Development and use of the nickel bioavailability assessment tool (Draft)

by

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

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Health and safety statement
WARNING. Working in or around water is inherently dangerous; persons using this standard should be familiar with normal laboratory and field practice. This published monitoring system does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national regulatory guidelines.

It is also the responsibility of the user if seeking to practise the method outlined here, to gain appropriate permissions for access to water courses and their biological sampling.

EXECUTIVE SUMMARY

Chronic Biotic Ligand Models (BLM) have been developed for nickel (Ni) by the Nickel Producers Environmental Research Association (NiPERA). These models were used in the fulfilment of the requirements under the Existing Substances Regulation (793/93). There are four BLMs in all, one for fish, algae and two for cladocerans. The models can account for Ni bioavailability in freshwaters and can be used to assess potential aquatic risks. However, they are relatively complicated to use and require considerable resource and skill to interpret the outputs.

NiPERA recently commissioned Karel De Schamphelaere of the University of Ghent to develop an integrated version of the NiBLMs and which incorporates some recent developments in the understanding of nickel behaviour and increases the breadth of physico-chemical boundaries over which the models apply. Nevertheless, this integrated version of the BLM is still relatively complex in terms of data manipulation and output interpretation.

It was therefore considered necessary to develop a simplified version of the integrated NiBLM to facilitate the wider use of bioavailability-based assessments for Ni.

This project developed a Ni Bioavailability Tool (NiBAT) for use by both the regulated and regulator communities. The NiBAT mimics the NiBLM, but runs in Microsoft Excel™ and requires data input for site-specific dissolved organic carbon, pH and calcium. The NiBAT uses an algorithm and constants which can be readily automated within Laboratory Information Management Systems (LIMS). The performance of this NiBAT compared with the integrated NiBLM is reviewed and discussed in this report.

Bioavailability Tools have been developed for other metals (copper, manganese and zinc) and are readily accepted by regulatory organisations. Guidance on the use of the NiBAT and interpretation of the outputs from the tool, including screenshots, is also provided here.

Finally, a face-value assessment (i.e. without considering confidence of failure statistics) is provided for matched monitoring data from samples and sites in England and Wales by applying the EU-wide generic $EQS_{bioavailable}$ of 4µg Ni L^{-1} and NiBAT to account for bioavailability. The results of this assessment show that of the 235 sites assessed, 44 sites gave a face-value exceedance of the $EQS_{bioavailable}$ on at least one sampling occasion when the measured concentration is adjusted for bioavailability using NiBAT.

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GLOSSARY

BioF	The bioavailability factor. The BioF is based on a comparison between the expected bioavailability at the reference site and that relating to site-specific conditions. Through the use of a BioF, differences in (bio)availability are accounted for by adjustments to the monitoring data but the EQS remains the same. It is calculated by dividing the Generic or Reference EQS by the calculated site-specific EQS.
BLM	Biotic Ligand Model. This is a predictive tool that can account for variations in metal toxicity and calculates a site-specific PNEC using information on the chemistry of local water sources, i.e. pH, calcium concentrations, hardness, dissolved organic carbon, etc.
MBAT or BAT	Metal Bioavailability Tool or Bioavailability Tool. Effectively is a simplified version of the BLM. It performs the same calculations as the BLM, but is run in MS Excel, requires fewer data inputs, and gives outputs that are precautionary relative to the full BLM but that are readily interpretable in the context of basic risk management and EQS compliance assessment.
HC5	Hazardous Concentration to 5 percent of tested aquatic organisms.
DOC	Dissolved organic carbon. The input to the screening tool for DOC should be site-specific median concentrations from at least eight sampling occasions. Default waterbody values of DOC are available for some waterbodies[1].
EQS	Environmental Quality Standard. A term used for long-term water qualitu standard in Europe, using the annual average concentration of a substance.
Generic EQS	Generic Predicted No Effect Concentration, sometimes also termed the reference or generic EQS. This is representative of conditions of high bioavailability and is expressed as "bioavailable" metal concentration.
PEC	Predicted Environmental Concentration. These are usually replaced in the screening tool with measured environmental concentrations of dissolved nickel in the waters of interest.
PNEC	Predicted No Effect Concentration. This concentration is derived from the ecotoxicological data and site-specific water quality data using the BLM.
RCR	Risk Characterisation Ratio, also sometimes called the risk quotient. This is calculated by dividing the PEC by the PNEC. Values equal to or greater than 1 present a potential risk.

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[1] ENVIRONMENT AGENCY. 2010. The importance of dissolved organic carbon in the assessment of environmental quality standard compliance for copper and zinc. Draft final report SC080021/SR7a. Environment Agency, Bristol, UK.

1 INTRODUCTION

This report describes an approach to account for Nickel (Ni) bioavailability that is consistent with Water Framework Directive (WFD) requirements and implementable in routine regulatory systems for the UK. Specifically, in this report we:

- Describe the development of a Metal Bioavailability Tool (BAT) for Ni in freshwaters.

- Explain how the BAT can be used and implemented in regulatory frameworks.

- Assess compliance against the proposed EQS value for Ni, using freshwater monitoring data from England, Wales and Scotland.

In this introduction we briefly describe the background to the development of a BAT for Ni before outlining what the tool is, and how and where it should be used. In Section 2 we describe in more detail the construction and testing of the tool, and how the Ni Biotic Ligand Model (BLM) has been used. Section 3 gives instructions on the use of the MBAT, data requirements, inputting data, and the interpretation of outputs. The results of the EQS face-value compliance assessment for Ni for England and Wales are given in Section 4. Finally, in Section 5 we provide a brief comparison between NiBAT and the other freely available user-friendly tools for accounting for metal bioavailability, Bio-Met[2] and PNECpro[3].

1.1 Background

Nickel ecotoxicity in freshwater systems is well documented, in part driven by research programmes undertaken by NiPERA and the completion of the Risk Assessment Report under of Existing Substances Regulation (793/93/EEC)(EC 2008). This has led to a recent revision of the Ni EQS under the Water Framework Directive, where Ni is identified as a Priority Substance. The annual average EQS has been changed from 20 µg Ni L^{-1} to an $EQS_{bioavailable}$ of 4 µg Ni L^{-1} (EC 2012). Therefore, in order to implement this EQS in national regulation for compliance, classification and permitting it is imperative to have a methodology to account for Ni bioavailability.

