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Biotic Ligand Models: availability, performance and applicability for water quality assessment

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1203842-000

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Beknopte Nederlandse samenvatting

De Kaderrichtlijn Water (KRW) schrijft voor om de effecten te beoordelen die contaminanten veroorzaken op het aquatisch ecosysteem. Voor zware metalen koper, nikkel en zink zijn hiertoe Biotic Ligand Modellen (BLM) ontwikkeld. Deze zijn in Europees kader erkend als een bruikbare methodiek in de tweedelijns beoordeling. In de afgelopen tijd zijn operationele tools ontwikkeld, die vereenvoudigingen zijn van het originele concept. Het doel van deze studie is om te achterhalen in hoeverre deze vereenvoudigde tools een acceptabele schatting geven van effecten op organismen, en daarmee kunnen voldoen als gangbaar toepasbare beoordelingsmethode.
De volgende tools werden getest in deze studie:

1	BLM EU-RAR	In Europese Risk Assessment Reports gevalideerde BLM modellen voor Cu, Ni en Zn. Uitgebreid en geoperationaliseerd door Deltares.
2	BLM (HydroQual)	Een op de EU-RAR gebaseerde en vereenvoudigde tool (versie 2.2.3) die gebruik maakt van statistische functies. Ontwikkeld door HydroQual, USA.
3	BLM (WCA)	Een op de EU-RAR gebaseerde en vereenvoudigde tool (versie 8) die via statistische functies een schatting maakt van biobeschikbare fracties. Ontwikkeld door WCA Environment ltd, UK.
4	Ni-BLM (ARCHE)	Een vereenvoudigde methode voor de berekening van potentieel No-effect concentraties (PNEC) voor nikkel (versie 10). Ontwikkeld door ARCHE, België.
5	Transfer functions (STOWA)	Nulde-orde relaties van het verband tussen HC5 waarden en opgelost koolstof (DOC), gebaseerd op lokale monitoringsgegevens. Gepubliceerd door STOWA.

Voor deze studie is een dataset samengesteld waarin 2575 monitoringsgegevens zijn opgenomen uit de landelijke database iBever, aangevuld met waterkwaliteitsgegevens van verschillende waterschappen. De gegevens zijn landsdekkend en representeren de meeste watertypen uit de KRW (inclusief 14% rijkswateren). Ook is een toxiciteit-database gebouwd bestaande uit chronische NOEC waarden van een groot aantal aquatische organismen (19 tot 27 soorten van verschillende trofische niveaus, afhankelijk van het metaal) met bijbehorende chemische samenstelling van de betreffende testen. De uitkomsten van de gevalideerde EU-RAR BLM methoden (inclusief chemische speciatie berekening en normalisatie) werden gebruikt als referentie.

Het voordeel van de hier geteste tools is dat ze allen zeer toegankelijk een eenvoudig toepasbaar zijn. Enkele tools vereisen weinig input parameters. Dat veronderstelde voordeel is echter beperkt of geheel afwezig, omdat de parameters die nodig zijn om volledige BLMs te gebruiken inmiddels zijn voorgeschreven in het KRW monitoringsprotocol (gepubliceerd in Staatscourant 5615, 14 april 2010) en dus al beschikbaar zijn in de verplichte meetprogramma's voor rapportage.

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Project	**Reference**	**Pages**
1203842-000	1203842-000-BGS-0006	36

Deze studie toont aan dat geen van de geteste tools voldoet aan de gestelde betrouwbaarheidscriteria. Incidenteel zijn er wel trend-correlaties gevonden, maar dit is bij lange na niet voldoende om te volstaan als tweedelijns beoordelingsmethode voor de KRW rapportage. Om dit te realiseren zijn de volgende activiteiten noodzakelijk:

1. Op basis van de in deze studie vervaardigde waterkwaliteit- en toxiciteit databases en de geoperationaliseerde BLMs, kunnen statistisch betrouwbare rekenregels worden geconstrueerd die representatief zijn voor bijna alle voorkomende Nederlandse watertypen en een ruim scala aan ecologische soorten. Deze rekenregels kunnen dan worden opgenomen als tweedelijns beoordelingsmethode ten behoeve van de KRW rapportage.
2. Het uitwerken van een added-risk methode voor meerdere metalen tegelijk.
3. Voor overgangswateren (zoet-zout) moet worden uitgezocht of de bestaande biotic ligand modellen toepasbaar zijn op deze watertypen, of hoe deze toepasbaar gemaakt kunnen worden.

Summary

Biotic ligand models (BLMs) for heavy metals were developed in past years as tools for water quality assessment, following the Water Framework Directive (WFD). The essence of BLMs is that chemical speciation is incorporated in the assessment of ecotoxicological risks for aquatic species. For WFD purposes, BLMs are recognized as useful concepts to determine site-specific risks, and are allowed as second-tier assessment method. Concepts have been made operational, resulting in tools that make the approach available to a larger audience. As a consequence, these tools are in general simplifications of the original, validated concept.

The goal of this study is to test the performance of available tools that aim at predicting risks, or related water quality assessment, based on the concept of biotic ligand modelling, in order to aid in the recommendation for second-tier assessment method for heavy metals in surface waters.
The tools that were tested in this study are:

1	BLM EU-RAR	Validated BLM models for Cu, Ni and Zn, used in the European (Voluntary) Risk Assessment Reports. Extended and operationalized by Deltares, NL.
2	BLM (HydroQual)	A simplified tool (version 2.2.3) based on the BLM EU-RAR, using statistical functions. Developed by HydroQual, USA.
3	BLM (WCA)	A simplified tool (version 8) based on the BLM EU-RAR which makes use of statistical functions to determine bioavailable fractions. Developed by WCA Environment ltd, UK.
4	Ni-BLM (ARCHE)	A simplified method to calculate potential no effect concentrations for Ni. Version 10, developed by ARCHE, BE.
5	Transfer functions (STOWA)	Statistical zero-order relations of HC5 values and DOC concentrations, based on local data sets. Published by STOWA, NL.

For the five tools that were tested in this study, a large dataset of water composition measurements was composed that was used as input for all selected models. Data for water chemistry and metal concentrations were collected from the iBever National monitoring database. This database was supplemented with data from various monitoring programs of different local water managers. The database contained a grand total of 2575 records with geographical information and surface water compositions in the Netherlands. These data covered most of the water types that are described in the Water Framework Directive. The database was constructed to contain all parameters that are required to perform the chemical speciation calculations and the BLM modeling.

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1203842-000	1203842-000-BGS-0006	36

A database was composed containing toxicity data that largely originate from the EU risk assessment reports of Cu, Ni and Zn, mentioned earlier. These toxicity data contain chronic No Effect Concentrations (NOECs), expressed as dissolved concentrations, for a large number of species of various taxa. Information from original studies are added, containing data on chemical composition of the test media. The database of Cu contained 136 test results of 27 aquatic species, tested for various toxic endpoints (e.g., reproduction and mortality). The Zn database contained 19 species (132 test records), the Ni database contained 24 species (128 test records). Fish, invertebrates, algae, insects, mollusks and amphibians were present in the database.

The BLM EU-RAR is the reference-of-quality in the comparison between these tools. As explained earlier, these biotic ligand models were validated within the risk assessment reports. In the comparison of tools, we used the statistical t-test for comparison of means and variances. Since the input parameters were always the same (records from one database), the calculated results are regarded as paired observations. The paired sample population is assumed to have equal variances. Results were also tested for correlation.

The various tools differ in the requirement of the necessary input (from very detailed to only one input parameter), but also in output. The latter makes comparison on a quantitative level in some cases impossible.
The tested tools share the same advantage: they are easily accessible and easy to operate. The advantage of a limited number of input parameters, however, is very small or even absent, since most parameters that are needed to run full BLMs are already adapted in the WFD monitoring protocols.

The results of this study show that none of the tested models meet the quality criteria for the defined reliability. There are however some occasional agreements based on trend correlation.
For implementation as a second tier method for WFD reporting, it remains necessary to address the following activities:

1 To derive statistically-sound transfer functions for a large dataset, representative for the majority of WFD water types and a wide range of taxonomic groups. The combination of the water quality database used for this study, and the supplemented toxicological database used in the European risk assessment reports, qualifies for this purpose.
2 Work out a method for added-risk, based on a multi-metal approach.
3 Analyze the possibility for a BLM approach for transition waters (brackish, salt).

Version	Date	Author	Initials	Review	Initials	Approval	Initials
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		Anja Verschoor					

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final

Content

Appendices

1 Introduction

Background
The Water Framework Directive (Directive 2000/60/EC of the European Parliament (EC, 2000) commits European Union member states to achieve good qualitative and quantitative status of all water bodies by the year 2015. The WFD prescribes certain steps in order to reach common goals, rather than adopting the generic limit value approach. Although the first-tier assessment consists of the comparison of total dissolved water concentrations to a generic environmental quality standard (EQS), it is recognized that compliance of annual average concentrations with EQS is not sufficient to guarantee a good level of ecological protection.

To overcome this shortcoming, the concept of bioavailability of toxic compounds was introduced as a second tier. However, the WFD gives no recommendations concerning the use of specific methods.

Biotic ligand models (BLMs) have been developed for several heavy metals in past years. The essence of BLMs is that chemical speciation is incorporated in the assessment of ecotoxicological risks for aquatic species. For WFD purposes, BLMs are recognized as useful concepts to determine site-specific risks, and are allowed as second-tier assessment method as exemplification of monitoring results. Concepts have been made operational, resulting in tools that make the approach available to a larger audience. As a consequence, these tools are generally simplifications of the original, validated concept. This study aims at testing the performance of these tools, and comparing the quality of outcomes to the original concept.

Goal
The goal of this study is to test the performance of available tools that aim at predicting risks, or related water quality assessment, based on the concept of biotic ligand modelling. The results are used in the recommendation for second-tier assessment method for heavy metals in surface waters.

2 Methods

2.1 Concept of biotic ligand models

Environmental quality standards for heavy metals in sediments and surface waters have been developed to protect the ecosystem from adverse effects. These quality standards are generic, which means that they apply to all surface waters. The importance of explicitly considering bioavailability in the development of water and sediment quality criteria for metals has been recognized for some time (DiToro et al., 1991; Allen & Harsen, 1996; Ankley et al., 1996). Criteria that incorporate this concept are being recommended to, and are being considered for application by, regulatory authorities (Bergman & Dorward-King, 1997; Renner, 1997). A long history of experiments demonstrates the importance of water chemistry on the degree of toxicity of metals. What has been missing is a practical modelling implementation that can predict these variations in toxicity with some degree of generality and reliability (Di Toro et al., 2001).

