**Practicum 7: Bioavailability of metals**

## 7.1. Introduction

Metal bioavailability and toxicity have long been recognized to be dependent on water chemistry. It is increasingly being accepted that ambient water chemistry characteristics such as water hardness, the concentration of dissolved organic carbon (DOC) and pH may influence metal toxicity. For example, as water hardness increases at a fixed free metal ion (Me2+) concentration, the higher Ca2+ concentration will compete increasingly with the free metal ion for binding sites at a biologically sensitive receptor, the biotic ligand. A higher free metal concentration is therefore required to achieve the same toxic effect in the presence of an elevated Ca2+ concentration. Similarly, a high concentration of DOC will form an increasing amount of metal complexes with the metal ion and therefore reduce binding to the biotic ligand. In addition, certain inorganic metal species, such as MeOH+ and MeCO3, are not or less able to bind to the biotic ligand, and thus metal speciation is an important factor that may influence toxicity. As a consequence, the water chemistry of the surface water ultimately governs the bioavailability of metals (Figure 7.1).



**Figure 7.1 Reactions considered in the biotic ligand model (BLM) for predicting metal toxicity**

The biotic ligand model (BLM) can provide a normalization that removes the influence of test medium chemistry when comparing results of individual toxicity tests, that may have been performed using different water chemistries. It does so by taking into account both metal speciation (the distribution of all metal in the dissolved phase over the free metal ion and the various inorganic and organic metal complexes) and competitive effects of cations, such as Ca2+, Mg2+, Na+ and H+. This can be accomplished with the BLM software and ensures that all toxicity data within a given species sensitivity distribution (SSD) are evaluated on an equivalent basis (i.e. normalized - or ‘translated’ - to the same target water chemistry). Second, the BLM also takes into account the competitive and H+, on the uptake of metal ions at the site of action on the organisms.

**7.2. Explanation Hydroqual BLM software**

The BLM software can be used to calculate the chemical speciation of metal in the dissolved phase, including complexation with inorganic and organic ligands, and the biotic ligand. The biotic ligand represents a discrete receptor or site of action on an organism where accumulation of metal leads to toxicity. The BLM can therefore be used to predict the amount of metal accumulation at this site for a variety of chemical conditions and metal concentrations i.e., the inorganic, organic, and biotic speciation of metals in an aquatic environment. According to the conceptual framework of the BLM, accumulation of metal at the biotic ligand at a critical threshold concentration leads to a given degree of toxicity. For instance, if the accumulation of metal on the biotic ligand reaches the so-called LA50 (the Lethal Accumulation for 50% of the test population) it is predicted that 50% of the population will die. The LA50 is expressed in units of nmol of metal per gram wet weight of the biotic ligand. Since the BLM includes inorganic and organic metal speciation and competitive interactions with the biotic ligand, the amount of dissolved metal required to reach this threshold will depend on the water chemistry. Therefore, in addition to calculating chemical speciation, the BLM can also be used to predict the concentration of metal that would result in acute toxicity within a given aquatic system.

The use of site-specific water chemistry input data in the BLM results in model calculations of metal bioavailability, which can be applied to the reference toxicity database to generate site-specific toxicity values (e.g. NOEC, EC10), which are used in an SSD to calculate HC5-values and PNECs.

In this PC-LAB we will use the Hydroqual BLM software platform:

* to learn about the main principles of the biotic ligand model to predict metal toxicity to aquatic organisms
* to learn about concepts behind its application in regulatory frameworks such as setting water quality criteria (WQC) or carrying out risk assessments.

**2.1 How does the Hydroqual BLM software operate?**

The software is designed for two types of calculations, depending on the *mode* in which it is run:

* ***Speciation mode:***

In this mode the speciation of the metal is calculated based on the water chemistry that is given in the input-file.

* ***Toxicity mode:***

This mode calculates toxic effect concentrations (e.g., LC50) for an organism based on the water chemistry and using information about metal binding to the ‘biotic ligand’ of that organism (contained in the parameter file, \*.dat, see below).

**7.2.2 Data-files required to run the Hydroqual BLM software**

* ***Thermodynamic database (\*.dbs)***

This contains stability constants for complexation reactions of metals with inorganic ligands and humic and fulvic acid. This thermodynamic database-file was originally taken from the original WHAM V publication (Tipping, 1994). It is the core of the speciation module of the biotic ligand model.