The Risk Assessment Report for Ni and subsequent publications detail the development of four NiBLMs for three trophic levels[4]. These models predict the chronic ecotoxicity of Ni to aquatic organisms in freshwaters as a function of water physico-chemistry. The models that were derived are consistent with models of the toxicity of other metals to

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[2] http://bio-met.net/
[3] www.pnec-pro.com
[4] http://www.nipera.org/~/media/Files/NiperaFactSheet4/EU%20Ni%20RA%20Fact%20Sheet%204%202012%20June.ashx (and reference therein).

aquatic organisms: divalent cations can act as competitors to Ni toxicity in fish and invertebrates, and protons act as competitors to Ni toxicity in algae. The models are able to predict Ni ecotoxicity to test organisms to within a factor of two in most cases.

The validated boundary conditions of the NiBLMs for pH have recently been extended upwards in order to increase the applicability domain of freshwaters (from pH 8.2 to 8.7) (Nys et al. 2012). This was undertaken in response to requests from Member States (e.g. Hommen and Rüdel 2012) who considered the ranges were too narrow and so now the NiBLMs cover the upper 97th percentile of pH values waters in the EU (as determined using FOREGS[5]). These new boundary conditions have been incorporated into an Excel-based tool developed by Karel De Schamphelaere of the University of Ghent that can be used with WHAM VI[6]. This tool includes an updated database which is used to normalise all the chronic Ni ecotoxicity dataset to water chemistry conditions in the ranges outlined in Table 1.1 and to derive HC5s.

Table 1.1   Validated boundary ranges

Metal	pH	Calcium, mg L^{-1}	DOC, mg L^{-1}
Ni	6.5-8.7	2.0-88*	30

* These upper limits reflect only where the protective effect of Ca ceases, so above this value the EQS is not going to be underprotective (No Type II errors).

However, the calculation process remains a relatively complex one, with an initial step to convert water chemistry determinands to molar concentrations for input into WHAM VI to calculate the speciation. This is followed by a number of steps which use the WHAM VI outputs in the Excel-based tool to perform the normalisation for each species in the SSD and then a final step in WHAM VI to convert these outputs back into dissolved nickel concentrations. The process requires considerable technical skill to use and interpret and is also extremely time consuming when applied to moderately large datasets. It is therefore unlikely to be widely used in a routine regulatory context. However, it is possible to develop simplified versions of the process (e.g. Environment Agency 2009).

1.2   What is a Bioavailability Tool (BAT)?

Bioavailability Tools have been developed to provide a simple, straightforward and easily implementable methodology to account for metal bioavailability in freshwaters[7]. Bioavailability can mean a number of different things depending on the area of science, but in relation to the BAT it is considered to be a combination of the physico-chemical factors governing metal behaviour at the biological receptor, i.e. its specific

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[5] http://weppi.gtk.fi/publ/foregsatlas/
[6] http://www.ceh.ac.uk/products/software/wham/
[7] http://www.wfduk.org/reference/environmental-standards

pathophysiological characteristics (such as route of entry, and duration and frequency of exposure). Effectively this means that a measure of bioavailability will reflect what the organism in the water column actually "experiences". This is important as it has long been established that measures of total metal in waters have limited relevance to potential environmental risk (Campbell 1995; Niyogi and Wood 2004).

One way to account for bioavailability is through the use of BLMs. Unlike many other speciation-based approaches, the BLMs have been rigorously tested in the laboratory and field; and they routinely predict ecological effects to many aquatic taxa across a wide range of water chemistries to within a factor of two. Recent European guidance recommends that where bioavailability models exist they should be used in setting and assessing EQS for metals under the WFD (EC 2011). However, there are some major drawbacks in implementing the BLMs in a routine regulatory context. Specifically, the model complexity, runtime per sample, input data requirements and the level of operator skill needed to interpret the outputs mean that few regulatory organisations have adopted BLMs.

A user-friendly tool has been developed that utilises calculations from the full BLM as the basis for determining the appropriate bioavailable EQS for a given combination of pH, hardness, and DOC. This is a web-based tool available at www.bio-met.net. While bio-met offers straightforward use, the user still has to manually enter the relevant water quality data. For situations like the UK, which utilises a central analytical laboratory and information system, the process can be simplified even further by incorporating the predictive capacity offered by the bio-met tool (and hence the full BLM) directly into the central laboratory system.

It is against this backdrop that MBAT, also available for zinc, manganese and copper, was developed[8]. Bioavailability Tools provide an implementable methodology to account for metal bioavailability. They maximise the use of current understanding of metal fate and behaviour in freshwaters, but are practical regulatory tools with few data inputs. Detailed descriptions of the development of the BAT for zinc and manganese are provided in previous Environment Agency Science Reports and the open literature (2009; 2010; Comber et al. 2008; Peters et al. 2011). Generally, the BAT estimates are typically within a factor of 2 compared to the full BLMs[9]. Recent regulatory investigations by Germany and France have also assessed the ease of implementation of user-friendly tools (including MBAT) to account for bioavailability (Geoffroy et al. 2010; Hommen and Rudel 2012).

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[8] http://www.wfduk.org/reference/environmental-standards
[9] http://www.bio-met.net/

