EXECUTIVE SUMMARY

The main purpose of this study was to quantify, more accurately, the risk that ptaquiloside and other bracken toxins may pose to drinking water supplies in England and Wales using existing data available from the published literature.

The objectives of the study were, in relation to bracken toxins, to identify a small number of high risk catchments for both public and private water supplies; to estimate likely concentrations in raw water sources using predictive modelling; to estimate likely concentrations in water after treatment; and to assess the risks to humans based on knowledge of toxicity.

High risk catchments were identified by overlaying spatially-referenced datasets relating to key factors influencing the presence of ptaquiloside in drinking waters. These were: bracken coverage (Landcover 2000), abstraction points (Environment Agency data), soil type (NATMAP Soilscapes) where soilscapes were assigned a risk depending on organic matter (OM) content (high OM = low risk), pH and sand content (very acid = low risk; acid sand = high risk), rainfall (Worldclim database), and slope (100 m resolution DEM). Data were overlayed on a catchment basis rather than grid-square. Two categories of high risk catchment were proposed: Risk A = a catchment with > 50% bracken coverage, mean annual rainfall > 800 mm, at least 1 abstraction point, > 50% medium or high risk soil, slope = steep or very steep; Risk B = as Risk A but including moderate slopes.

A small number of high risk catchments were identified in Wales, the Lake District and a few in the Pennines. However, it was noted that the lack of data on the location of private water supplies, which are likely to be most common in rural areas where bracken grows, was a substantial limitation. Repeating the high level risk assessment, but excluding 'abstraction point' as a variable, yielded a much higher number of catchments in Wales and the Lake District being identified as potentially at risk.

The published literature was reviewed for data relating to ptaquiloside in bracken. The ptaquiloside content of bracken has been shown to vary widely depending on species, growth stage, and altitude; it can also vary between and within stands grown in similar conditions. Very few measurements have been made for UK bracken. Data from Scotland were used as a measure of ptaquiloside concentrations in bracken (2138 µg/g of frond). Bracken biomass in the UK has been studied in greater detail than ptaquiloside concentrations, primarily due to the fact that an understanding of the growth cycle and energy stores of bracken is paramount to optimising methods of bracken control. Much of the data referred to levels of peak biomass thus the 90th percentile of the literature values was used (biomass = 973 g/m^2). This gives an estimated ptaquiloside load of 2.08 g/m^2.

The concentration of ptaquiloside in raw water was estimated by adapting the PEARL model which is normally used at the European level to predict the concentration of pesticides in groundwater. The model has relevant weather scenarios and describes the adsorption and degradation of organic compounds well. Four representative soil types were modelled. The output of the model was an annual average and maximum ptaquiloside concentration in percolate water at a given depth (dependent on soil type) each year over a period of 20 years. The 90th percentile values were calculated. This gave concentrations of 4.2, 6.1, 4.5 and 11.5 µg/L. The soil showing the highest risk was used as a measure of raw water concentrations (11.5 µg/L).

The total annual amount of ptaquiloside contained in the percolate for the high risk soil was between 1.5 and 30.9 g ha^{-1} for the different years. This is less than 1% of the amount of ptaquiloside that was released from the plants (between 4.6 and 8.5 kg ha^{-1}) indicating that substantial sorption and/or degradation occurs in the soil. Limitations of the modelling included very few measurements to calculate the sorption and degradation potential of ptaquiloside, and that only vertical flow was modelled. For soils expected to contain horizontal flow, ptaquiloside concentrations were calculated to the depth at which horizontal flow would occur, but the model could not predict any further dissipation occurring during horizontal flow to surface waters, thus the risk could potentially be overestimated. However, bracken is often found on steep valley sides so there is little time for dissipation to occur under these circumstances, and runoff, which could contribute to the transport of ptaquiloside to surface waters was also not modelled. On balance therefore, PEARL could be expected to reasonably predict the concentrations of ptaquiloside in ground and surface water. Indeed, the concentrations predicted are highly comparable to measured data from drinking water wells in Denmark and Sweden, 4 – 6 µg/L and 45 µg/L respectively providing confidence in the model output.

The lack of data relating to the quantity of ptaquiloside that is available for leaching was a substantial limitation of the model. There is no simple relationship between the ptaquiloside content of bracken and that in the soil, thus the plant ptaquiloside content could not be used as a measure. The quantity released was based on a single experiment where a known amount of water was sprayed onto fronds and the ptaquiloside concentration in the drip water was quantified. Drip water contained between 99 and 734 µg/L of ptaquiloside and a value of 700 µg/L was used in the model as a worse case scenario. However, it is not known whether continued rainfall will give sustained removal of ptaquiloside. It is known that ptaquiloside can be found in bracken litter so it was assumed that it was available for removal all year round, even when the bracken had senesced. There are great uncertainties associated with these assumptions and the precautionary approach has been adopted in the absence of any data. Further model runs using a lower ptaquiloside release value of 100 µg/L reduced raw water concentrations by a factor of ~ 8 giving a 90^{th} percentile maximum concentration of 1.5 µg/L.

Four representative treatment processes were considered: 1) Simple private – chlorination; 2) Advanced private – filtration + ultraviolet disinfection; 3) Municipal upland treatment - coagulation-flocculation, clarification, filtration and chlorination; 4) Municipal advanced treatment - coagulation-flocculation, clarification, ozonation and granular activated carbon + chlorination. The removal of ptaquiloside by each of the processes is estimated based on knowledge of the structure of ptaquiloside and its sorption properties, and the underlying processes of each treatment. Assuming a raw water concentration of 11.5 µg/L, the concentration in drinking water subject to the treatments 1-4, above were predicted to be 1.5, 8.6, 0.86 and 0.09 µg/L respectively. The primary data limitation in assessing the fate of ptaquiloside in treatment plants is the uncertainty surrounding its breakdown due to chlorine. This would require experimental data to validate the assumptions made in this study.