The conceptual framework for the BLM is an adaptation of the gill surface interaction model, originally proposed by Pagenkopf (Pagenkopf et al., 1974; Pagenkopf, 1983) and more recently utilized by many others (e.g., Playle et al., 1993; Janes & Playle, 1995; Hollis et al., 1996; Playle, 1998; Richards & Playle, 1998; Wood et al., 1999), and the free ion activity model of toxicity, extensively reviewed by e.g., Morel (1983) and Campbell (1995). The general framework is illustrated in Figure 1. The model is based on the hypothesis that toxicity is not simply related to total aqueous metal concentration but that both metal–ligand complexation and metal interaction with competing cations at the site of action of toxicity need to be considered (Pagenkopf, 1983; Meyer, 1999). Mortality occurs when the concentration of metal bound to the biotic ligand exceeds a threshold concentration. The BLM simply replaces the fish gill as the site of action with a more generally characterized site, the biotic ligand. The reason for this replacement is to emphasize that this model should be applicable to other aquatic organisms, like crustaceans, for which the site of action is not readily accessible to direct measurement. It is likely that these principles apply to any organism for which the site of action is directly in contact with the external aqueous environment.

The role of metal complexation is critical because formation of organic and inorganic metal complexes renders a significant fraction of the total metal non-bioavailable. In fact, this modelling framework defines bioavailability of metals. As shown in Figure 1, dissolved metal exists in solution partially as free metal ion. This species is hypothesized to be the bioavailable species in more simplified versions of the free ion activity model of toxicity. The rest of the metal exists as non-bioavailable metal complexes that result from reactions of the metal with organic and inorganic ligands.

Biotic ligand models were developed and validated for copper, nickel and zinc. Efforts to develop BLMs for other metals (such as cobalt (e.g., Richards & Playle, 1998) and cadmium (e.g., Niyogi et al. (2008)) are undertaken. For cadmium, a bioavailability correction factor based on water hardness has been suggested (e.g., Meyer, 1999) and implemented in WFD.

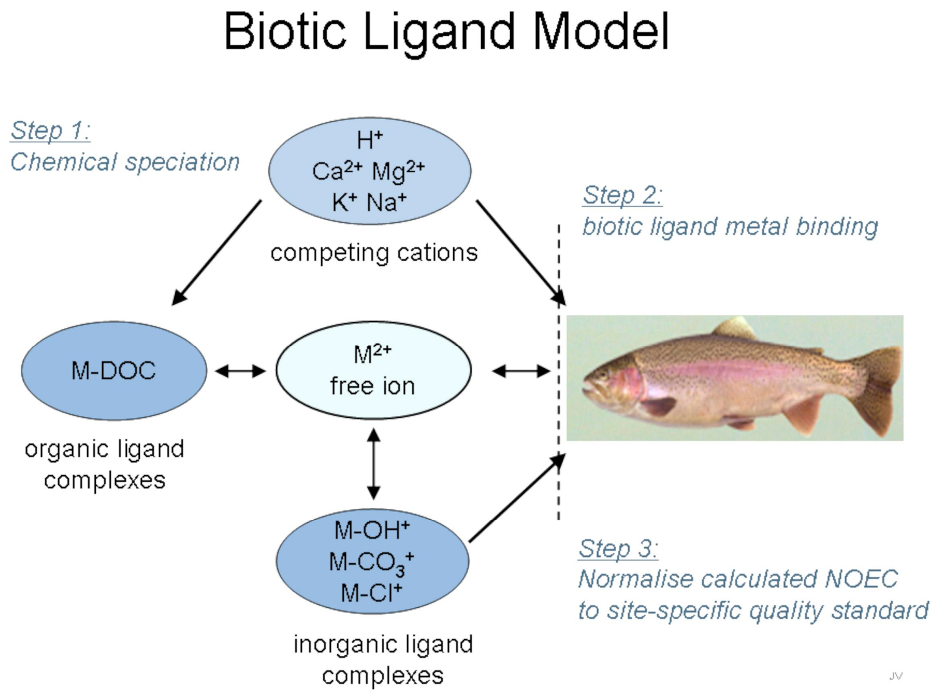

Fig. 1. Concept of biotic ligand model, which includes calculation of chemical speciation, binding to biota and a normalisation procedure to calculate site-specific quality standards.

2.2 Used models

In this study, 5 different tools were tested and compared:

Table 1. Tools tested in this study.

Nr.	Name	Description
1	BLM EU-RAR	Validated BLM models for Cu, Ni and Zn, used in the European (Voluntary) Risk Assessment Reports. Extended and operationalized by Deltares, NL.
2	BLM (HydroQual)	A simplified tool (version 2.2.3) based on the BLM EU-RAR, using statistical functions. Developed by HydroQual, USA.
3	BLM (WCA)	A simplified tool (version 8) based on the BLM EU-RAR which makes use of statistical functions to determine bioavailable fractions. Developed by WCA Environment ltd, UK.
4	Ni-BLM (ARCHE)	A simplified method to calculate potential no effect concentrations for Ni. Version 10, developed by ARCHE, BE.
5	Transfer functions (STOWA)	Empirical zero-order relations of HC5 values and DOC concentrations based on local data sets. Published by STOWA, NL.

In the following sections, detailed information on the concepts and the handling of the models and tools are given.

2.2.1 BLM EU-RAR

These are the models that were developed, optimized and validated for the European (Voluntary) Risk Assessment Reports (RAR). Within the EU-framework, much effort is put into the preparation of RARs for individual priority substances. In case of heavy metals, RARs were developed for copper (EC, 2008a), nickel (EC, 2008b) and zinc (EC, 2008c). BLMs proved to be one of the promising methods for use in the risk assessment procedures.

The available BLM models and toxicity databases were extended and compiled within the modelling framework R (version 2.10; FSC,1999) by Deltares, The Netherlands, in 2010. It is now an operational tool that processes the necessary speciation and biotic ligand calculations, following the validation procedures in the various RARs.

Toxicity data

For this study, a database was composed containing toxicity data that largely originate from the EU risk assessment reports of Cu, Ni and Zn, mentioned earlier. These toxicity data contain chronic No Effect Concentrations (NOECs) for a large number of species of various taxa. Information from original studies is added, containing data on chemical composition of the test media, e.g., temperature and concentrations of DOC, H^+, Ca^{2+}, Mg^{2+}, Na^+, K^+, OH^-, Cl^-, SO_4^{2-} and HCO_3^-.

The database contains the following toxicity data (Table 2 and Appendix A):

Table 2. Composition of toxicity data in the used database.

Metal	**Number of toxicity tests**[1]	**Number of aquatic species**	**Taxonomic Groups**[2]
Cu	136	27	8
Ni	128	24	8
Zn	76	19	6

[1] Toxic enpoints: acute and chronic data on mortality, reproduction and (cell) growth.

[2] Taxonomic groups: Fish, invertebrates, algae, mollusks, insects, amphibians.

Speciation modeling

The toxicity database also includes the necessary information on the composition of the test media in which exposure tests were performed. For these test media, as well as for the surface water samples, the free ion concentrations were calculated in order to perform the normalisation procedure discribed hereafter. WHAM6 (Tipping, 1998) was used for this purpose. The model was modified by using updated metal stability constants from the NIST-database (Martell & Smith, 2004).

To calculate metal binding to dissolved organic matter, we obtained reactive fractions of fulvic and humic acids in natural surface waters by calibration of calculated to measured binding (Cheng et al., 2005; Bryan et al., 2002; Guthrie et al., 2005; Deleebeeck et al., 2005). The fulvic reactive material contribution to DOM approximates 50%.

HC5 calculations

The EU risk assessment reports present biotic ligand models for chronic toxicity of Cu, Ni and Zn (European communitie, 2008a; 2008b; 2008c). BLMs were available for three taxonomic groups algae, crustacean and fish. For Cu, the algae-BLM was based on an empirical relation between toxicity, metal activity and pH (De Schamphelaere et al., 2003). A BLM for crustacea was used that considered the competition of Cu^{2+} with H^{+} and Na^{+} for binding to the biotic ligand (De Schamphelaere & Janssen, 2002; De Schamphelaere & Janssen, 2004). For Ni, chronic algae, crustacea and fish-BLMs are based on the competition between Ni, H^{+} Ca^{2+} and Mg^{2+} for binding to the ligand (Keithly et al., 2004; Deleebeeck et al., 2005; 2008; 2009a; 2009b). For Zn, a chronic algae BLM was used based on an empirical relation between toxicity, metal activity and pH (De Schamphelaere et al., 2005). Chronic Zn-BLMs are available for crustacea (Heijerick et al., 2005) and fish (De Schamphelaere & Janssen, 2004) in which Zn competes with H^{+}, Ca^{2+}, Mg^{2+} and Na^{+} for binding to the biotic ligand.

For nickel and zinc, free ion concentrations are processesed in the BLM. For copper however, the species CuOH and CuCO3 also participate in the Cu-BL binding. For this reason it is important to perform speciation calculations that include CuOH and CuCO3 as dissolved species. The normalization procedure of NOEC is as follows:

NOEC.X[i,j]<- EffectConcentration[i] * (1 + Inorganics.X[j]) /
((K.CuBL + (K.CuOHBL * K.CuOH * OHAct.X[j]) + (K.CuCO3BL * K.CuCO3 * CO3Act.X)[j]))
For i,j being the test and sample medium, respectively, and X the biological species of interest.

Species Sensitivity Distributions (SSD) are constructed based on site-specific NOECs (Posthuma et al., 2002). Finally, the HC5s, expressed as free ion activity, were derived from the site-specific SSDs and subsequently transformed to site-specific total dissolved HC5, using the same speciation model in a reversed mode. HC5 values are defined as the dissolved metal concentration that protects 95% of the ecosystem, taking into account the speciation and competition effects of other ions on the specific site. By expressing the HC5 as a total dissolved metal concentration, a straightforward comparison between HC5 and measurements and generic quality standards is enabled.

<table>
<tr><th colspan="2">BLM EU-RAR</th></tr>
<tr><th>Input</th><th>Output</th></tr>
<tr><td>Cu concentration
Ni concentration
Zn concentration
Temperature
pH
DOC
Ca
Mg
Na
K
SO4
Cl
HCO3-

Data processing capacity: unlimited</td><td>Full chemical speciation Cu, Ni, Zn
NOEC (calculated for 6-8 taxonomic groups representing 18-27 biota, depending on type of metal)
HC5
Species sensitivity distributions Cu, Ni, Zn
Risk characterisation ratio (RCR) Cu, Ni, Zn</td></tr>
</table>

2.2.2 BLM (HydroQual)

Version 2.2.3 (2007), available at http://www.hydroqual.com/blm, was used in this study. This tool uses empirical models for copper, silver, cadmium and zinc.