1.3 Why and where should the bioavailability tool be used?

The MBAT can be used in an early tier within a tiered risk-based framework (Figure 1.1) or as part of the approach to determine site-specific issues for dischargers. The use of the MBAT in a tiered approach is consistent with classic risk assessment paradigms in that analyses in early tiers are precautionary, but simple to perform with large numbers of sites. As progress is made through the tiers the site numbers are reduced and the levels of precaution and uncertainty decrease. A description of the activity within each tier shown in Figure 1.1 is given below (updated from Environment Agency 2009, Paul Whitehouse, Environment Agency pers. comm.). It may be possible that at Tier 3, as part of the local refinement, the Excel-based normalisation tool could be used to further investigate an exceedance of the EQS. As indicated above this may deliver a slightly less precautionary assessment (Section 2.3). The NiBAT would be used in Tier2.
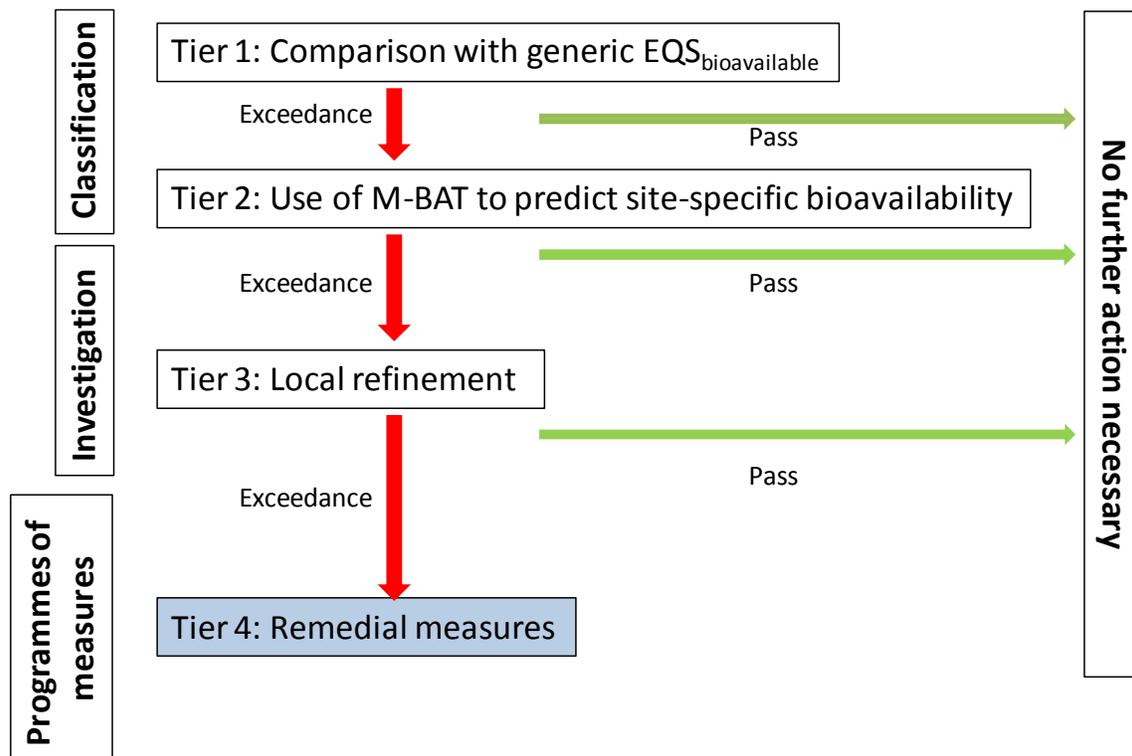
Figure 1.1 **Flow diagram of the stages of a tiered EQS compliance assessment under the Water Framework Directive.**

Tier 1. The first tier in the scheme considers a direct comparison of the annual average concentration from monitoring data with the proposed generic 100% "bioavailable" Ni EQS (4 µg L^{-1}). Although the EQS is expressed as a "bioavailable" concentration, it is compared to dissolved metal measurements. This means that the assessment is

conservative and false negatives are minimised. This tier is applicable to all waterbodies that will be monitored, so that additional supporting parameters (such as pH, DOC, and Ca) are not required in order to undertake analysis in this initial tier of the assessment. Sites, or samples, failing at this tier progress to the second tier of the assessment, in which information on additional supporting parameters (pH, DOC, and Ca) are required as inputs to the screening tool. The generic EQS$_{bioavailable}$ can be precautionary as its use is part of a tiered risk-based framework, so "failure" at this tier leads to further analysis and not to more expensive regulatory action.

Tier 2. This tier makes use of the NiBAT. Samples failing at this level progress to Tier 3 and the consideration of local refinements.

Tier 3. This tier is a local refinement of the assessment for sites (waters) that have exceeded the EQS. This refinement might include consideration of the ambient natural background concentrations (ABCs) of Ni, collection of local physico-chemical data (for example a waterbody default DOC may have been used in the NiBAT) to account for the site-specific characteristics, or perhaps use of the full NiBLM (Section 2.1), which will give a value of improved of accuracy over the NiBAT.

Tier 4. At this tier the failure of a site to achieve good chemical status has been clearly determined. Consideration of a programme of measures to mitigate the situation, within the appropriate cost/benefit framework, may be required. The advantage of using the bioavailability-based approach at an earlier tier is that causal factors may be identified which provide a focus for the programme of measures.

2 THE DEVELOPMENT OF THE BIOAVALABILITY TOOL FOR NICKEL

As described in Section 1.1 the current process to use the integrated NiBLM is a relatively complex and resource intensive one. Therefore, in order to provide a practical method by which to use the new Ni EQS in Europe and assess potential Ni risks on a site-specific basis, a simplified or User-friendly bioavailability tool has been developed which relates the water quality conditions, expressed as the pH, DOC, and Ca concentrations, directly to an ecologically acceptable Ni concentration under those conditions.

2.1 The NiBLM

The BAT for Ni in freshwaters was developed from the dataset of 420 NiBLM simulations in the Bio-Met tool[10] which cover the range of water quality conditions shown in Table 1.1. However, this dataset represents relatively few calculations compared to those used for other BATs (e.g. for copper 8400 were used). There is of course a balance to be struck between the desire for improved accuracy of predictions with more simulations and the effort required to perform those simulations. For copper the integrated Hydroqual BLM was used which, while data intensive, can produce many more simulations than the current process with the NiBLM.

Nevertheless, the Project Team considered it necessary to undertake further simulations within the resource constraints of the project. The NiBLM, as described in Section 1.1, was used to calculate HC5 values for dissolved Ni (in µg L^{-1}) with the input values for pH, DOC, and Ca for a further 216 simulations extracted from a UK 'matched' monitoring dataset, and covering the ranges of conditions observed in UK waters and the conditions of the model (Environment Agency 2009). The concentrations of other major ions required to perform the chemical speciation calculations (Mg, Na, K, Cl, SO$_4$, and alkalinity) were calculated based on relationships with Ca concentrations established from European surface waters (Peters et al. 2010). Temperature data was unavailable and therefore the assumption that all waters were at 285K was applied.

This resulted in a dataset of 636 simulations from which the algorithm was developed.

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[10] http://www.bio-met.net/

2.2 Development of the algorithm

The algorithm was developed using Matlab[11] and polyfitn[12]. This approach allows the creation of models with more than one independent variable. The basic premise of the model is to predict the HC5 produced from the NiBLM based on the pH, DOC and Ca of the water sample with relevant constants fitted to provide the best fit of the predicted results to the NiBLM calculated results (cf. Environment Agency 2009). The algorithm and the respective constants from this development process are shown in the Appendix.

