Proposed Quality Standards for Iron in Freshwaters Based on Field Evidence *(For consultation)*

by

Water Framework Directive - United Kingdom Technical Advisory Group (WFD-UKTAG)

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Executive summary

Iron has a potentially complex chemistry in freshwaters due to the oxidation of Fe(II) to Fe(III), and the precipitation of Fe(III) to form colloidal or fine particulate material. In addition iron may interact with dissolved organic carbon (DOC), either by direct binding of free Fe ions or through associations between DOC and precipitated forms of iron. Many historic ecotoxicity tests are considered to have effectively tested the "toxicity" of a suspension of precipitated material, and often have limited detail on the actual exposure conditions. This means that interpretation of the results of most of the available test data is uncertain: do they show direct toxic effects or adverse effects due to precipitated material? As a result of the uncertainties surrounding the available ecotoxicity data, this project has focused on the use of field data with matched monitoring for both ecology and chemistry. These datasets have been used to derive thresholds for iron concentrations which are consistent with the ability of benthic macroinvertebrate communities to achieve particular predefined Ecological Status objectives under the Water Framework Directive (WFD).

Analyses of data for fish, macrophyte, and diatom communities did not show any statistically significant decline in the maximum achievable ecological quality with increasing total iron exposures. Assessments based on benthic macroinvertebrate communities did show a statistically significant decline in response to increasing total iron exposures. Thresholds have been derived on both a whole macroinvertebrate community basis, for direct comparison with ecological quality standards, and also for the most sensitive fraction of the community. Thresholds have been derived for the boundary between Good and Moderate ecological status (GMB).

The ten invertebrate taxa identified as being the most sensitive to iron, in order of decreasing sensitivity, are (common name in parentheses): Goeridae (Caddisflies), Gyrinidae (Beetles), Polycentropodidae (Caddisflies), Perlodidae (Stoneflies), Rhyacophilidae (Caddisflies), Ephemeridae (Mayflies), Caenidae (Mayflies), Elmidae (Beetles), Ephemerellidae (Mayflies), and Heptageniidae (Mayflies).

Thresholds for the protection of sensitive macroinvertebrate taxa and for the protection of benthic macroinvertebrate communities have been derived. The thresholds are not normalised for water quality conditions. Both of these thresholds have been derived to be consistent with the Good/Moderate boundary (GMB) for ecological status as defined under the WFD. The proposed thresholds are 0.73 mg l^{-1} total iron for the protection of sensitive taxa, and 1.84 mg l^{-1} total iron for the protection of the whole community (using community metrics agreed for use in classification under the WFD). As these thresholds are not normalised for possible differences in iron toxicity under different water quality conditions they may not necessarily be protective of iron exposures under sensitive conditions.

Thresholds which relate directly to defined measures of ecological status under the WFD can therefore be proposed which are expected to be protective of sensitive conditions and can also be adjusted through the use of an empirical relationship between DOC concentrations, water hardness, and iron toxicity to invertebrates where conditions are less sensitive. Thresholds which are normalised in this way have been derived only for the whole community and the value relating to the Good/Moderate boundary (GMB) for ecological status is 0.78 mg l^{-1} total iron under sensitive conditions of low DOC and low hardness. This threshold is considered to be applicable to waters with a pH of greater than 7, but there is considerable uncertainty surrounding its relevance to waters of lower pH.

The GMB thresholds derived for both the most sensitive taxa (0.73 mg l^{-1}), and for the whole community under sensitive conditions (0.78 mg l^{-1}) are both slightly higher than the NOEC (No Observed Effect Concentration) values from the most sensitive ecotoxicity tests, but are below the LOEC (Lowest Observable Effect Concentration) values from these tests. They are therefore considered to be broadly consistent with the existing laboratory ecotoxicity data.

The EQS proposal is 0.73 mg l^{-1} total iron, and is derived from analyses of the abundance of the most sensitive taxa. This approach is considered to be the most consistent with the current approach towards EQS derivation where protection of the most sensitive species is assumed to ensure the protection of ecosystem structure and function. Taking account of the effect of water chemistry on the sensitivity of invertebrates to iron suggests that the ability of communities to achieve good ecological status will not be compromised, even under the most sensitive water chemistry conditions. This is considered as supporting information for the proposed EQS value.

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Contents

1. Introduction

An Environmental Quality Standard (EQS) is required for the effective regulation of discharges of iron into surface freshwaters. There is currently a UK statutory EQS for iron of 1 mg l^{-1} dissolved iron. A revision of the current EQS was previously derived in accordance with guidance for EQS derivation current at the time (Environment Agency 2007a). This resulted in a proposed PNEC of 16 μg l^{-1} dissolved iron but as this concentration is below typical background levels of iron in many surface freshwaters, it was not adopted for regulatory use.

Guidance for EQS derivation (JRC 2009) has been developed further in recent years, and has acknowledged the difficulties in deriving standards for substances which may cause physical effects due to the formation of mineral precipitates, such as is the case for iron, aluminium, and some other metals. It is stated that in these cases the use of field data may be more important than the results of standard laboratory ecotoxicity tests, which are not designed to take account of physical effects.

For chemical toxicants, evidence from mesocosm and field studies is generally used in support of the derivation of thresholds, for example, in the decision as to the size of the assessment factor. However, given that much of the historic ecotoxicity data derived for iron may have effectively tested the “toxicity” of a suspension of iron hydroxide precipitates, questions have been raised about the relevance of using such data for deriving toxicity thresholds for use in classification and EQS setting.

In recent years datasets have become available which combine ecological monitoring data with chemical monitoring data, and potentially allow the effects of common environmental contaminants on benthic macroinvertebrate communities to be assessed. In addition, other ecological assessment methods have also been developed to assess the quality of diatom, macrophyte, and fish communities in surface freshwaters. An important aspect of these ecological assessment methods is that acceptable levels of ecological quality have been defined, which makes it possible to derive critical concentrations of chemical contaminants, above which a predefined level of ecological quality is unlikely to be achieved.

This report describes the behaviour of iron in freshwaters (Section 2), summarises available ecotoxicity studies for iron (Section 3) and then uses field data to derive thresholds that can form the basis of an iron EQS for UK freshwaters (Section 4).

2. Behaviour of Iron in Freshwaters

Iron has a particularly complex chemistry in natural waters and can exist in two oxidation states, Fe(II) and Fe(III). Fe(II) is the dominant form of iron under reducing conditions, whereas Fe(III) is the dominant form of iron under oxidising conditions. The rate of oxidation of Fe(II) to Fe(III) in oxic aqueous environments largely depends on pH, with shorter half-lives at higher pH. Half-lives summarised by Skeaff (2004) are in the order of days at pH 6, minutes to hours at pH 7, and seconds at pH 8. An experimental study into the binding of iron by organic matter (Weber 2006) identified an influence of organic matter on the redox speciation in solution, which exerts control over the Fe(II):Fe(III) ratio. The authors observed a relatively consistent ratio of Fe(II):Fe(III) of between 2 and 3.5 over a range of pH.

The test media used for experiments of iron oxidation rates summarised by Skeaff (2004) were all inorganic solutions. Any potential controls which organic matter may exert over the oxidation state of iron would therefore not be observed in these experiments. It has been suggested from other studies that organic matter may exert a degree of control over both the oxidation state and size of Fe species in natural waters (Gaffney et al. 2008). A reduction in the proportion of Fe(II) which became oxidised in experiments containing organic ligands, and the extent of Fe(II) oxidation, appeared to be influenced by the ratio of iron to organic carbon. A reduction in the ratio of Fe:organic carbon resulted in a reduction in the oxidation of Fe(II). The authors suggest that there may be the potential for a significant proportion of the iron load in natural waters to remain in a reduced form, even when the waters are well oxygenated.

Fe(III) is extensively hydrolysed in slightly acidic to neutral freshwaters, which can result in the formation of precipitates due to the low solubility of $Fe(OH)_3$. The solubility products of precipitates such as amorphous iron oxide, hydrous ferric oxide and ferrihydrite are in the range $10^{2.5}$ to 10^5 (Tipping et al. 2002). The lower values represent aged materials, whereas higher values are more typical of freshly precipitated material. A range of solubilities can therefore be anticipated for natural systems. Some modelling studies have suggested that organic matter can increase the apparent solubility of precipitated hydrous ferric oxides (Weber et al. 2006). This effect was identified through the need to use higher LogK_S (solubility product) values when modelling the speciation of iron in model systems. Fe(II) is also expected to form hydroxide precipitates under the conditions of many natural waters, although oxidation to Fe(III) would be expected, but may be slow at low pH.

Iron can also be bound to organic matter, in a manner similar to that of many other trace metals, and the complexation can be predicted using models such as WHAM (NERC 2001). It is often necessary for the concentrations of iron species in solution to be estimated by assuming that they are at equilibrium with a solid phase, such as ferrihydrite. The two iron ions (Fe(II) and Fe(III)) differ in their binding affinities to humic and fulvic acids. These differences may be attributed principally to their charge, with Fe(III) showing much stronger binding than Fe(II), due to electrostatic effects. As a result of this, virtually all dissolved phase Fe(III) would be expected to be bound to organic matter throughout the range of pH which is relevant to natural waters. Fe(II), on the other hand, would be expected to show steadily increasing DOC binding with increasing pH, up to a maximum at around pH 7.8.

There have been essentially two types of investigations into the fate of iron in natural waters: studies of the oxidation kinetics of Fe(II) to Fe(III), and size fractionation studies to consider the distribution of iron (Gaffney et al. 2008). Gimpel et al. (2003) compared filtration, dialysis and Diffusive Gradients in Thin films (DGT) for the measurement of iron concentrations in several natural freshwaters. The authors found that concentrations measured by DGT, and also in most cases by dialysis, were considerably lower than concentrations determined by filtration (0.45 μm). This may be due to colloidal forms of iron that pass a 0.45 μm filter but not dialysis systems nor the permeable gel of DGT. Gimpel and co-workers further suggested that there may be small, reactive forms of Fe(III), which are removed on storage by polymerisation and aggregation processes, but are continually produced in the field by oxidation of Fe(II).

2.1 Effect of water quality conditions on the behaviour of iron

Several studies have considered the interactions between DOC, usually as extracted humic acids, and iron oxide colloids or particles. In many cases these studies were primarily concerned with the effect of such interactions on the speciation and partitioning of other metals (e.g. Vermeer et al. 1999). Measured dissolved concentrations (0.45 μm filtered) of iron in oxic surface waters are commonly appreciably higher than the concentrations that would be predicted to be truly dissolved by assuming that Fe(III) concentrations are controlled by the formation of a mineral phase such as amorphous iron hydroxide (Lofts et al. 2008). The presence of additional "dissolved" iron is often attributed to dissolved organic complexes, or the presence of small colloidal mineral particles.

Gunnars et al. (2002) studied the formation of iron oxyhydroxide colloids in freshwater and brackish seawater, and found that aggregation and precipitation of the colloidal iron particles formed was more rapid under the higher ionic strength conditions found in brackish seawater (6 to 33 parts per thousand salinity). They also found that the rate of removal of the particles was around 4 to 5 times higher under these conditions than in freshwater. Higher concentrations of both carbon and nitrogen (assumed to be due to organic matter) were found in particles formed in freshwaters, suggesting that interactions between natural organic matter (NOM) and iron colloids may be stronger at low ionic strength. It was noted that the organic matter appeared to stabilise the colloidal iron particles, which may lead to precipitates being retained in suspension for greater periods of time in the presence of organic matter.

A study of the sorption of simple organic acids on particles of goethite, a crystalline form of iron oxide (Evanko and Dzombak 1998), found the sorption to be greater at low pH than at high pH. The relative degree of sorption of the organic acids was greatest when they were present at lower concentrations. Increasing numbers of carboxylic acid functional groups on the organic acids resulted in a greater degree of sorption at the same pH. The sorption of a commercial humic acid was comparable to the sorption characteristics of some of the simple organic acids, with reduced sorption to the mineral surfaces observed at high pH.

These studies clearly demonstrate the importance of interactions between DOC and precipitated iron minerals, although the effect that this may have on the potential to cause adverse effects is unclear. Activities of Fe(III) can be estimated according to an empirical relationship with pH (Lofts et al. 2008), which indicates diminishing activities with increasing pH. However, this study noted that Fe(II) accounted for an average of 24% of the truly dissolved iron concentrations. In some cases high concentrations of Fe(II) were observed in samples with pH values above 7.5, which are conditions where oxidation of Fe(II) to Fe(III) would be expected to be relatively rapid.

3. Summary of Ecotoxicity Studies on Iron

There have been several reviews of the toxicity of iron to aquatic organisms in recent years (Environment Agency 2007, OECD 2007, Vangheluwe and Versonnen 2004, EPRI 2004). These reviews suggest that it is difficult to assess clearly whether the observed effects from exposure to iron are due to chemical toxicity or physical effects. Many chronic fish tests have essentially been conducted with a suspension of ferric hydroxide (EPRI 2004). Vangheluwe and Versonnen (2004) noted that most of the observed effects appear to be due to particulate iron hydroxides and that no clear evidence of chemical toxicity had been identified.

Dissolved concentrations of metals are typically considered to be most relevant to any evidence of ecological effects. However, this may not be the case for iron, as indicated by these reviews of traditional ecotoxicity test data. If the mode of action of iron is not usually exerted via chemical toxicity then other expressions of iron concentration may be required. Total or particulate iron concentrations may be more relevant to ecological effects if the mode of action of iron is usually via physical effects, such as smothering.

3.1 Laboratory Studies

Vangheluwe and Versonnen (2004) identified *Daphnia magna* as a sensitive organism in a review of acute toxicity data, although the mayfly *Ephemerella subvaria* was noted as being the most sensitive organism in acute tests, with a reported LC50 of 0.32 mgl^{-1}. Another review of acute toxicity data for iron (EPRI 2004) also identified the same mayfly (*Ephemerella subvaria*) as the most sensitive species to acute iron exposures. A review by the Environment Agency (2007) identified *Daphnia magna* as the most sensitive species in chronic tests, with a chronic NOEC equivalent for reproduction of 0.16 mg l^{-1} (Dave 1984), this study was assessed as being reliable with restrictions (Klimisch code 2), but is likely to have assessed exposure to a suspension of iron precipitates, rather than truly dissolved iron, as the pH of the test was in the range 7 to 8. Although this test was reported as having been based on measured iron concentrations it was actually based on nominal concentrations, with no analyses of the exposure solutions having been carried out during the tests performed. Furthermore, the test was performed on freshly made solutions which were replaced regularly. A similar test, using aged solutions which were replaced less frequently (Beisinger and Christensen 1972) showed very low toxicity of iron to the same test organism.