The number of organisms that can be tested is limited: models can be run only for (up to) three species of fish (*Fathead minnow*, Rainbow trout) and three crustacean (*Daphnia magna, Daphnia pulex, Ceriodaphnia dubia*), depending on the metal of choice. The tool offers user-defined options if LA50-values are known.

The BLM interface application allows the user to run the BLM either in toxicity mode or in the speciation mode. When run in the toxicity mode, for the metal and organism specified by the user, the BLM will predict the amount of metal required to cause acute mortality in the water specified by the user. However, when the BLM is run in the speciation mode, for the metal concentration specified by the user, the BLM will predict the organic and the inorganic speciation in the water column.

The applicability range of this tool is shown in table 3. Input and output parameters that are out of range are automatically marked in the files.

Table 3. Applicability range of BLM (HydroQual).

Parameter		Lower boundary	Upper boundary
Temperature	^{0}C	10	25
pH		4.9	9.2
DOC	mg/l	0.05	29.6
Humic Acid Content	%	10	60
Ca	mg/l	0.204	120.2
Mg	mg/l	0.024	51.9
Na	mg/l	0.16	236.9
K	mg/l	0.039	156
SO_4	mg/l	0.096	278.4
Cl	mg/l	0.32	279.7
Alkalinity	mg/l	1.99	360
DIC	mmol/l	0.056	44.9

The input manager of this tool, which allows for the entry of data in any dimension (moles or grammes) is very practical, and makes data conversions obsolete.

The set-up of this tool becomes limiting in two cases:

4 When using a large amount of data;
5 When testing multiple metals and multiple organisms.

The maximum data processing capacity is 1000 records, so for the database used in this study (2575 records, see section 2.3) the tool had to be run three times. Furthermore, the user can only run one metal, and one biotic species combination at a time. This results in repeated model runs and meticulous data input and output management. Multiple output files are generated (.sim; .det), and the user should keep close track of the mode (speciation, toxicity), metal and biotic species that was selected prior to calculations.

BLM (HydroQual)	
Input	**Output**
Cu concentration Ag concentration Cd concentration Zn concentration Temperature pH DOC Humic acid content Ca Mg Na K SO4 NO3 (for Ag) Cl Alkalinity Sulfide (optional) Data processing capacity: max 1000	Chemical species Cu, Ag, Cd, Zn LC50 (calculated for 2 taxonomic groups representing 3-4 biota, depending on type of metal) Instantaneous water quality criteria WQC (Cu) Acute toxic unit (Cu)

2.2.3 BLM (WCA)

This tool (version 8.0, March 2009, developed by WCA-Environment, UK) is an automated version of the published biotic ligand models for copper and zinc. It predicts the risk posed by metals expressed as a Predicted No Effect Concentration (PNEC), based on a calculated, non-defined “bioavailable concentration”. It is unclear for which taxonomic groups or species these calculations are performed.

The tool detects any data that are out of the boundary conditions for which the BLM was developed. These conditions are automatically highlighted, and no results will be calculated in those cases for that row of input.

The PNEC is set to the generic value for Cu or Zn, and the bioavailable fraction (BioF) is assumed to be 1 (=100%).

Table 4. Generic PNEC concentrations for Cu and Zn.

Metal	**Generic PNEC Concentration (µg l^{-1})**
Copper	1
Zinc	7.8
Zinc (soft water)	3.1

The risk characterisation ratio (RCR, = PEC/PNEC) is related to the generic PNEC concentration of copper and zinc. The bioavailability factor (BioF) is based on a comparison between the expected bioavailability at the reference site and that relating to site-specific conditions. By using a BioF, differences in bioavailability are accounted for by adjustments to the monitoring data but the EQS remains the same. It is calculated by dividing the generic EQS by the calculated site-specific EQS.

BLM (WCA)	
Input	**output**
Cu concentration Zn concentration pH DOC Ca Data processing capacity: unlimited	Bioavailable concentration Cu, Zn Bioavailable fraction Cu, Zn (BioF) Estimated PNEC Risk characterisation ratio (RCR) Cu, Zn

2.2.4 Ni-BLM (ARCHE)

This tool (version 10) resembles the BLM (WCA) in terms of interface and user applications, and was constructed in corporation with WCA. It predicts the risk posed by nickel expressed as a Predicted No Effect Concentration (PNEC), based on a calculated, non-defined “bioavailable concentration”. It is unclear for which taxonomic groups or species these calculations are performed.

This tool is promoted as a screening tool, based on estimations made from the biotic ligand models for nickel.

The tool detects any data that are out of the boundary conditions for which the nickel BLM was developed. Sensitive conditions are automatically highlighted, and additional information is given for these cases (e.g., that the PNEC has been calculated with an upper or lower boundary value, and that these results have to be regarded as tentative). These data have to be discarded, if desired, manually.

The risk characterisation ratio (RCR, = PEC/PNEC) is related to the generic PNEC concentration of nickel. The help-function of this tool mentions a “generic bioavailable PNEC” of 2.0 µg/l which is in contrast with the PNEC of 20 µg/l mentioned by EU (2008). The PEC (Predicted Environmental Concentration) is replaced by the input of dissolved Ni-concentrations. The bioavailability factor (BioF) is based on a comparison between the expected bioavailability at the reference site and that relating to site-specific conditions. By using a BioF, differences in bioavailability are accounted for by adjustments to the monitoring data but the EQS remains the same. It is calculated by dividing the generic EQS by the calculated site-specific EQS.

Like the BLM (WCA), the required input of this tool is very limited (in fact, only pH, DOC and Ca is required, indicating the use of empirical functions. No separate output files are generated.

Ni-BLM (ARCHE)	
Input	**output**
Ni concentration pH DOC Ca Hardness (optional) Na (optional) Data processing capacity: unlimited	Bioavailable concentration Ni Bioavailable fraction Ni (BioF) Estimated PNEC Risk characterisation ratio (RCR) Ni

2.2.5 Transfer functions (STOWA)

This method (STOWA, 2007) is based on empirical, linear correlations between calculated Hazard Concentrations at 5% of affected species (HC5) and dissolved organic carbon (DOC) concentrations in surface waters.

The calculations for HC5 concentrations were performed with the published BLMs for Cu, Ni and Zn (DiToro et al., 2001; Paquin et al., 2002; Niogyi and Wood, 2004; De Schamphelaere et al., 2005), using the toxicity base that was used in the EU risk assessment reports (EC 2008a; 2008b; 2008c) for a range of biological taxa. NOEC data were normalized via the BLM procedure and HC5 concentrations were derived from species sensitivity curves. These were correlated with water characteristics of a limited number of observations in waters mainly of the types brooks and streams (WFD R-types).

The dataset that was used for the derivation of transferfunctions are compiled from monitoring programmes of various local water boards in the Netherlands (Dommel, Hunze en Aa's, Regge en Dinkel, Schieland, Valei en Eem, Velt en Vecht) (STOWA, 2007). The dataset comprised of 216 measurements, of which 200 contained measurements of copper, nickel and zinc.

The applicability domain and median values are given in table 5. From this dataset, and the calculated HC5 values, linear correlations were derived for copper, nickel and zinc, giving the following equations:

Cu: HC5 (µg/l) = 3.0 × DOC (mg/l) + 3.5
Ni: HC5 (µg/l) = 1.8 × DOC (mg/l) + 12.6
Zn: HC5 (µg/l) = 4.2 × DOC (mg/l) + 15.6

Table 5. Dataset and applicability domain for the transfer functions (STOWA).

Parameter		Lower boundary	Upper boundary	Median dataset	n
pH		5.5	9.1	7.5	216
DOC	mg/l	1.7	45	12	216
Ca	mg/l	14	170	63	216
Mg	mg/l	3.7	38	7.8	216
$CaCO_3$	mg/l	51	482	192	216
Na	mg/l	10	182	37	216
K	mg/l	2.9	91	12	216
Cl	mg/l	19	220	53	216
SO_4	mg/l	10	240	52	216
Cu diss	µg/l	<0.7	8.3	2	200
Cu tot	µg/l	<0.7	31	2.6	200
Ni diss	µg/l	<1	33	3.8	200
Ni tot	µg/l	<1	34	4.2	200
Zn diss	µg/l	<4	170	9	200
Zn tot	µg/l	<4	590	14	200

Diss = dissolved, filtrated over 0.45µm

Tot = including suspended matter; values not corrected.

These functions can be incorporated in any data processing environment.
According to the authors, the method may provide a first estimation of risks, provided that other water characteristics are within the ranges of application. Further analysis of these correlations, and in particular the variations in the HC5 prediction of these functions, revealed that pH contributed to a large part of these variations. Predicted HC5 concentrations with the copper BLM and zinc BLM at pH values < 6.5, respectively < 7, were significantly lower than the predicted HC5 values obtained with the linear functions. For nickel, the opposite effect was found for waters with pH values > 7.5.

Transfer functions (STOWA)	
Input	**Output**
DOC Data processing capacity: unlimited	HC5 Cu, Ni, Zn

2.3 Water quality database

For the five tools that were tested in this study, a large dataset of water measurements was composed that was used as input for all selected models. Data for water chemistry and metal concentrations were collected from the iBever National monitoring database. This database was supplemented with data from various monitoring programs of different local water managers.

It should be noted that the STOWA transferfunctions were empirically derived from a different dataset from local monitoring programmes in brooks and streams for which these functions apply.

The database contained a grand total of 2575 records with geographical information and surface water compositions in the Netherlands. These data covered most of the water types that are described in the Water Framework Directive. The database was constructed to contain all parameters that are required to perform the chemical speciation calculations and the BLM modeling. Data below the limit of detection were assigned the value 0.5 times the limit of analytical detection of that compound. In cases of multiple temporal measurements, e.g., repeatedly over various points in time, the data were aggregated to monthly average concentrations.

Table 6 gives an overview of the water types, following the WFD characterisation and terminology, that are represented in the used database. Table 7 gives an impression of the range of water characteristics in this database.

Table 6. Water types represented in the database.