2.3 Testing the algorithm

In order to assess the performance of the algorithm it is necessary to use a dataset of waters not considered in the development of the algorithm that also represent realistic combinations of physico-chemical conditions that were within the ranges used to develop the algorithm. A validation dataset was produced using the reported conditions of 98 sites in England and Wales for the prediction of the Ni HC5. Only England and Wales data was used in this dataset as typically Scottish data has been demonstrated to be less sensitive to divalent cation exposures (Environment Agency 2009). The physico-chemical characteristics of this dataset are shown in Table 2.1. These data are site-specific, but have been summarised according to the way they will be used in compliance assessment. Nevertheless, the data represent a wide range of physico-chemical conditions within the validation range of the model.

Table 2.1   Physico-chemical characteristics of validation dataset of waters from England and Wales

	Mean pH	Median DOC*, mg L^{-1}	Mean Ca, mg L^{-1}
Min - Max	6.20-8.64	0.68-20.95	1.39-390
Mean	7.71	5.71	74.20

*Site-specific median values of DOC have been selected for inputs to the User-friendly tools rather than means due to the log-normal distribution of DOC in freshwaters.

The generic EQS for Ni is 4 µg L^{-1} and this is an EQS$_{bioavailable}$ and is set without the addition of an assessment factor on the HC5 from the derivation process (EC 2011). The NiBLM, as described earlier, can give an HC5 for the specific water conditions as dissolved Ni. By inputting the same physico-chemical data into the NiBAT a site-specific PNEC$_{dissolved}$ is calculated as one of the model outputs.

Figure 2.1 shows a comparison of the outputs from both the NiBLM and NiBAT for the 98 sites in England and Wales. The figure shows that the all but one of the NiBAT estimations are within a factor 2 of those from. There is a slight tendency towards protective predictions, although for the most sensitive sites the predictions are under

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[11] http://www.mathworks.co.uk/products/matlab/
[12] https://www.mathworks.co.uk/matlabcentral/fileexchange/34765-polyfitn

protective. The single site for which NiBAT is under protective by more than a factor of 2 is a highly sensitive water. This waterbody has a high pH (pH 8.6), low hardness (Ca 8.5 mg l^{-1}), and low DOC (1.8 mg l^{-1}). These conditions represent a situation where competition for nickel binding to the biotic ligand from both protons (H$^+$) and alkali earth cations (Ca^{2+} and Mg^{2+}) is very low, and complexation of nickel by DOC is also low, resulting in highly sensitive conditions for potential nickel toxicity. The full BLM HC5 value is below the generic EQS of 4 μg l^{-1}, indicating the extreme nature of this water chemistry.
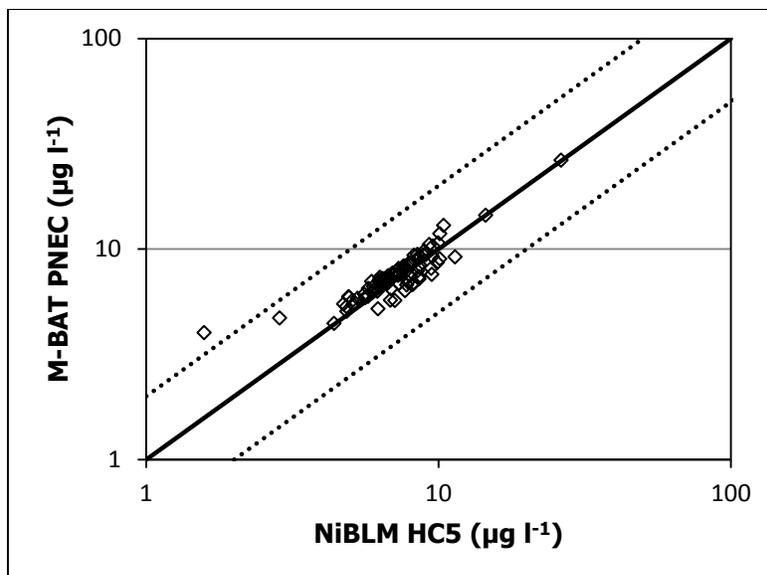
Figure 2.1 **'Full' NiBLM (x-axis) HC5 predictions against NiBAT HC5 predictions (y-axis) for 98 UK waters.**

The NiBAT performance is in line with those produced for other metals as shown in Figure 2.2 for copper. Furthermore, it should be noted that the model has been produced with relatively few data compared to the other BATs, but that even these have been shown, under certain conditions, to return values outside a factor of 2.

The precision and accuracy of predictions could be improved further by increasing the size of the training dataset used to derive the NiBAT prediction model. This may also enable more reliable application of the model over a broader range of water chemistry conditions.
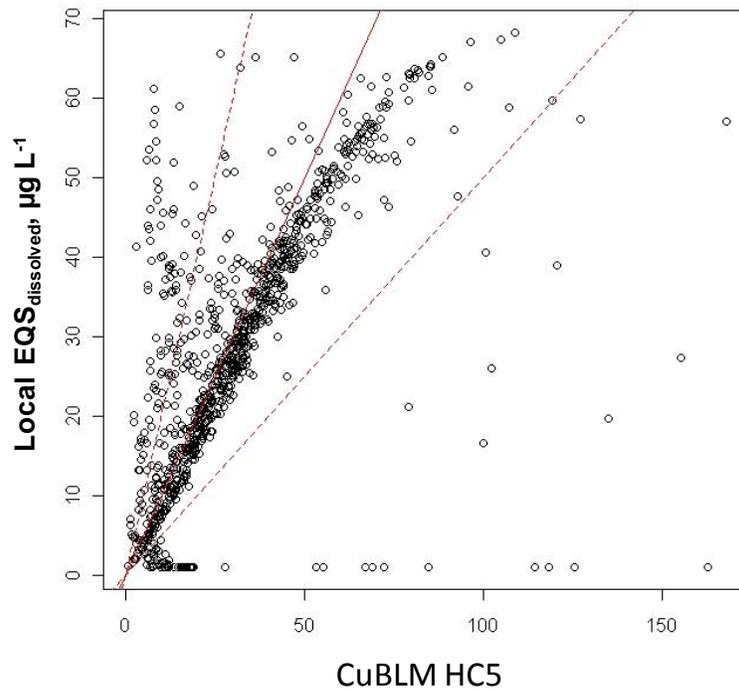
Figure 2.2 **Comparison of HC5 values for Cu calculated using the CuBLM and the CuBAT for a Swedish dataset. The solid line indicates a 1:1 relationship, and the dashed lines indicate a factor of 2 from the true result.**

3 USING THE NICKEL BIOAVAILABILITY TOOL

This section describes how to use the NiBAT to assess the potential aquatic risks of Ni. The data input requirements are outlined along with what to do to get started. The NiBAT will operate in versions of Excel™ from 2003 onwards. Supplementary information on this section is available from the report of the Water Framework Directive Workshop on bioavailability of metals held in June 2011[13].