The Iron Platform (an iron industry group) has attempted to derive a species sensitivity distribution (SSD) for iron based on available laboratory ecotoxicity data. The dataset comprises data for both Fe(II) and Fe(III), although the majority of the data are for Fe(III). Under circumneutral, oxic conditions Fe(II) would be expected to be oxidised within a relatively short timescale (minutes to hours) to Fe(III), which would then be expected to hydrolyse to form a precipitate of iron (oxy)hydroxides. It is likely that the majority of the tests (if not all of them) have tested the effects of these precipitates. For this reason the data are not viewed as useful for PNEC derivation under REACH or EQS derivation under the WFD, as the test substance has, in all cases, been tested at levels which are greatly in excess of its limit of water solubility in the test system.

Some tests were excluded from the assessment specifically because the effects were reported to be due to the presence of precipitated material in the test system, although the exclusion of such tests may not be necessary when proposing a PNEC for possible adoption as an EQS, for which protection against physical effects may be necessary. Some of the studies not considered in the Iron Platform preliminary hazard assessment, because of effects due to precipitated material, have also been considered in more detail here. However, their reliability for PNEC derivation is considered to be questionable (although both of these studies have previously been considered to be of reliability rating 2 in some reviews (Dalzell and Macfarlane 1999, Randall et al. 1999)).

The most sensitive result used in the Iron Platform review was a 33 day test performed on the fathead minnow (*Pimephales promelas*) reported by Birge et al. (1985). It has not been possible to obtain a copy of the original report, although this study has been reviewed in several other studies of iron toxicity (Vangheluwe and Versonnen 2004, OECD 2007) and assessed as reliability rating 2. The most sensitive endpoint assessed was survival (NOEC 0.32 mg l^{-1}, LOEC 1.01 mg l^{-1}), whereas the more common endpoints for chronic studies, length and weight, were less sensitive than survival. The NOEC value for length was 1.0 mg l^{-1} (LOEC 1.6 mg l^{-1}), and the NOEC value for weight was 1.6 mg l^{-1} (LOEC 2.8 mg l^{-1}). The survival endpoint was used in the Iron Platform hazard assessment, but was not considered to be a true chronic endpoint in the other reviews. The test was performed in reconstituted laboratory water at a pH of 7.5, 100 mg l^{-1} ($CaCO_3$) hardness, and a DOC concentration of <2 mg l^{-1}. Total iron concentrations were measured.

The next most sensitive endpoint was a NOEC of 0.63 mg l^{-1} iron for both total offspring and brood size in a 21 day test on *Daphnia pulex* (LOEC 1.3 mg l^{-1}) from the same study (Birge et al. 1985). The NOEC from this study has been reported as 0.7 mg l^{-1} in some reviews (e.g. OECD 2007). The NOEC for *Daphnia longispina*, reported by Randall et al. (1999), is also lower than this value, although the LOEC from this test was 2 mg l^{-1}.

Dalzell and Macfarlane (1999) tested the toxicity of two different grades of iron sulphate to brown trout, and studied the effects on the gills. Effects were considered to be due to the deposition of iron precipitates on the gill surfaces and, as a result of this, the study was previously considered not to be useful for PNEC derivation (Iron Platform 2009). The acute tests reported by Dalzell and Macfarlane (1999) considered five concentrations in addition to a control, but sub-lethal (14 day) tests considered only a single concentration of each of the two grades of iron sulphate. The acute tests showed a trend of increased levels of iron accumulated on the gills with increasing exposure concentrations. Fish exposed to 7.4 mg l^{-1} iron (commercial grade), or 6.7 mg l^{-1} iron (analytical grade) for 14 days did not show any significant accumulation of iron on the gills relative to control fish. These levels of iron exposure were below the lowest exposure concentration tested in acute tests (10 mg l^{-1} nominal, 12.2 and 13.0 mg l^{-1} total iron). This suggests an unbounded NOEC of >7 mg l^{-1} total iron, although the test duration is relatively short, and so this is not considered to be a valid test for PNEC derivation.

This study was not included in the Iron Platform PNEC derivation due to effects having been reported to be as a result of precipitate formation. However, more important shortcomings for PNEC derivation are the short duration of the study and the fact that only a single exposure concentration was tested in the sub-lethal tests. The concentrations of dissolved iron in the acute exposures were in the order of 100 times lower than total concentrations.

Randall et al. (1999) conducted both acute and chronic toxicity tests on field collected *Daphnia longispina*. Exposure to dissolved iron in acute tests did not cause any significant mortality, although exposure to particulate iron did cause mortality at concentrations of greater than 8 mg l^{-1}. Effects were observed in chronic 21 day tests with concentrations of particulate iron in excess of approximately 2 mg l^{-1}. The chronic tests resulted in a NOEC of 0.5 mg l^{-1}, and a LOEC of 2 mg l^{-1} for particulate iron, although no statistical analysis was presented and considerable variability of the response was observed for higher exposure concentrations. Adverse effects were also observed in tests with china clay particles, which were assumed not to be caused by toxicity but by particle effects.

This study also considered the effect of exposures on the filtering rate of the test organisms, and the frequency of thoracic beats. A NOEC for thoracic beats can be identified at 0.5 mg l^{-1}, and a LOEC at 1 mg l^{-1} particulate iron, with further decreases in the rate of thoracic beats observed at higher exposure concentrations. A safe limit of 1.69 mg l^{-1} particulate iron was calculated for the daphnids, from the results of both acute and chronic tests, although sufficient information to confirm this was not included in the paper. As a result of these limitations in details of the tests and results this study is not considered to be sufficiently robust for PNEC derivation.

There are insufficient taxonomic groups included in the dataset to meet the criteria set in the TGD for the application of an SSD to derive a PNEC, although an SSD approach was considered in the Iron Platform preliminary hazard assessment. Applying the data set compiled by the Iron Platform review results in HC5 values of between 0.23 to 0.30 mg l^{-1} total iron, depending on whether all individual values or species geometric means are used, and whether or not the two additional tests are included (Dalzell and Macfarlane 1999, Randall et al. 1999). The lowest HC5 value results from using species geometric mean data and including the two additional tests, whereas the highest value results from taking the individual values without the two additional test results. All of these analyses assume that the 33 day NOEC for fish mortality is suitable for chronic PNEC derivation.

The lowest NOEC values are 0.32 mg l^{-1} (NOEC) for fish survival (Birge et al. 1985), 0.5 mg l^{-1} (NOEC) for *Daphnia* reproduction (Randall et al. 1999), and 0.63 mg l^{-1} (NOEC) for *Daphnia* reproduction (Birge et al. 1985). The LOEC values from all of these studies were above 1 mg l^{-1} total iron.

3.2 Field Studies

Gerhardt and Westermann (1995) studied the effect of iron precipitations on the mayfly (Ephemeroptera) *Leptophlebia marginata* (L.). Nymphs of *L. marginata* were exposed to Fe in two different streams over three months. In a clearwater stream (known as the Wispe, Niedersachen, Germany) Fe was present as permanent Fe_2O_3 precipitations on the streambed, and in a DOC rich stream (the Mullra, Southern Sweden) Fe was present as $Fe(OH)_3$-humus precipitations. Groups of 20 nymphs were kept in net cages containing local sediment and fine detritus for food. The cages were dug into the stream sediment; the construction ensured that the animals obtained sufficient water and oxygen even if the nets were clogged with Fe-precipitate. Cages were collected 8 and 12 weeks after exposure.

Speciation of Fe may be controlled by chemical oxidation processes in the Wispe, but microbial processes in the Mullra due to high microorganism concentrations in the sediment. Fe was found adsorbed to the bodies of the nymphs as well as internally. Fe body loads were higher in the Wispe; however, internal uptake of Fe was higher in the Mullra. This may be because Fe is present as Fe_2O_3 precipitations in the Wispe, but as $Fex(OH)_y$ co-precipitations with humic matter in the Mullra and is therefore more available for dietary uptake. Fe precipitation occurred randomly onto different body parts of the mayflies and moulting removed the Fe crusts. Neither Fe concentrations up to 1.64 mg $Fe_{tot}l^{-1}$ in water nor Fe precipitations of different chemical forms on the sediment affected *L. marginata* measurably over the three month exposure period. Fine particle feeders like *L. marginata* may be less affected by Fe on sediment than grazers such as *Epeorus sylvicola*, *Rhitrogena iridina*, and *Baetis* sp., which were not found downstream of iron-rich groundwater influx.

Reviews of the toxicity of iron to aquatic organisms indicate that invertebrates are likely to be the most sensitive group of organisms, suggesting that an assessment of the effects of iron on invertebrate communities in the field may provide a reliable indication of the effects of iron on aquatic ecosystems.

A field study of the effects of iron on benthic invertebrates (Rasmussen and Lindegaard 1988) provides some evidence of effects on aquatic ecosystems. Water samples were taken between 1979 and 1980 from the River Vidaa, Denmark. The samples were analysed for total iron, dissolved iron and pH. Concentrations of annual average dissolved iron from 28 sites ranged from 0 to 32 mg Fe(II) l^{-1}. Dissolved iron was reported to consist almost entirely of Fe(II), and consequently dissolved (i.e. <0.45 μm filtered) iron concentrations were assumed to be Fe(II). Samples of benthic invertebrates were taken at the same sites. pH was between 6.7 and 8.8 at the different sites. Numbers of taxa were correlated to the concentrations of different iron components. The number of taxa was negatively correlated to iron concentrations expressed as annual average Fe(II), maximum recorded Fe(II), annual average total iron and winter average Fe(II). At concentrations below 0.2 mg Fe(II) l^{-1} 67 taxa were collected, and between 0.2 and 0.3 mg Fe(II) l^{-1} 53 taxa were recorded. Taxa that were eliminated were primarily grazers that feed on biofilm. Up to concentrations of 10 mg Fe(II) l^{-1} taxa continued to be eliminated, with 10 taxa left at this concentration. It is not clear from this study whether dissolved iron is genuinely a better metric of iron exposure, and other potential pressures on the benthic macroinvertebrate communities were not considered. A further limitation is that no established thresholds or guidelines for the quality of benthic macroinvertebrate communities were used to derive the critical concentrations of iron reported.

Some studies have also been conducted on fish (Teien et al. 2008) to investigate the association of iron with fish gills, which may be the "target organ". These studies have considered the transformation of iron from Fe(II) to Fe(III) and the formation of Fe(III) precipitates. It appears from these studies that there may be transient forms of iron which can be relatively toxic to fish. These forms of iron appear to be associated with a decrease in Fe(II) and an increase in Fe(III), and also with a shift from low molecular mass forms of iron to higher molecular mass forms (possibly indicating the formation of solid, or colloidal, precipitates). These changes in the behaviour of iron coincided with an increase in gill iron concentrations and also with some mortality in test fish.

The tests conducted by Teien et al. (2008) were performed over relatively short timescales (96 hours), and fish were exposed to iron which had been added to the water shortly before exposure of the fish. Different sets of fish were exposed to iron which had been able to react for different periods of time, and the reaction times of the iron to which the fish were exposed were tightly controlled. A limited range of exposure concentrations was considered, as the study was designed to investigate the effects of iron species during oxidation and precipitation transformations. The study is therefore not directly useful for PNEC derivation, although it does provide evidence of the potential for effects under certain environmental conditions when iron concentrations are relatively low (around 1 mg l^{-1}). The effects observed in these tests are considered to be most applicable to situations where waters rich in Fe(II) and with relatively low pH mix with water with a higher pH, causing oxidation and subsequent precipitation of iron.

The results of the tests reported by both Teien et al. (2008) and Dave (1984) indicate that under situations where iron is undergoing a transformation (possibly due to hydrolysis) it may be considerably more toxic than it is under circumstances where such transformations have already occurred. It is possible that the more toxic iron species are hydrolysis products, such as $FeOH^{2-}_{(aq)}$ and $Fe(OH)_2^{-}{}_{(aq)}$, which are essentially transient species in the formation of iron hydroxide precipitates ($Fe(OH)_{3(s)}$). The formation of these hydrolysis products is also associated with the generation of acidity. These tests suggest that invertebrates may be more sensitive than fish to the effects of iron, although direct comparisons between the results of the two studies are difficult due to the different kinds of procedures used in each.

3.3 Relevance of iron exposure metrics

An EQS for iron needs to be adequately protective of effects from both chemical toxicity and physical effects due to the formation of precipitates. However, the behaviour of iron in environmental systems leads to considerable difficulty in assessing the most relevant exposure metric for iron. The analyses in this report focus on total iron concentrations for three reasons. First, total iron concentrations should include any potentially hazardous forms of iron, although this may also include inactive components. Second, dissolved iron concentrations have been observed to co-vary with dissolved organic carbon concentrations in several UK datasets (see Figure 3.1). Third, a previous analysis of field data on benthic invertebrate communities (Rio Tinto 2009) which considered total, dissolved and particulate iron concentrations did not identify any of the different exposure metrics as more likely than others to be responsible for the observed effects.

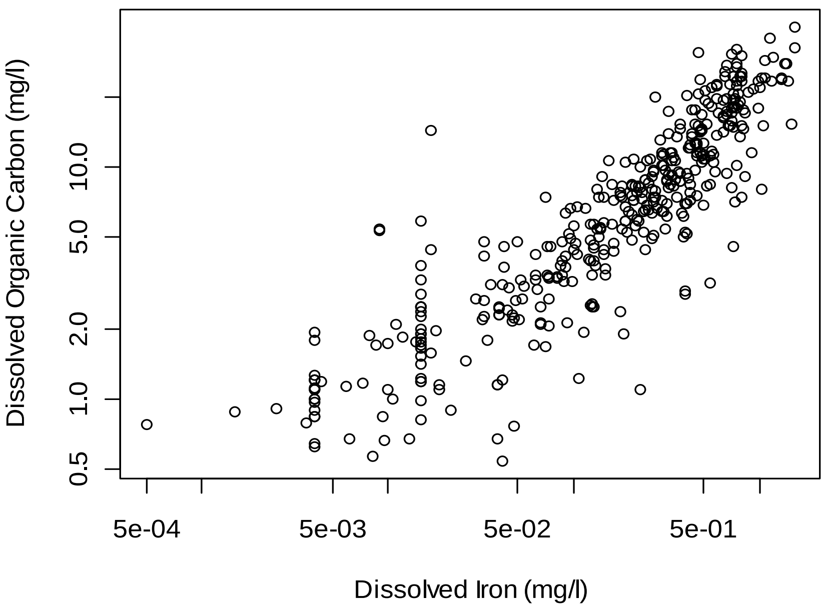

Figure 0.1 Dissolved iron and dissolved organic carbon concentrations in 407 samples from the UK.