WFD Watertype	**% covered in database**
I Large rivers	14
II Canals, lakes	6
III Streams, brooks	48
IV Ditches	25
V Sandy springs	7
VI Small acid ponds	0

Table 7. Concentration ranges and statistics of the database

	Temp (C)	**pH**	**DOC**[*] mg/l	**Na** mg/l	**Mg** mg/l	**K** mg/l	**Ca** mg/l	**Cu** µg/l	**Ni** µg/l	**Zn** µg/l	**Cl** mg/l	**SO_4** mg/l	**HCO_3**[**] mg/l
Average	12.9	7.48	11.3	108	17.6	13.4	70.1	2.55	7.98	31.1	191	76.6	177
Min	0.4	4.6	0.05	3	0.5	1	1.7	0.25	0.25	0.5	1.34	5	2.5
Max	23.8	10.1	100	8100	910	351	380	28	180	1200	15000	1580	738
Median	14	7.5	9.8	33.2	8.8	12	62	2.2	3.9	11	50	60	180
n	2573	2573	2573	2573	2573	2573	2573	1554	1616	1457	2573	2573	2573

* Dissolved organic carbon

** In cases where no measurements were available, HCO_3^- was calculated from total $CaCO_3$ (diss) and/or pH.

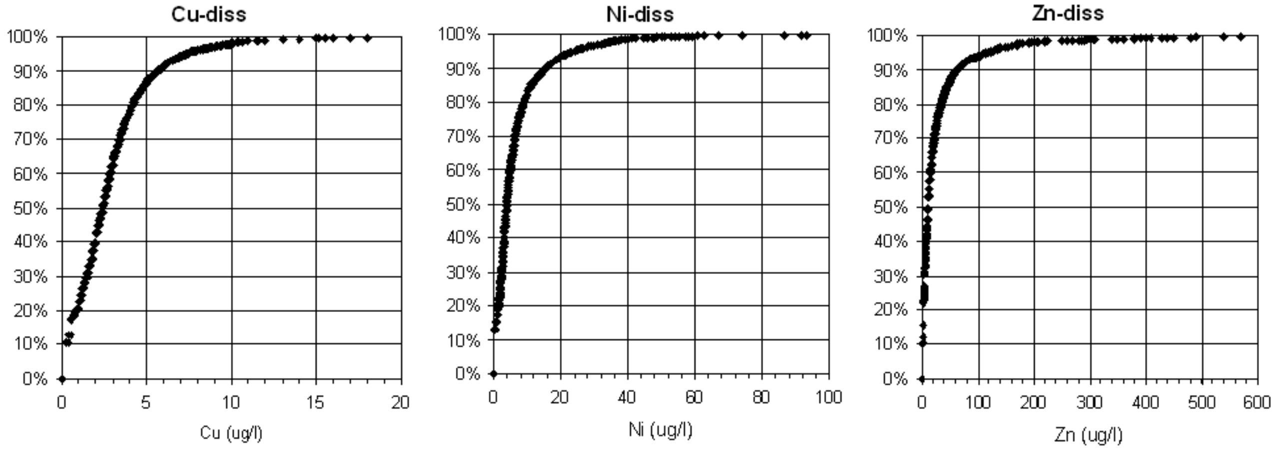

Fig 2. Percentile distribution of dissolved concentrations in the database for copper, nickel and zinc.

Table 7 shows that there is a large variation in the water characteristics, with sometimes some orders of magnitude between minimum and maximum values.
Based on metal concentrations of Cu, Ni and Zn, the general water quality of all locations presented in this database is poor. The majority of the waters (76%) exceed generic environmental quality standards (EQS) for copper. For zinc, 62% of all locations exceed the generic EQS. Nickel shows the least problems; only 8% of all locations exceed the generic EQS.

Generic water quality standards are:

Cu:	1.5 µg/l	(RIVM, 2010)
Ni:	20 µg/l	(EU, 2008)
Zn:	7.8 µg/l	(EU, 2008c)

2.4 Criteria for comparison of performance

The BLM EU-RAR is the reference-of-quality in the comparison between these tools. As explained earlier, these biotic ligand models were validated within the risk assessment reports for copper and zinc. We complied with the same procedures and models that were used for these validations, including the use of the chemical speciation model WHAM, and the toxicity database for a large number of taxa. By using the same database for all models, covering most water types of the Netherlands and covering a large range of boundary limits, the variance in output data can only be subscribed to the design of the tool in question.

From the input-output tables, presented in each paragraph of the used tools, it is clear that there is no real uniformity in computed outputs. Some tools generate chemical speciation, i.e. free ion and complexed species, other generate non-defined bioavailable fraction. Also, the indicator for risks (NOEC / PNEC, HC5, LC50, RCR, BioF, TU) varies largely. This makes one-on-one comparison, a-priori, in many cases difficult or impossible.

For this reason, only output parameters that may be regarded as being “comparable” were tested. In some cases, the comparable sets were derived for the chemical speciation output (e.g., free ion concentrations). In other cases, the goodness of prediction was derived from the toxicological output parameters, such as HC5 or the risk characterization ratio (RCR).

In the comparison of tools, we used the statistical t-test for comparison of means and variances. Since the input parameters were always the same (records from one database), the calculated results are regarded as paired observations. The paired sample population is assumed to have equal variances. The zero hypothesis (H0) assumes that the mean difference between sample populations is zero. H0 is tested two-sided. The two-tail values are therefore used for the analyses. If this hypothesis is accepted, the two data sets are comparable and no significant differences between the calculated outcomes exist within certain reliability (in this case the 95% confidence level, alpha = 0.05). The zero hypothesis is accepted if the calculated statistical p-value is larger than 0.05, or when the calculated statistical t-value is smaller than the critical value ($t_{stat} < t_{crit}$) for the two-tail test.

When two (paired) calculation sets are regarded as being not similar, there still can be a correlation between the two. This means that larger values of one population (=calculation results of method 1) tend to lead to larger values of the second population (=calculation results of method 2). Therefore, the results were also tested for correlation. Correlation is regarded as being significant if the correlation coefficient (sum of squares, R^2) is larger than the value of 0.5.

A suitable tool should also meet some user-defined criteria. In practice, these are mainly dictated by the WFD reporting requirements. For example, the tool must be able to process large amount of data, and the input requirements should match the characteristics that are measured in periodic monitoring programmes.

3 Results and discussion

3.1 BLM EU-RAR

Table 8 shows the results of the speciation calculations. WHAM6 generates almost all possible species. Only the free ion concentrations for copper, nickel and zinc are shown since these were used as input in the BLM calculation and normalization procedure. For copper, also $CuOH^+$ and $CuCO_3$ concentrations are shown, since these species contribute in the total biotic ligand binding, as explained in section 2.2.1. In terms of magnitude of concentrations, it becomes clear that these species dominate the labile fraction in solution.

Table 8. Statistics for chemical species that are used for input in the BLM EU-RAR calculations.

	Cu2+ µg/l	**Ni2+ µg/l**	**Zn2+ µg/l**	**CuOH+ µg/l**	**CuCO3 µg/l**
Average	9.55E-04	4.50	7.36	4.78E-04	0.029
St dev	3.13E-03	7.81	23.7	2.06E-03	0.183
min	7.16E-08	0.07	0.0048	8.76E-08	4.27E-08
max	5.43E-02	110	402	0.048	6.16
median	2.55E-04	1.92	1.28	6.14E-05	1.74E-03
n	1554	1616	1457	1554	1554

Figure 3 shows the percentile distribution of calculated free ion activities for the entire dataset. These curves show how concentrations are distributed in x % of the cases, including extreme values (min/max). By including the total-dissolved (filtrated) data from figure 3, the relative ratios of total and free dissolved concentrations become visible. In general, the contribution of free nickel and zinc concentrations to total dissolved concentrations is much larger than in the case of copper.

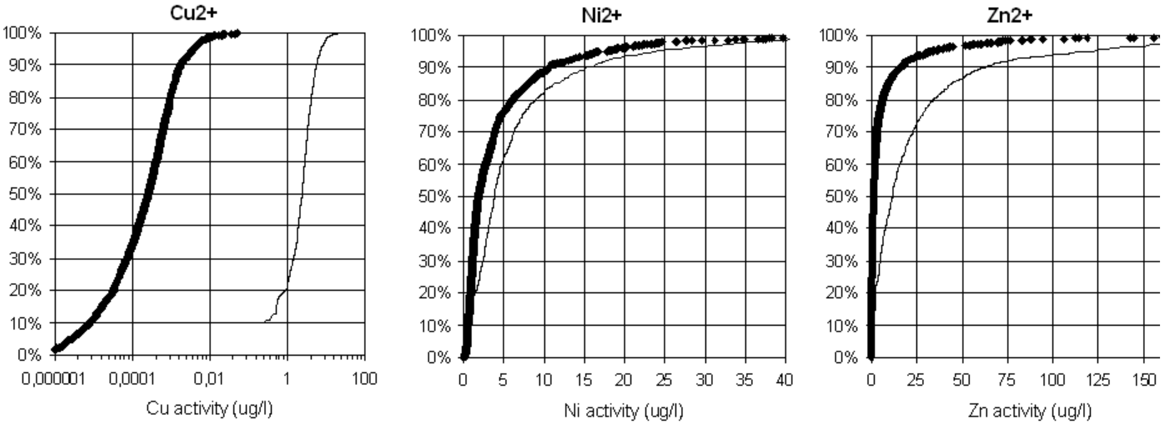

Fig 3.Percentile distribution (free ion activities in µg/l; monitoring database, BLM EU-RAR). For comparison, the distributions of total dissolved concentrations from fig 2 are added (thin solid line).

Figure 4 gives the results of the computed NOEC data for zinc that are compiled into species sensitivity distributions (SSD), based on free ion concentrations. HC5-values are derived by reading the 5% affected fraction cut-off with the X-axis. These data were transformed into total dissolved concentrations by using the speciation calculations in reverse mode. The results are presented in table 9.

SSD curves are expressed as the summation of NOECs (mostly of the most sensitive toxic endpoint). The ranges of these curves clearly show the variation of sensitivity to biological species. Curves on the left hand site generally represent sensitive waters (low HC5), curves on the right are the more robust cases (high HC5). The curves also give an excellent insight as to which taxa or biological species are at risk. This is not further discussed here, since none of the other tools that are tested in this study are able to present SSDs.

