, Japan. Bracken dienone was shown to form adducts with sulphur-containing aminoacids – cysteine, methionine and glutathione – as well as with nucleosides and nucleotides, *via* its reactive cyclopropylidene ring [125]. Under physiological conditions, the dienone alkylates the DNA to form adducts through the N-3 of adenine and/or the N-7 of guanine, leading to DNA depurination and breakage at the adenine but not guanine sites [126]. This DNA cleavage activity was sequence-selective as follows: 5′-A̲T > 5′-A̲G > 5′-A̲C > 5′-A̲A for the 3′-flanking nucleotides and 5′-A A̲ > 5′-T A̲ > 5′-G A̲ > 5′-C A̲ for the 5′-flanking nucleotides (sites of cleavage are underlined). The most preferable sequence was estimated to be 5′-A̲AAT [126]. The DNA-damaging properties of bracken extracts were demonstrated using the comet assay [127,128] and the same technique was recently used to study the genotoxicity of ptaquiloside towards human peripheral blood mononuclear cells [129]. The DNA-alkylating properties of ptaquiloside (**1**) were demonstrated by another group, who used a ^{32}P-postlabelling assay to identify DNA adducts formed *in vitro* by ptaquiloside (**1**) [130]. DNA-adducts formed *in vivo* were also detected in the ileum of calves fed bracken [131], in mammary carcinomas of rats dosed with bracken dienone [132] and in mice treated with bracken spores, extracts or with bracken dienone [133–135]. Prakash et al. [131] found that ptaquiloside (**1**) alkylated the H-*ras* proto-oncogene at adenine sites, resulting in an activating mutation (an adenine to pyrimidine transversion) at codon 61, and proposed that such mutations constituted the basis for bracken-induced carcinogenesis. This hypothesis was further supported by the finding of H-*ras* activating mutations on rat mammary carcinomas induced by bracken dienone [136]. The genotoxicity of ptaquiloside (**1**) thus seemed to depend upon its DNA-alkylating properties. The genotoxicity of ptaquiloside (**1**) was further demonstrated on the rat hepatocyte primary culture/DNA-repair test [137], on *Salmonella typhimurium* [38–138] and also by Matsuoka et al. [139] who showed it to induce structural chromosomal aberrations on Chinese hamster lung cells. The genotoxic properties of ptaquiloside (**1**) were expressed in alkaline conditions and were much reduced at pH < 7.4 [38,138,139]. Recent studies on human peripheral blood mononuclear cells [129] have shown that ptaquiloside (**1**) is an aneugenic as well as clastogenic toxin and that it also induces sister-chromatid exchanges. These results indicate that ptaquiloside (**1**) is not only a DNA-alkylating agent, but exerts its genotoxicity through multiple mechanisms including clastogenesis, aneugenesis and the not entirely understood mechanism underlying SCE induction. The genotoxic action of ptaquiloside (**1**) against soil microorganisms was also demonstrated by Schmidt et al. [140].

3.5.3. The carcinogenicity of ptaquiloside

The carcinogenicity of ptaquiloside (**1**) results from its mutagenicity, as previously described in Section 3.5.2. The carcinogenicity of ptaquiloside (**1**) was proved soon after its isolation by the Nagoya group [117,122,141] and repeatedly confirmed [132,136]. As observed with bracken, the target organs for ptaquiloside's carcinogenicity seem to be dependent on the administration route, ptaquiloside (**1**) dose and on the exposure time. Only mammary carcinomas were obtained by intravenous administration of a low bracken dienone dose for a short period [136], while the induction of ileal and bladder tumours required prolonged oral administration of higher doses [117,122]. One interesting observation is that some target organs, such as the ileum of rodents, often have alkaline pH, which is thought to reflect ptaquiloside (**1**) activation and formation of the more reactive bracken dienone. Recently, intraperitoneal ptaquiloside administration to CD-1 mice resulted in high incidence of urinary bladder dysplasia and a B-cell lymphoproliferative malignancy [142]. Lymphoproliferative lesions had already been found [114] in bracken-fed Swiss mice; these recent results confirmed that ptaquiloside is the carcinogen responsible for such lesions. Immunohistochemical analysis of dysplastic bladder lesions showed them to maintain normal adhesion molecules

(E-cadherin and β-catenin) expression and significantly increased proliferation (Ki-67) indices [143].

3.5.4. The immunomodulatory effects of ptaquiloside

Early studies of bracken toxicity reported severe neutropaenia as one of its effects [95]. Monocytosis and increased TNFα levels were reported as long-term effects of bracken dienone [132], 30 weeks after the last intravenous administration. More recently, bracken extracts were shown to reduce natural killer (NK) cells activity and to induce splenic white pulp atrophy [144]. Ptaquiloside (**1**) was also shown to reproduce these effects, which were fully reversible by selenium co-treatment or post-treatment [145]. These findings suggest that ptaquiloside (**1**) may also promote carcinogenesis by reducing immune surveillance against (bracken-induced or otherwise) newly arising tumours.

3.5.5. Upper digestive tract carcinomas

In cattle, bovine papillomavirus type 4 (BPV-4) infects the mucosa of the upper gastrointestinal tract leading to the formation of papillomas [146,147]. Bovine gastrointestinal tumours have been found in several world locations, including Brazil, Bolivia, the Nasampolai Valley of Kenya, the Scottish Highlands and southern Italy [148,149]. In the presence of bracken, BPV-4-induced papillomas undergo malignant transformation into carcinomas (papilloma–carcinoma transition) [147,150,151]. It has been argued that this may be partly due to an immunosuppressive effect induced by bracken toxins (Fig. 2), which would stop the host's immune system from eradicating the infection and eliminating the papillomas. Persistent papillomas would then be at increased risk of suffering malignant transformation. Some recent findings [144,145] certainly supported this hypothesis by showing that ptaquiloside (**1**) reduces NK cell function, therefore colliding with the host's immunosurveillance against arising tumours.

Papillomaviruses transform host cells by expressing several oncoproteins (E5, E6 and E7) [150]. Quercetin, a flavonoid found in bracken, was shown to interact *in vitro* with the long control region (LCR) of BPV-4 [152,153] and human papillomavirus type 18 (HPV-18), but not HPV-16, through a conserved quercetin response element in the viral genome [154]. Prof. Saveria Campo's group, at the University of Glasgow, demonstrated that this interaction results in a dose-dependent increase in the expression of viral oncoproteins E6 and E7. Partially transformed BPV-4-infected cells were shown to suffer full malignant transformation with a single quercetin dose, acquiring immortality and tumourigenicity [155]. It remains to be known whether this *in vitro* synergy mechanism remains valid in field conditions [150].

Quercetin was also shown to exhibit dose-dependent *in vitro* [156] but not *in vivo* [157] genotoxicity. This discrepancy is, most likely, due to hepatic metabolic inactivation by catechol-o-mertyltransferase [158]. Thus, although some authors proposed that quercetin-induced mutations might also contribute to the appearance of a malignant phenotype *in vivo* [150,159], this seems unlikely. Indeed, quercetin and other flavonoids act as antioxidants capable to reduce oxidative stress-induced mutagenesis and quercetin is even used as a dietary supplement [160].

The mutagenic and carcinogenic properties of ptaquiloside (**1**) are likely to be implicated in bovine gastrointestinal cancers. This compound may inactivate tumour-suppressor genes (such as p53) and activate proto-oncogenes (such as H-*ras*), thus driving the papilloma-carcinoma transition [161].

3.5.6. Bovine enzootic haematuria

Haematuria has long been enzootically associated with cattle feeding over 2 or 3 years in bracken-infested areas and results from haemorrhages due to bracken-induced bladder tumours [1,21,106,121,151] rather than haemostasis disturbances [162]. A recent report refers the occurrence of a similar syndrome in buffaloes [163]. Cattle with enzootic haematuria show an increased incidence of structural chromosomal aberrations in peripheral blood lymphocytes [164]. The disease has been recognised worldwide and has been especially well characterised in the Portuguese islands of the Azores [165–167]. The symptoms and its underlying neoplastic bladder lesions were experimentally reproduced by feeding bracken to cows [108,110], laboratory rats and guinea pigs [108,120]. Ptaquiloside (**1**) is believed to cause bovine enzootic haematuria, since its administration also induced bladder tumours in laboratory animals [141] though its importance in field conditions remains to be confirmed. The oncogenesis of ptaquiloside-induced bladder lesions has not been thoroughly investigated, but a number of studies have addressed its morphologic and molecular features [168–180].

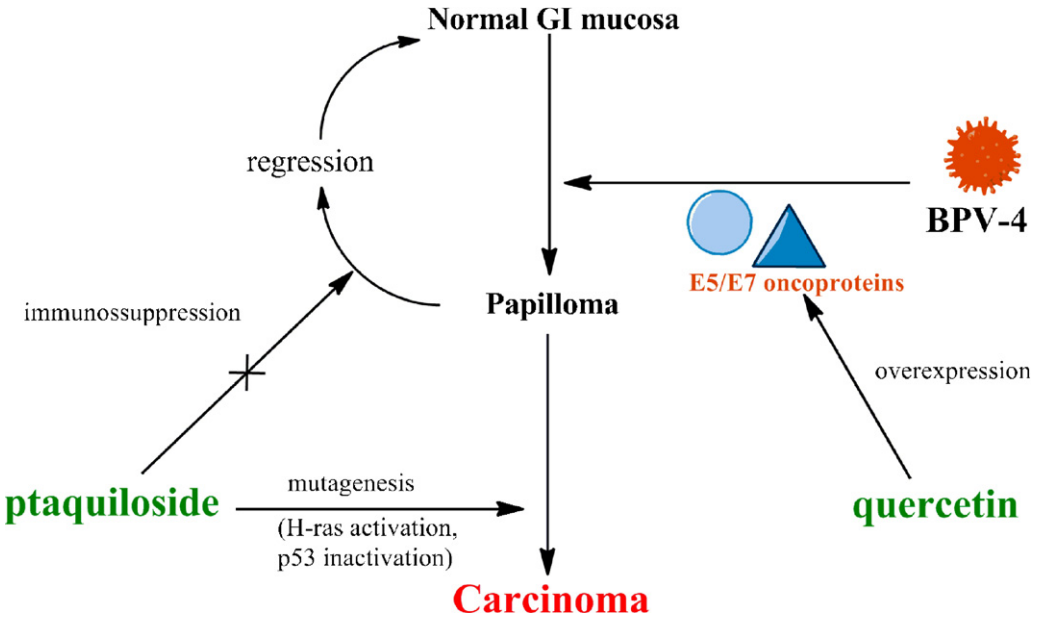


Fig. 2. How BPV-4 and bracken fern are thought to cooperate in the development of gastrointestinal tumours in cattle.