4. Derivation of Quality Standards from Field Evidence

This section of the report focuses on the analysis of field datasets of both ecological and chemical quality, and the interpretation of such data to derive thresholds for iron exposures which may provide a suitable basis for proposing a PNEC for iron in freshwaters.

4.1 Precedents for quality standards derived from field evidence

Two water quality standards have been set in the UK on the basis of field evidence. These are for dissolved oxygen and ammonia (UKTAG 2008a). These standards have been derived as the 90th percentile concentration of the pollutant at High quality and Good quality sites, based on the status of benthic macroinvertebrate communities. Both of these standards were derived from data for benthic macroinvertebrate communities, because this was considered to be the most sensitive trophic level (out of plants, invertebrates, and fish), although routine biological monitoring should be able to detect any deterioration in other trophic levels in order to ensure that an adequate level of protection is afforded by the standards. This suggests that there is a precedent for deriving Environmental Quality Standards from field data, and that the focus should be on organisms in the ecosystem which are believed to be the most sensitive to a particular pressure. The existing standards have been derived to be consistent with ecological quality at High and Good Status sites, i.e. on the basis of the whole benthic macroinvertebrate community, rather than on a (sensitive) subset of the community.

The approach taken for the derivation of the standards for dissolved oxygen and ammonia suggests:

- standards must be derived from field data for the organisms which can reasonably be expected to be the most sensitive to the pressure under consideration, although this has not always been the case (see notes on ammonia standard below);
- the thresholds used to derive standards must be consistent with the definition of ecological quality (i.e. they should relate to a similar degree of impact relative to the reference condition); and
- where a technique such as quantile regression analysis is used, assessments based at the 90thquantile would be most consistent with other standards derived from field evidence.

The standard derived for dissolved oxygen on the basis of benthic macroinvertebrate communities was considered to be protective of fish communities, because the available evidence does not suggest that fish are likely to be more sensitive than invertebrates. The standard for ammonia was also derived from data for benthic macroinvertebrates alone, although a previous review of laboratory toxicity data (Environment Agency 2007) states that "*Fish are clearly the most sensitive species with regard to both chronic and acute effects of ammonia.*" This information does not appear to have been considered in the derivation of the standard for ammonia, although it has been stated that further work will be undertaken during the first cycle of River Basin Management Plans to confirm that fish are adequately protected by the standard.

4.2 Statistical analysis

A quantile regression approach was taken for the assessment of field data in order to derive field based limit values. The quantile regression approach considers a limiting function and is particularly useful in cases where it is not possible to remove all of the potentially confounding factors. It has been applied to the concept of limiting factors as constraints on organisms (Scharf et al. 1998, Cade et al. 1999, Cade and Noon 2003). The approach taken in this study was similar to that applied in previous assessments of field data (Pacheco et al. 2005, Linton et al. 2007, Crane et al. 2007). An advantage of statistical techniques such as quantile regression is that they do not require the datasets to be screened for potentially interfering pressures.

Quantile regression was applied to the datasets using a log linear model (log(response) = a + b.(exposure)) using the quantreg package (Koenker 2009) in R (Version 2.9.1; http://cran.r-project.org). The quantile regression was applied to the 90th, 95th and 99th quantiles of the datasets, although in some cases the model could not be fitted to all of the quantiles assessed. Higher quantiles are potentially more susceptible to outliers in the data, although both 90th quantiles (Linton et al. 2007) and 99th quantiles (Crane et al. 2007) have been successfully applied previously. 95% confidence intervals for estimates of the thresholds were calculated by bootstrapping using 2000 resamples.

The principle of the quantile regression approach to derive a limiting function is shown in Figure 4.1. Data with unimpacted responses at low exposures serve as controls, and impacted data at low exposures are effectively not considered in the analysis. A decline in the maximum response with increasing exposure is assumed to be due to the contaminant of interest.

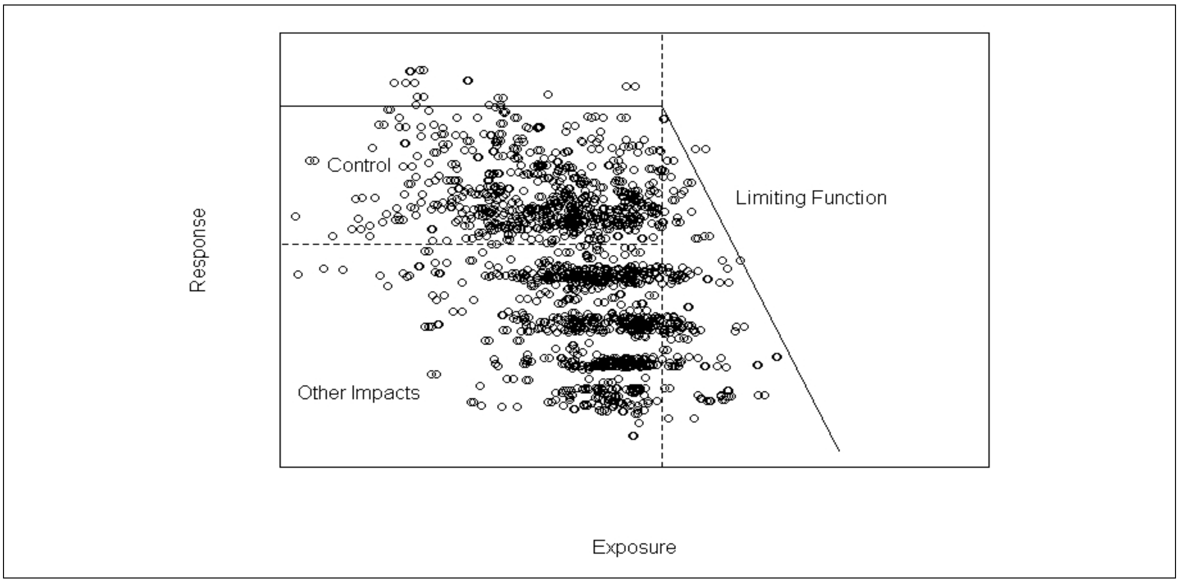

Figure 0.1 Illustration of the interpretation of combined chemical and ecological field data.

One potential limitation of this approach is that other contaminants, whose concentrations co-vary with the contaminant of interest, may affect the slope of the dose-response curve. This could have the effect of resulting in a steeper dose response curve being identified for the limiting function, with the consequence of the derived threshold being lower than it would be in the absence of additional pressures.

4.3 Interpretation of ecological thresholds

The effect threshold used in the data analyses relates to the Good/Moderate Boundary (GMB) for Ecological Status. This is consistent with the Water Framework Directive threshold for invertebrate communities (when assessed in terms of Ecological Quality Index [EQI] values). The GMB is taken to be at EQI values of 0.74 for the number of taxa (N-Taxa; the same values are also used for assessments based on the EQI for the Biological Monitoring Working Party [BMWP] score), and 0.90 for EQI ASPT (Average Score Per Taxon). These thresholds are calculated assuming an EQI value of 1 under low exposure conditions. It is questionable, given the less stringent thresholds for a change in ecological communities whether a 10% effect level can be robustly identified as an effect threshold, when the derivation is based on ecological data of this nature. EC10 values are therefore not derived here.

4.4 Identification of potentially sensitive taxa

A dataset of matched chemical and ecological data, compiled by the Centre for Intelligent Environmental Systems (Staffordshire University) for the Environment Agency was used for the derivation of thresholds from field evidence. This dataset included ecological samples taken in 1995 and 2003, and associated chemical monitoring data, expressed as a median for the 3 years preceding the ecological sampling. The dataset includes 3397 samples covering 1617 sites, of which approximately 90% were suitable for River Invertebrate Classification Tool (RICT) predictions (UKTAG 2008b). Iron exposures ranged from 17 $\mu g\ l^{-1}$ to 11.7 mg l^{-1}. The previously proposed EQS for iron of 16 $\mu g\ l^{-1}$ (Environment Agency 2007a) is below the lowest iron exposure concentration in this dataset.

Initial analyses were based on the identification of a decline in the abundance of taxa with increasing iron exposure concentrations, in a manner comparable to previous analyses of the effects of iron on aquatic invertebrates (Linton et al. 2007). However, this approach did not take into account the fact that not all taxa would be expected to be found in all types of habitats, and the results suggested that relatively rare taxa are more sensitive to the effects of iron than relatively abundant taxa. In order to refine the analysis RIVPACS III+ (Clarke et al. 2003) was used to enable observations of invertebrate presence and abundance to be expressed relative to a predicted reference condition.

Potentially sensitive taxa were identified by calculating Observed to Expected ratios for each individual taxon at each site, and selecting those taxa with the lowest average O/E values for the highest quartile of the total iron exposure data (total iron concentrations greater than 797 $\mu g\ l^{-1}$). The O/E values based on measurements and predictions of taxon abundance were also treated in a similar manner. This was in order to assess whether taking account of the abundance of each taxon can increase the sensitivity of the analysis relative to assessments which are based on presence or absence only (as the community based EQI metrics are). The most sensitive 25% of taxa identified according to this method are shown in Table 4.1, and their relative rankings based on presence or absence and abundance are shown in Figure 4.2. This procedure was performed for both spring and autumn data. Separate spring and autumn analyses were undertaken as RIVPACS cannot calculate predicted abundance values from combined spring and autumn data. These analyses should therefore be able to identify whether the benthic invertebrate communities are more sensitive in one of these seasons.

Table 0.1 Sensitivity Ranking of the 25% most sensitive taxa according to RIVPACS normalised presence (or absence), and abundance, at the highest 25% of total iron exposures in both Spring and Autumn.

	Spring		**Autumn**	
Taxon	**Presence**	**Abundance**	**Presence**	**Abundance**
Goeridae	1	2	1	4
Gyrinidae	3	6	2	1
Polycentropodidae	4	5	3	3
Perlodidae	2	1	6	10
Rhyacophilidae	5	3	10	7
Ephemeridae	7	11	4	6
Caenidae	10	8	8	5
Elmidae	12	4	14	2
Ephemerellidae	11	12	5	9
Heptageniidae	9	7	11	11
Sericostomatidae	8	10	15	15
Taeniopterygidae	19	19	7	8
Perlidae	14	16	12	14
Haliplidae	15	18	13	13
Leptophlebiidae	16	15	16	16
Limnephilidae	13	13	19	18
Nemouridae	6	9	32	23
Piscicolidae	24	31	9	12
Lepidostomatidae	21	22	17	17
Sialidae	17	21	20	24
Leuctridae	18	14	28	26
Odontoceridae	23	26	18	19
Tipulidae	20	17	30	25
Hydropsychidae	25	20	26	22
Valvatidae	29	32	23	20

Notes: Taxa identified in bold were also identified as sensitive by Linton et al. (2007).

This method may not only identify those taxa which are sensitive to iron exposure, because the dataset contains sites which are impacted by a wide variety of pressures. By selecting those taxa which show low O/E scores at high iron exposures it should be possible to increase the sensitivity of the analyses to this specific contaminant. Five of the eight taxa identified by Linton et al. (2007) as being sensitive to iron in a study of US data have also been identified as occurring in the 25% most sensitive taxa in these analyses. This suggests that although the approaches taken for the analyses differ (this analysis considers the occurrence of taxa relative to a predicted reference condition) broadly comparable results are obtained by both methods.

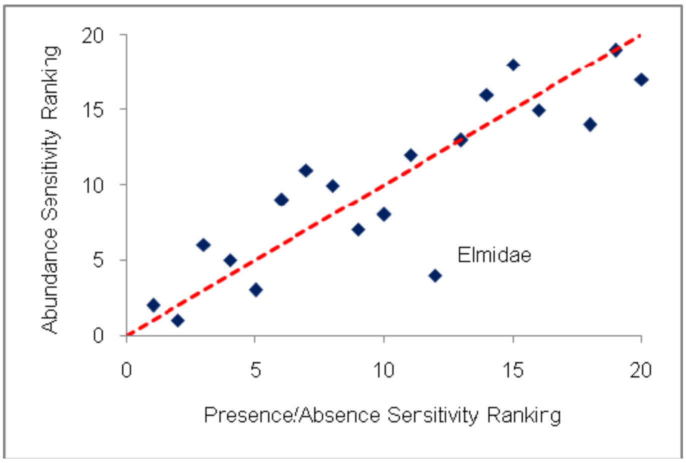

Figure 0.2 Relative ranking of the 25% most sensitive taxa according to Presence or Abundance in the Spring.

Quantile regressions were performed at the 90th quantile based on O/E for both the 25% and 10% most sensitive taxa. These were based on presence or absence (e.g. Figure 4.3), and abundance (e.g. Figure 4.4). The 25% most sensitive taxa showed a similar sensitivity to EQI BMWP (see Figure 4.5) when based on presence or absence, and a greater sensitivity when based on abundance. Analyses were therefore also performed on O/E for the 10% most sensitive taxa, for both presence or absence, and abundance (see Figure 4.4). A further increase in sensitivity was observed in both cases relative to O/E for the 25% most sensitive taxa.