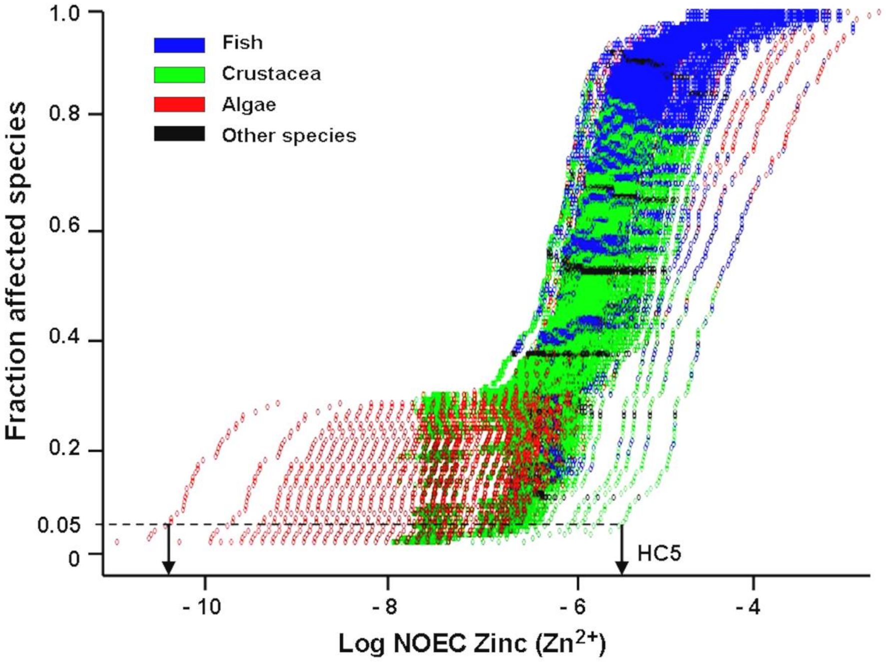

Fig 4. Species sensitivity distributions of zinc based on free ion concentrations.

Table 9. Statistics for toxicity calculations. HC5=Hazard concentration 5%, RCR=Risk characterisation ratio.

	HC5.Cu	**RCR.Cu**	**HC5.Ni**	**RCR.Ni**	**HC5.Zn**	**RCR.Zn**
	ug/l	-	ug/l	-	ug/l	-
Average	35.1	0.12	15.2	0.75	49.1	0.87
St dev	26.7	0.14	15.9	1.23	25.8	1.89
Min	0.53	0	6.6	0	13.9	0
Max	477	2.66	239	17.9	389	25.2
Median	30.3	0.08	11.48	0.32	43.9	0.28
n	2573	1554	2573	1616	2573	1457

The risk characterization ration (RCR) is defined as:

$$RCR = \frac{[C_{metal}]}{HC_5}$$

In which C_{metal} is the filtrated, total dissolved concentration of the metal of interest, and HC5 is the Hazard concentration at which up to 5% of species are affected (95% is unaffected and therefore regarded as protected).

Table 9 shows that the average value of the calculated RCR for copper is 0.12. This value is well below 1. This indicates that the majority of sites from the database (sd = 0.14) pose no ecotoxicological risk. For zinc however, the average is 0.87, with a standard deviation of 1.89. Results showed that in 53% of the cases the RCR is larger than 1. Hence, in the majority of cases, zinc poses a significant potential risk to the aquatic environment.

3.2 BLM (HydroQual)

This tool was tested for copper and zinc, but not for silver and cadmium since no other tool tested here provides comparable output for these elements. Cadmium calculations are most probably based on the procedure for hardness correction, but this is not documented in the tool.

When run in the speciation mode, this model provides fairly detailed information on the actual speciation of Cu and Zn. Table 10 gives a summary of the outcome of chemical calculations.

Table 10. Statistics for calculated chemical speciation by BLM (HydroQual).

	Cu2+ (µg/l)	**Zn2+ (µg/l)**
Average	1.2E-03	6.71
Stdev	3.0E-03	41.5
Min	1.0E-06	0.0039
Max	0.11	1769
Median	6.0E-4	1.14
n	1554	2203

The results from table 10 can directly be compared to the speciation calculations performed with BLM EU-RAR (using WHAM6), since the free ion concentration is an operationally defined parameter. Figure 5 shows the plotted results of both tools.

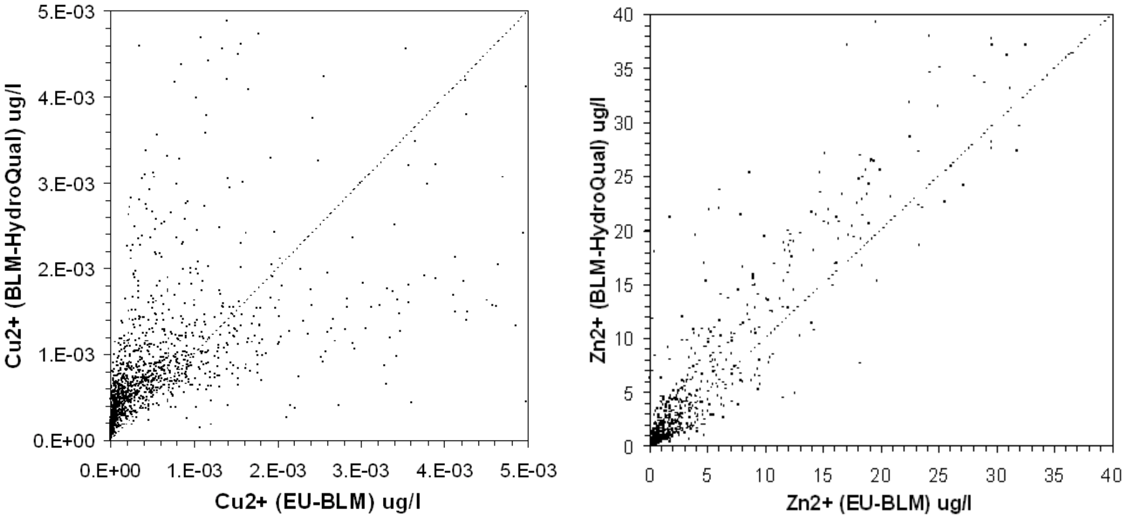

Fig 5. Plotted results of the free ion concentrations calculated by the BLM EU-RAR and BLM (HydroQual). The dotted line is the ideal 1:1 fit.

As discussed in section 2.4, the outcomes of the various tools were statistically tested for differences in means and variances. Table 11 provides the statistical results for the data presented in figure 5 (calculation of free ion concentrations). Also, no significant correlation was found for Cu or Zn ($R^2 = 0.05$ and 0.21, respectively).

Table 11. Statistics for comparison of variance for calculated free ion concentrations by BLM EU-RAR and BLM (HydroQual) at the 95% confidence level.

Statistical parameter	**Calculation of Cu^{2+}**	**Calculation of Zn^{2+}**
t-stat	-2.389	-1.778
t-critical two tail	1.961	1.962
P (T<=t) two tail	0.017	0.076
H0 hypothesis (variance=0)	reject	accept
Sign. correlation	no	no

From Table 11 it is concluded that the data for free ion concentrations, generated by BLM EU-RAR and BLM (HydroQual), are in agreement for zinc, but not for copper. Hence, both models have comparable performance for the speciation calculation of Zn, and not for Cu.

Table 12 shows an example of calculated toxicity parameters for fish (*Fathead minnow*). For Cu, the tool generates various parameters. The output for Zn is restricted to a predicted LC50. No significant relation between HC5 (calculated with BLM EU-RAR) and LC50 for zinc could be found.

Table 12. Statistics for toxicity calculations for the example Fathead minnow (fish).

	LC50, Cu µg/l	**CMC[1], Cu µg/l**	**Cu Toxic Units**	**LC50, Zn µg/l**
Average	1510	61.3	0.11	987
Stdev	2058	64.3	0.27	1146
Min	26.7	0.07	0.0016	29.4
Max	36667	707	6.94	12582
Median	1048	41.5	0.068	549
n	2571	2570	1540	2571

[1] CMC is defined as the "Instantaneous Water Quality Criterium", or site-specific WQC. It is calculated as the Final Acute Value (FAV) divided by 2.

The site-specific quality standard for copper is represented by the CMC from table 12. The same definition is valid for site-specific HC5 values, which are determined for the same reason, i.e., site-specific risk assessment. Although the means and variances of the two parameters differ (average HC5 = 35.12, average CMC = 61.3), the predictive performance was tested. In figure 6, these two parameters are compared by plotting the outcome of the BLM EU-RAR with the CMC.

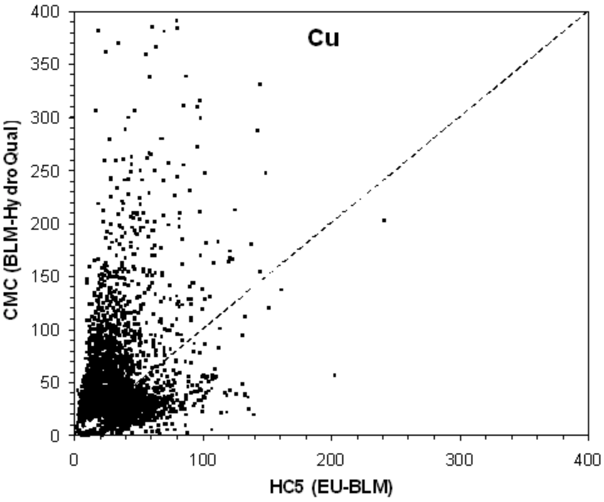

Fig 6. Plotted results of HC5 calculated by the BLM EU-RAR and the site-specific Water Quality Criterium (CMC) calculated by BLM (HydroQual). The dotted line is the ideal 1:1 fit.

From figure 6, no significant relationship can be derived between the calculated site-specific HC5 values and the instantaneous water quality criterion for copper. It should be noted however that the HC5 values are calculated from a toxicological database representing a variety of biological taxa. With tool 2.2.3, only one species can be tested at a time. If it is desired to find the most sensitive quality standard for the water quality data base, or each individual water sample, the available species in this tool should all be tested separately.

Table 13. Statistics for comparison of variance for calculated site specific environmental quality standard by BLM EU-RAR (HC5) and BLM (HydroQual) (CMC) at the 95% confidence level.

Statistical parameter	Calculation of site spec. EQS for Cu
t-stat	-21.075
t-critical two tail	1.960
P (T<=t) two tail	4.4E-91
H0 hypothesis (variance=0)	reject
Sign. correlation	no

3.3 BLM (WCA)

The output of this tool is summarized in table 14. For the calculated Risk Characterization Ratio (RCR), the same trend is observed as in the BLM EU-RAR results: in virtually all cases, the RCR for copper is smaller than 1, indicating no potential risk to the aquatic environment. For zinc however, the calculated RCR is >1 in the majority of cases. Also note that the EQS for zinc is 7.8 µg/l (EU, 2008c), which is a standard for filtrated, total dissolved concentrations. The average bioavailable concentration, which is smaller than total dissolved at all times, is approximately 1.5 times the generic EQS at average.

Table 14. Results of calculations for BLM (WCA).