A possible synergism between ptaquiloside (**1**) and bovine papillomavirus type 2 (BPV-2) is a hypothesis that has been gaining support over the years [150,170,181,182]. Bovine papillomavirus type 2 DNA (but no nuclear capsids) was shown to be present in the normal bladder mucosa in up to 50% of sampled cattle in both Italy [170] and Romania [183], probably reflecting a latent infection. Expression of the BPV-4 major oncoprotein, E5, which induces malignant transformation by activating the platelet-derived growth factor receptor β (PDGFR β) *in vitro* [184], was implicated in bladder carcinogenesis [170,185]. The real contribution of bovine papillomaviruses for bladder carcinogenesis and the mode of their possible interaction with ptaquiloside (**1**) or other bracken toxins are still in debate [150].

In the year 2000, 28% of cattle herds from São Miguel Island contained one or more affected animals and bladder tumours accounted for the rejection of 14% of all slaughtered cattle [167]. The economic impact of bovine enzootic haematuria can be appreciated from the 4 220 443.7 euros on state compensations and insurances paid to affected milk producers in the Azores between 2000 and 2006 [186], though this may be an underestimate.

4. Bracken and its potential impact on human health

Bracken has long been used by man [81]. Historically, bracken has been used for animal bedding, for soap production [187] and for medicinal uses [81]; the starch-rich rhizomes were used as food in times of hunger [13,88]. Bracken's potential public health risks have been long recognised, especially since the discovery of ptaquiloside (**1**) [33–35] and its carcinogenic properties [117,122]. Nowadays, bracken is still used, for instance in the United Kingdom, for the production of a peat substitute for horticultural use [187]. In 1992 and 1993 approximately 1500 m^3 of bracken were composted and the amount doubled in the following year. Composting was reported to be effective in destroying ptaquiloside (**1**) in bracken [188]. The worldwide increase in bracken-covered land, which reaches up to 11,120 kg bracken biomass per hectare [46], leads to a closer contact between this plant and human communities and their animals. It is feared that the toxicity of bracken towards domestic animals may, directly or indirectly, affect people too, particularly in the case of ptaquiloside-induced carcinogenesis.

4.1. Bracken as food

Some human populations, namely in Japan, in the Ouro Preto area in Brazil, and in Canada also eat bracken crosiers as a delicacy, known as *warabi*, as *broto de samambaia* and as fiddleheads, respectively [189–191]. Cooked or salted bracken was demonstrated to retain some of its carcinogenic potential [192] and bracken-eating Brazilians showed increased chromosomal aberrations in peripheral blood leukocytes, compared with non-bracken eating controls [193]. Interestingly, a similar observation was made on cattle affected by bovine enzootic haematuria [164]. Since the Japanese *warabi* production was insufficient to meet local demands, as much as 13,000 tons, which may contain 13–169 kg of pure ptaquiloside (**1**), were imported yearly, mostly for the prefectures of central Honshu island [62]. Significantly, the first reports to connect bracken with human cancer were by Japanese authors [194,195], establishing an association between increased oesophageal cancer risk and bracken consumption. Oesophageal cancer risk was found to be respectively, 2.1- and 3.7-fold higher in men and women who consumed *warabi* in a daily basis, when compared with controls [195]. However, in contrast with previous studies on farm and laboratory animals, no association was observed between bracken fern consumption and high bladder cancer mortality in New England [41,196] or increased bladder cancer risk in Canada [197], where bracken crosiers are known as fiddleheads.

4.2. Inhalation of bracken spores

The inhalation of bracken spores has raised concerns as a possible exposure route to bracken carcinogens [198]. A spatial association between bracken and cancer in Great Britain was proposed [199] and an epidemiological study in Wales [200] pointed out a possible relation between bracken-contaminated foodstuffs or air-borne bracken spores and gastric cancer. Bracken spores may pose a risk to human health as they were shown to be mutagenic *in vitro* [127] and *in vivo* [133,134], but they have not yet been demonstrated to contain ptaquiloside (**1**).

4.3. Ptaquiloside-contaminated milk

Two studies in South and Central America associated increased digestive tract cancer risk with exposure to bracken [201–203]. In Costa Rica, the frequency of gastric and oesophageal neoplasms was found to be higher on high-altitude, bracken-infested areas, compared with low-altitude, bracken-free areas (odds ratios of 2.50 and 2.73 for males and females, respectively) and to co-localise with bovine enzootic haematuria [201,202]. In Venezuela, increased mortality due to gastric cancer was associated with high-altitude, bracken-infested areas where bovine enzootic haematuria is prevalent, compared with low-altitude, bracken-free areas (odds ratio 3.64), but no association was found with oesophageal cancer [203].

In fact, 8.6 ± 1.2% of the ptaquiloside (**1**) ingested by bracken-fed cows was shown to be excreted dose-dependently in milk [204]. This important discovery explained earlier findings [116] reporting that milk from bracken-fed cows showed carcinogenic activity in mice. The contamination of milk may be an important route for human exposure to ptaquiloside (**1**) among rural populations who consume milk directly from bracken-grazing cows. Urban populations consuming industrially processed milk from stabled animals fed on controlled diets should be at lower risk. Any ptaquiloside-contaminated milk entering the industrial circuit will, most likely, be diluted in milk from stabled animals and will be pasteurised before being further used [205]. Pasteurisation was found to decrease ptaquiloside (**1**) concentrations in milk by 50% and boiling by about 75% [206]. In view of these findings, boiling the milk before drinking it may be a useful recommendation for rural populations suspected of being exposed to considerable amounts of ptaquiloside (**1**).

4.4. Ptaquiloside residues in meat

The presence of ptaquiloside in products used for human consumption has raised considerable concerns [207] and a governmental report by the British Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) has recently addressed the risks placed by ptaquiloside-contaminated foodstuffs, in particular by milk [208] and considered it prudent to regard bracken as potentially carcinogenic at all levels of ingestion. This Committee also called for further studies on ptaquiloside (**1**) residues that may be present in foods derived from bracken-fed animals and on the determination of safety intervals for ptaquiloside (**1**) clearance from tissues and milk [208].

Very recent findings [209] indicate that ptaquiloside (**1**) is present in skeletal muscles (0.42 μg/g) and liver (0.32 μg/g) of bracken-fed cattle, fifteen days after bracken consumption ended. Bracken represented 19% of these animals diet, corresponding to 1.8 mg ptaquiloside (**1**) and 4.0 mg ptesculentoside (**5**) daily intakes, per kilogram of body weight. The same authors suggest that other illudane-type molecules such as ptesculentoside (**5**) may leave more residues than ptaquiloside (**5**). As mentioned on Section 1.2.1, the toxicity of the other ptaquiloside-like illudane compounds in bracken is still poorly understood. However, in a

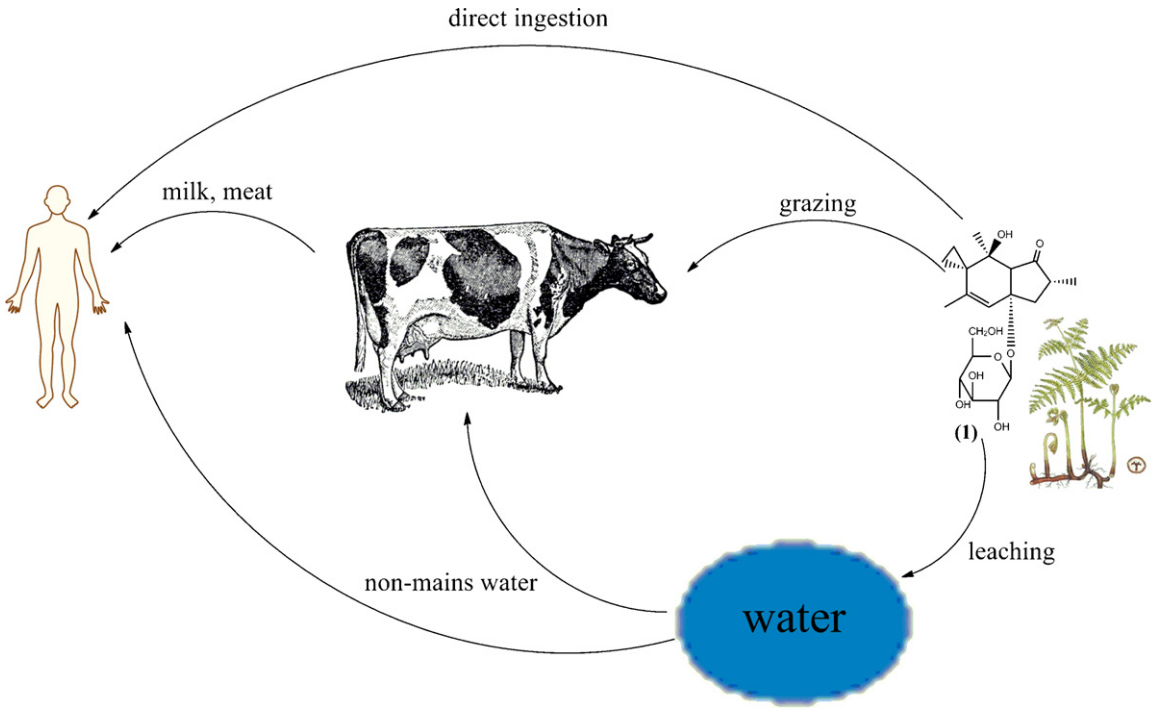


Fig. 3. Ptaquiloside (**1**) flow through the environment and human exposure routes.

toxicity assay [51] against *Artemia salina*, ptaquiloside Z (**5**) and ptaquiloside (**1**) showed the same lethal concentrations (LC50) at 24 and 48 h (62.5 μg/ml and 7.8 μg/ml, respectively). These findings support the hypothesis that such bracken ptaquiloside-like compounds possess identical toxicological properties, since their chemical reactivity is very similar, and the presence of their residues in foodstuffs should be of concern.