*Important note: Default Stability constants for inorganic metal complexes are not the same as in either Visual Minteq or in the NIST database. The largest differences occur for the Me-HCO3 complexes. Although these are less important for determining Me2+ concentrations in DOC-rich waters, where DOC-complexes dominate the speciation, the difference may be important in low DOC – high alkalinity waters (Bryan et al., 2002). We will not go deeper into this issue, however. It is our feeling, however, that constants for inorganic metal complexes can be adjusted to their NIST values provided that one knows what he is doing.*

* ***Parameterfile(\*.dat) – contains the full description of the problem that is going to be run, including info about metal binding to the biotic ligand***
	+ Contains stability constants (log K) for metal (e.g., Cu) and competitive cation (Ca, Mg, Na) binding to the biotic ligand
	+ Contains a reference to the thermodynamic database that is to be used (cited next to [THERMO] )
	+ Contains the so-called ‘critical gill-concentration or ‘biotic-ligand concentration of the metal’ [CRITICAL].
		- This is the amount of accumulation of metal to the biotic ligand that results in a well-defined effect (e.g., 50% mortality). In this case the critical accumulation is also termed the ‘LA50’. It means that 50% of the individuals in the test population will die at this accumulation. Within the BLM-concept, this critical gill accumulation is independent of the water chemistry! This forms the basis of the model!
		- In ‘*speciation mode*’ the software will calculate not only metal speciation, but also the gill concentration of metal for the problem that is defined (water chemistry + metal concentration). If the dissolved metal concentration inserted into the model is the LC50, the model output will yield the ‘critical accumulation’, i.e., the LA50.
		- In ‘*toxicity mode*’ the software will calculate how much dissolved Cu is needed to obtain this LA50. This dissolved Cu concentration is then the predicted LC50.
		- *Important note: For Cu, the model works with a binding site density of the biotic ligand of 30 nmol Cu/g wet wt. This means for example that a critical fractional biotic ligand occupancy (f50%) of 30%, would correspond to a critical accumulation (LA50) of 9 nmol/g wet wt when using the software. This is important to know because in literature both LA50’s and f50% values are being reported to indicate ‘critical accumulation’.*
* ***Inputfile (\*.blm) – contains the water chemistry of a problem***
	+ It contains the water chemistry with which the software will calculate. Each row represents a different ‘problem’ (different water chemistry)

**7.3. Practical exercise**

**7.3.1. Case study 1: effects of water chemistry on acute copper toxicity to fathead minnows**

*Aim: learning to work with speciation and toxicity mode in order to know how to tackle the problem presented in case study 2*

We want to know the speciation of 25 µg Cu/L in an aquatic system with the water characteristic presented in Table 1 (and ‘input1.blm’). The inorganic parameters in this water are ‘typical’ for EU surface waters. The DOC concentration is about the lower 5th percentile of EU surface waters. Data come from a 1991-1996 monitoring database of large rivers and lakes in the EU.

Table 1 Water chemistry for case study 1

|  |  |  |
| --- | --- | --- |
| **Variable** | **Unit** | **Value** |
| T | °C | 20 |
| pH |  | 7.8 |
| Cu | µg/L | 25 |
| DOC | mg/L | 2 |
| HA | % | 10 |
| Ca | mg/L | 51.2 |
| Mg | mg/L | 5.7 |
| Na | mg/L | 17.2 |
| K | mg/L | 2.4 |
| SO4 | mg/L | 39.8 |
| Cl | mg/L | 30.5 |
| Alkalinity | mg/L | 124.2 |
| S | mg/L | 1 |

1. **Stepwise approach**

The problem is solved by following the **steps below**. The students will be guided through this process by by demonstration via the projection system.