	Copper				Zinc			
	Estimated PNEC[1] (µg/l)	BioF[2]	Bioavailable Concentration (µg/l)	RCR[3]	Estimated PNEC[1] (µg/l)	BioF[2]	Bioavailable Concentration (µg/l)	RCR[3]
Average	23.44	0.08	0.21	0.21	30.91	0.32	11.15	1.46
Stdev	17.40	0.09	0.35	0.35	16.70	0.17	25.96	3.38
Min	1.00	0.01	0.01	0.01	3.10	0.04	0.12	0.01
Max	152	1.00	8.80	8.80	184	1.00	351	45.1
median	17.88	0.06	0.13	0.13	28.19	0.28	3.65	0.47
n	2516	2516	1528	1528	2573	2573	1457	1457

[1] PNEC = Predicted no-effect concentration; [2] BioF = Bioavailable fraction ($C_{bioavailable}$ / $C_{Total\ dissolved}$);

[3] RCR = Risc characterization ratio ($C_{Total\ dissolved}$ / PNEC).

Figure 7 gives the plotted results of chemical availability. Section 2.2.1 discussed the chemical availability of copper, discussing the fact that –OH and $–CO_3$ species of Cu also contribute to the labile fraction and are therefore incorporated in the BLM. So, to be able to compare the chemical speciation results of BLM EU-RAR to the ill-defined “bioavailable” concentration, the sum of [Cu^{2+}], [$CuOH^-$], and [$CuCO_3^0$] were used, the latter being the most dominant species. Nevertheless, the “bioavailable” concentration is overestimated by approximately a factor of 6.

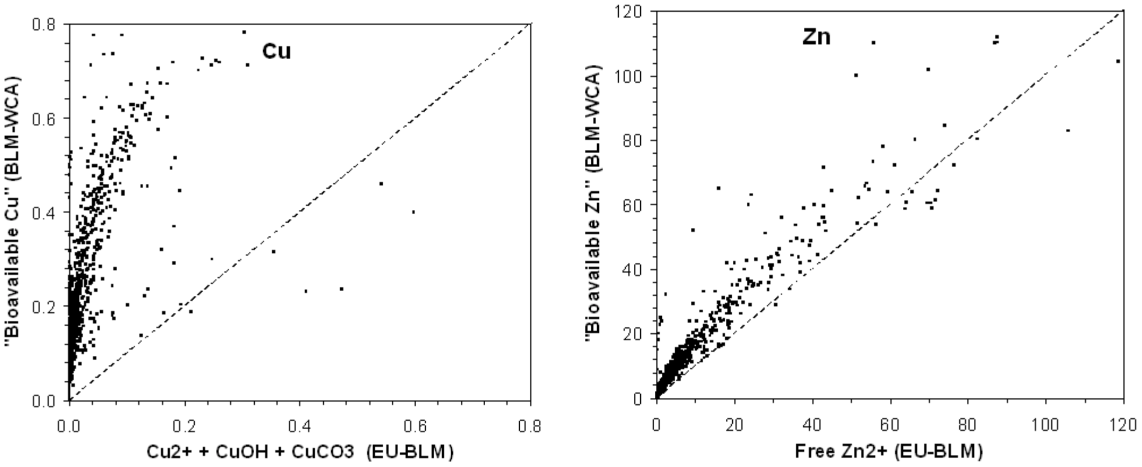

Fig 7. Plotted results of the labile concentrations calculated by the BLM EU-RAR and BLM (WCA). The dotted line is the ideal 1:1 fit.

For Zinc, there appears to be a closer relationship than in the case of copper. The Pearson correlation of the two variables is 0.914, and a simple linear relation of $Y=X+3.8$ ($R^2 = 0.84$) was derived. This means that larger values of one population tend to lead to larger values of the second population. However, the two-sided test of variance shows that there still is a significant difference between the two sets at the 95% confidence level. Agreement on similarity (i.e., accept H0) is only reached at the 70% confidence level, which is outside the quality criterion discussed in section 2.4.

Table 15. Statistics for comparison of variance for calculated labile fractions by BLM EU-RAR and BLM (WCA) at the 95% confidence level.

Statistical parameter	**Calculation labile Cu**	**Calculation of Zn^{2+}**
t-stat	-22.032	-13.723
t-critical two tail	1.962	1.962
P (T<=t) two tail	1.23E-93	2.14E-40
H0 hypothesis (variance=0)	reject	reject
Sign. correlation	no	yes

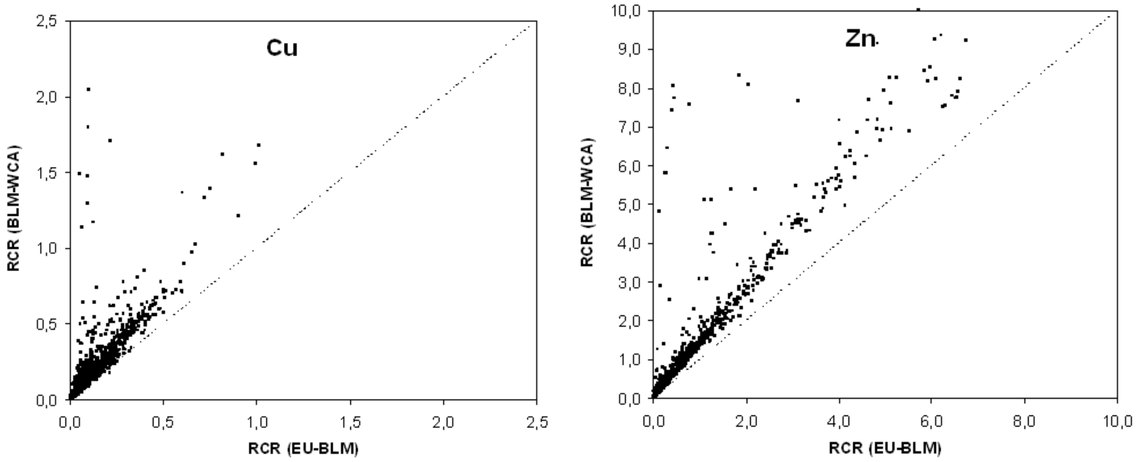

Fig 8. Comparison of risk ratios calculated by BLM EU-RAR and BLM (WCA) for copper and zinc.

Figure 8 shows the vast majority of predicted RCRs are above the 1:1 line for both metals, indicating that the BLM (WCA) systematically over-predicts the risks of copper and zinc. The results may therefore be regarded as worst-cases, and the chance of being under-protective for the aquatic environment is therefore small.
In this study we do not aim at explaining outliers like those close to the Y-axis in figure 8. Analyzing these would however give more insight in the model's effectiveness to predict critical compositions or water types.

Table 16. Statistics for comparison of variance for calculated risk characterization ratios by BLM EU-RAR and BLM (WCA) at the 95% confidence level for copper and zinc.

Statistical parameter	**RCR Cu**	**RCR Zn**
t-stat	-11.777	-12.871
t-critical two tail	1.962	1.962
P (T<=t) two tail	1.05E-30	5.54^{E}-36
H0 hypothesis (variance=0)	reject	reject
Sign. correlation	no	no

3.4 Ni-BLM (ARCHE)

Table 17 summarizes the output of calculations performed with this tool.

Table 17. Results of calculations for Ni-BLM (ARCHE).

	Nickel			
	Estimated PNEC (ug/l)	**BioF**	**Bioavailable concentration (ug/l)**	**RCR**
Average	13.22	0.20	1.40	0.70
Stdev	6.98	0.11	2.05	1.03
Min	2.47	0.02	0.05	0.02
Max	88.05	0.81	28.91	14.45
Median	12.10	0.17	0.80	0.40
N	2573	2573	1616	1616

The tool calculates a “bioavailable concentration” for nickel. This value, however, is operationally undefined, and it is therefore unclear whether this represents a free ion concentration or activity, or some sort of labile fraction. Since the tool does not make use of a chemical speciation programme, the bioavailable fraction is therefore calculated from empirically derived transfer functions. Figure 9 shows the relation between dissolved Ni concentrations in the database and calculated bioavailable concentrations. The high correlation (R^2=0.92) suggest a simplified transfer function based on total dissolved concentration and a limited amount of parameters.

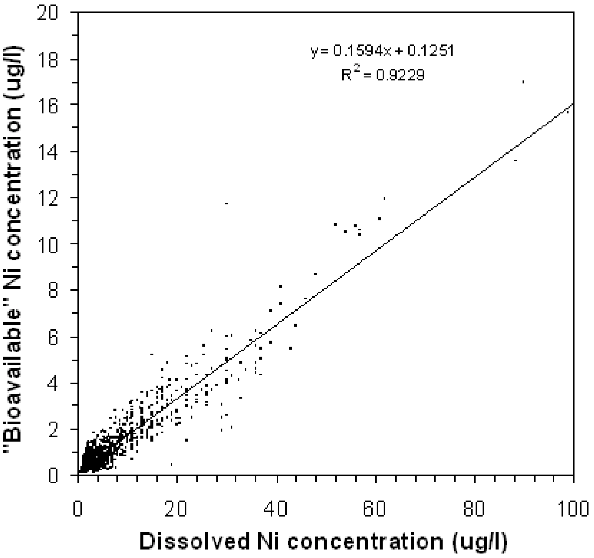

Fig 9. Dissolved vs bioavailable nickel concentration calculated by BLM (ARCHE).

Figure 10 shows the comparison of calculated free ion concentrations, performed by BLM EU-RAR and the calculated bioavailable concentration by Ni-BLM (ARCHE)

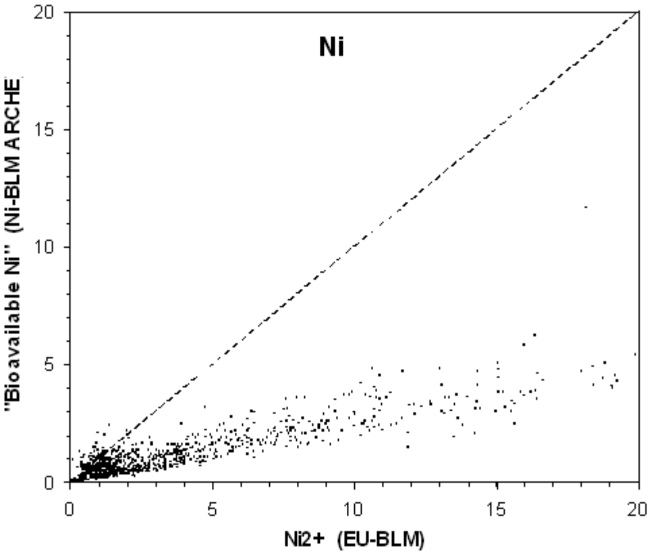

Fig 10. Ni free ion concentration calculated by BLM EU-RAR versus bioavailable nickel concentration calculated by BLM (ARCHE).