3.1 Data inputs

The BAT accounts for Ni bioavailability for specific locations through the use of local water chemistry data, specifically pH, DOC (mg L^{-1}) and Ca (mg L^{-1}). These estimates can be based on a single sampling occasion or, in accordance with the requirements of the WFD, from monitoring data from 12 monthly sampling occasions over a period of one calendar year.

A hazard assessment can be performed if no measured Ni data are available, i.e. the tool will give an indication of the relative sensitivity of waters to potential Ni exposure. However, if a risk or EQS compliance assessment for Ni is to be undertaken then monitoring data for dissolved Ni are required (at an appropriate level of detection). For a compliance assessment, the annual average of the respective measured metal data needs to be calculated and entered into the NiBAT.

Columns are also available in the BAT for entry of sample ID, location, water body code and date (Figure 3.2), although none of these need to be entered for the Tool to work.

3.2 What if data for some of the fields are absent?

The BAT requires data inputs for pH, DOC and Ca. Without these, the BAT will not run (and you will be prompted for an input). Dissolved organic carbon is a determinand that is not routinely monitored in freshwaters in England and Wales or many other European Member States. However, in the past a large amount of DOC data were collected across most Environment Agency regions. These historical data allow estimation of DOC default values for many waterbodies and most hydrometric areas in England and Wales (Environment Agency 2009). Importantly, as shown in Figure 1.1, only sites that progress through Tier 1 will require the collation of additional data, such as DOC.

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[13]http://circa.europa.eu/Public/irc/env/wfd/library?l=/framework_directive/implementation_conventio/bioavailability&vm=detailed&sb=Title

3.3 Getting started

The NiBAT runs in Excel™ and upon opening it is imperative to ensure that macros are _enabled_, otherwise the tool will not work. The first page that you should see is shown in Figure 3.1, once macros have been enabled.

The following are step-by-step instructions on how to run the Tool. These are the same instructions that are given on the front page of the Tool.

1. Click the Start button on the Introduction Page. This will open the BAT (Figure 3.2).

2. This sheet contains an empty table (if it isn't empty, click the Clear Data button to empty it).
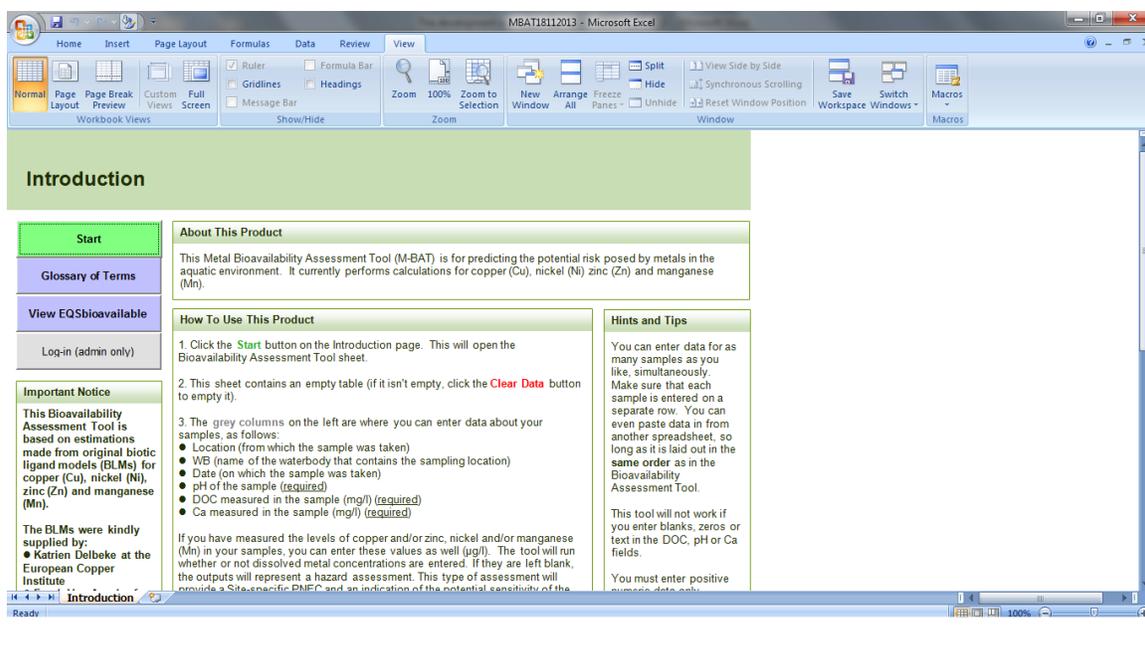
Figure 3.1   Screenshot of the introduction page of BAT.
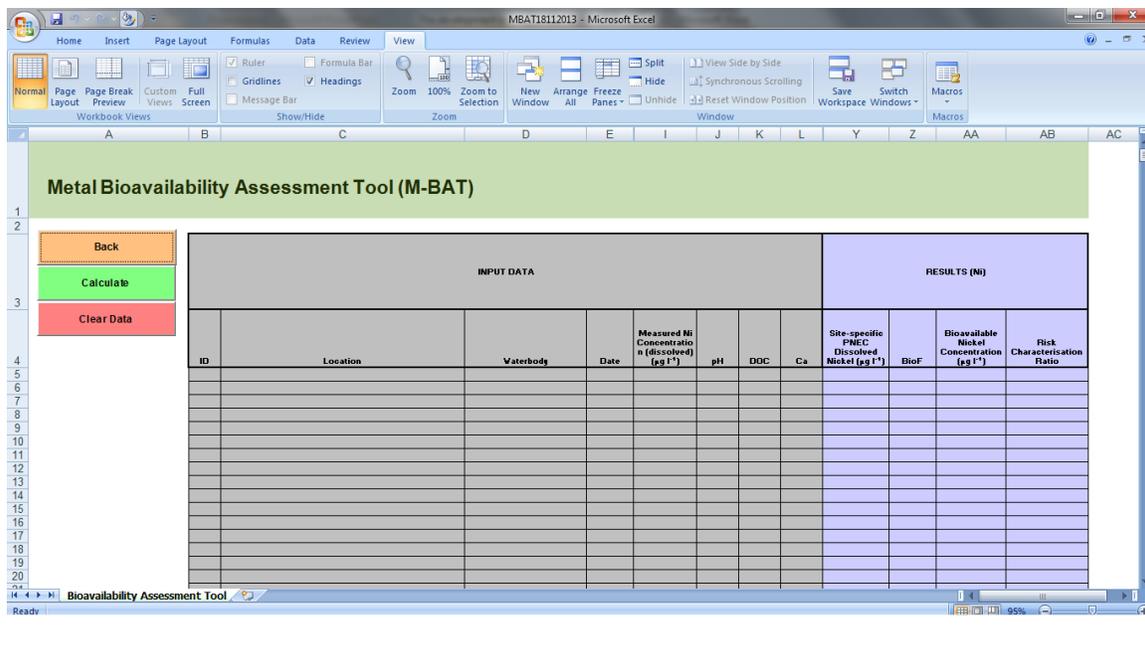
Figure 3.2   Screenshot of the BAT page for nickel.