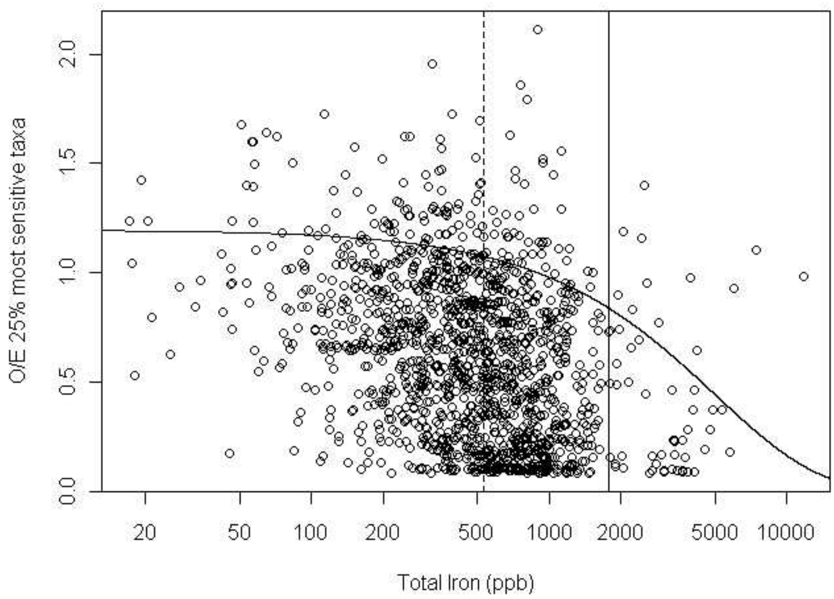

Figure 0.3 O/E for the 25% most sensitive taxa, based on presence or absence in the spring. The dose response curve is based on the 90th quantile of the dataset; the solid vertical line indicates the $GMB_{90th\%ile}$.

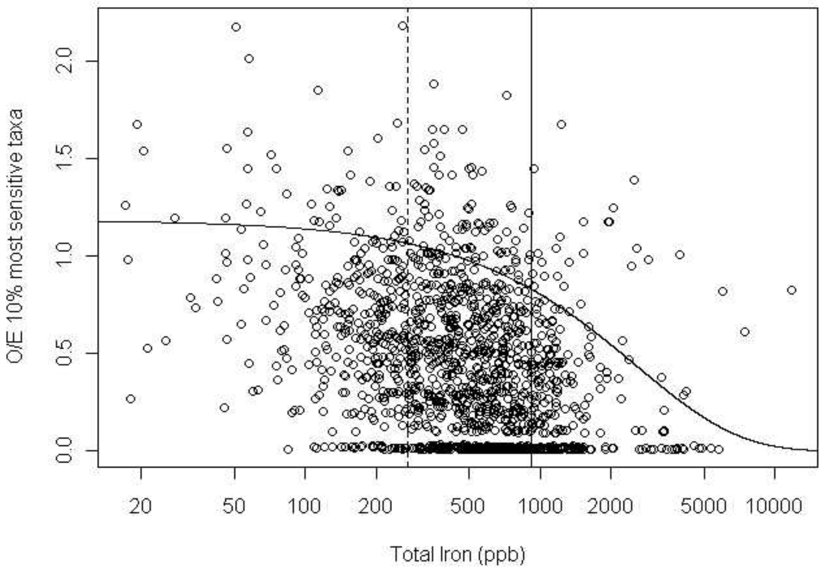

Figure 0.4 O/E for the 10% most sensitive taxa, based on abundance in the spring. The dose response curve is based on the 90th quantile of the dataset; the solid vertical line indicates the $GMB_{90th\%ile}$.

The Good Moderate Boundary ($GMB_{90th\%ile}$) values that are derived increase in the order 10% most sensitive taxa, 25% most sensitive taxa, EQI BMWP. Relatively high scores are observed for some high exposure sites when the 10% and 25% most sensitive taxa are assessed. This may be due to the occurrence of a taxon which is not expected to be found (i.e. where the predicted probability of capture is low). Analyses based on taxa abundance may be less sensitive to this effect.

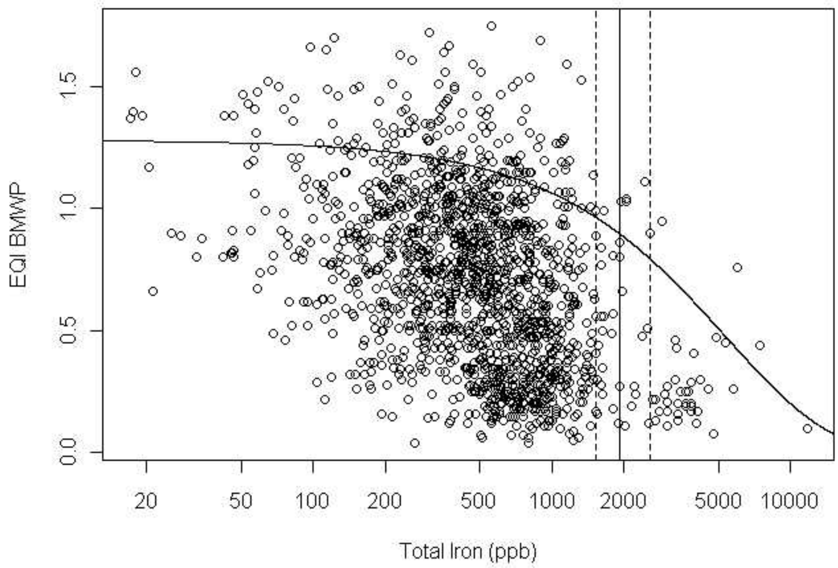

Figure 0.5 EQI BMWP for spring data. The solid vertical line indicates the $GMB_{90th\%ile}$ and the dashed vertical lines indicate the lower and upper 95% confidence interval around this estimate.

Using information on the abundance of taxa, rather than simply whether or not they were present in the sample, appears to provide a more sensitive response to iron exposure. Whilst the use of O/E for the 25% most sensitive taxa does not appear to be appreciably more sensitive than the benthic community as a whole (e.g. when expressed as EQI BMWP), the use of the 10% most sensitive taxa does appear to increase sensitivity.

Analyses based on the most sensitive fraction of the community all show relatively high O/E scores for the highest exposure conditions (0.61 to 0.83). These metrics, which are based on a limited number of taxa, appear to be more sensitive than the whole community metrics such as EQI BMWP to the presence or absence of an individual taxon. Statistical analyses which are based on extreme quantiles of the dataset are likely to be particularly susceptible to this variation. Consequently the use of the 90^{th} quantile is probably most reliable for deriving a limiting function for benthic invertebrates as a result of iron exposure, and is also consistent with the approach used to derive standards for dissolved oxygen and ammonia from field evidence. Co-variation of iron and DOC concentrations may mean that some sites which receive relatively high iron exposures do not exhibit effects, due to a potential protective effect of DOC on iron toxicity, however it is elicited (see Section 4.7).

Analyses based on either the 25% or the 10% most sensitive taxa for each situation, either in the spring or autumn, or when assessed on the basis of either presence or abundance, do not result in quite the same taxa being included in the analyses. It is possible that some taxa could be more sensitive at particular times of the year, if there are particularly sensitive life stages. However, there is substantial consistency in the relative sensitivity ranking of the taxa in each of the analyses (see Table 4.1). Goeridae and Gyrinidae were consistently identified amongst the most sensitive taxa, whereas other taxa such as Leptophlebidae, Perlidae, and Tipulidae were consistently identified as being less sensitive. Therefore, in order to make the analyses of the different ways of expressing the data more consistent the Spring and Autumn average ranking of each taxon was calculated and the ten taxa with the lowest average rankings were selected to comprise an ecological metric for assessing iron exposure.

This metric was defined according to Equation 1

$$O/E_{S10} = (\Sigma O_i + O_j + O_k,...+ 0.1) / (\Sigma O_i + O_j + O_k,...+ 0.1) \qquad \text{Eq. 1}$$

The ten taxa included were (common name and original BMWP score in parentheses): Goeridae (Caddisflies, 10), Gyrinidae (Beetles, 5), Polycentropodidae (Caddisflies, 7), Perlodidae (Stoneflies, 10), Rhyacophilidae (Caddisflies, 7), Ephemeridae (Mayflies, 10), Caenidae (Mayflies, 7), Elmidae (Beetles, 5), Ephemerellidae (Mayflies, 10), and Heptageniidae (Mayflies, 10).

Quantile regression analyses were performed at the 90^{th}quantile for RIVPACS normalised datasets from both the spring and autumn, and considering both presence or absence (PA), and abundance (AB). The results of these analyses for the GMB are provided in Table 4.2, along with the lower (LCL) and upper (UCL) 95 percent confidence limits.

Table 0.2 $GMB_{90th\%ile}$ values derived for the 10 most sensitive taxa (µg l^{-1}).

Analysis	$GMB_{90th\%ile}$	LCL	UCL
Spring PA	993	958	7881
Autumn PA	849	832	1082
Spring AB	768	754	944
Autumn AB	692	644	1405

Notes: PA – Presence/Absence, AB - Abundance

The analyses based on the presence or absence of the most sensitive ten taxa in the spring show considerable uncertainty in the estimation of the threshold values. This is indicated by the range of the confidence intervals surrounding the estimates, which suggest that the uncertainties tend to lie above the derived value. This is likely to be due to the chance occurrence of one of the taxa included in the metric, which would not have been expected to be present. Analyses based on the abundance of the ten most sensitive taxa are shown in Figure 4.6 for Spring, and Figure 4.7 for Autumn.

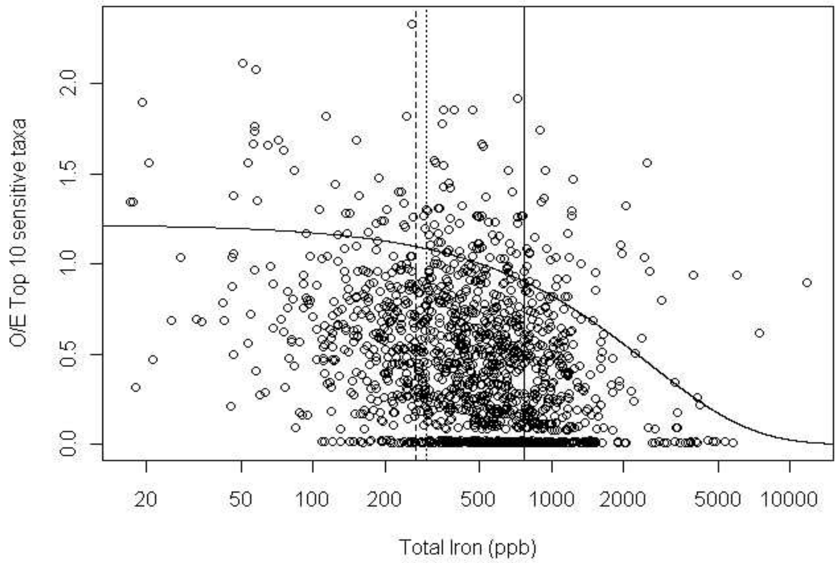

Figure 0.6 Abundance of the 10 most sensitive taxa in the spring; the solid vertical line indicates the GMB

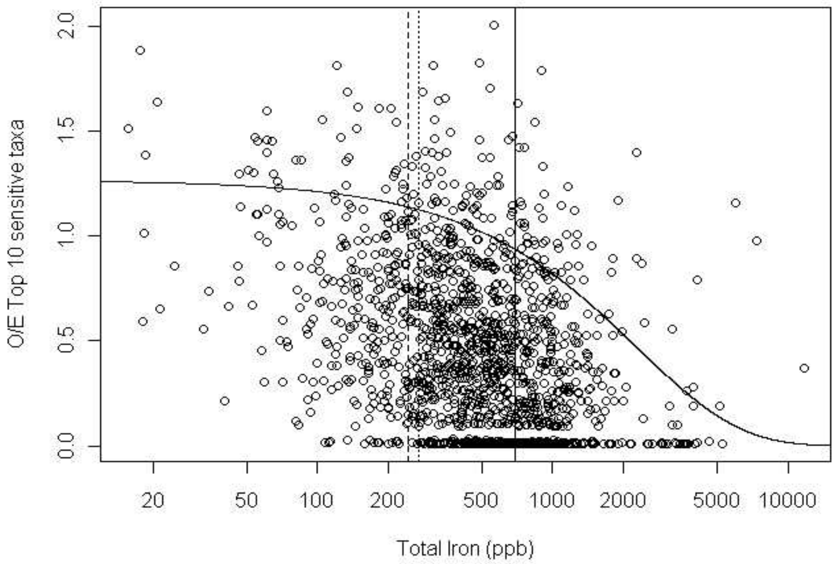

Figure 0.7 Abundance of the 10 most sensitive taxa in the autumn; the solid vertical line indicates the GMB

Analyses based on the abundance of the ten most sensitive taxa are more sensitive than those based on presence or absence, and may therefore be preferable for quality standard derivation, where the protection of sensitive taxa, rather than the whole community, is required. Whilst it appears that these taxa may be more sensitive in the autumn than in the spring the difference is relatively slight, and may not be significant. The average GMB for analyses based on abundance in the Spring and Autumn is 730 $\mu g\ l^{-1}$ (Total Iron), and is recommended as an appropriate threshold for the protection of sensitive benthic macroinvertebrate taxa.

4.5 Analysis using established WFD ecological thresholds

Performing analyses to derive thresholds for iron which are aligned with established ecological quality boundaries under the WFD should enable a better degree of comparability between ecological quality and quality assessed as concentrations of Specific Pollutants. The latter also contribute to the Ecological Quality component of WFD classification. If chemical standards are not aligned to the ecological boundaries there is the potential for a mismatch between the different indicators of “ecological quality”. Where quality standards for pollutants are more stringent than those for ecological quality then a reduction in emissions may not necessarily lead to an improvement in ecological quality. It is generally considered that where a chemical quality standard is achieved the ecological quality should be of good status or higher, in the absence of additional pressures.

It is worth noting that whilst WFD classification of ecological quality appears to accept some degree of disturbance or change in biological communities (e.g. the loss of some taxa) before they fail to achieve good status, WFD chemical standards are usually derived to ensure protection of the most sensitive (tested) species, assuming that if the most sensitive species are protected then community structure (and hence also function) will also be protected (EC 2003).

Analyses of the effects of iron on the whole benthic macroinvertebrate community were performed for the same dataset as for the previous analyses, with Ecological Quality Ratios (EQR values) for each site calculated using RICT (River Invertebrate Classification Tool). The dataset was analysed separately for both Spring and Autumn data using both EQR N-Taxa and EQR ASPT. In addition, O/E BMWP was also analysed, although this metric is not used for classification, so the results of this analysis are not aligned to ecological thresholds as defined under the WFD. The results of analyses based on EQR ASPT are shown in Figures 4.8 and 4.9 for Spring and Autumn respectively. Analyses based on EQR N-Taxa are shown in Figure 4.10 for the Spring, and Figure 4.11 for the Autumn analysis. The results of analyses based on O/E BMWP are shown in Figure 4.12 for the Spring, and Figure 4.13 for the Autumn. The derived thresholds (including lower 95% confidence limits (LCL) and upper 95% confidence limits (UCL)) are shown in Table 4.3.