* *Save the BLM folder which is available at Minerva to your personal H-drive*
* *Open the command line by typing ‘cmd’ in the field just above the ‘Start’ button. A black screen will pop up*
* *Change the directory to the H-disk by typing* ***h:***
* *Change the directory to the BLM folder by typing* ***cd BLM***
* *Now type the following*

***blm\_212 CuFish.dat input1.blm/W/A1/QQ***

*explanation:*

 *blm\_212: this is the software that is used*

 *CuFish.dat: this is the parameter file with constants that is used*

 *input1.blm: this is the water chemistry input as in Table1*

 *W/A1/QQ: option to let the model be run in ‘****speciation mode’***

* *Two output-files will be generated:*
	+ *“input1.sim.txt”: a simple output file*
	+ *“input1.det.txt”: a detailed output file*
* *In the file “input1.sim.txt” we find*
	+ *The inputs that were used*
	+ *The free ionic Cu concentration, [Cu2+], “free Cu”*
	+ *The concentration of Cu bound to DOC, “TOrgCu”*
	+ *The concentrations of Cu bound to the biotic ligand = sum of Gill-Cu and Gill-CuOH*
		- *The BLM accounts for binding of both Cu2+ and CuOH+ to the biotic ligand and thus assumes that CuOH+ also contribute to the toxicity*
* *In the file“input1.det.txt” we can find, amongst many other outputs*
	+ *The concentrations of all inorganic Cu species*
1. **Questions to be solved**
2. What is the species distribution of Cu in this water (percentage of each Cu-species)?
3. How much Cu is bound to the gills? Do you expect fathead minnows to die in this water within 96 hours of exposure (if you know that the 96h-LA50 is 7.32 nmol Cu/g wet wt)?
4. How does the species distribution and metal binding to the gill change and what do you expect about the toxicity to fathead minnow if…
	1. The DOC is increased to 10 mg/L?
	2. The pH is reduced to 6?
	3. The pH is increased to 8.5?
	4. Ca is reduced to 10 mg/L?
	5. Ca is increased to 300 mg/L?
	6. Based on this information: in which water will toxicity be highest (most ‘sensitive’ water) Why?

*First predict this without calculations. To check the answers to question 3, use the input file “input2.blm” in the command line*

1. **Additional question to be solved**
2. What is the most ‘sensitive’ water, calculated using the ‘toxicity mode’? Why?

The problem to determine the ‘most sensitive water’ can also be solved by predicting the LC50 in this water as follows:

* Open the command line and set the correct directory
* Now type the following:

 ***blm\_212 CuFish.dat input2.blm/L/W/A1/QQ***

 *explanation:*

 *blm\_212: this is the software that is used*

 *CuFish.dat: this is the parameter file for the BLM pedicting acute 96hLC50s for fathead minnow (Santore et al., 2001)*

 *input2.blm: this is the water chemistry input for the 6 different waters*

 *L/W/A1/QQ: this makes sure the model is run in ‘toxicity mode’*

* *The output-file “input2.sim.txt” will now yield the predicted 96h-LC50-values for fathead minnows for these waters. These values can be found in the column “Dis Cu (mol/l)”. You will also see that the sum of the Gill-Cu and Gill-CuOH in each row is identical. This is because the software has calculated the dissolved Cu level that was needed in each water to reach the critical level of Cu on the biotic ligand (i.e., the LA50).*

**7.3.2. Case study 2: normalization of toxicity data from literature for regulatory purposes**

*Aim: to learn how ecotoxicity data can be used to derive water quality criteria and how the BLM can be used to account for differences in metals bioavailability in setting such criteria for metals.*

A common approach to derive water quality criteria (WQC), environmental quality standards (EQS) or predicted no effect concentrations (PNEC) for data-rich substances (such as Zn) is to construct so-called species-sensitivity distributions (SSD) (Posthuma *et al.,* 2002).

Briefly, toxicity data (LC50’s or NOEC’s) are collected from literature for a range of aquatic species (organisms) and a statistical distribution is fitted to these data (e.g., log-normal distribution) to derive a concentration of a chemical that affects less than x% of the species (see also practicum 6). A frequently-used approach is that it is considered acceptable that only 5% of the species are affected, while 95% are protected. The corresponding concentration is termed the ‘hazardous concentration for 5% of the organisms’, the HC5. This 95% protection level is the basis of many chemicals regulations, such as in the EU risk assessment for Zn. In practicum 6 we have applied the most commonly used distribution for the derivation of the HC5 values, i.e. the log-normal distribution. Methodological details can be found in practicum 6.