4.5. Ptaquiloside contamination of soil and water

Human exposure to bracken toxins, namely to ptaquiloside (**1**), was also proposed to occur through contamination of underground waters [210–216]. These studies demonstrated that ptaquiloside (**1**) can be quite stable in the soil environment, especially in clay-rich soils [213] and is present in some soils after 72 days, with a half-life of 150–180 h. Between 0.05% and 0.25% of total ptaquiloside (**1**) present on bracken fronds is liable to leach into the surrounding soil due to the action of rain [211]. This toxin was shown to have the potential to contaminate underground waters, especially when leaching into lightly acidic, sandy soils, poor in organic matter, during rainy and cold seasons [213–215]. In a recent report by the British Food and Environmental Research Agency, human exposure to ptaquiloside (**1**) *via* drinking water in the United Kingdom was estimated to be minimal, even though the ultimate risk arising from contaminated water consumption could not be quantified [217]. These authors estimated that ptaquiloside (**1**) concentrations in underground waters could reach up to 11.5 μg/L, but the overall risk posed by contaminated water was estimated to be lower than that posed by contaminated foodstuffs [217]. These values agree with previous results [213] reporting ptaquiloside (**1**) concentrations between 4 and 6 μg/L in Denmark and up to 45 μg/L in Sweden. The differences are likely to be due to differences in soil composition, bracken coverage and ptaquiloside (**1**) concentration in bracken. Ptaquiloside (**1**) was also shown to have a considerable impact on soil microbial communities as reflected by the inhibition of soil microbial respiration [140], an effect which helps prolonging its persistence in soil and underground waters.

4.6. Bracken and ptaquiloside: human carcinogens?

The International Agency for Research on Cancer (IARC) classified bracken as carcinogenic to animals and possibly carcinogenic to humans (group 2B) and considered ptaquiloside (**1**) unclassifiable as to its carcinogenicity (group 3) [218] but a later report from the Dutch government stated that ptaquiloside (**1**) should be considered as a human carcinogen [219]. The scientific community's interest in bracken was considerably excited by the discovery of ptaquiloside (**1**) and its carcinogenicity and a number of reviews on bracken-associated risks were published in recent years [13,36,82,220–223]. Despite the role that quercetin may play in papillomavirus-induced carcinogenesis *in vivo*, ptaquiloside (**1**) has emerged, since its discovery, as the main bracken carcinogen. The existence of different routes for human exposure to this toxin (Fig. 3) cannot but raise concerns about its impact on public health. New epidemiological studies addressing the association of bracken exposure with gastric and oesophageal cancers should be undertaken, taking into account other factors, such as *Helicobacter pylori* infection.

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References

[1] K.E. Panter, D.R. Gardner, S.T. Lee, J.A. Pfister, M.H. Ralphs, B.L. Stegelmeier, L.F. James, Important poisonous plants of the United States, in: R.C. Gupta (Ed.), Veterinary Toxicology: Basic and Clinical Principles, Academic Press, New York, 2007.