Figure 10 clearly shows that the calculated “bioavailable Ni” gives a significant under-prediction of free ion concentrations by a factor of 4. This is a remarkable phenomenon; labile fractions are defined as being at least the free ion concentration, in some cases added by very labile ion pairs like salts or easily dissociated complexes. For example, in the case of copper, –OH and –CO_3 species also contribute to the labile fraction and are therefore incorporated in the BLM. The data show a strong linear correlation between Ni^{2+} and “bioavailable” concentrations: $Y=0.25X + 0.25$ ($r^2 = 0.93$). Note however that from a mechanistic viewpoint, such relation does not make any sense; it is obvious that the free ion concentration is the most labile fraction, and adding other labile fractions (to achieve the “bioavailable pool”) should result in higher concentrations.

Figure 11 shows the comparison of the risk characterization ratio’s calculated for nickel. The data correlate well with the predicted RCRs of the validated BLM EU-RAR (R^2=0.92), indicating strong agreement in the prediction of low and high risk. The majority of data however, are below the 1:1 line, which indicates a general prediction that may lead to being under-protective as a risk indicator for the aquatic environment.

Although the RCR predictions correlate well, the two-sided test of variance shows that there is a significant difference between the two sets at the 95% confidence level. Agreement on similarity (i.e., accept H0) is only reached at the 65% confidence level, which is outside the quality criterion discussed in section 2.4.

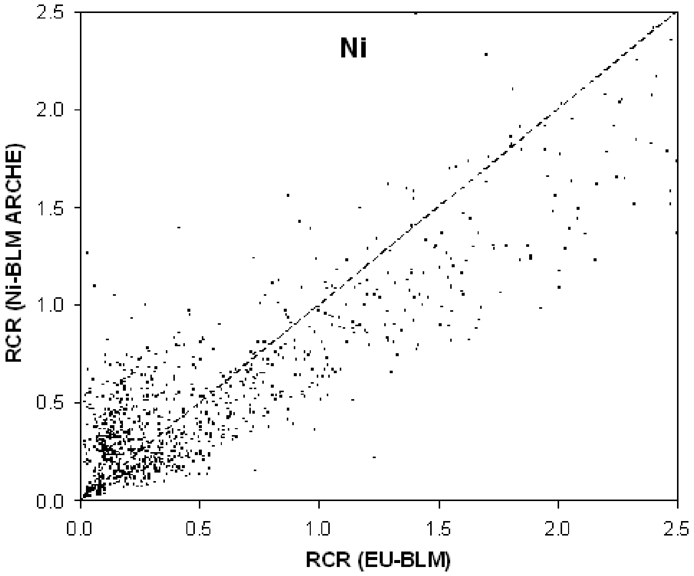

Fig 11. Comparison of calculated risk ratios.

Table 18. Statistics for comparison of variance for calculated Risk Characterization Ratio by BLM EU-RAR and Ni-BLM (ARCHE) at the 95% confidence level.

Statistical parameter	Calculation of RCR Ni
t-stat	5.117
t-critical two tail	1.961
P (T<=t) two tail	3.47E-07
H0 hypothesis (variance=0)	reject
Sign. correlation	yes

3.5 Transfer functions (STOWA)

In section 2.2.5, the derivation of these transfer functions were discussed. Although the functions are empirically derived, the calculations of HC5 values followed the procedures as described in the EU-RAR for Cu, Ni and Zn.

PNEC and NOEC values are derived from single combinations of one metal and one biological species. The HC5 value however can only be derived from a species sensitivity distribution (SSD) which has to be constructed from an array of NOECs for various species. Unlike the other tools, that make simplifications in the calculation procedure, this method keeps the integrity of the calculation procedure in tact, and derives empirical descriptions from the results of a given dataset. It may be clear that there are both advantages and disadvantages to this approach.

Table 19. Results of calculations with the transfer functions (STOWA).

	HC5 Cu µg/l	HC5 Ni µg/l	HC5 Zn µg/l
Average	37.5	33.0	63.2
St dev	22.9	13.7	32.0
Min	3.7	12.7	15.8
Max	304	193	436
Median	32.9	30.2	56.8
N	2573	2573	2573

Table 19 summarizes the results of calculations. HC5 values are the only output of this tool.

Figure 12 shows the plot results of the linear transfer functions, using the 2573 DOC data from the water quality data base. The intercept of these lines may be regarded as a “baseline” of toxicity of that specific metal, since it is assumed that the presence of DOC has a de-toxifying effect of metals, via larger amount of metal binding and, consequently, decreasing bioavailability (Guthrie et al., 2005; Dwane and Tipping, 1998; Tipping, 1998; Kramer et al., 2001). So when DOC concentrations are zero (= the intercept), the following HC5 would represent the maximum induced toxicity of that metal. In table 20, this “toxicity baseline” or maximum toxicity (lowest HC5) is compared to the generic environmental quality standards of Cu, Ni and Zn. It shows that for copper and zinc, this baseline is *at all times* larger than the generic EQS. This observation cannot be matched with the NOEC data from the toxicity database.

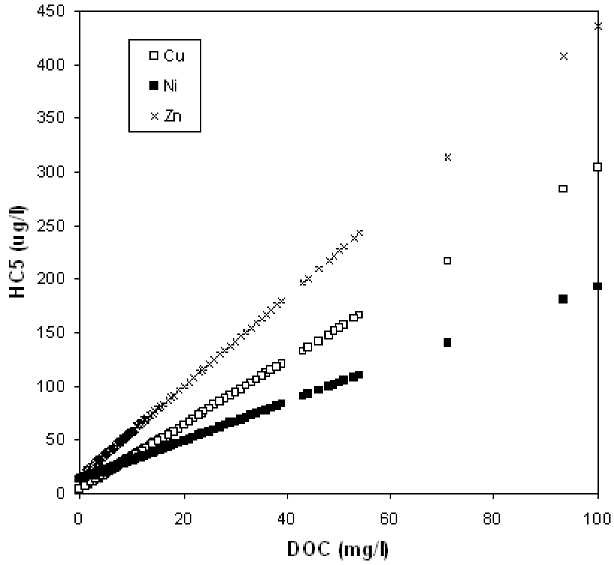

Fig 12. Plot results of the transfer functions (STOWA).

Table 20. Comparison of intercept and environmental quality standards.

	Cu	Ni	Zn
Baseline HC5 (=intercept)	3.5	12.6	15.6
Generic HC5 (=EQS)	1.5	20	7.8

The angle of the linear functions represents the response of HC5 to changes in DOC concentrations. These results show that the response for zinc – and therefore the sensitivity to species – to increasing DOC concentrations is larger than for copper and nickel. This observation cannot be matched with thermodynamic and experimentally derived binding coefficients of these metals to DOC, generally increasing from Ni ≤ Zn < Cu (Tipping, 1998; Guthrie et al., 2005).

In figure 13, the HC5 values calculated with this tool and the BLM EU-RAR are compared.

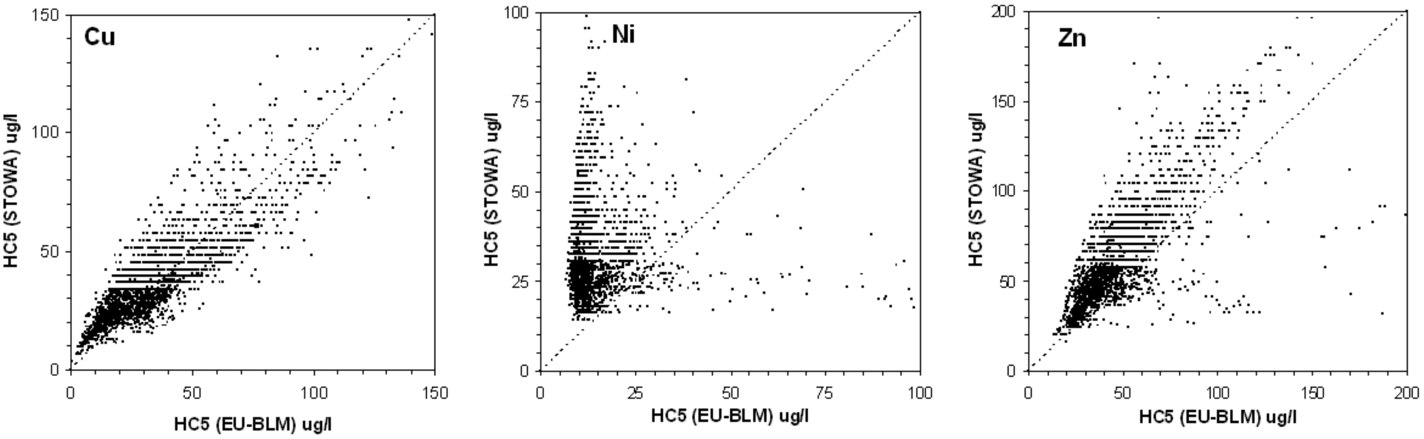

Fig 13. Comparison of calculated Hazard Concentrations for copper, nickel and zinc.

Table 21. Statistics for comparison of variance for calculated HC5 values by BLM EU-RAR and Transfer functions (STOWA) at the 95% confidence level.

Statistical parameter	HC5 Cu	HC5 Ni	HC5 Zn
t-stat	-9.847	-42.795	-32.532
t-critical two tail	1.961	1.962	1.961
P (T<=t) two tail	2.2E-16	2.2E-16	2.2E-16
H0 hypothesis (variance=0)	reject	reject	reject
Sign. correlation	yes	no	no

Statistical variance testing shows that calculated HC5 values differ significantly from the BLM EU-RAR calculations for all metals. For copper, a positive correlation was found ($R^2 = 0.79$), indicating a similarity in trend of prediction.

4 Synthesis and concluding remarks

By incorporating chemical speciation of heavy metals in the assessment of ecotoxicological risks for aquatic species, site-specific variations are made visible that remain undetected with generic EQS testing. A long history of experiments has provided sufficient evidence for the importance of water chemistry on the adverse effects (toxicity) of heavy metals and other compounds to aquatic organisms.

Biotic ligand models were developed over past years, incorporating site-specific water characteristics in the water quality assessment procedure. Conceptual models were constructed and validated within the European Risk Assessment Reports, using a large number of preselected toxicity tests representing an array of biological taxonomic groups (e.g., algae, crustacean, insects, mollusks, snails, fish, and amphibians).