Table 2 Selected chronic toxicity values of Zn for case study 2. These data are also available in the worksheet SSD-data.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Code** | **Species** | **Taxonomic group** | **Endpoint** | **NOEC – Zn (µg/L)** | **pH** | **DOC (mg/L)** | **Ca (mg/L)** |
| 95 | *D. magna* | Crustacean - waterflea | Reproduction | 491 | 8.0 | 4.3 | 63.6 |
| 96 | *D. magna* | Crustacean - waterflea | Reproduction | 63 | 6.8 | 17.3 | 28.3 |
| 98 | *D. magna* | Crustacean - waterflea | Reproduction | 244 | 6.0 | 5.4 | 3.7 |
| 148 | *S. trutta* | Fish | Hatching | 250 | 8.0 | 7.5 | 52.7 |
| 48 | *P. jenkinsi* | Snail | Growth | 72 | 7.5 | 1 | 12.9 |
| 119 | *J. floridae* | Fish | Growth | 26 | 6.4 | 4.1 | 32.0 |

However, if the water chemistry at which the NOEC value of each species was obtained, is consulted, one will notice that considerable difference exists. For example, pH varies between 6 and 8, DOC between 1 and 17.3 mg/L, Ca between 3.7 and 63.6 mg/L, etc… It is therefore obvious that the NOEC values for the different species were obtained at different levels of bioavailability.

As a consequence, the above constructed SSD and the derived HC5-value reflects the species sensitivity at a mixture of ‘bioavailability levels’. This does hamper a straight-forward interpretation of the derived HC5-value and queries its ecological relevance.

In order to obtain a true SSD, with species sensitivities valid for one and the same water chemistry, all toxicity data should first be ‘translated’ to this water chemistry, the so-called ‘target’ water chemistry. This can for example be the chemistry of a river or a lake for which you want to estimate the HC5.

The BLM allows to derive the critical accumulation for a species when the NOEC (CANOEC) is known, together with the water chemistry of the test water in which the toxicity of this species was tested.

NOEC of species A (µg/l) obtained in test water X

CANOEC of species A

Water chemistry of water X

This first step of the translation (normalization) can be achieved with the ‘speciation mode’ in the command line, as demonstrated in case study 1. In the second step, this CANOEC of species A, which is *by definition* independent of water chemistry, can be used to predict the NOEC for that species A in any other water chemistry of a target water Y:

 CANOEC of species A

 NOEC of species A (µg/l) in water Y

Water chemistry of target water Y

This step can be achieved with the ‘toxicity mode’ of the command line. To do this, we first have to create separate parameter files (\*.dat) for every species, by using the CANOEC that we will calculate in the ‘speciation mode’. This \*.dat file is then used in the ‘toxicity mode’ to calculate the normalized NOECs. It is important to note that *an important assumption with this approach is that the stability constants describing interactions between Zn2+, Ca2+, Na2+, Mg2+ and H+ are identically the same for all aquatic species.*

With this approach in mind, the following exercise will be made. NOECs obtained in test waters will be normalized to the water chemistry of the Rhine (Van Sprang et al, 2009) (Table 3 and ‘input3.blm’)

Table 3 Water chemistry of the Rhine from Van Sprang et al. 2009

|  |  |  |
| --- | --- | --- |
| **Variable** | **Unit** | **Rhine** |
| T | °C | 20 |
| pH |  | 7.9 |
| Zn=(PEC50) | µg/L | 6.4 |
| DOC | mg/L | 2,5 |
| HA | % | 0.01 |
| Ca | mg/L | 55.0 |
| Mg | mg/L | 13.3 |
| Na | mg/L | 19.98 |
| K | mg/L | 3.54 |
| SO4 | mg/L | 38.47 |
| Cl | mg/L | 31.43 |
| Alkalinity | mg/L | 17.8 |
| S | mg/L | 0.1 |

1. **Questions to be solved**
2. ‘Normalize’ the NOECS from *D. magna, S. trutta, P. jenkinsi* and *J. floridae* from Table 2 to the water with chemistry given in table 3.