[2] B.L. Smith, The toxicity of bracken fern (genus *Pteridium*) to animals and its relevance to man, in: F.J.P. D'Mello (Ed.), Handbook of Plant and Fungal Toxicants, CRC Press, New York, 1997, pp. 63–76.
[3] R.M. Tryon, A revision of the genus *Pteridium*, Rhodora 43 (1941) 1–31 (37–67).
[4] G.A. Cooper-Driver, Chemotaxonomy and phytochemical ecology of bracken, Bot. J. Linn. Soc. 73 (1976) 35–46.
[5] A.R. Smith, K.M. Pryer, E. Schuettpelz, P. Korall, H. Schneider, P.G. Wolf, A classification for extant ferns, Taxon 55 (2006) 705–731.
[6] J.A. Thomson, Morphological and genomic diversity in the genus *Pteridium* (Dennstaedtiaceae), Ann. Bot. 85 (2000) 77–99.
[7] J.A. Thomson, Towards a taxonomic revision of *Pteridium* (Dennstedtiaceae), Telopea 10 (2004) 793–803.
[8] J.A. Thomson, M.E. Alonso-Amelot, Clarification of the taxonomic status and relationships of *Pteridium caudatum* (Dennstaedtiaceae) in Central and South America, Bot. J. Linn. Soc. 140 (2002) 237–248.
[9] C.N. Page, Structural variation in Western European bracken: an updated taxonomic perspective, in: R.T. Smith, J.R. Taylor (Eds.), Bracken, an Environmental Issue, International Bracken Group Occasional Publication, Aberystwyth, 1995, pp. 13–15.
[10] P.G. Wolff, E. Sheffield, J.A. Thomson, R.B. Sinclair, Bracken taxa in Britain: a molecular analysis, in: R.T. Smith, J.R. Taylor (Eds.), Bracken, an Environmental Issue, International Bracken Group Occasional Publication, Aberystwyth, 1995, pp. 16–20.
[11] J.A. Thomson, P.H. Weston, M.K. Tan, A molecular approach to tracing major lineages in *Pteridium*, in: R.T. Smith, J.R. Taylor (Eds.), Bracken, an Environmental Issue, International Bracken Group Occasional Publication, Aberystwyth, 1995, pp. 21–28.
[12] C.N. Page, Taxonomic evaluation of the fern genus *Pteridium* and its active evolutionary state, in: R.T. Smith, J.R. Taylor (Eds.), Bracken Biology and Management, Australian Institute of Agricultural Science Occasional Publication, Sidney, 1990, pp. 23–34.
[13] B.L. Smith, A.A. Seawright, Bracken fern (*Pteridium* spp.) carcinogenicity and human health – a brief review, Nat. Toxins 3 (1995) 1–5.
[14] E. Sheffield, P.G. Wolf, C.H. Haufler, How big is a bracken plant, Weed Res. 29 (1989) 455–460.
[15] R.C. Miatto, I.A. Silva, D.M. Silva-Matos, R.H. Marrs, Woody vegetation structure of Brazilian Cerrado invaded by *Pteridium arachnoideum* (Kaulf.) Maxon (Dennstaedtiaceae), Flora 206 (2006) 757–762.
[16] R.J. Pakeman, R.H. Marrs, Vegetation development on moorland after control of *Pteridium aquilinum* with asulam, J. Veg. Sci. 3 (2009) 707–710.
[17] K. Roos, H.G. Rödel, E. Beck, Short- and long-term effects of weed control on pastures infested with *Pteridium arachnoideum* and an attempt to regenerate abandoned pastures in South Ecuador, Weed Res. 51 (2010) 165–176.
[18] R.C. Robinson, Invasive and problem ferns: a European perpective, Int. Urban Ecol. Rev. 4 (2009) 83–91.
[19] G.A. Cooper-Driver, Defence strategies in bracken. *Pteridium aquilinum* (L.) Kuhn, Ann. Missouri. Bot. Gard. 77 (1990) 281–286.
[20] J. Villalobos-Salazar, A. Meneses, J.L. Rojas, B. Pashov, The carcinogenic effects of bracken spores, in: R.T. Smith, J.R. Taylor (Eds.), Bracken: an Environmental Issue, International Bracken Group Occasional Publication, Aberystwyth, 1995, pp. 102–103.
[21] P-P. Bringuier, C. Jean-Blain, Hématurie chronique des bovins: étiologie et épidemiologia, Point Vet. 19 (1987) 393–403.
[22] R.W. Soeder, Fern constituents: including occurrence chemotaxonomy and physiological activity, Bot. Rev. 51 (1985) 442–536.
[23] G.R. Fenwick, Bracken (*Pteridium aquilinum*): toxic effects and toxic constituents, J. Sci. Food Agr. 46 (1988) 147–173.
[24] T. Murakami, N. Tanaka, Occurrence, structure and taxonomic implications of fern constituents, in: W. Herz, H. Grisebach, G.W. Kirby, C. Tamm (Eds.), Progress in the Chemistry of Organic Natural Products, vol. 54, Springer-Verlag, Wien, 1988.
[25] U. Castillo, A.L. Wilkins, D.R. Lauren, B.L. Smith, M. Alonso-Amelot, Pteroside A2 – a new illudane-type sesquiterpene glycoside from Pteridium caudatum L. Maxon, and the spectrometric characterization of caudatodienone, J. Agric. Food Chem. 51 (2003) 2559–2564.
[26] N.V. Kovganko, Z.N. Kashkan, S.N. Krivenok, Bioactive compounds of the flora of Belarus, Chem. Nat. Compd. 40 (2004) 227–229.
[27] B.M. Fraga, Natural sesquiterpenoids, Nat. Prod. Rep. 27 (2010) 1681–1708.
[28] D-W. Ouyang, X. Ni, H-Y. Xu, J. Chen, P-M. Yang, D-Y. Kong, Pterosins from *Pteris multifida*, Planta Med. 76 (2010) 1896–1900.
[29] Z-J. Zhan, Y-M. Ying, F-Y. Zhang, C-P. Li, W-G. Shan, Three new illudalane sesquiterpenoids from *Pteris semipinnata*, Helv. Chim. Acta 93 (2010) 550–554.
[30] B.M. Fraga, Natural sesquiterpenoids, Nat. Prod. Rep. 28 (2011) 1580–1610.
[31] W.C. Evans, T. Korn, S. Natori, K. Yoshihira, M. Fukuoka, Chemical and toxicological studies on Bracken fern. *Pteridium aquilinum* var. latiusculum. VIII. The inability of Bracken extracts containing pterosins to cause cattle Bracken poisoning, J. Pharmacobio. Dyn. 6 (1983) 938–940.
[32] Y-H. Chen, F-R. Chang, M-C. Lu, P-W. Hsieh, M-J. Wu, Y-C. Du, Y-C. Wu, New benzoyl glycosides and cytotoxic pterosin sesquiterpenes from *Pteris ensiformis* Burm, Molecules 13 (2008) 255–266.
[33] H. Niwa, M. Ojika, K. Wakamatsu, K. Yamada, I. Hirono, K. Matsushita, Ptaquiloside a novel norsesquiterpene glycoside from Bracken *Pteridium aquilinum* var. latiusculum, Tetrahedron Lett. 24 (1983) 4117–4120.
[34] H. Niwa, M. Ojika, K. Wakamatsu, K. Yamada, S. Ohba, Y. Saito, I. Hirono, K. Matsushita, Stereochemistry of ptaquiloside, a novel norsesquiterpene glucoside from Bracken *Pteridium aquilinum* var. latiusculum, Tetrahedron Lett. 24 (1983) 5371–5372.
[35] J.C.M. van der Hoeven, W.A. Lagerwij, M.A. Posthumus, A. van Velduizen, H.A.J. Holterman, A. Aquilide, a new mutagenic compound isolated from bracken fern (*Pteridium aquilinum*), Carcinogenesis 4 (1983) 1587–1590.
[36] K. Yamada, M. Ojika, H. Kigoshi, Ptaquiloside, the major toxin of bracken, and related terpene glycosides: chemistry biology and ecology, Nat. Prod. Rep. 24 (2007) 798–813.
[37] M. Ojika, H. Kigoshi, H. Kuyama, H. Niwa, K. Yamada, Studies on *Pteridium aquilinum* var. latiusculum IV. Isolation of three p-hydroxystyrene glycosides and an efficient method for the isolation of ptaquiloside, an unstable bracken carcinogen, J. Nat. Prod. 48 (1985) 634–637.
[38] M. Matoba, M. Saito, K. Saito, K. Koyama, S. Natori, T. Matsushima, M. Takimoto, Assay of ptaquiloside, the carcinogenic principle of bracken. *Pteridium aquilinum*, by mutagenicity testing in *Salmonella typhimurium*, Mutagenesis 2 (1987) 419–423.
[39] K. Saito, T. Nagao, M. Matoba, K. Koyama, S. Natori, T. Murakami, Y. Saiki, Chemical assay of ptaquiloside the carcinogen of *Pteridium aquilinum*, and the distribution of related compounds in the Pteridaceae, Phytochemistry 28 (1989) 1605–1611.
[40] P.B. Oelrichs, J.C. Ng, J. Bartley, Purification of ptaquiloside a carcinogen from *Pteridium aquilinum*, Phytochemistry 40 (1995) 53–56.
[41] P.W. Burkhalter, P.M. J.Groux, U. Candrian, P. Hübner, J. Lüthy, Isolation, determination and degradation of ptaquiloside, a bracken fern (*Pteridium aquilinum*) carcinogen, J. Nat. Toxins 5 (1996) 141–159.
[42] F. Bonadies, G. Borzacchiello, S. Dezzi, R. Nicoletti, S. Roperto, Mass spectrometric analysis of ptaquiloside the toxic sesquiterpene from bracken fern, Rapid Commun. Mass Spectrom. 18 (2004) 825–828.
[43] P.H. Jensen, O.S. Jacobsen, H.C.B. Hansen, R.K. Juhler, Quantification of ptaquiloside and pterosin B in soil and groundwater using liquid chromatography–tandem mass spectrometry (LC–MS/MS), J. Agric. Food Chem. 56 (2008) 9848–9854.
[44] F. Bonadies, G. Berardi, R. Nicoletti, F.S. Romolo, F. De Giovanni, R. Marabelli, A. Santoro, C. Raso, A. Tagarelli, F. Roperto, V. Russo, S. Roperto, A new very sensitive method of assessment of ptaquiloside, the major bracken carcinogen, in the milk of farm animals, Food Chem. 124 (2011) 660–665.
[45] M.E. Alonso-Amelot, S. Rodulfo-Baechler, R. Jaimes-Espinoza, Comparative dynamics of ptaquiloside and pterosin B in the two varieties (caudatum and arachnoideum) of neotropical bracken fern (*Pteridium aquilinum* L. Kuhn), Biochem. Syst. Ecol. 23 (1995) 709–716.
[46] B.L. Smith, A.A. Seawright, J.C. Ng, A.T. Hertle, J.A. Thomson, P.D. Bostock, Concentration of ptaquiloside, a major carcinogen in bracken fern (*Pteridium* spp.) from eastern Australia and from a cultivated worldwide collection herd in Sidney, Aust. Nat. Toxins 2 (1994) 347–353.
[47] Y. Hayashi, M. Nishizawa, T. Sakan, Structure of hypacrone a novel secoilludoid, possible biological precursor of pterosins in Hypolepis punctata Mett, Chem. Lett. 2 (1973) 63–66.
[48] K. Koyama, S. Takatsuki, S. Natori, A. Dennstoside, and analogue of ptaquiloside from Dennstaedtia scabra, Phytochemistry 30 (1991) 2080–2082.
[49] K. Saito, T. Nagao, S. Takatsuki, K. Koyama, S. Natori, The sesquiterpenoid carcinogen of Bracken fern, and some analogues from the Pteridaceae, Phytochemistry 29 (1990) 1475–1479.
[50] U.F. Castillo, A.L. Wilkins, D.R. Lauren, B.L. Smith, N.R. Towers, M.E. Alonso-Amelot, J. Jaimes-Espinoza, Isoptaquiloside and caudatoside illudane-type sesquiterpene glucosides from *Pteridium aquilinum* var. caudatum, Phytochemistry 44 (1997) 901–906.
[51] U.F. Castillo, M. Ojika, M.E. Alonso-Amelot, Y. Sakagami, Z. Ptaquiloside, a new instable sesquiterpene glucoside from the neotropical bracken fern *Pteridium aquilinum* var. caudatum, Bioorgan. Med. Chem. 6 (1998) 2229–2233.
[52] U. Castillo, Y. Sakagami, M.E. Alonso-Amelot, M. Ojika, Pteridanoside, the first protoilludane sesquiterpene glucoside as a toxic component of the neotropical bracken fern *Pteridium aquilinum* var. caudatum, Tetrahedron 55 (1999) 12295–12300.
[53] M-M. Li, K. Wang, Z-H. Pan, X-Q. Chen, L-Y. Peng, Y. Li, X. Cheng, Q-S. Zhao, Two new sesquiterpene glucosides from Dennstaedtia scabra (Wall.) Moore, Chem. Pharm. Bull. 57 (2009) 1123–1125.
[54] M.T. Fletcher, P.Y. Hayes, M.J. Somerville, J.J. de Voss, Ptesculentoside, a novel sesquiterpene glucoside from the Australian bracken fern *Pteridium esculentum*, Tetrahedron Lett. 51 (2010) 1997–1999.
[55] M.T. Fletcher, I.J. Brock, K.G. Reichmann, R.A. McKenzie, B.J. Blaney, Norsesquiterpene glycosides in bracken ferns (*Pteridium esculentum* and *Pteridium aquilinum* subsp. wightianum) from eastern Australia: reassessed poisoning risk to animals, J. Agric. Food Chem. 59 (2011) 5133–5138.
[56] M. Ojika, K. Wakamatsu, H. Niwa, K. Yamada, I. Hirono, Isolation and structures of two new p-hydroxystyrene glycosides ptelatoside-A and ptelatoside-B from bracken *Pteridium aquilinum* var. latiusculum and synthesis of ptelatoside-A, Chem. Lett. 3 (1984) 397–400.
[57] I. Hirono, Carcinogenic principles isolated from bracken fern, Crit. Rev. Toxicol. 17 (1986) 1–22.
[58] M.E. Alonso-Amelot, A. Oliveros-Bastidas, Kinetics of the natural evolution of hydrogen cyanide in plants of neotropical *Pteridium arachnoideum* and its ecological significance, J. Chem. Ecol. 31 (2005) 315–331.