3. The grey columns on the left (Figure 3.2) are where you must enter data about your samples, as follows:

- Location (from which the sample was taken);

- WB (name of the waterbody that contains the sampling location);

- Dissolved Ni concentration (MBAT can also account for Cu, Mn and Zn bioavailability). These data are not necessary to running the BAT and you can undertake a hazard assessment without the measured metals data and this can provide an indication of the sensitivity of waters to exposures of the respective metals;

- Date (on which the sample was taken);

- pH of the sample (this should be an annual average) (*required*);

- DOC measured in the sample (this should be an annual median or a default value in mg L^{-1}) (*required*);

- Ca measured in the sample (this should be an annual average mg L^{-1}) (*required*).

4. When you have entered your data, click Calculate to continue. A box will pop up to tell you when calculation is complete (Figure 3.3). Click OK to continue.
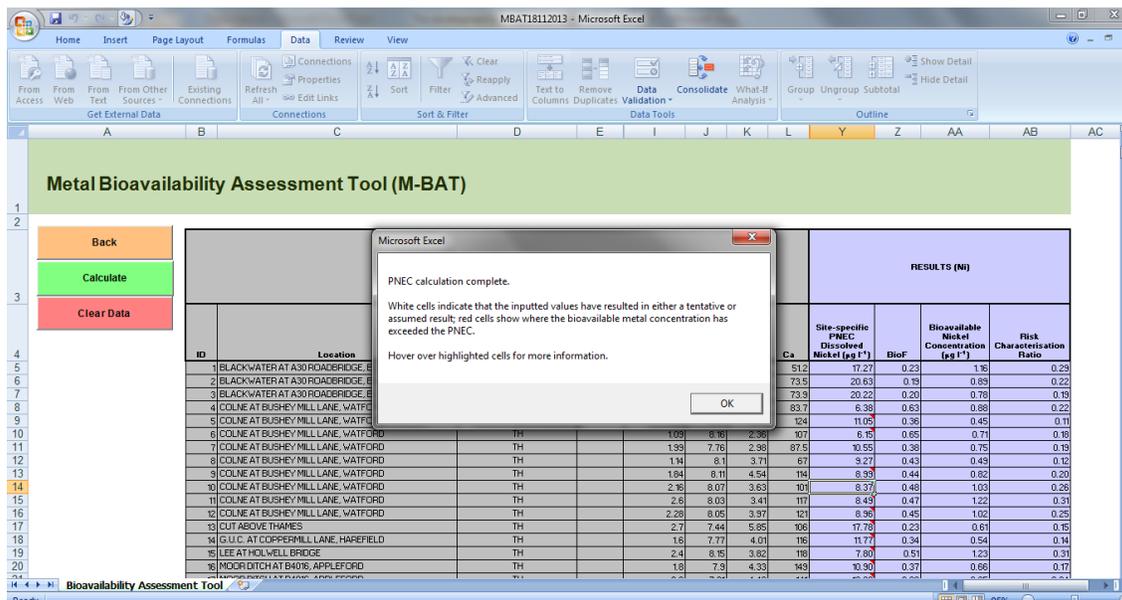
Figure 3.3   Screenshot of the MBAT page when data have been added and the calculation is complete.

5. The results are displayed in the blue columns on the right-hand side of the table.

6. In all cases, the following results are shown:

- Estimated Site-specific $PNEC_{dissolved}$ for each site ($\mu g\ L^{-1}$)

- BioF for each metal (calculated using the reference $EQS_{bioavailable}$ for Ni of 4 $\mu g\ L^{-1}$)

7. Where you have entered data on the measured dissolved concentrations of Ni, the following results are also shown:

- Bioavailable concentration for Ni ($\mu g\ L^{-1}$)

- Risk Characterisation Ratio for Ni at each site

8. Some results could be highlighted in red or marked with comments boxes. Hover your cursor over the highlighted cells or comments box, and the comment will appear. This will explain why the result has been flagged. It will be for at least one of the following reasons:

- The red cells indicate where the dissolved nickel concentrations are greater than the EQS giving an exceedance (a risk characterisation ratio (RCR) greater than 1.

- The comments boxes will show when the water chemistry conditions under consideration represent; conditions of relatively high bioavailability for the respective metal or fall outside the validation ranges. Comments boxes will also show when advice should be sort in relation to the calculation.

You can enter data for as many samples as required, simultaneously. Make sure that each sample is entered on a separate row. You can even paste data in from another spreadsheet, so long as it is laid out in the same order as in the NiBAT (also make sure you paste 'as value').

This tool will not work if you enter blanks, zeros or text in the DOC, pH or Ca fields.

You must enter positive numeric data only. If you edit any of the input data after running the programme, the results will not adjust automatically. You will have to click Calculate again, even if you have only changed one row. If you want to re-run the spreadsheet with a completely new set of input data, as if from the beginning, click Clear Data and start again.