The toxicity of bracken and its constituent, ptaquiloside has been investigated in studies of farm and laboratory animals and in human epidemiological studies. The focus of these studies has primarily been on exposure via the oral route. Bracken causes a range of well-defined syndromes in farm animals. These include thiamine deficiency of monogastric animals, acute haemorrhagic syndrome (AHS) associated with bone marrow aplasia and upper alimentary ulceration, a progressive retinal degeneration (PRD) called “bright blindness” and two neoplastic disease syndromes: bovine enzootic haematuria and upper alimentary carcinoma.

The mutagenic and carcinogenic potential of bracken, extracts of bracken components, and ptaquiloside have been studied extensively in laboratory animals. In oral carcinogenicity

studies in mice, rats, guinea-pigs and cows, bracken induced benign and malignant intestinal tumours in all species, except cows. Bracken also induced bladder carcinomas in rats, guinea-pigs and cows, lymphocytic leukaemias in mice and mammary carcinomas in rats. Oral administration of bracken processed for human consumption produced intestinal cancers, but at a lower rate of incidence than unprocessed bracken. Whereas starch made from bracken rhizomes did not produce tumours in rats, oral administration of boiling water extracts of bracken to rats induced intestinal and bladder tumours. Oral administration of bracken in mice included maternal toxicity, some embryotoxicity and some minor abnormalities in offspring. Oral administration of ptaquiloside isolated from bracken in rats produced mammary, intestinal and bladder tumours and unscheduled DNA synthesis in primary liver hepatocytes.

There are several epidemiological studies of human populations in Japan, Brazil, Venezuela, Costa Rica and Wales that show an association between exposure to bracken toxins and the development of cancers of the stomach and oesophagus. Although a strong association between eating bracken and cancer in *cattle* has been established, the strength of association is less in human studies, and no study has identified a definitive link. Human studies have shown an association between eating bracken and human stomach cancers, but the studies lack the statistical power to demonstrate a clear dose-response relationship. A Japanese study has shown a higher risk of oesophageal cancer in people who ate bracken regularly than in those who ate it only rarely. Another Japanese study has suggested an association between eating wild plants (mainly bracken) and pancreatic cancer in men.

In experimental animal studies, bracken was found to be a genotoxic carcinogen that exhibits organ specificity with respect to its carcinogenic properties, inducing intestinal and bladder tumours in cattle and rats. Bracken contains a genotoxic carcinogen, ptaquiloside, and activation of ptaquiloside into a mutagen was reported to occur more favourably under alkaline conditions in genotoxicity tests (van der Hoeven, *et al*., 1983).

Following an evaluation of the carcinogenic potential of bracken, IARC concluded that there was sufficient evidence for the carcinogenicity of bracken in experimental animals and limited evidence for the carcinogenicity of ptaquiloside. They concluded, however, that there was inadequate evidence for the carcinogenicity of bracken in humans, and they classified bracken in Group 2B: possibly carcinogenic to humans. This classification was based on the conclusion that although available epidemiological evidence for carcinogenicity in humans was inadequate, there was sufficient evidence of carcinogenicity of bracken in animals.

Potential human exposure to ptaquiloside via ingestion of drinking water from (a) a private supply (b) an upland municipal supply and (c) an advanced treatment works was estimated for high-end (95^{th} and 99^{th} percentile) consumers across different age groups. Estimates of the potential lifetime average daily intake of ptaquiloside from high-end ingestion of drinking water (direct or indirect via the use of water for food or drink preparation) indicate that exposure to ptaquiloside via this route is likely to be minimal. Assuming that the highest expected concentration of ptaquiloside of 8.6 μg/l would be associated with a private water supply, the worst-case estimate of the potential lifetime average daily intake of ptaquiloside was 0.5μg/kg bw/day for high-end (99^{th} percentile) consumers between the ages of 21 and 64. Estimated lifetime average daily intakes in younger age groups would be orders of magnitude lower. Water treatment carried out at municipal upland or advanced treatment works potentially reduce the concentration of ptaquiloside in water ingested by consumers.

The outcome of the human health risk assessment is wholly dependent on the results of the environmental modelling which was limited by the available data. Additional assessments were made, varying the value of the initial raw water concentration of ptaquiloside.

Whilst bracken is a potential human genotoxic carcinogen, there is evidence that ptaquiloside is carcinogenic in animal models, but the quality of carcinogenicity studies is insufficient to allow a reliable identification of a clear no-observed-adverse-effect level, benchmark dose, or a dose-response relationship. Therefore it is not possible to identify a dose of ptaquiloside from the ingestion of drinking water to which consumers may not be at increased risk of developing cancer. The worst-case potential lifetime average daily intake of ptaquiloside from private water supplies, municipal upland supplies and an advanced treatment works were however lower than the potential intakes from high-end consumption of milk from bracken-fed cows for the elderly and toddlers estimated in a study by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. Although the risk from drinking water cannot be quantified, on the basis of the exposure assessment it is unlikely that exposure to ptaquiloside from the ingestion of water will pose a more significant risk to human health than other potential routes of exposure (e.g. dietary intake).