[59] A.J. Oliveros-Bastidas, M.E. Alonso-Amelot, Cyanogenic polymorphism in brackens. *Pteridium arachnoideum* and *P. caudatum*, from the northern Andes, Quim. Nova 33 (2010) 1520–1524.
[60] M.E. Alonso-Amelot, A. Oliveros, A method for the practical quantification and kinetic evaluation of cyanogenesis in plant material. Application to *Pteridium aquilinum* and *Passiflora capsularis*, Phytochem. Anal. 11 (2000) 309–316.
[61] R.M. Gil da Costa, M.M.S.M. Bastos, P.A. Oliveira, C. Lopes, Isolation of carcinogenic and cyanogenic bracken (*Pteridium aquilinum*) constituents from mainland Portugal specimens, in: Proceedings of the 9th European Congress of Toxicological Pathology, Uppsala, Sweden, 2011, p. 233.
[62] M.E. Alonso-Amelot, The chemistry and toxicology of bioactive compounds in bracken fern (*Pteridium* spp.) with special reference to chemical ecology and carcinogenesis, Stud. Nat. Prod. Chem. 26 (2002) 685–739.
[63] F. Imperato, Flavonol glycosides from *Pteridium aquilinum*, Phytochemistry 40 (1995) 1801–1802.
[64] F. Imperato, Kaempferol 3-o-(5″-feruloylapioside) from *Pteridium aquilinum*, Phytochemistry 43 (1996) 1421–1423.
[65] F. Imperato, Kaempferol 7-o-rhamnoside-4′-o-glycoside from *Pteridium aquilinum*, Phytochemistry 47 (1998) 911–913.
[66] G.A. Cooper-Driver, S. Finch, T. Swain, Seasonal variation in secondary plant compounds in relation to the palatability of *Pteridium aquilinum*, Biochem. Syst. Ecol. 5 (1977) 177–183.
[67] M.N. Sebastian, S.I. Baskin, S.E. Czerwinski, Renal toxicity, in: R.C. Gupta (Ed.), Veterinary Toxicology: Basic and Clinical Principles, Academic Press, New York, 2007, pp. 171–172.
[68] M.E. Alonso-Amelot, A. Oliveros, M.P. Calcagno, Phenolics and condensed tannins in relation to altitude in neotropical *Pteridium* spp. A field study in the Venezuelan Andes, Biochem. Syst. Ecol. 32 (2004) 969–981.
[69] A.M. Pamucku, W. Ching-Yung, J. Hatcher, G.T. Bryan, Carcinogenicity of tannin and tannin-free extracts of bracken fern, J. Natl. Cancer Inst. 65 (1980) 131–136.
[70] M.E. Alonso-Amelot, A. Oliveros, M.P. Calcagno, E. Arellano, Bracken adaptation mechanisms and xenobiotic chemistry, Pure Appl. Chem. 73 (2001) 549–553.
[71] J. Méndez, Dihydrocinnamic acids in *Pteridium aquilinum*, Food Chem. 93 (2005) 251–252.
[72] I. Hirono, K. Fushimi, N. Matsubara, Carcinogenicity test of shikimic acid in rats, Toxicol. Lett. 1 (1977) 9–10.
[73] I.A. Evans, M.A. Osman, Carcinogenicity of bracken and shikimic acid, Nature 250 (1974) 348–349.
[74] A.J. Ngomuo, R.S. Jones, Genotoxicity studies of quercetin and shikimate in vivo in the bone marrow of mice and gastric mucosal cells of rats, Vet. Hum. Toxicol. 38 (1996) 176–180.
[75] P. Meyer, Thiaminase activity and thiamine content of *Pteridium aquilinum*, Equisetum ramonissimum. *Malva parviflora*, *Pennisetum clandestinum* and *Mendicago sativa*, Onderstepoort J. Vet. Res. 56 (1989) 145–146.
[76] T. Saito, D. Mochizuki, Isolation of two active glucosides braxin A1 and A2, from rhizomes of bracken fern, J. Toxicol. Sci. 11 (1986) 15–27.
[77] T. Saito, K. Takeno, S. Nakamura, M. Uehara, Acute bracken poisoning with braxin A1 a bracken glucoside, in Guinea pigs, Jpn. J. Vet. Sci. 49 (1987) 181–183.
[78] M. Yoshida, T. Saito, Acute toxicity of braxin C a bracken toxin, in Guinea pigs, J. Toxicol. Sci. 19 (1994) 17–23.
[79] M. Yoshida, T. Saito, Non-urotoxic induction of haemorrhagic cystitis by braxin C a bracken toxin, in Guinea pigs, J. Toxicol. Sci. 19 (1994) 55–59.
[80] R.F.S. Tjatur, T. Saito, H. Satoh, The haemolytic activity of bracken extracts on Guinea pigs, J. Vet. Med. Sci. 61 (1999) 129–133.
[81] N. Culpeper, The English Physician or an Astrologo-Physical Discourse of the Vulgar Herbs of this Nation, William Bentley, London, 1652.
[82] M. Shahin, B.L. Smith, A.S. Prakash, Bracken carcinogens in the human diet, Mutat. Res. 443 (1999) 69–79.
[83] J. Vetter, Toxicological medicinal aspects of the most frequent fern species, *Pteridium aquilinum* (L.) Kuhn, in: A. Kumar, H. Fernandez, M.A. Revilla (Eds.), Working with Ferns: Issues and Applications, Springer, New York, 2010, pp. 361–375.
[84] I.A. Evans, D.J. Humphreys, I. Goulden, A.J. Thomas, W.C. Evans, Effect of bracken rhizomes on the pig, J. Comp. Pathol. Ther. 73 (1963) 229–243.
[85] B.L. Smith, C.A. Pinto, Bracken (genus *Pteridium*) and its carcinogen, ptaquiloside as a worldwide cause of animal health problems, in: Proceedings of the International Symposium Intractable Weeds & Plant Invaders, University of the Azores, Ponta Delgada, 2006, pp. 13–14.
[86] W.C. Evans, Bracken thiaminase-mediated neurotoxic syndromes, Bot. J. Linn. Soc. 73 (1976) 113–131.
[87] H.J. Bakker, J. Dickson, P. Steele, M.C. Nottle, Experimental induction of ovine polyoencephalomalatia, Vet. Rec. 107 (1980) 464–466.
[88] M.E. Alonso-Amelot, M. Avendaño, Human carcinogenesis and bracken fern: a review of the evidence, Curr. Med. Chem. 9 (2002) 675–686.
[89] E. Fernandes, K. Orita, Bracken as a risk factor in digestive tract tumours: state of the art, Ann. Ist. Super. Sanita 27 (1991) 275–280.
[90] L.R. Xu, Bracken poisoning and enzootic haematuria in cattle in China, Res. Vet. Sci. 53 (1992) 116–121.
[91] F.M. Sunderman, Bracken poisoning in sheep, Aust. Vet. J. 64 (1987) 25.
[92] C.A. Pinto, M.C. Peleteiro, M.A. Lobo, J. Machado, L. Silva, Intoxicação aguda pelo feto comum [*Pteridium aquilinum* (L.) Kühn] em bovinos, Rev. Port. Ciênc Vet. 102 (2007) 289–298.
[93] J.M. Naftalin, G.H. Cushnie, Haematology of experimental bracken poisoning of cattle. I. Changes in blood and bone marrow. II. Attempts to modify the course of the bone marrow damage, J. Comp. Pathol. 66 (1956) 354–372.
[94] F.E. Moon, M.A. Rafat, The experimental production of bracken poisoning in sheep, J. Comp. Pathol. 61 (1951) 88–100.
[95] I. Hirono, Y. Kono, K. Takahashi, K. Yamada, H. Niwa, M. Ojika, H. Kigoshi, K. Niiyama, Y. Uosaki, Reproduction of acute bracken poisoning in a calf with ptaquiloside a bracken constituent, Vet. Rec. 115 (1984) 375–378.
[96] K.C. Barnett, W.F. Blakemore, J. Mason, Bracken retinopathy in sheep, Trans. Ophtal. Soc. UK 92 (1972) 741–744.
[97] W.A. Watson, K.C. Barnett, S. Terlecki, Progressive retinal degeneration (bright blindness) in sheep: a review, Vet. Rec. 91 (1972) 665.
[98] W.A. Watson, S. Terlecki, D.S. Patterson, D. Sweasey, C.N. Herbert, J.T. Done, Experimentally produced progressive retinal degeneration (bright blindness) in sheep, Br. Vet. J. 128 (1972) 457–469.
[99] I. Hirono, M. Ito, S. Yagyu, M. Haga, K. Wakamatsu, T. Kishikawa, O. Nishikawa, K. Yamada, M. Ojika, H. Kigoshi, Reproduction of progressive retinal degeneration (bright blindness) in sheep by administration of ptaquiloside contained in bracken, J. Vet. Med. Sci. 55 (1993) 979–983.
[100] Y. Yasuda, T. Kihara, H. Nishimura, Embryotoxic effects of feeding bracken fer (*Pteridium aquilinum*) to pregnant mice, Toxicol. Appl. Pharm. 28 (1974) 264–268.
[101] M. Fujimoto, H. Ogino, I. Hirono, Influence of ptaquiloside on the development of newborn mice, J. Toxicol. Sci. 12 (1987) 135–145.
[102] L.P. Bignold, Initiation of genetic instability and tumour formation: a review and hypothesis of a non-genotoxic mechanism, Cell. Mol. Life Sci. 60 (2003) 1107–1117.
[103] L.P. Bignold, B.L. Coghlan, H.P. Jersmann, Cancer morphology carcinogenesis and genetic instability: a background, Experientia 96 (2006) 1–24.
[104] P.A. Oliveira, A. Colaço, R. Chaves, H. Guedes-Pinto, P.L.F. de la Cruz, C. Lopes, Chemical carcinogenesis, An. Acad. Bras. Ciênc. 79 (2007) 593–616.
[105] C.A. Iacobuzio-Donahue, Epigenetic changes in cancer, Annu. Rev. Pathol. 4 (2009) 229–249.
[106] G. Rosenberger, W. Heeschen, Adler-farn (*Pteris aquilina*) – die Ursache des Sog. Stalrotes der Rinder, Deut. Tierärtz Woch. 67 (1960) 201–208.
[107] G. Rosenberger, Längere Aufnahme von Adlerfarn (*Pteris aquilina*) – die Ursache der chronischen vesikalen Haematurie des Rindes, Wien Tierärztl Monat. 52 (1965) 415–421.
[108] I.A. Evans, J. Mason, Carcinogenic activity of bracken, Nature 208 (1965) 913–914.
[109] A.M. Pamucku, J.M. Price, Induction of intestinal and urinary bladder cancer in rats by feeding bracken fern (*Pteris aquilina*), J. Natl. Cancer Inst. 43 (1969) 275–281.
[110] J.M. Price, A.M. Pamucku, The induction of neoplasms of the urinary bladder of the cow and the small intestine of the rat by feeding bracken fern (*Pteris aquilina*), Cancer Res. 28 (1968) 2247–2251.
[111] A.M. Pamucku, S.K. Goksoy, J.M. Price, Urinary bladder neoplasms induced by feeding bracken fern (*Pteris aquilina*) to cows, Cancer Res. 27 (1967) 917–924.
[112] I.A. Evans, The radiomimetic nature of bracken toxin, Cancer Res. 28 (1968) 2252–2261.
[113] A.M. Pamucku, S. Yalciner, J.M. Price, G.T. Bryan, Effects of the coadministration of thiamine on the incidence of urinary bladder carcinomas in rats fed bracken fern, Cancer Res. 30 (1970) 2671–2674.
[114] A.M. Pamucku, E. Erturk, J.M. Price, G.T. Bryan, Lymphatic leukaemia and pulmonary tumors in female Swiss mice fed bracken fern (*Pteris aquilina*), Cancer Res. 32 (1972) 1442–1445.
[115] I. Hirono, Human carcinogenic risk in the use of bracken fern, in: Y. Hayashi (Ed.), Diet, Nutrition and Cancer, Japan Science Society Press, Tokyo, 1986, pp. 139–145.
[116] A.M. Pamucku, E. Erturk, S. Yalciner, U. Milli, G.T. Bryan, Carcinogenic mutagenic activities of milk from cows fed bracken fern (*Pteridium aquilinum*), Cancer Res. 38 (1978) 1556–1560.
[117] I. Hirono, K. Yamada, H. Niwa, Y. Shizuri, M. Ojika, S. Hosaka, T. Yamaji, K. Wakamasa, H. Kigoshi, K. Niiyama, Y. Uosaki, Separation of carcinogenic fraction from bracken, Cancer Lett. 21 (1984) 239–246.
[118] I. Hirono, S. Aiso, S. Hosaka, T. Yamaji, M. Haga, Induction of mammary cancer in CD rats fed bracken diet, Carcinogenesis 4 (1983) 885–887.
[119] I. Hirono, S. Hosaka, K. Kuhara, Enhancement by bracken of induction of tumors of the upper alimentary tract by N-propyl-N-nitrosourethan, Br. J. Cancer 46 (1982) 423–427.
[120] P-P. Bringuier, E. Piaton, N. Berger, F. Debruyne, P. Perrin, J. Schalken, M. Devonec, Bracken fern-induced bladder tumors in Guinea pigs. A model for human neoplasia, Am. J. Pathol. 147 (1995) 858–868.
[121] R.K. Dawra, N.P. Kurade, O.P. Sharma, Carcinogenicity of the fern *Pteridium aquilinum* collected from enzootic bovine haematuria-free hilly area in India, Curr. Sci. 83 (2002) 1005–1009.
[122] I. Hirono, S. Aiso, T. Yamaji, H. Mori, K. Yamada, H. Niwa, M. Ojika, K. Wakamatsu, H. Kigoshi, K. Niiyama, Y. Uosaki, Carcinogenicity in rats of ptaquiloside isolated from bracken, Jpn. J. Cancer Res. (Gann) 75 (1984) 833–836.
[123] A.M. Pamucku, S. Yalciner, J.F. Hatcher, G.T. Bryan, Quercetin a rat intestinal and bladder carcinogen present in bracken fern (*Pteridium aquilinum*), Cancer Res. 40 (1980) 3468–3472.
[124] H. Takanashi, S. Aiso, I. Hirono, T. Matsushima, T. Sugimura, Carcinogenicity test of quercetin and kaempferol in rats by oral administration, J. Food Saf. 5 (1983) 55–60.