3.4 What do the outputs from the bioavailability tool mean?

The BAT will account for Ni bioavailability for specific locations through the use of local water chemistry data, specifically pH, DOC (mg L^{-1}) and Ca (mg L^{-1}). If only data for pH, DOC and Ca are entered into the BAT then results will appear under the column headers estimated Site-specific $PNEC_{dissolved}$ and BioF. If dissolved Ni concentrations are added, in addition to the abiotic parameters, then bioavailable Ni and risk characterisation will also be calculated. How these outputs are calculated and what they mean is discussed below.

3.4.1 Estimated Site-specific PNEC $_{dissolved}$ and BioF

The estimated Site-specific $PNEC_{bioavailable}$ is calculated from the relationships shown in Section 2.2 that were developed on the basis of the BLM outputs. The Site-specific $PNEC_{bioavailable}$ can be considered as a site-specific EQS, and is useful in ranking sites in terms of their sensitivity to Ni toxicity.

The BioF is calculated by dividing the generic $EQS_{bioavailable}$ (4 µg Ni L^{-1}) by the estimated Site-specific $PNEC_{bioavailable}$. This step enables the use of a single EQS, but allows account to be taken of bioavailability at each individual site. The BioF is then used in the next stage of calculations, if dissolved metal data have been added in the columns to the left. Values of the BioF should always be below 1 in this tool.

3.4.2   Bioavailable Ni concentration and risk characterisation ratio

If measured dissolved Ni data have been added to the sheet in the left hand column then there is an opportunity to assess potential risks at individual sites and undertake an EQS compliance assessment. The bioavailable Ni concentration value and risk characterisation ratio will be calculated, the former by multiplying the measured data by the BioF and the latter by dividing the measured metal concentration by the Site-specific $PNEC_{dissolved}$.

The bioavailable Ni concentration gives an estimate of the amount of Ni in the sample that is biologically active and of ecological relevance. The risk characterisation ratio, or risk quotient, provides an indication of whether the site being assessed has passed or failed to meet the Ni EQS and by what extent. The risk characterisation ratio is a commonly used metric in screening risk assessments, and a value of equal to, or above, unity indicates a potential risk. It is information in this final column that can be used to determine which sites progress into Tier 3, as shown in Figure 1.1 (and perhaps consideration of using the integrated NiBLM), and which sites exit the compliance process and require no further action.

4 ASSESSMENT OF NICKEL COMPLIANCE IN ENGLAND AND WALES

Any EQS regime needs to reflect the real risk to the environment and the protection goals being sought in order to avoid either unnecessary costs to society or possible environmental impacts. However, an EQS regime also needs to be as simple as possible to minimise regulatory burdens. The need to strike this balance between precision and practicality for an EQS regime is helped by taking account of metal bioavailability. This section provides a bioavailability assessment, using the NiBAT, of 235 sites and 2648 samples in England and Wales.

4.1 The datasets

The dataset used was a subset of Environment Agency monitoring data from all the formerly defined regions of England and Wales from the years 2009-2012. The sites assessed were matched to annual average data for pH, DOC and Ca (the annual median value was used for DOC) and dissolved Ni concentration. These physico-chemical data were then used as inputs to predict the bioavailability of Ni using the NiBAT.

4.2 The results

The results from the assessment do not constitute a compliance assessment as no confidence of failure calculation has been made, so this assessment is based on face-values without any statistical consideration. This tends to make the findings more precautionary than a full regulatory assessment (monitoring data in England and Wales also tend to be targeted towards potentially hazardous sites, rather than gaining a routine assessment of all sites from all areas). Table 4.1 shows the sites and samples per Environment Agency region and the percentages of samples that fell outside the validated boundary conditions of the NiBAT tool shown in Table 1.1. Also given is the overall percentage of samples to fall outside those ranges for England and Wales.

Table 4.1 **The sites and samples used in the face value compliance assessment per region and the percentages of sites outside the validation boundaries for Ca and pH of the NiBAT.**

Region	Sites (samples)	Ca (% of samples) < 2mgL^{-1}, >88 mgL^{-1}	pH (% of samples) < 6.5, >8.7
Midlands	74 (505)	0, 37	1.6, 4
North East	15 (183)	0, 0.5	2.2, 1
North West	32 (227)	0, 32	0, 3
Southern	34 (530)	0, 9.4	0.5, 2.8
South West	39 (785)	0, 38	4.8, 0.5
Thames	11 (50)	0, 66	0, 0

Wales	30 (368)	3, 2	6.5,0
Overall	**-**	**0.4,24**	**3.0, 1.9**

From Table 4.1 it is clear that very few of the waters fall outside the validation boundaries for NiBAT at the lower Ca limit or for pH. However, nearly a quarter of all waters in England and Wales fall outside the upper limit set for Ca of 88 mg L^{-1}.

Importantly, the upper boundary for Ca concentrations is applied in the NiBAT (and the integrated NiBLM) because further increases in Ca concentration _do not_ result in a further decrease in organism sensitivity (e.g. Heijerick et al. 2002; Deleebeeck et al 2008). This is due to saturation (or near saturation) of the "biotic ligand" with Ca, meaning that further increases in the Ca concentration will not result in appreciably greater occupancy of the "biotic ligand" by Ca. Consequently, limiting the input Ca concentration to the upper boundary limit should provide reasonable predictions under higher Ca conditions. This means that NiBAT may reasonably used in hard waters, such as those observed in Thames region, without reducing the protection of the resulting EQS. Where Ca concentrations are in excess of approximately 80 mg l^{-1} the available sites on the biotic ligand will have been saturated (or nearly saturated) by Ca.

The balance of exceedances are shown Table 4.2, for samples exceeding the generic EQS (Figure 1.1) and also exceeding the bioavailability consideration at Tier 2.

Table 4.2   The percentage of samples with exceedances at Tiers 1 and 2, and the number of sites with exceedances at Tier 2, as calculated using NiBAT.

Region	Exceedance at Tier 1 (%)	Exceedance at Tier 2 (%)	Number of sites showing exceedance at Tier 2
Midlands	52	18	22
North East	19	3.2	4
North West	23	1.3	3
Southern	16	0.75	3
South West	24	11	8
Thames	20	0	0
Wales	19	11	4

The results in Table 4.2 all show considerable reductions in exceedance between Tiers 1 and 2, i.e. when bioavailability is considered. This was especially marked for Midlands, North West and Thames regions. For five of the regions the number of Tier 2 exceedances were <5, but for Midlands 22 of the sites showed an exceedance, with the next highest at 8 for South West. These percentages may be reduced when confidence of failure is taken into consideration.