**Step A – Reduce the observed variability in NOEC data via normalization: *Daphnia magna***

1. We will first calculate the intrinsic sensitivity (CANOEC) by working in the ‘**speciation mode’**
* *Open the command line in the correct directory and file*
* *Use the “ZnDaphnia.dat” file as the parameter file, and the “ssdDaphnia.blm” file as the water chemistry input*
* *Run the model in* ***speciation*** *mode*
* *Two output-files are created:*
	+ *“ssdDaphnia.sim.txt” and “ssdDaphnia.det.txt”*
* *In the “ssdDaphnia.sim.txt” file, the “Gill-Zn (nmol/gw)” is the amount of Zn that is bound to the Gills. This is the* **CANOEC** *of the species*
* *Now we have to create a parameter file (\*.dat) for every organism by filling out this LA50 value as the [critical] value*
* *Open the “ZnDaphnia.dat” file and fill in the* CANOEC *as the [critical] value*
* *Save the file as follows: the test label of each species followed by .dat: e.g. “Zn95.dat”, and save under ‘alle bestanden’*
* *Do this for the three Daphnia datapoints*
1. Now we will calculate the NOEC of the species in the target water by working in the ‘**toxicity mode’**
* *Open the command line in the correct directory and file*
* *Use the first \*.dat file that you just created (e.g. Zn95.dat) as the parameter file, and the “input3.blm” file as the water chemistry input*
* *Run the model in* ***toxicity*** *mode*
* *Now rename your output by typing*

*rename input3.sim.txt* ***Zn95****.out*

 *This should be the same as your input \*.dat file*

* *You will get the output-file ‘Zn95.out’, were we find:*
	+ *The ‘Dis Zn’ in mol/L, which is the normalized NOEC of that species*
* *Copy this normalized NOEC to your output Excel and convert to µg/l*
* *Now repeat these steps for the other Daphnia species (different parameter files, Zn96.dat and Zn98.dat), using the same target water*

**Step B - Depending on the species a different BLM is to be used: *Salmo trutta***

Calculate the intrinsic sensitivity (LA50) by working in the ‘speciation mode’ and calculate the normalized NOEC by working in the ‘toxicity mode’

* *Repeat the above for a fish species: S. trutta*
* *This time use the “ZnFish.dat” file as the parameter file, and the “ssdTrutta.blm” file as the water chemistry input*
* *Run in model in speciation mode and retrieve the* CANOEC *of the species from the output file*
* *Create your own parameter file by filling in this LA50 in the ‘ZnFish.dat’ parameter file, and give it the appropriate file-name (Zn148.dat)*
* *Now calculate the normalized NOEC for target water 1 (input3.blm)*
* *Copy this normalized NOEC to your output Excel and convert to µg/l*

**Step C -An intrinsically more sensitive species may appear less sensitive*: J. floridae vs. P. jenkinsi***

* *Repeat the above for a fish (J. floridae) and a snail (P. jenkinsi)*
* *For P. jenkinsi (a snail): use the “ZnDaphnia.dat” file as the parameter file, and the “ssdJenkinsi.blm” file as the water chemistry input*
* *For J. floridae (a fish): us the “ZnFish.dat” file as the parameter file, and the “ssdFloridae.blm” file as the water chemistry input*
* *Calculate the LA50s and create the parameter files*
* *Then calculate the normalized NOECs for target water 1 (input3.blm)*
* *Copy these normalized NOECs to your output Excel and convert to µg/*l
1. **Questions to be solved**
2. Compare the normalized NOECs with the original NOECs. Do they increase or decrease? Why? Has the rank order of sensitivity of the organisms changed? Why?
3. Calculate the HC5 for the water from Table 3.
4. Calculate HC5 values for a water with a different DOC-value (Table 4. and ‘input4.blm’)

Table 4 Water chemistry of the Rhine from Van Sprang et al. 2009

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Unit** | **Rhine-DOC2** | **Rhine-DOC3** |
| T | °C | 20 | 20 |
| pH |  | 7.9 | 7.9 |
| Zn=(PEC50) | µg/L | 6.4 | 6.4 |
| DOC | mg/L | 11,5 | 27.45 |
| HA | % | 0.01 | 0.01 |
| Ca | mg/L | 55.0 | 55.0 |
| Mg | mg/L | 13.3 | 13.3 |
| Na | mg/L | 19.98 | 19.98 |
| K | mg/L | 3.54 | 3.54 |
| SO4 | mg/L | 38.47 | 38.47 |
| Cl | mg/L | 31.43 | 31.43 |
| Alkalinity | mg/L | 17.8 | 17.8 |
| S | mg/L | 0.1 | 0.1 |

1. Are the aquatic ecosystems in Table 3 and 4 at risk when bioavailability is taken into account / when bioavailability is not taken into account?
2. Perform a risk assessment, taking into account the measured exposure data (see practicum 6). Compare the outcomes of the calculations with and without taking into account bioavailability.