[125] M. Ojika, K. Wakamatsu, H. Niwa, K. Yamada, Ptaquiloside a potent carcinogen isolated from bracken fern *Pteridium aquilinum* var. latiusculum: structure elucidation based on chemical and spectral evidence and reactions with amino acids nucleosides and nucleotides, Tetrahedron 43 (1987) 5261–5274.
[126] T. Kushida, M. Uesugi, Y. Sugiura, H. Kigoshi, H. Tanaka, J. Hirokawa, M. Ojika, K. Yamada, DNA damage by ptaquiloside a potent bracken carcinogen: detection of selective strand breaks and identification of DNA cleavage products, J. Am. Chem. Soc. 116 (1994) 479–486.
[127] S.E. Simán, A.C. Povey, T.H. Ward, G.P. Margison, E. Sheffield, Fern spore extracts can damage DNA, Br. J. Cancer 83 (2000) 69–73.
[128] L.O. Pereira, L.S. Bicalho, L.M. Campos da Paz, T.M. de Sousa, S.N. Báo, M.F.M. Almeida Santos, M.J. Fonseca, DNA damage and apoptosis induced by *Pteridium aquilinum* aqueous extract in the oral cell lines HSG and OSCC-3, J. Oral Pathol. Med. 38 (2009) 441–447.
[129] R.M. Gil da Costa, P. Coelho, R. Sousa, M.M.S.M. Bastos, B. Porto, J.P. Teixeira, I. Malheiro, C. Lopes, Genotoxicity/clastogenicity of ptaquiloside, the bracken (*Pteridium aquilinum*) carcinogen, towards human peripheral blood lymphocytes, in: Proceedings of the 21st Meeting of the European Association for Cancer Research, Oslo, 2010, p. 219.
[130] B.L. Smith, G. Shaw, A.S. Prakash, A.A. Seawright, Studies in DNA adduct formation by ptaquiloside, the carcinogen of bracken ferns (*Pteridium* spp.), in: S.M. Colgate, P.R. Dorling (Eds.), Plant Associated Toxins, CAB International, Wallingford, 1994, pp. 167–172.
[131] A.S. Prakash, T.N. Pereira, B.L. Smith, G. Shaw, A.A. Seawright, Mechanism of bracken fern carcinogenesis: evidence for H-ras activation via initial adenine alkylation by ptaquiloside, Nat. Toxins 4 (1996) 221–227.
[132] M. Shahin, B.L. Smith, S. Worran, M.R. Moore, A.A. Seawright, A.S. Prakash, Bracken fern carcinogenesis: multiple intravenous doses of activated ptaquiloside induce DNA adducts monocytosis, increased TNFα levels and mammary gland carcinoma in rats, Biochem. Biophys. Res. Commun. 244 (1998) 192–197.
[133] A.C. Povey, I.A. Evans, J.A. Taylor, P.J. O'Connor, Detection of DNA adducts by 32P-postlabelling in mice treated with bracken extract and bracken spores, in: R.T. Smith, J.R. Taylor (Eds.), Bracken: an Environmental Issue, International Bracken Group Occasional Publication, Aberystwyth, 1994, pp. 95–98.
[134] A.C. Povey, D. Potter, P.J. O'Connor, 32P-postlabelling analysis of DNA adducts formed in the upper gastrointestinal tissue of mice fed bracken extract or bracken spores, Br. J. Cancer 74 (1996) 1342–1348.
[135] R.N. Freitas, P.J. O'Connor, A.S. Prakash, M. Shahin, A.C. Povey, Bracken (*Pteridium aquilinum*)-induced DNA adducts in mouse tissues are different from the adduct induced by the activated form of the bracken carcinogen ptaquiloside, Biochem. Biophys. Res. Commun. 281 (2001) 589–594.
[136] M. Shahin, M.R. Moore, S. Worran, B.L. Smith, A.A. Seawright, A.S. Prakash, H-ras activation is an early event in the ptaquiloside-induced carcinogenesis: comparison of acute and chronic toxicity in rats, Biochem. Biophys. Res. Commun. 250 (1998) 491–497.
[137] H. Mori, S. Sugie, I. Hirono, K. Yamada, H. Niwa, M. Ojika, K. Wakamatsu, H. Kigoshi, Genotoxicity of ptaquiloside a bracken carcinogen, in the hepatocyte primary culture/DNA-repair test, Mutat. Res. 143 (1985) 75–78.
[138] T. Nagao, K. Saito, E. Hirayama, K. Uchikoshi, K. Koyama, S. Natori, N. Morisaki, S. Iwasaki, T. Matsushima, Mutagenicity of bracken the carcinogen in bracken, and its related illudane-type sesquiterpenes. Mutagenicity testing in *Salmonella typhimurium*, Mutat. Res. 215 (1989) 173–178.
[139] A. Matsuoka, A. Hirosawa, S. Natori, S. Iwasaki, T. Sofuni, M. Ishidate, Mutagenicity of ptaquiloside the carcinogen in bracken, and its related illudane-type sesquiterpenes II. Chromosomal aberration tests with cultured mammalian cells, Mutat. Res. 215 (1989) 179–185.
[140] B. Schmidt, L.H. Rasmussen, G.W. Svendsen, F. Ingerslev, H.C. Hansen, Genotoxic activity and inhibition of soil respiration by ptaquiloside a bracken fern carcinogen, Environ. Toxicol. Chem. 24 (2005) 2751–2756.
[141] I. Hirono, H. Ogino, M. Fujimoto, K. Yamada, Y. Yoshida, M. Ykagawa, M. Okumura, Induction of tumors in ACI rats given a diet containing ptaquiloside a bracken carcinogen, J. Natl. Cancer Inst. 79 (1987) 1143–1149.
[142] R.M. Gil da Costa, P.A. Oliveira, M. Vilanova, M.M.S.M. Bastos, C.C. Lopes, C. Lopes, Ptaquiloside-induced B-cell lymphoproliferative and early-stage urothelial lesions in mice, Toxicon 58 (2011) 543–549.
[143] R.M. Gil da Costa, P.A. Oliveira, M. Vilanova, M.M.S.M. Bastos, C.C. Lopes, C. Lopes, Ptaquiloside from bracken (*P. aquilinum*) induces a B-cell lymphoproliferative malignancy and urothelial dysplasia in mice, in: Proceedings of the 9th European Congress of Toxicological Pathology, Uppsala, 2011, p. 232.
[144] A.O. Latorre, M.S. Furlan, M. Sakai, H. Fukumasu, I.M. Hueza, M. Haraguchi, S.L. Górniak, Immunomodulatory effects of *Pteridium aquilinum* on natural killer cell activity and on select aspects of the cellular immune response of mice, J. Immunotoxicol. 6 (2009) 104–114.
[145] A.O. Latorre, B.D. Caniceiro, H.L. Wysoki Jr., M. Haraguchi, D.R. Gardner, S.L. Górniak, Selenium reverses *Pteridium aquilinum*-induced immunotoxic effects, Food Chem. Toxicol. 49 (2011) 464–470.
[146] M.S. Campo, M.H. Moar, W.F.H. Jarrett, H.M. Laird, A new papillomavirus associated with alimentary cancer in cattle, Nature 286 (1980) 180–182.
[147] E. Tsirimonaki, B.W. O'Neil, M.S. Campo, Extensive papillomatosis of the bovine upper gastrointestinal tract, J. Comp. Pathol. 129 (2003) 93–99.