What has been missing is a practical modelling implementation that can predict these variations in toxicity with a certain degree of reliability. For good chemical and biological purposes (e.g., reporting), described in the Water Framework Directive, BLMs are recognized as valuable and useful concepts to determine site-specific risks, and are allowed as second-tier assessment method. The demand for simple, operational concepts by end users to test water quality monitoring data has resulted in several tools. These tools are generally simplifications of the original, validated BLM concept. In order to implement these tools as generic testing tools, it is important to have an insight in the reproducibility and reliability of such tools.

The tools that were tested in this study are all operational. This means that the methods that are used are automated, and are shielded from the user. The advantage is that these tools generally show a high degree of reproducibility (although the user has no insight in the type of calculations or assumptions that are used in the tool). Reliability, however, is directly associated with the method of use. Simplifications of - or any deviation from - the validated BLM concept, will irrevocably result in deviations in results. In screening tools, deviations from the “truth” are generally accepted up to a point: acceptance is directly related to 1) the degree of deviation; 2) the direction of deviation. For the degree of deviation, a confidence interval of 95% was used as a quality criterion. Basically this means that in 5% of the cases the calculated result is “wrong”. In the next paragraphs, the performance of the various tools, tested against the quality criteria discussed earlier, are summarized.

4.1 Performance

The results of this study show that none of the tested models meet the quality criteria for the defined reliability for both chemical availability and risk indicators. Only BLM (HydroQual) produces statistically significant (reliable) results for the chemical speciation calculation of zinc. For copper however, there is no agreement with the WHAM6 speciation routine. Also no agreement was found in the prediction of toxic indicators of copper and zinc.

Based on trend correlation, there are some occasional agreements with the various tools:

- BLM (WCA) shows a significant correlation of calculated “bioavailability” with free ion concentrations for zinc, but not for copper.

- The Ni-BLM (ARCHE) shows a good correlation with the calculation of the risk characterization ratio (RCR), but systematically underestimates these values. From the viewpoint of general protection to aquatic systems, systematic underestimation in not desired, due to the lack of an intrinsic safety factor.
- The empirical functions (STOWA) show significant correlation with calculated HC5 for copper, but not for zinc.

Table 22 summarizes the results that were discussed for each tool in chapter 3. Performance" is based on statistical testing. "Selling points" and "Limitations" include aspects on both conceptual soundness and completeness, and user-defined criteria discussed in section 2.4.

Table 22. Summary of performance, tested against BLM EU-RAR.

	BLM (HydroQual) v. 2.3.3	**BLM (WCA) V. 8.0**	**Ni-BLM (ARCHE) v. 10**	**Functions (STOWA) 2007**
Performance[1]	Agreement on speciation calculation of Zn^{2+}, but not of Cu^{2+}. No agreement on risk indicator CMC vs HC5.	No agreement on chemical speciation. No agreement on Risk characterization Ratio (RCR).	No agreement on chemical speciation. No agreement on Risk characterization Ratio (RCR).	No speciation testing possible. No agreement on calculation of HC5
Correlation[2]	No significant correlations found	Significant correlation for calculation of "bioavailable Zn", not for Cu.	Significant correlation of Risk Characterization Ratio (RCR), but systematic underestimation.	Significant correlation for Cu (not Ni, Zn)
Selling points	Detailed output on chemical speciation; Test modules for silver and cadmium.	Few input data needed; Unlimited data processing capacity.	Few input data needed; Unlimited data processing capacity.	Based on the procedure of EU-RAR. Based on variety of taxa. Only DOC required for input variable.
Limitations	No testing for Ni; Large amount of input data required; Only 1 to 1-parameter testing possible (metal, organism); Limited biota testing; Elaborate data management; Limited data processing capacity.	No testing for Ni. Undefined chemical speciation. Underlying representation for species or taxa unknown.	No testing for Cu, Zn. Undefined chemical speciation. Underlying representation for species or taxa unknown.	No chemical speciation as output; Limited underlying water data; Limited water types (local streams).

[1] Statistical test for equality of results, based on means and variances at the 95% confidence level. The BLMs for Cu, Ni and Zn that were validated in the EU-RARs were used as a reference of quality. [2] Correlated trends, R^2>0.5 (linearity).

4.2 Applicability for WFD monitoring reporting

For the purpose of implementing fast and user friendly screening tools, regulators or water managers may decide to accept a decrease in the level of confidence. As a consequence, the reliability of the tool is affected. In that case, the direction of deviation generated by the tool in question becomes critical. These cases may generate, systematically, both fals-positive and fals-negative results. False-positives are results that predict - unjustly - no risk. False-negatives are results that are marked as risk, but are in fact not. Generally, under-estimations result in the generation of false-positives. From a protective viewpoint, this must be regarded as not acceptable.

The tested tools share the same advantage: they are easily accessible and easy to operate. The advantage of a limited number of input parameters, however, is very small or even absent, since most parameters that are needed to run full BLMs are already adapted in the WFD monitoring protocols. Tools that are viable for implementation into WFD reporting procedures need not necessarily be a simplification of the original BLM concept. Reliability also means that the chance for fals-positives is reduced or eliminated.

In order to assess good ecological status, a risk assessment tool should cover a wide range of species from various taxa (i.e., algae, crustacean, insects, fish, etcetera; appendix A). For example, the PNEC is based on single-species toxicity data, and protection to the entire ecosystem is based under the assumed that ecosystem sensitivity depends on the most sensitive species and that protecting ecosystem structure (i.e. species composition) protects community function. For site-specific assessment however, it is near to impossible to know at forehand which species are the most sensitive, and for which type of metal this is a valid assumption. Testing water quality on one trophic level is simply not sufficient to assure protection of the aquatic ecosystem. The ability to create species sensitivity distributions (SSDs) from the most sensitive no-effect concentrations (NOECs) for a variety of species, as is shown in section 3.1, allows for the possibility to analyze the impact on an ecosystem level, which is obviously a major advantage.

Accepting this criterion a priori excludes the tools that are based on a limited amount or limited variety of toxicological data.

Aspirant tools should also be able to address both the chemical and the ecological “good quality” criterion of the WFD. If toxicological indicators are unreliable, or cannot be applied, compared or verified, the insight in chemical available fractions (sometimes indicated as bioaccessibility) becomes a necessity for the interpretation of site specific risks in multi-metal mixtures. Tools that lack chemical modules, or are poor at predicting so, should be excluded as screening candidates.

Simplifications of the original validated biotic ligand models should be carried out with the utmost caution. Validated concepts are generally based on data that pass certain quality assurance criteria both for toxicological measurements, i.e., exposure tests, and water monitoring data. These monitoring data should in any case cover, and be representative for a large range of water types. Empirically or statistically derived transfer functions should represent the majority of WFD water types, including large flowing and stagnant water systems.

4.3 Recommendations

As discussed, none of the tested tools meet the criteria for the defined reliability for both chemical availability and risk indicators. For implementation as a second tier method for WFD reporting, it remains necessary to address the considerations discussed above. Priority activities are:

1 For water management and WFD reporting purposes, the aim should be to develop a full WHAM/BLM model for multi metals, addressing risks for a large number of taxonomic groups, and allowing for ecological structure analysis via species sensitivity distributions.

2 On the short term, it is recommended to derive statistically-sound transfer functions to improve the prediction of local HC5-values. These functions must be based on a large dataset, representative for the majority of WFD water types and a wide range of taxonomic groups. The combination of the water quality database used for this study, and the supplemented toxicological database used in the European risk assessment reports, qualifies for this purpose.

3 Work out a method for added-risk, based on a multi-metal approach.

4 Analyze the possibility for a BLM approach for transition waters (brackish, marine).

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A Species and taxa represented in the Deltares toxicity database

(n) = number of data

Copper	Nickel	Zinc
	Algae	
Chlamydomonas reinhardtii (4) *Chlorella vulgaris (17)* *Pseudokirchneriella subcapitata (12)*	*Ankistodesmus falcatus (2)* *Chlamydomos sp (2)* *Chlorella sp. (2)* *Coelastrum microporum (4)* *Desmodesmus spinosus (4)* *Pediastrum duplex (2)* *Pseudokircheneriella subcapitata (12)* *Pseudokirchneriella sp. (2)* *Scenedesmus accumitus (2)*	*Chlorella sp.(5)* *Pseudokircheneriella subcapitata (30)*
	Crustacea	
Ceriodaphnia dubia (14) *Daphnia magna (9)* *Daphnia pulex (9)* *Gammarus pulex (1)* *Hyalella azteca (6)*	*Alona affinis (2)* *Ceriodaphnia dubia (10)* *Ceriodaphnia pulchella (4)* *Ceriodaphnia quadrangula (8)* *Daphnia longispina (4)* *Daphnia magna (32)* *Hyalella azteca (1)* *Peracantha truncata (4)* *Simocephalus vetulus (8)*	*Ceriodaphnia dubia (8)* *Daphnia longispina (2)* *Daphnia magna (39)* *Hyalella azteca (1)*
	Fish	
Catostomus commersoni (2) *Esox lucius (2)* *Ictalurus punctatus (2)* *Noemacheilus barbatulus (1)* *Oncorhynchus kisutch (5)* *Oncorhynchus mykiss (7)* *Perca fluviatilis (2)* *Pimephales notatus (3)* *Pimephales promelas (12)* *Salvelinus fontinalis (12)*	*Brachydanio rerio (1)* *Oncorhynchus mykiss (5)*	*Cottus bairdi (2)* *Danio rerio (9)* *Jordanella floridae (2)* *Oncorhynchus mykiss (23)* *Phoxinus phoxinus (2)* *Pimephales promelas (1)* *Salvelinus fontinalis (1)* *Salmo trutta (2)*
	Other taxa	
Brachionus calyciflorus(rotifer) (4) *Campeloma decisum (mollusc) (2)* *Chironomus riparius (insect) (1)* *Clistoronia magnifica (insect) (2)* *Dreissenia polymorpha (bivalve) (2)* *Juga plicifera (mollusc) (1)* *Lemna minor L.(plant) (1)* *Paratanytarsus parthenogeneticus (insect) (2)* *Villosa iris (bivalve) (1)*	*Bufo terrestris (toad) (5)* *Gastrophryne carolensis(toad) (5)* *Hydra littoralis (hydrozoa) (1)* *Xenopus laevis(frog) (6)*	*Anuraeopsis fissa (rotifer) (1)* *Brachionus rubens (rotifer) (1)* *Dreissena polymorpha (mollusc) (1)* *Ephoron virgo (insect) (1)* *Potamopyrgus jenkinsi (mollusc) (1)*