Table 0.3 Thresholds derived for the Good/Moderate Boundary ($\mu g\ l^{-1}$).

Response Metric	Threshold	LCL	UCL
ASPT Spring	3126	2095	3869
ASPT Autumn	2274	1632	3844
N-Taxa Spring	1737	1574	3007
N-Taxa Autumn	1946	1729	3163
BMWP Spring	1189	1158	2183
BMWP Autumn	1258	1183	2329

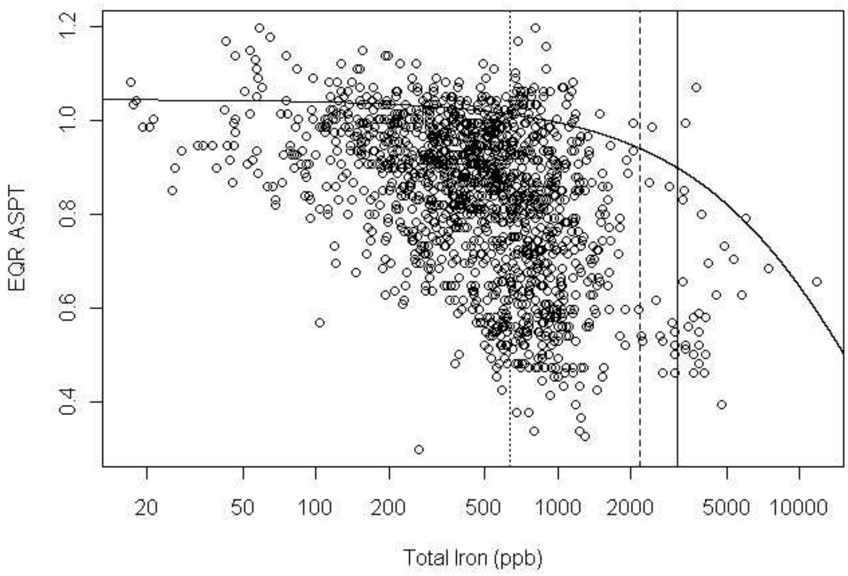

Figure 0.8 Response of EQR ASPT to iron exposure in the Spring; the solid vertical line indicates the GMB.

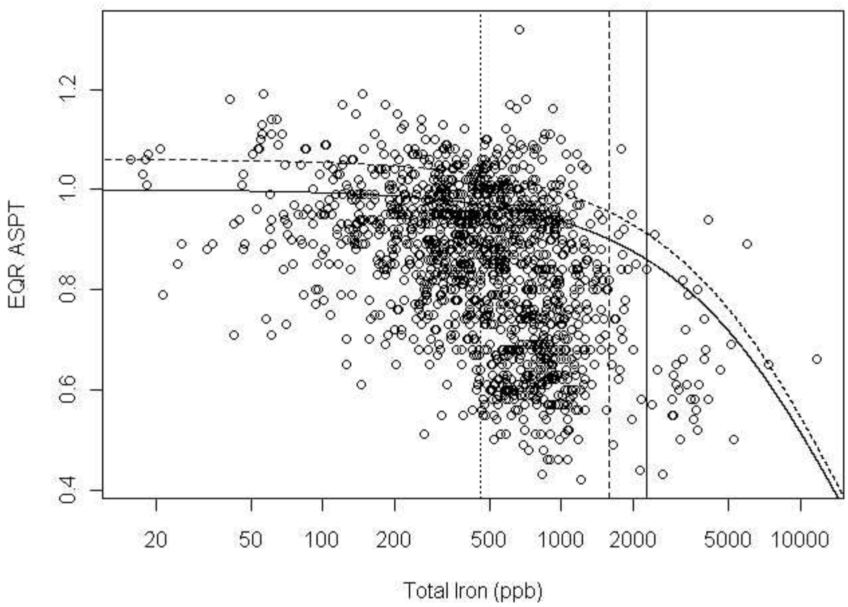

Figure 0.9 Response of EQR ASPT to iron exposure in the Autumn; the solid vertical line indicates the GMB.

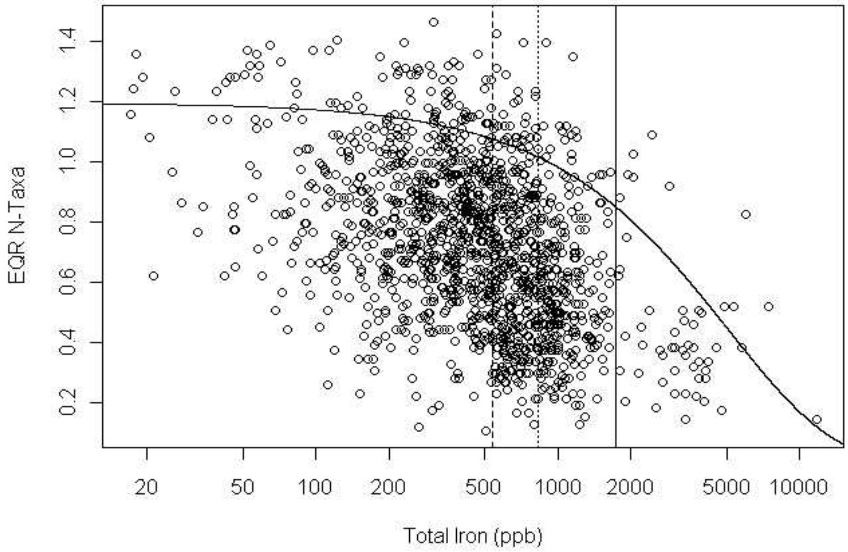

Figure 0.10 Response of EQR N-Taxa to iron exposure in the Spring; the solid vertical line indicates the GMB.

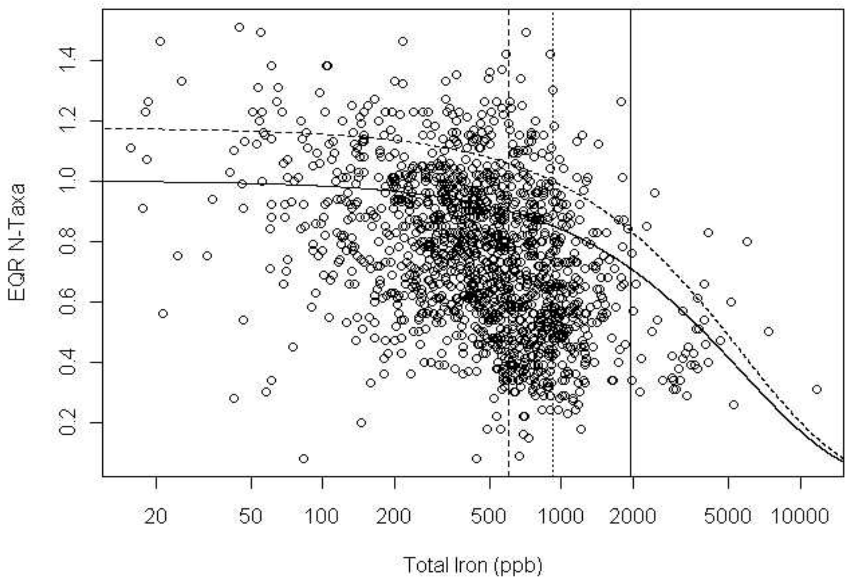

Figure 0.11 Response of EQR N-Taxa to iron exposure in the Autumn; the solid vertical line indicates the GMB.

The O/E BMWP ecological response metric is not used for ecological classification purposes, but has been included here for completeness and because previous analyses have indicated that it may be the most sensitive of the available ecological metrics to the effects of iron exposure. The ecological threshold values applied in the analyses are the same as those for EQR N-Taxa, although it is possible that if BMWP was used for ecological classification then lower thresholds would be established due to the greater apparent variability in this response metric. The thresholds derived on the basis of O/E BMWP are lower than those derived for either EQR ASPT or EQR N-Taxa, but are not directly related to ecological boundaries set under the WFD.

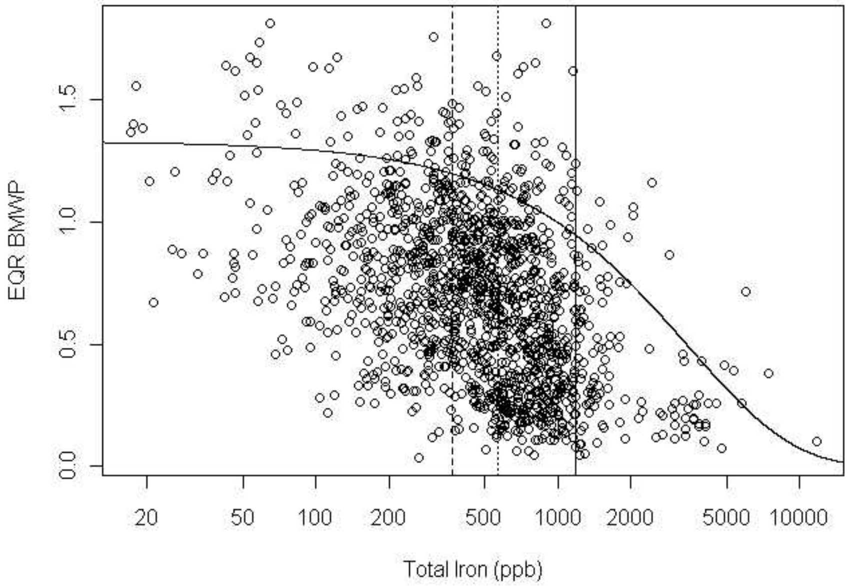

Figure 0.12 Response of O/E BMWP to iron exposure in the Spring; the solid vertical line indicates the GMB.

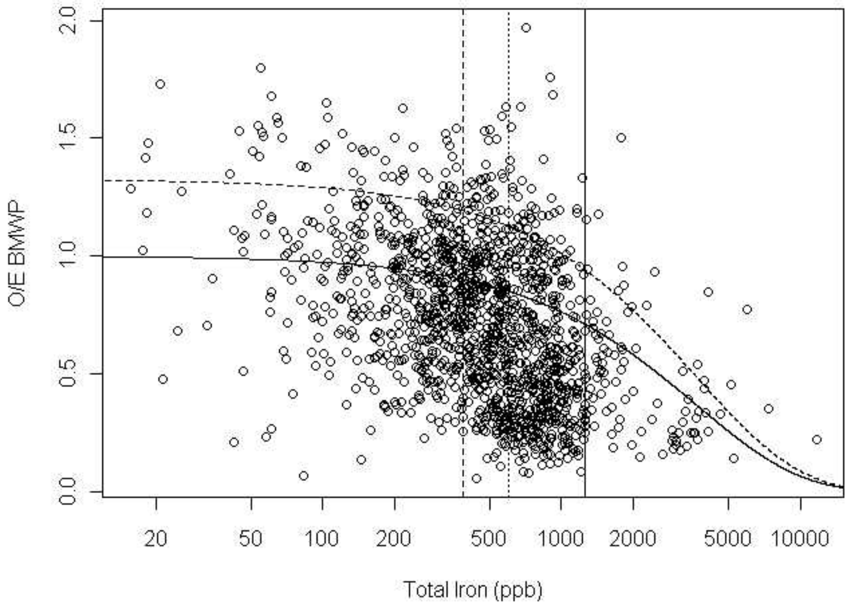

Figure 0.13 Response of O/E BMWP to iron exposure in the Autumn; the solid vertical line indicates the GMB.

Similar datasets for sites with matched iron exposure data and ecological quality for fish, macrophytes, and diatoms have also been assessed in a similar manner to benthic macroinvertebrates. The data are shown in Figure 4.14 for fish, Figure 4.15 for macrophytes, and Figure 4.16 for diatoms. The datasets are less extensive than those for invertebrates, and no statistically significant declines in the maximum achievable ecological quality with increasing iron exposure were observed. In all cases some sites had high ecological quality at relatively high exposure levels. Although these results for the other three measures of ecological quality are less conclusive than analyses based on benthic macroinvertebrates, they do suggest that fish, macrophyte, and diatom communities are not likely to be more sensitive than benthic macroinvertebrates to adverse effects due to iron.

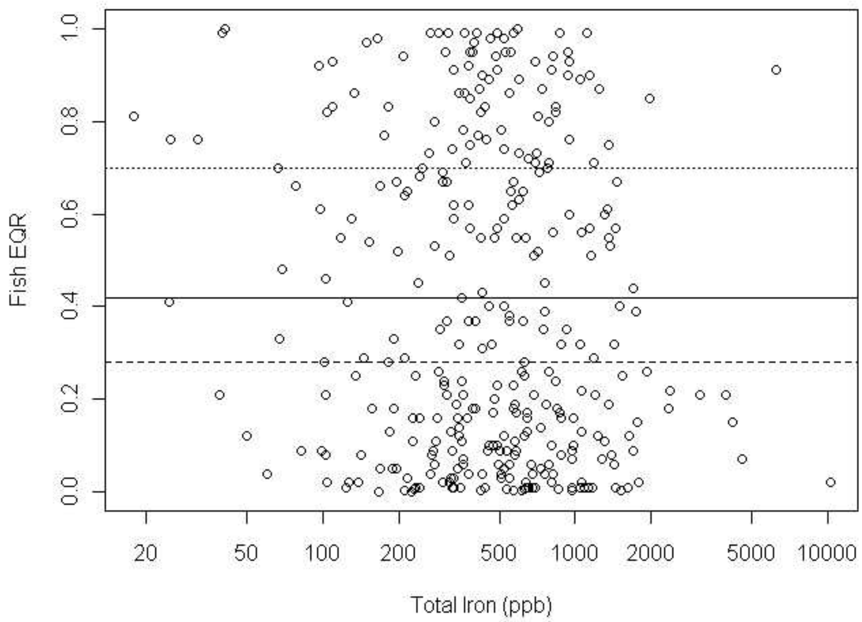

Figure 0.14   Fish EQR as a function of total iron exposure;horizontal lines indicate the HGB (dotted), the GMB (solid), and the MPB (dashed).