[148] E. Marrero, C. Bulnes, L.M. Sánchez, I. Palenzuela, R. Stuart, F. Jacobs, J. Romero, *Pteridium aquilinum* (bracken fern) toxicity in the humid Chaco of Tarija, Bolivia, Vet. Hum. Toxicol. 43 (2001) 156–158.
[149] G. Borzacchiello, V. Ambrosio, S. Roperto, F. Poggiali, E. Tsirimonaki, A. Venuti, M.S. Campo, F. Roperto, Bovine papillomavirus type 4 in oesophageal carcinomas of cattle from the South of Italy, J. Comp. Pathol. 128 (2003) 203–206.
[150] G. Borzacchiello, F. Roperto, Bovine papillomaviruses, papillomas and cancer in cattle, Vet. Res. 39 (2008) 45.
[151] R.B. Lucena, D.R. Rissi, G.D. Kommers, F. Pierezan, J.C. Oliveira-Filho, J.T. Macedo, M.M. Flores, C.S. Barros, A retrospective study of 586 tumours in Brazilian cattle, J. Comp. Pathol. 145 (2011) 20–24.
[152] J.A. Conolly, I.M. Morgan, M.E. Jackson, M.S. Campo, The BPV-4 co-carcinogen quercetin induces cell cycle arrest and up-regulates transcription from the LCR of BPV-4, Oncogene 16 (1998) 2739–2746.
[153] R.G. Beniston, I.M. Morgan, V. O'Brien, M.S. Campo, Quercetin, E7 and p53 in papillomavirus oncogenic transformation, Carcinogenesis 22 (2001) 1069–1076.
[154] R.G. Beniston, M.S. Campo, HPV-18 transformed cells fail to arrest in G1 in response to quercetin treatment, Virus Res. 109 (2005) 203–209.
[155] W.D. Pennie, M.S. Campo, Synergism between bovine papillomavirus type 4 and the flavonoid quercetin in cell transformation in vitro, Virology 190 (1992) 861–865.
[156] A. Rahman, F. Fazal, J. Greensill, K. Ainley, J.H. Parish, S.M. Hadi, Strand scission in DNA induced by dietary flavonoids: role of Cu(I) and oxygen free radicals and biological consequences of scission, Mol. Cell. Biochem. 111 (1992) 3–9.
[157] D. Utesch, K. Feige, J. Dasenbrock, T.H. Broschard, M. Harwood, B. Danielewska-Nikiel, T.C. Lines, Evaluation of the potential in vivo genotoxicity of quercetin, Mutat. Res. 654 (2008) 38–44.
[158] B.T. Zhu, E.L. Ezell, J.G. Liehr, Catechol-o-methyltransferase catalyzed rapid o-methylation of mutagenic flavonoids. Metabolic inactivation as a possible reason for their lack of carcinogenicity in vivo, J. Biol. Chem. 269 (1994) 292–299.
[159] A.M. Leal, O.P. Ferraz, C. Carvalho, A.C. Freitas, R.G. Beniston, M.S. Campo, W. Beçak, R.C. Stocco dos Santos, Quercetin induces structural chromosome aberrations and uncommon rearrangements in cells transformed by the E7 protein of BPV-4, Vet. Comp. Oncol. 1 (2003) 15–21.
[160] M. Harwood, B. Danielewska-Nikiel, J.F. Borzelleca, G.W. Flamm, G.M. Williams, T.C. Lines, A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity including lack of genotoxic/carcinogenic properties, Food Chem. Toxicol. 45 (2007) 2179–2205.
[161] L. Scobie, M.E. Jackson, M.S. Campo, The role of exogenous p53 and E6 oncoproteins in in vitro transformation by bovine papillomavirus type 4 (BPV-4): significance of the absence of an E6 ORF in the BPV-4 genome, J. Gen. Virol. 78 (1997) 3001–3008.
[162] A. Di Loria, D. Piantedosi, L. Cortese, S. Roperto, C. Urraro, O. Paciello, J. Guccione, D. Britti, P. Ciaramella, Clotting profile in cattle showing chronic enzootic haematuria (CEH) and bladder neoplasms, Res. Vet. Sci., in press, doi:10.1016/j.rvsc.2011.07.011.
[163] R. Somvanshi, Papillomatosis in buffaloes: a less-known disease, Transbound. Emerg. Dis. 58 (2011) 327–332.
[164] M.B. Lioi, R. Barbieri, G. Borzacchiello, S. Dezzi, S. Roperto, A. Santoro, V. Russo, F. Roperto, Chromosome aberrations in cattle with chronic enzootic haematuria, J. Comp. Pathol. 13 (2004) 1233–1236.
[165] C. Pinto, R. Lima, A.C. Louzã, V. Almeida, M. Melo, Y. Vaz, I. Neto Fonseca, D.R. Lauren, B.L. Smith, Bracken fern induced bovine enzootic haematuria in São Miguel Island, Azores, in: J.A. Taylor, R.T. Smith (Eds.), Bracken Fern: Toxicity, Biology and Control, International Bracken Group Occasional Publication, Aberystwyth, 2000, pp. 136–140.
[166] C.A. Pinto, A.C. Louzã, V. Almeida, M. Melo, Y. Vaz, M.C. Peleteiro, B.L. Smith, Caracterização epidemiológica da ocorrência de tumores da bexiga em populações de bovinos leiteiros da ilha de São Miguel, Rev. Port. Ciênc Vet. 537 (2001) 11–19.
[167] C. Pinto, T. Januário, M. Geraldes, J. Machado, D.R. Lauren, B.L. Smith, R.C. Robinson, Bovine enzootic haematuria on São Miguel Island – Azores, in: T. Akamovic, C.S. Stewart, T.W. Pennycott (Eds.), Poisonous Plants and Related Toxins, CABI Publishing, Wallingford, 2004, pp. 564–574.
[168] G. Borzacchiello, V. Ambrosio, P. Galati, F. Poggiali, A. Venuti, F. Roperto, The pagetoid variant of urothelial carcinoma in situ of urinary bladder in a cow, Vet. Pathol. 38 (2001) 113–116.
[169] G. Borzacchiello, V. Ambrosio, P. Galati, A. Perillo, F. Roperto, Cyclooxigenase-1 and -2 expression in urothelial carcinomas of the urinary bladder in cows, Vet. Pathol. 40 (2003) 455–459.
[170] G. Borzacchiello, G. Iovane, M.L. Marcante, F. Poggiali, F. Roperto, S. Roperto, A. Venuti, Presence of bovine papillomavirus type 2 DNA and expression of the viral oncoprotein E5 in naturally occurring urinary bladder tumours in cows, J. Gen. Virol. 84 (2003) 2921–2926.
[171] D. Sardon, I. de la Fuente, E. Calonge, M.D. Perez-Alenza, M. Castaño, S. Dunner, L. Peña, H-ras immunohistochemical expression and molecular analysis of urinary bladder lesions in grazing adult cattle exposed to bracken fern, J. Comp. Pathol. 132 (2005) 195–201.
[172] T. Carvalho, C. Pinto, M.C. Peleteiro, Urinary bladder lesions in bovine enzootic haematuria, J. Comp. Pathol. 134 (2006) 336–346.
[173] T. Carvalho, A.P. Elias, T. Nunes, M.C. Peleteiro, S. Dias, Chemo-angiogenic profile of bovine urinary bladder tumors distinguishes urothelial carcinomas from hemangiossarcomas, Vet. Immunol. Immunopathol. 121 (2007) 344–358.
[174] S. Roperto, G. Borzacchiello, R. Casellato, P. Galati, V. Russo, S. Sonnino, F. Roperto, Sialic acid and GM3 ganglioside expression in papillomavirus-associated