5　　A COMPARISON BETWEEN NiBAT AND OTHER USER-FRIENDLY TOOLS

A brief comparison was made of the estimated outputs between the three user-friendly tools that are currently freely available: Bio-Met, PNECpro and MBAT. The input dataset used has was for 496 routine monitoring sites in England and Wales.

The results of this comparison in Figure 5.1 show that for PNECpro predictions below the generic $EQS_{bioavailable}$ of 4 µg L^{-1} are possible, whereas for Bio-met and MBAT this is not possible. The dots in the top left portion of the graph, above the line, show situation in which the tools predictions are not protective compared to the integrated NiBLM, the converse is true for dots below the line in the bottom right hand side of the figure. All the models show a similar spread of data about the line.
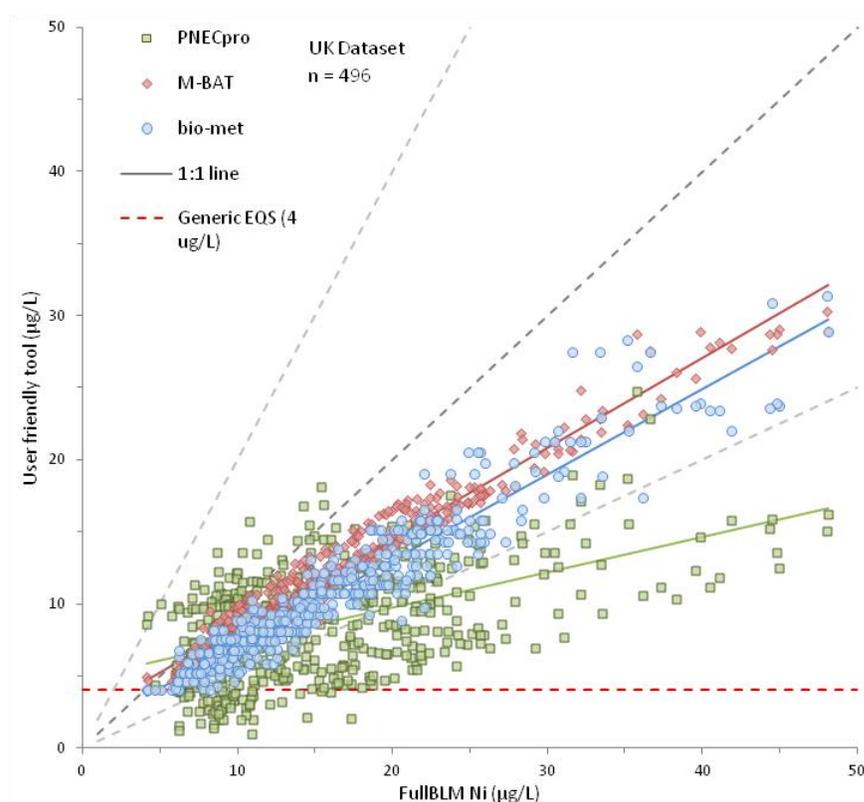
Figure 5.1　　**Comparison of the predictions from PNECpro, NiBAT and Bio-Met against the results from the integrated NiBLM for waters from 98 sites across England and Wales.**

A more extensive statistical analysis of the performance of all of the currently available 'User-friendly' tools is being performed for the fulfilment of EU Guidance on the implementation of bioavailability-based EQs for metals in mid 2014.

6   RECOMMENDATIONS

A user-friendly NiBAT has been developed which provides comparable results than the similar models developed for other metals. The current NiBAT is therefore appropriate for use as a screening tool, but it is recommended that routine spot checking of the predictions is performed using the NiBLM in order to help to identify any particularly under protective or over protective predictions.

Further development of the NiBAT would be expected to improve both the precision and accuracy of the model, although validation against full NiBLM calculations for a set of UK water chemistries indicates that virtually all predictions would be expected to be within a factor of 2 of the true result. This would require a significant investment of time in order to perform the NiBLM calculations, but could result in an improved model with better applicability to performing reliable screening assessments over a broader range of water chemistry conditions.

An alternative option would be to reconfigure the current version of the NiBLM tool so that instead of performing the normalisation for each species in the SSD at once for a single sample, the model calculates the normalisation for a single species in multiple samples. This alternative calculation approach requires that the normalisation process is performed separately for each species in the SSD, and is a more time consuming process for performing calculations for a single site but because large numbers of samples can be processed simultaneously it is an efficient method for large sample numbers. Where the number of samples to be processed is small relative to the number of species in the SSD the current calculation method would still be the most efficient calculation approach.

A model based on this alternative calculation approach could be validated against the existing model for a number of calculations, in order to ensure that the calculations performed are consistent. The end result would also provide greater flexibility for users wishing to process significant numbers of NiBLM calculations in the future, although in practice the use of the reconfigured model is likely to be somewhat more complex (i.e. requiring a greater degree of user interaction) than the current model and may therefore present practical challenges to over-stretched regulatory users.

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APPENDIX. ALGORITHM FROM THE NiBAT

The equation for the estimation of the Ni HC5 using the BAT tool is displayed below:

$$
\begin{aligned}
= & -0.56967982318224 * pH^\wedge 3 + -0.546442220881186 * pH^\wedge 2 * DOC \\
& + 0.017873574212145 * pH^\wedge 2 * Ca + 13.9139170475463 * pH^\wedge 2 \\
& + 0.000677478793063272 * pH * DOC^\wedge 2 + -0.00235133997132463 * pH \\
& * DOC * Ca + 8.1312683310178 * pH * DOC + 0.000743787798609306 \\
& * pH * Ca^\wedge 2 + -0.408903859243256 * pH * Ca + -114.841714537138 \\
& * pH + 0.0000207135900987445 * DOC^\wedge 3 + 0.0000263556571728847 \\
& * DOC^\wedge 2 * Ca + -0.00768857333627623 * DOC^\wedge 2 \\
& + 0.000389205532491767 * DOC * Ca^\wedge 2 + -0.0245331646917477 * DOC \\
& * Ca + -28.075457573396 * DOC + -0.0000600450945465155 * Ca^\wedge 3 \\
& + 0.00124576134400644 * Ca^\wedge 2 + 1.98617171918137 * Ca \\
& + 322.209425623404
\end{aligned}
$$

This is the equation that sits in the MBAT and is what will be incorporated into the Environment Agency's National Laboratory Service information system.