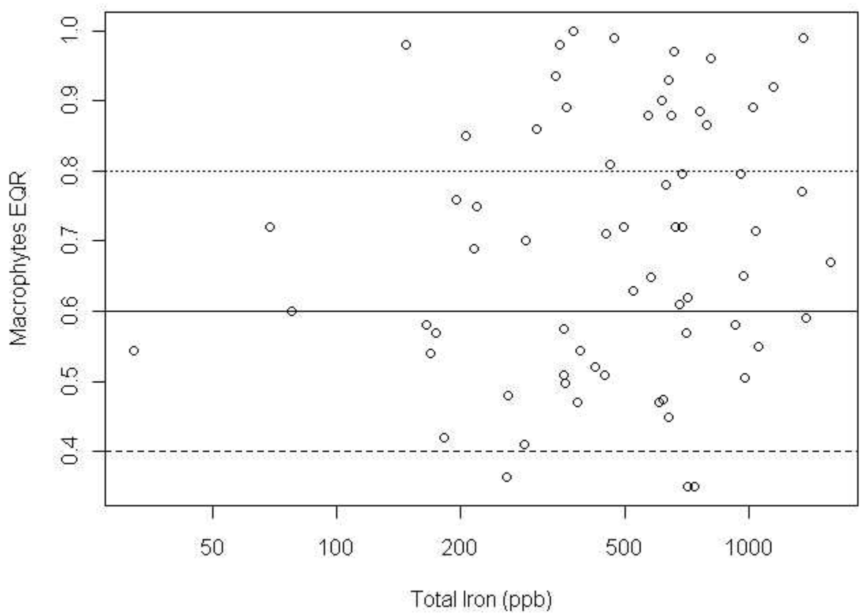

Figure 0.15 Macrophyte EQR as a function of total iron exposure;horizontal lines indicate the HGB (dotted), the GMB (solid), and the MPB (dashed).

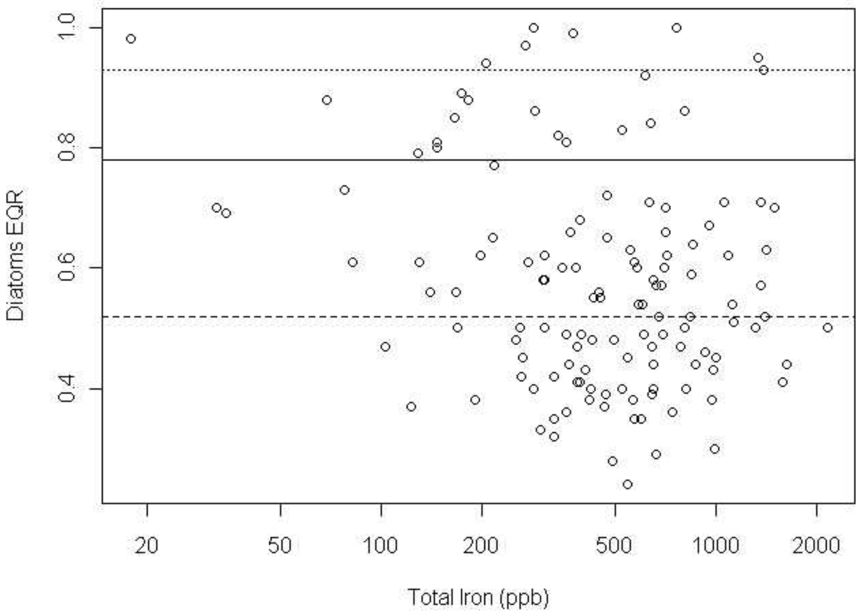

Figure 0.16 Diatom EQR as a function of total iron exposure;horizontal lines indicate the HGB (dotted), the GMB (solid), and the MPB (dashed).

If an EQS for iron is based on ecological data for benthic macroinvertebrates then further analyses of fish, macrophyte and diatom communities in relation to iron exposure conditions should be performed as greater quantities of data become available, in order to ensure that these communities are adequately protected by the standard.

4.6 Covariation of chemical pressures

Correlations were observed between iron exposure and exposure to several trace metals (Cd, Cu, Ni, and Zn) in the dataset used for analyses of the effect of iron on invertebrates. The strength of the correlation depends on how the data are processed for analysis, i.e. whether all pair-wise observations are included for a particular combination of parameters, or whether a subset of data which contains measurements for all of the parameters of interest is analysed, and whether the data are log transformed before analysis. All of these different approaches consistently identified Cu and Zn as correlated with total iron concentrations. Ni and Cd were also identified in some analyses, along with the sewage related parameters ammonia and biochemical oxygen demand.

Dissolved Cu, Ni, and Zn had correlation coefficients of 0.51, 0.48, and 0.36 with total iron in a subset of samples in which all of these parameters had been measured and log transformed prior to analysis. The potential for these contaminants to affect the analysis was considered in more detail by estimating the “bioavailable” concentrations of these metals using information on pH, DOC and Ca concentrations for a separate subset of samples from the same dataset. DOC monitoring data were not available for the dataset, so samples for which default DOC concentrations had been derived by the Environment Agency were used.

Comparisons of total iron concentrations against “bioavailable” concentrations of Cu, Ni, and Zn revealed either a weakly negative correlation (Cu and Zn), or no significant relationship (Ni). This suggests that effects due to Cu, Ni, and Zn are not likely to have been particularly important pressures at high iron exposures, and are therefore not likely to have had a significant influence on the derived dose-response.

4.7 Abiotic factors affecting the apparent toxicity of iron to benthic macroinvertebrates

There are indications that some abiotic factors, such as DOC, can affect the toxicity of iron to freshwater life, and efforts were made to assess this possibility through analysis of the field dataset. This analysis was undertaken by matching sites in the field monitoring dataset to water bodies for which default DOC values have been derived (Environment Agency 2010). The default DOC values were established as the 25th percentile of available DOC monitoring data in order to be generally protective, without being unnecessarily overprotective. A set of 493 samples was available with matched data for total iron, pH, default DOC, and EQI scores. These were sorted into four datasets covering different ranges of default DOC concentrations (number of samples in parenthesis), less than 2 mg l^{-1} (126), 2 to 4 mg l^{-1} (152), 4 to 6 mg l^{-1} (181), and greater than 6 mg l^{-1} (34).

The four data subsets were each analysed using quantile regression analysis to identify whether there was a significant decline in the observed ecological quality, expressed as EQI BMWP. This metric of ecological response was selected for these analyses because it shows a clear decline in relation to increasing iron exposures when the whole dataset is analysed, and is less uncertain than analyses based on the most sensitive identified taxa.

The data subsets for default DOC concentrations below 4 mg l^{-1} both showed a significant decline in the maximum achievable ecological quality with increasing total iron concentrations. In contrast, the data subsets for default DOC concentrations above 4 mgl^{-1} did not show a statistically significant decline in the maximum achievable ecological quality with increasing total iron concentrations. The thresholds identified on this basis were lower by a factor of 2 for sites with default DOC concentrations of less than 2 mg l^{-1} than they were for sites with default DOC concentrations in the range 2 to 4 mg l^{-1}. This suggests that there may be a protective effect of DOC on adverse effects caused by iron exposure, although evidence for this is not sufficiently robust for application in the proposed standard in the absence of any additional supporting data.

The thresholds derived for the data subset for default DOC concentrations between 2 and 4 mg l^{-1} are very similar to those derived on the basis of the entire dataset when DOC concentrations are not taken into account. An $EC10_{90th\%ile}$ of 0.64 mg l^{-1} total iron was derived for this data subset, and compares well with an $EC10_{90th\%ile}$ of 0.62 mg l^{-1} total iron derived from an analysis of the effect of total iron on EQI BMWP for the complete dataset. The analysis therefore indicates an increase in the thresholds of approximately a factor of two for an approximate two-fold increase in DOC concentrations, although it must be borne in mind that the actual DOC concentrations at the monitoring sites are unknown.

Vuorinen et al.(1999) studied the effects of iron, aluminium, humic material and pH on fish. In a study with grayling (*Thymallus thymallus*) yolk-sac fry, iron was added as $FeCl_3$ and $FeSO_4$, at concentrations of 0, 1, 2 and 5 mg l^{-1}, with aluminium added as $AlSO_4$ at concentrations of 0, 100, 200, 400 and 800 µg l^{-1}. Dissolved humic material was added at 0 and 10 mg l^{-1} and exposures at three pH values were performed at 5.0, 5.5 and 6.0 over 8 days (192 hours). Test solutions were renewed every 2 – 3 days. Ten fry were kept per jar and the test temperature was 10°C. Mortality and swimming activity of the fry were measured. Fry were also exposed to natural waters taken from different locations, used to simulate the situation in spring, when peat production waters flow from a clarification basin to a brook, where aluminium-rich meltwaters also flow. Dissolved humic material reduced the toxicity of iron and aluminium to yolk-sac fry. Iron and aluminium still decreased swimming activity and exchangeable body sodium concentrations where humic acid was present up to pH 6. Ion balance was disturbed at pH 5.0 before any observed decrease in swimming activity. Fry kept in natural water with high aluminium and iron concentrations had increased mortality. Effects were not detected in the water with the greatest amount of humic material. Swimming activity also decreased when natural water that was non-toxic was acidified.

A second study with one-summer-old grayling involved exposing them to iron (2 mg l^{-1}) and aluminium (250 µg l^{-1}) in artificial water either containing humic material (15 mg l^{-1}) or not containing humic material, at pH values of 5 and 6. Fish were exposed for three days at 10°C. Over three days, 23% of fish exposed to iron (2 mg l^{-1}) died at pH 5 and none died at pH 6. When exposed to iron and aluminium, 69% of fish died at pH 5 and 8% died at pH 6. Grayling were also exposed to iron concentrations of 1 mg l^{-1}, aluminium concentrations of 100 µg l^{-1}, and humus at 15 mg l^{-1}, at pH 5 and at 3°C and 13°C. The test lasted six days, followed by a seven day recovery period. Test solutions were renewed once or twice a day and fish were not fed. Following exposure and the recovery period, oxygen consumption was measured, and blood samples were taken for the determination of plasma chloride concentrations. Gill samples were also taken and studied. Over six days, 50% of grayling exposed to 1 mg l^{-1} iron and 100 µg l^{-1} aluminium at pH 5.5 died. The gills of the fish exposed in this way had deteriorated and the plasma chloride concentration and oxygen consumption were lower than the control. With humic material added, no grayling died, gill damage was reduced and ionoregulation was not disturbed. After seven days in control water the gills were almost completely recovered and ionoregulation had recovered at 13°C, but not at 3°C.

Peuranen et al. (1994) studied the effect of iron in acute tests on brown trout (*Salmo trutta*) in the presence of humic acid (15 mg l^{-1}) at low pH. Only minimal information on the exposure conditions was provided. Iron exposures were to a mixture of ferrous (as $FeSO_4$) and ferric (as $FeCl_3$) iron, and only a single exposure concentration of 2 mg l^{-1} Fe was tested at both pH 5 and 6. Measured iron concentrations were similar to nominal concentrations (>80%) in the presence of humic acid at both pH levels, and also at low pH in the absence of humic acid. At pH 6 in the absence of humic acid, measured iron concentrations were around 40% of the nominal concentrations, possibly indicating precipitation of iron hydroxides under these conditions. Higher pH conditions and the presence of humic acid both resulted in reduced effects on the fish during the tests. This was considered to be due to reduced deposition of iron on gill surfaces. At pH 5 the addition of 15 mg l^{-1}humic acid reduced, but did not prevent, the accumulation of iron on gills. It is not clear whether the low pH conditions tested were also a source of additional stress to the test subjects in this study.

The limited available evidence suggests that there may be a protective effect of DOC on the potential for iron to cause effects on fish and invertebrates, although the manner in which the laboratory tests have been undertaken, with additional pressures such as low pH and aluminium, means that it is not currently possible to take such an effect into account in the derivation of a PNEC for iron in freshwaters. Some studies are currently being undertaken by the iron industry to consider the effects of DOC on iron toxicity in standard laboratory tests with invertebrates (K. Jackson, Iron Platform, personal communication), and the results of these studies may be helpful in taking account of such effects.

The ecological thresholds derived in this report have not been normalised or corrected for DOC concentrations, principally due to a lack of DOC monitoring data for the field dataset. The thresholds derived are not therefore expected to reflect tolerable iron concentrations under conditions of low DOC (approximately 1 mg l^{-1} or less). Where

DOC concentrations are low the relevant threshold concentrations are expected to be lower than those derived here.

The influence of abiotic factors such as the DOC concentration and water hardness on the effects of iron on aquatic organisms was further assessed. The available dataset of matched ecological and chemical data was supplemented to include measured DOC concentrations for the same sites where this information was available from Environment Agency monitoring databases. Where DOC concentrations had been monitored on several occasions at the sites the median value was used. This resulted in a set of 1082 samples from the CIES dataset with matched monitoring data for total iron, DOC, and hardness, of which 885 had EQI values available from ecological monitoring. A total of 461 of the samples could be matched to ecological monitoring data for which EQR values were available (calculated using RICT).

Two approaches were taken to assess the possible influence of these factors on the effects of iron on benthic invertebrate communities. The first approach involved splitting the data into different ranges of DOC concentrations and analysing each subset individually to derive thresholds for iron. A similar treatment of the data is shown in Figure 4.17, which clearly shows that a broader range of iron exposures occur at low DOC concentrations, whereas at higher DOC concentrations the range of iron exposure concentrations is much more restricted. Particularly noticeable is the absence of samples with low iron exposures, when DOC concentrations are relatively high.