urinary bladder tumours of cattle with chronic enzootic haematuria, J. Comp. Pathol. 137 (2007) 87–93.
[175] R. Brun, C. Urraro, C. Medaglia, V. Russo, G. Borzacchiello, F. Roperto, S. Roperto, Lymphoepithelioma-like carcinoma of the urinary bladder in a cow associated with bovine papillomavirus type-2, J. Comp. Pathol. 139 (2008) 121–125.
[176] E. Guidi, C. Uboldi, L. Ferreti, Molecular analysis of the fragile histidine tiad (FHIT) tumor suppressor gene in vesical tumors of cattle with chronic enzootic hematuria, Cytogenet. Genome Res. 120 (2008) 173–177.
[177] A. Corteggio, C. Urraro, S. Roperto, F. Roperto, G. Borzacchiello, Phosphatidylinositol-3-kinase-AKT pathway, phospho-JUN and phospho-JNK expression in spontaneously arising bovine urinary bladder tumours, J. Comp. Pathol. 143 (2010) 173–178.
[178] I. Pires, F. Silva, F.L. Queiroga, P. Rodrigues, R. Henriques, C.A. Pinto, C. Lopes, Epithelioid hemangiosarcomas of the bovine urinary bladder: a histologic immunohistochemical and ultrastructural examination of four tumours, J. Vet. Diagn. Invest. 22 (2010) 116–119.
[179] S. Roperto, R. De Tullio, C. Raso, R. Stifanese, V. Russo, M. Gaspari, G. Borzacchiello, M. Averna, O. Paciello, G. Cuda, F. Roperto, Calpain 3 is expressed in a proteolitically active form in papillomavirus-associated urothelial tumors of the urinary bladder in cattle, PloS One 22 (2010) 5.
[180] A. Corteggio, J. Florio, F.Roperto, G. Borzacchiello, Expression of gap junction protein connexin 43 in bovine urinary bladder tumours, J. Comp. Pathol. 144 (2011) 86–90.
[181] M.S. Campo, W.F.H. Jarrett, R. Barron, B.W. O'Neil, K.T. Smith, Association of bovine papillomavirus type 2 and bracken fern with bladder cancer in cattle, Cancer Res. 52 (1992) 6898–6904.
[182] A.R. Resendes, S. Roperto, F. Trapani, C. Urraro, A. Rodrigues, F. Roperto, G. Borzacchiello, Association of bovine papillomavirus type 2 (BPV-2) and urinary bladder tumours in cattle from the Azores archipelago, Res. Vet. Sci. 90 (2011) 526–529.
[183] L.G. Balcos, G. Borzacchiello, V. Russo, O. Popescu, S. Roperto, F. Roperto, Association of Bovine papillomavirus type-2 and urinary bladder tumours in cattle from Romania, Res. Vet. Sci. 85 (2008) 145–148.
[184] D. DiMaio, D. Mattoon, Mechanisms of cell transformation by papillomavirus E5 proteins, Oncogene 20 (2001) 7866–7873.
[185] G. Borzacchiello, V. Russo, F. Gentile, F. Roperto, A. Venuti, L. Nitsch, M.S. Campo, S. Roperto, Bovine papillomavirus E5 oncoprotein binds to the activated form of the platelet-derived growth factor β receptor in naturally occurring bovine urinary bladder tumours, Oncogene 25 (2006) 1251–1260.
[186] C.A. Pinto, Hematúria enzoótica bovina: contribuição para o seu estudo etiopatogénico, PhD Thesis, Faculty of Veterinary Medicine, Technical University of Lisbon, Lisbon, Portugal, 2010.
[187] H. Sanderson, H.D.V. Prendergast, Commercial Uses of Wild and Traditionally Managed Plants in England and Scotland, Centre for Economic Botany, Royal Botanic Gardens, Kew, 2002, pp. 53–56.
[188] D.M. Potter, R.M. Pitman, The extraction and characterisation of carcinogens from bracken and the effect of composting, in: R.T. Smith, J.A. Taylor (Eds.), Bracken: an Environmental Issue, International Bracken Group Occasional Publication, Aberystwyth, 1994, pp. 110–115.
[189] I. Hirono, Edible plants containing naturally occurring carcinogens in Japan, Jpn. J. Cancer Res. 84 (1993) 997–1006.
[190] C.A. Marlière, R.C. Santos, M.A.M. Galvao, J.F. Soares, Ingestão de broto de samambaia e risco de cancer de esófago e estômago na região de Ouro Preto, MG, Rev. Bras. Cancerol. 44 (1998) 225–229.
[191] N.S. Hojo-Souza, C.M. Carneiro, R.C. Santos, *Pteridium aquilinum*: what we know and what is yet to be learnt, Biosci. J. 26 (2010) 798–808.
[192] I. Hirono, C. Shibuya, M. Shimizu, K. Fushimi, Carcinogenic activity of processed bracken used as human food, J. Natl. Cancer Inst. 48 (1972) 1245–1250.
[193] R.C. Recouso, R.C. Stocco dos Santos, R. Freitas, R.C. Santos, A.C. d. Freitas, O. Brunner, W. Beçak, C.J. Lindsey, Clastogenic effect of bracken fern (*Pteridium aquilinum* v. arachnoideum) diet in peripheral lymphocyte of human consumers: preliminary data, Vet. Comp. Oncol. 1 (2003) 22–29.
[194] S. Kamon, T. Hirayama, Epidemiology of cancer of the oesophagus in Miye, Nara and Wakayama prefectures with special reference to the role of bracken fern, Proc. Jpn. Cancer Assoc. 34 (1975) 211.
[195] T. Hirayama, Diet and cancer, Nutr. Cancer 1 (1979) 67–81.
[196] L.M. Brown, S.H. Zahm, R.N. Hoover, J.F. Fraumeni, Bracken fern consumption and human bladder cancer, J. Epidemiol. Commun. Health 53 (1999) 653.
[197] G.R. Howes, J.D. Burch, A.B. Miller, G.M. Cook, J. Esteve, B. Morrison, P. Gordon, L.W. Chambers, G. Fodor, G.M. Winsor, Tobacco use occupation, coffee, various nutrients and bladder cancer, J. Natl. Cancer Inst. 64 (1980) 701–713.
[198] S.E. Simán, A.C. Povey, E. Sheffield, Human health risks from fern spores? A review, Fern Gaz. 15 (1999) 275–287.
[199] A.J. Wells, R. McNally, Appraisal of the spatial association of bracken and cancer in England and Wales, in: J.A. Taylor (Ed.), Bracken Toxicity and Carcinogenicity as Related to Animal and Human Health, International Bracken Group Special Publication, Aberystwyth, 1989.
[200] O.P. Galpin, C.J. Whitaker, R.H. Whitaker, J.Y. Kassab, Gastric cancer in Gwinned. Possible links with bracken, Br. J. Cancer 61 (1990) 737–740.
[201] J. Villalobos-Salazar, Carcinogenity of *Pteridium aquilinum* and high incidence of gastric cancer in Costa Rica, Rev. Costarric. Cienc. Méd. 6 (1985) 131–139.
[202] J. Villalobos-Salazar, J. Mora, A. Meneses, J.L. Rojas, A. Porras, M.V. Herrero, Bracken-derived carcinogens as affecting animal health and human health in Costa Rica, in: J.A. Taylor (Ed.), Bracken Toxicity and Carcinogenicity as Related to Animal and Human Health, International Bracken Group Occasional Publication, Aberystwyth, 1989, pp. 40–51.
[203] M.E. Alonso-Amelot, M. Avendaño, Possible association between gastric cancer and bracken fern in Venezuela: an epidemiologic study, Int. J. Cancer 91 (2001) 252–259.
[204] M.E. Alonso-Amelot, U. Castillo, B.L. Smith, D.R. Lauren, Bracken ptaquiloside in milk, Nature 382 (1996) 587.
[205] M.E. Alonso-Amelot, U. Castillo, B.L. Smith, D.R. Lauren, Excretion through milk, of ptaquiloside in bracken-fed cows. A quantitative assessment, Lait 78 (1998) 413–423.
[206] J. Villalobos-Salazar, H. Hernandez, A. Meneses, G. Salazar, Factors which may affect ptaquiloside levels in milk: effects of altitude, bracken fern growth stage and milk processing, in: J.A. Taylor, R.T. Smith (Eds.), Bracken Fern: Toxicity, Biology and Control, International Bracken Group Occasional Publication, Aberystwyth, 2000, pp. 68–75.
[207] L.R. Ferguson, M. Philpott, Nutrition and mutagenesis, Annu. Rev. Nutr. 28 (2008) 313–329.
[208] COT – Committee on the 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, Food Standards Agency, London, 2008.
[209] M.T. Fletcher, K.G. Reichmann, I.J. Brock, R.A. McKenzie, B.J. Blaney, Residue potential of norsesquiterpene glycosides in tissues of cattle fed austral bracken (*Pteridium esculentum*), J. Agric. Food Chem. 59 (2011) 8518–8523.
[210] L.H. Rasmussen, Ptaquiloside – an environmental hazard? Occurrence and fate of a bracken (Pteridium sp.) toxin in terrestrial environments, PhD Thesis, The Royal Veterinary and Agricultural University, Frederiksberg, 2003.
[211] L.H. Rasmussen, L.S. Jensen, H.C.B. Hansen, Distribution of the carcinogenic terpene ptaquiloside in Bracken fronds rhizomes (*Pteridium aquilinum*), and litter in Denmark, J. Chem. Ecol. 29 (2003) 771–778.
[212] L.H. Rasmussen, S. Kroghsbo, J.C. Frisvad, H.C.B. Hansen, Occurrence of the carcinogenic Bracken constituent ptaquiloside in fronds topsoils and organic soil layers in Denmark, Chemosphere 51 (2003) 117–127.
[213] L.H. Rasmussen, H.C.B. Hansen, D. Lauren, Sorption degradation and mobility of ptaquiloside, a carcinogenic bracken (*Pteridium* sp.) constituent, in the soil environment, Chemosphere 58 (2005) 823–835.
[214] K.B. Ayala-Luis, P.B. Hansen, L.H. Rasmussen, H.C. Hansen, Kinetics of ptaquiloside hydrolysis in aqueous solution, Environ. Toxicol. Chem. 25 (2006) 2623–2629.
[215] P. Engel, K.K. Brandt, L.H. Rasmussen, R.G. Ovesen, J.S. Ørensen, Microbial degradation and impact of bracken toxin ptaquiloside on microbial communities in soil, Chemosphere 67 (2007) 202–209.
[216] R.G. Ovesen, L.H. Rasmussen, H.C. Hansen, Degradation kinetics of ptaquiloside in soil and soil solution, Environ. Toxicol. Chem. 27 (2008) 252–259.
[217] C.T. Ramwell, W. van Beinum, A. Rowbothan, H. Parry, S.A. Parsons, W. Luo, G. Evans, Ptaquiloside and other bracken toxins: a preliminary risk assessment, Final Report, The Food and Environmental Research Agency, Sand Hutton, 2010.
[218] IARC – International Agency for Research on Cancer, Bracken fern (*Pteridium aquilinum*) and some of its constituents, Some Naturally Occurring and Synthetic food Components, Furocoumarins and Ultraviolet Radiation, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 40, supplement 7, International Agency for Research on Cancer, Lyon, 1986, p. 135.
[219] DECOS – Dutch Expert Committee on Occupational Standards, Scientific documentation on the Dutch list of occupational carcinogens (II), in: Bracken Fern: Kaempferol, Ptaquiloside, Quercetin and Shikimic Acid, Ministry of Social Affairs and Employment, Den Haag, 1995, pp. 14–23.
[220] D. Wilson, L.J. Donldson, O. Sepai, Should we be frightened of bracken? A review of the evidence, J. Epidemiol. Commun. Health 52 (1998) 812–817.
[221] D.M. Potter, M.S. Baird, Carcinogenic effects of ptaquiloside and related compounds, Br. J. Cancer 83 (2000) 914–920.
[222] C.C. Abnet, Carcinogenic food contaminants, Cancer Invest. 25 (2007) 189–196.
[223] J. Vetter, A biological hazard of our age: bracken fern (*Pteridium aquilinum* (L.) Kuhn) – a review, Acta Vet. Hung. 57 (2009) 183–196.