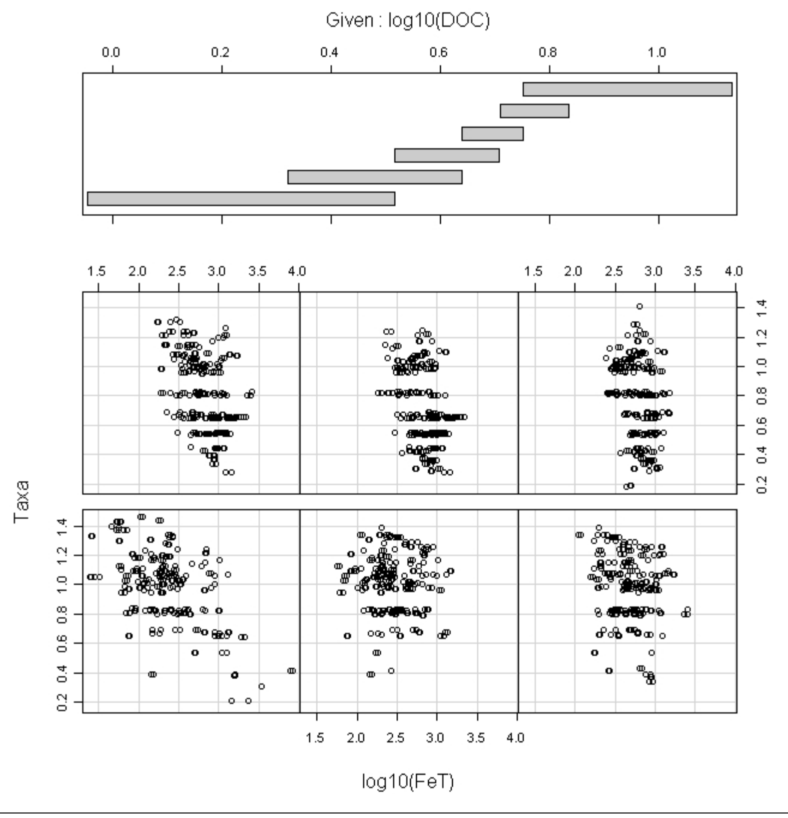

Figure 4.14 Co-plot of EQI N-Taxa as a function of total iron concentrations, for different ranges of DOC concentrations. The lowest DOC concentrations are at the bottom left, and the highest DOC concentrations are at the top right.

The results of these analyses of the data are rather uncertain, particularly for the higher DOC concentrations, because of the limited range of exposure conditions included in each of the subsets of the data, and the relatively small number of samples on which each analysis is based. Figure 4.18 shows the EC10 values derived for each subset of the data, as a function of the average DOC concentration for the subset. At low DOC concentrations an increase in the DOC concentration appears to result in an increase in the derived threshold values, although there is an apparent downward trend in the threshold values at DOC concentrations greater than 4 mg l^{-1}.

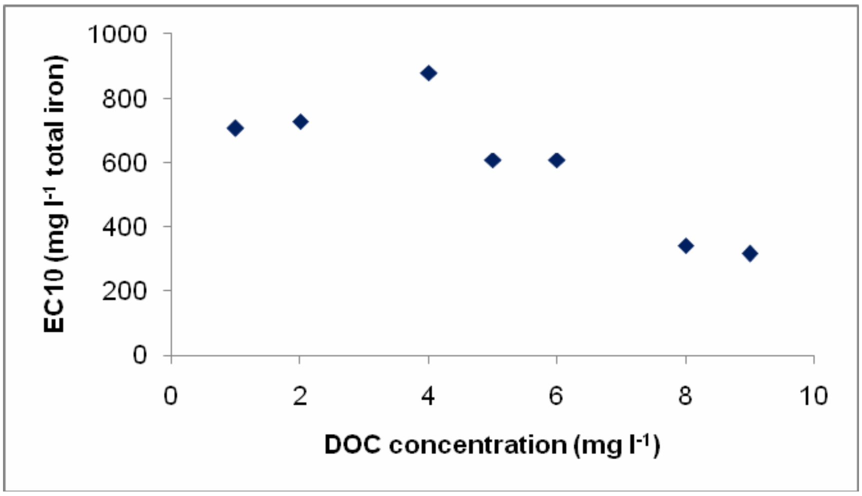

Figure 4.158 EC10 values for EQI N-Taxa derived from data subsets covering different ranges of DOC concentrations. The DOC concentrations represent the average DOC concentration for each subset of data.

A recent study performed on behalf of the Iron Industry to assess the influence of both DOC and water hardness on the effects of iron to *Ceriodaphnia dubia* (CIMM 2010), has shown an effect of both of these parameters on reproductive toxicity. A summary of the findings of these experiments is shown in Table 4.11. The results are expressed as nominal concentrations, and the actual concentrations are likely to be lower than these, although stability tests indicate that a consistent fraction of the nominal iron concentration can be expected to remain available for measurement as total iron. All of the experiments were conducted under relatively high pH conditions and so they may not be applicable to low pH conditions, where the behaviour of iron is expected to be different. However, the majority of UK surface waters are likely to have pH values above 7, although there may be regional variations, for example Scotland has a number of waters with low pH.

An empirical relationship was developed to describe the influence of these two parameters on the effects of iron on *C. dubia* reproduction. DOC appears to increase EC10 value for a given increase in DOC concentration. The sensitivity of *C. dubia* also appears to be affected by water hardness, in that higher hardness results in lower sensitivity to iron, although increased water hardness also appears to reduce the effectiveness of the DOC in protecting against iron. EC10 values were estimated according to Equation 2, which takes account of these effects in estimating the reproductive toxicity of iron to *C. dubia*. The EC10 values for *C. dubia* predicted using Equation 2 are shown against the results of the ecotoxicity tests in Figure 4.19. The outlier which was excluded from the model development is not included in this figure (the prediction for this test differs from the observed result by a factor of 2.34). The relative error for the predictions of the eight test results shown in Figure 4.19 varies between a factor of 0.9 and 1.3 from the observed result.

$$\textit{C. dubia}\ \mathrm{EC10} = (-0.16*\ln(H)+1.45)*DOC+(0.45*\ln(H)-0.27) \qquad \text{Equation 2}$$

Where DOC is the DOC concentration in mg l^{-1}, H is the water hardness in mg l^{-1} $CaCO_3$, and the EC10 is calculated in units of mg l^{-1} iron.

Table 4.2 Variation in *Ceriodaphnia dubia* EC10 for iron as a function of DOC and hardness concentrations in the exposure medium for the CIMM (2010) tests, iron concentrations are expressed as nominal concentrations

DOC	Hardness	*C. dubia* EC10	Estimated EC10
(mg l^{-1})	(mg l^{-1} $CaCO_3$)	(mg l^{-1} iron)	(mg l^{-1} iron)
0	10.6	0.74	0.79
1	10.6	NA	1.86
2	10.6	2.7	2.94
4	10.6	5.1	5.08
0	84	2.2	1.72
1	84	2.7	2.46
2	84	NA	3.21
4	84	5.1	4.69
0	252	2	2.22
1	252	*6.5* [1]	2.78
2	252	3.2	3.35
4	252	NA	4.48

Notes: [1]This data point omitted from model development.

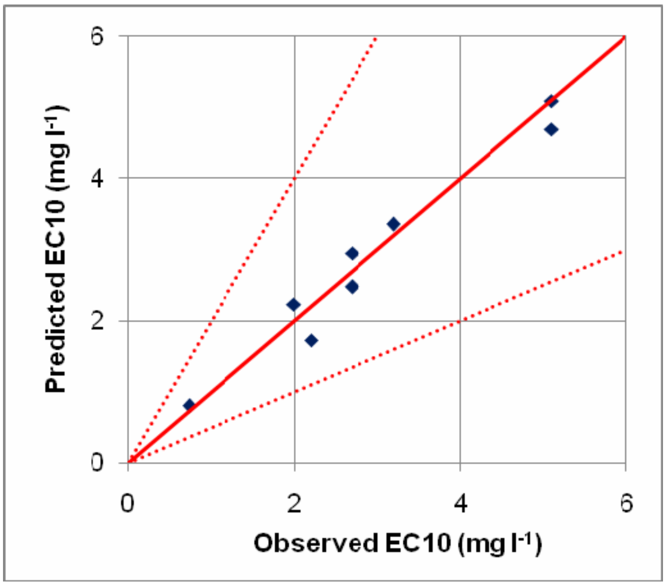

Figure 4.19 Prediction of the effects of iron on *Ceriodaphnia dubia* as a function of water hardness and DOC concentration. The solid red line is a 1:1 line, and the dotted lines indicate a factor of 2 from the true result.

Equation 2 was then used to reinterpret field data by expressing iron exposures on an equivalent basis, i.e. after normalisation for the effects of both DOC and water hardness. This normalisation was performed by calculating the *C. dubia* EC10 for each field sample on the basis of the measured DOC and hardness data, and then expressing these relative to the most sensitive conditions. The most sensitive conditions were low hardness (14.5 mg l^{-1} $CaCO_3$) and low DOC (1.1 mg l^{-1}). This resulted in a correction factor which could be applied to iron exposure concentrations to express them on an equivalent basis. This approach can be seen as conceptually equivalent to calculating a bioavailability factor to correct trace metal exposure concentrations for bioavailability. The resulting iron exposure concentrations are referred to as "Effective Iron" concentrations, and are expressed in the same units as the original iron exposure measurements ($\mu g\ l^{-1}$).

This method of analysis was applied to 885 samples for which both the required water quality parameters and RIVPACS predictions were available from the CIES dataset. A subset of these samples also had RICT predictions available (461 spring, 455 autumn). Figure 4.20 compares these data as either total iron concentration or the "Effective Iron" concentration.

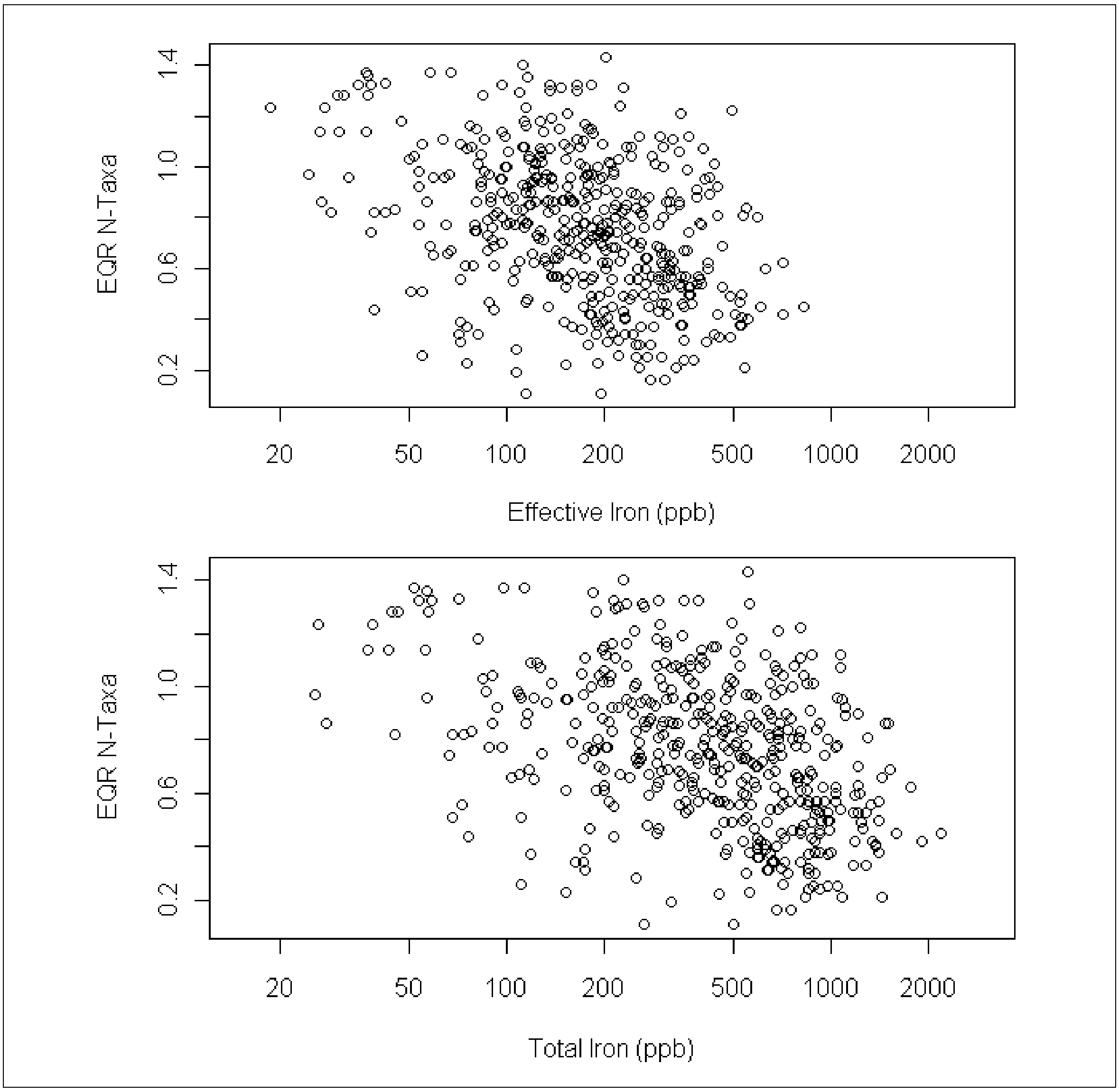

Figure 4.20 Comparison of the effects of iron on benthic macroinvertebrate communities with iron exposures expressed either as "Effective Iron" concentrations (upper) or total iron concentrations (lower) for a single set of data.

The thresholds derived on the basis of "Effective Iron" concentrations express the iron concentrations as an equivalent total iron concentration under conditions of high potential toxicity. These conditions are the combination of low DOC and low hardness. The most sensitive conditions in the datasets were 1.1 mg l^{-1} DOC and 14 mg l^{-1} ($CaCO_3$) hardness. Approximately 20 percent of surface waters in Great Britain could be expected to have lower water hardness, and approximately 5 percent could be expected to have lower DOC concentrations. More than 80% of the field samples have pH values above 7, and 45% have pH values above 7.5, indicating that the high pH conditions of the toxicity tests on which the corrections are based are likely to be applicable to these samples.

Ecological thresholds were derived on the basis of these analyses in which iron concentrations are expressed as an equivalent total iron concentration under conditions of high potential toxicity. The thresholds derived from the analyses of ecological quality calculated using RICT are shown in Table 4.12, and those derived from the CIES dataset are shown in Table 4.13.

Table 4.3 Ecological thresholds derived from analyses of RICT datasets with iron concentrations expressed in terms of "Effective Iron" ($\mu g\ l^{-1}$) for the GMB.

Response	Spring	Autumn
EQR N-Taxa	422	446
EQR ASPT	852	553

The thresholds derived from the same set of data for the GMB on the basis of total iron concentrations were 1120 (spring) and 940 (autumn) mg l^{-1} when based on EQR N-Taxa. This suggests that thresholds derived on the basis of total iron concentrations are approximately 2.5 times higher than those derived from data which have been normalised to conditions of high iron toxicity. The actual difference between the analyses is a factor of 2.65 in the spring and 2.11 in the autumn. A similar difference was also observed between the normalised and non-normalised results for the CIES data when assessed on the basis of EQI N-Taxa, although analyses based on O/E BMWP suggested a much smaller difference between the normalised and non-normalised results. The threshold values derived from analyses of the RICT and CIES data were quite different, with those derived from the CIES data approximately twice as high as those derived from the RICT data. The reasons for these differences are unclear, although both analyses suggest a similar modifying effect of DOC and Ca on the potential effects of iron on benthic macroinvertebrate communities.

Table 4.4 Ecological thresholds derived from analyses of RIVPACS datasets with iron concentrations expressed in terms of "Effective Iron" ($\mu g\ l^{-1}$).

Response	**GMB**
EQI N-Taxa	829
O/E BMWP	1624

We recommend use of the thresholds derived from larger, non-normalised, datasets for which ecological quality has been calculated using RICT, and application of a correction based on the difference between analyses of "Effective Iron" and total iron. This suggests that under conditions of high potential iron toxicity the thresholds are likely to be between 2.11 and 2.65 times lower than they appear to be when assessed in terms of total iron concentrations. These thresholds are proposed because of the unexplained differences between the threshold values derived from the different datasets for which suitable water chemistry information was available to undertake the analyses in which exposures were normalised to conditions of high potential iron toxicity, Thus the GMB (1737 and 1946 $\mu g\ l^{-1}$ for spring and autumn respectively) is corrected to 655 and 922 $\mu g\ l^{-1}$, depending on season. Taking averages of the summer and autumn results of the analysis results in a GMB of 774 $\mu g\ l^{-1}$ total iron for conditions of high potential iron toxicity. These thresholds could be corrected for differences in the potential iron toxicity due to differences in local water quality conditions (water hardness and DOC) through Equation 2.

4.8 Summary of the effects of iron on ecological communities

Analyses have been performed to identify the most sensitive benthic macroinvertebrate taxa to total iron exposures, and these have identified as follows: Goeridae (Caddisflies), Gyrinidae (Beetles), Polycentropodidae (Caddisflies), Perlodidae (Stoneflies), Rhyacophilidae (Caddisflies), Ephemeridae (Mayflies), Caenidae (Mayflies), Elmidae (Beetles), Ephemerellidae (Mayflies), and Heptageniidae (Mayflies). Substantial consistency has been observed between the results of this analysis and the results of similar analyses based on US datasets (Linton et al. 2007). A direct comparison of the two datasets (UK and US) is difficult because of the inability to normalise the observed ecological communities in the US dataset to a reference condition, which is performed using RIVPACS III+, or RICT, for the UK dataset.

Both this analysis and that of Linton et al. (2007) have identified mayflies (Ephemeroptera) as sensitive to the effects of iron, which is consistent with laboratory toxicity studies (Vangheluwe and Versonnen 2004). A concentration of 730 $\mu g\ l^{-1}$ total iron, which is the mean GMB threshold for analyses based on the abundance of sensitive invertebrates in spring and autumn with no corrections for DOC or hardness, is proposed as an appropriate threshold for the protection of the <u>most sensitive</u> macroinvertebrate taxa. This threshold is consistent with the standard approach towards EQS derivation, in that it aims to ensure protection of the most sensitive species. The threshold is also broadly consistent with the results of laboratory ecotoxicity tests which indicate low levels of effects on sensitive organisms at iron concentrations of approximately 1 $mg\ l^{-1}$.

Thresholds derived on a <u>whole community</u> basis using EQR ASPT and EQR N-Taxa values calculated using RICT are 2274 and 1946 $\mu g\ l^{-1}$ total iron respectively for the GMB. This suggests that the threshold proposed above will ensure the ability of these communities to achieve good ecological status if the standard is met. At the levels of iron exposure derived for the GMB of the whole community a significant reduction in the abundance of some sensitive taxa can reasonably be expected.

There are no indications of a significant decline in the maximum achievable ecological quality of fish, macrophyte, and diatom communities when assessed in a similar manner to the analyses performed for benthic macroinvertebrates. This provisionally suggests that these communities are likely to be protected by thresholds established on the basis of benthic macroinvertebrate communities. Further analyses of these trophic levels should be performed in order to ensure that this is the case, as suitable data become more abundant.

Taking account of the sensitivity of invertebrates to iron due to changes in water chemistry conditions results in a GMB of 774 $\mu g\ l^{-1}$ total iron for conditions of high potential iron toxicity. These thresholds could be corrected for differences in the potential iron toxicity due to differences in local water quality conditions using an empirical relationship for the influence of water hardness and DOC on iron toxicity to invertebrates. Currently, however, there is insufficient evidence to ensure that such effects would be protective of all trophic levels and there is a lack of validation of the approach in natural water samples. It is not, therefore, considered to be appropriate to apply a bioavailability correction for iron exposures based on the currently available evidence, although it is possible that such evidence may become available in the future.

One argument for the use of assessment factors in EQS derivation is that real environmental exposures are to complex mixtures of contaminants, whereas laboratory ecotoxicity tests typically consider exposure only to a single substance. A consequence of this is that a threshold or EQS which is derived from field data has been derived from exposures in the presence of other potential pressures, and whilst the nature of these other pressures may not be clear, the standard derived should take account of the resulting exposure to a complex mixture of contaminants at low levels in addition to exposure to the contaminant of interest.

Assessment factors are typically applied to derive standards from laboratory tests in order to account for “residual uncertainty”. This covers issues such as the range of species present in the dataset, the duration of the exposures, knowledge of the mode of action, uncertainties in the threshold estimates, and evidence from real (or simulated) ecosystems. The datasets used in this analysis consider all of the 76 BMWP scoring families of benthic macroinvertebrates, and has also considered field evidence for fish, diatoms, and macrophytes. There is therefore extensive taxonomic coverage of species which are relevant to the conditions in which the standard would be applied. The exposures are expressed as annual averages, and relate to the indigenous communities in freshwaters throughout Great Britain, so are considered to relate to long term exposure periods.

There is uncertainty surrounding the actual mode of action of iron, although it is considered likely that the adverse effects are due to physical effects and smothering of habitats rather than iron acting as a chemical toxicant. The proposed threshold is derived as the mean of two separate analyses for which the lowest of the lower 95% confidence intervals was 644 $\mu g\ l^{-1}$, which suggests a relatively low level of uncertainty although the range of the upper 95% confidence intervals was broader. This suggests that the uncertainties in the derivation of the threshold tend to lie above, rather than below, the derived value. All of the data used for the derivation of the thresholds in the present study were collected from real ecosystems and are therefore considered to be directly relevant to the conditions under which the standard may be applied.

The thresholds derived in the report are considered to be appropriate for application as Environmental Quality Standards without the need for an additional assessment factor. This is due to the extremely low residual uncertainty, and the fact that the thresholds have been derived in the presence of additional stressors.

5. Conclusions and Recommendations

Iron has a potentially complex chemistry in freshwaters, due to the oxidation of Fe(II) to Fe(III), and the precipitation of Fe(III) to form colloidal or fine particulate material. In addition, iron may interact with DOC, either by direct binding of free Fe ions or through associations between DOC and precipitated forms of iron. Many historic ecotoxicity tests are considered to have tested the “toxicity” of a suspension of precipitated material, and often have limited detail on the actual exposure conditions. This means that most of the available test data are rather uncertain.

Some ecotoxicity tests suggest that under conditions in which the form of iron is changing, due to oxidation or hydrolysis, aquatic organisms may be considerably more sensitive to iron toxicity, although the effects may be exerted in a different manner to those observed under more stable conditions. Such effects are likely to be transient in most circumstances and are therefore not taken into account in the derivation of a PNEC for chronic effects. Several ecotoxicity tests have indicated NOEC values for iron between 0.3 and 0.6 mg l^{-1}, although the LOEC values from these tests are greater than 1 mg l^{-1} in all cases. As a result of the uncertainties about available ecotoxicity data, this report has focused on the use of field data with matched monitoring for both ecology and chemistry. These datasets have been used to derive thresholds for iron concentrations which are consistent with the ability of benthic macroinvertebrate communities to achieve particular predefined Ecological Status objectives under the WFD. The thresholds derived are considered to be broadly comparable to the results of some of the more sensitive ecotoxicity test data.

5.1 Conclusions

Analyses of data for fish, macrophyte, and diatom communities did not show any statistically significant decline in the maximum achievable ecological quality with increasing total iron exposures. Assessments based on benthic macroinvertebrate communities did show a statistically significant decline in response to increasing total iron exposures, and thresholds have been derived on both a whole community basis, for direct comparison with ecological quality standards, and also for the most sensitive fraction of the community. Thresholds have been derived for the boundary between High and Good ecological status (HGB), the boundary between Good and Moderate ecological status (GMB), and also for a 10% effect level (EC10).

Thresholds have been derived, which are not normalised for water quality conditions, for the protection of sensitive macroinvertebrate taxa, and for the protection of benthic macroinvertebrate communities. Both of these thresholds have been derived to be consistent with the Good/Moderate boundary (GMB) for ecological status as defined under the WFD. The proposed threshold is **0.73 mg l^{-1} total iron** for the protection of sensitive taxa. A threshold of **1.84 mg l^{-1} total iron** was also derived for the protection of the whole community (using community metrics agreed for use in classification under the WFD), although compliance with such a threshold would not ensure protection of the most sensitive species.

As these thresholds are not normalised for possible differences in iron toxicity under different water quality conditions they may not necessarily be protective of iron exposures under sensitive conditions. Therefore, ecological thresholds which have been normalised for differences in potential iron toxicity on the basis of DOC and water hardness have been derived in this report and compared to thresholds derived for non-normalised analyses of the same datasets. Thresholds which relate directly to defined measures of ecological status under the Water Framework Directive can therefore be proposed which are expected to be protective of sensitive conditions and can also be adjusted through the use of an empirical relationship between DOC concentrations, water hardness, and iron toxicity to invertebrates where conditions are less sensitive. Thresholds which are normalised in this way have been derived only for the whole community, and not for the most sensitive taxa, and the value relating to the Good/Moderate boundary (GMB) for ecological status is **0.77 mg l^{-1} total iron** under sensitive conditions of low DOC and low hardness. This value is derived from analyses of the whole community in both spring and autumn which have been corrected to account for the increased sensitivity to iron under low DOC and low Ca conditions. The value of 0.77 mg l^{-1} is the mean of the corrected GMB values for N-Taxa from spring and autumn analyses.

The threshold derived for the whole community under sensitive conditions suggests that although the proposed standard may not be fully protective of all of the most sensitive species under conditions of high potential iron toxicity, it would be protective of the community overall. This supports the use of the threshold derived from the most sensitive taxa as the standard.

This threshold derived in the present study is considered to be applicable to waters with a pH of greater than 7, but there is considerable uncertainty about its relevance to waters of lower pH. Ecological monitoring of benthic macroinvertebrate communities, with a particular focus on the most iron sensitive taxa, is recommended in order to ensure that the standard is adequately protective under more acidic conditions.

5.2 Recommendations

The proposed threshold is **0.73 mg l^{-1} total iron** and is derived to be protective of the most sensitive invertebrate taxa, without making any correction for mitigating effects of hardness or DOC. Analyses of the whole community, and taking account of differences in iron toxicity under different water chemistry conditions suggest that this threshold will also be protective of the whole community under the most sensitive conditions, although a reduction in the abundance of the most sensitive taxa may be possible. Figure 5.1 shows the use of analyses based on sensitive taxa, and analysis based on the whole community taking into account the effect of differences in water chemistry on the sensitivity of invertebrates to iron.

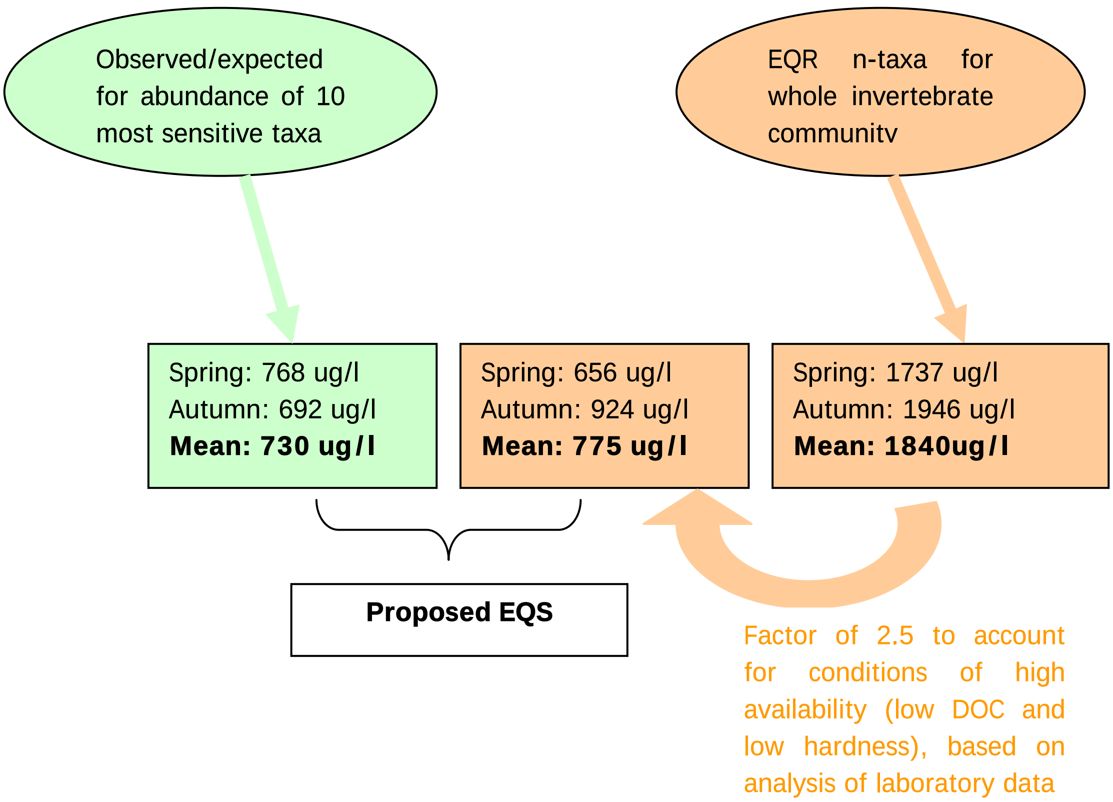

Figure 5.1 Derivation of the EQS proposal for iron.

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