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Simplification of Biotic Ligand Models of Cu, Ni, and Zn by 1-, 2-, and 3-Parameter Transfer Functions

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ABSTRACT

Biotic ligand models for calculation of watertype-specific no effect concentrations are recognized as a major improvement in risk assessment of metals in surface waters. Model complexity and data requirement, however, hamper the regulatory implementation. To facilitate regulatory use, biotic ligand models (BLM) for the calculation of Ni, Cu, and Zn HC5 values were simplified to linear equations with an acceptable level of accuracy, requiring a maximum of 3 measured water chemistry parameters. In single-parameter models, dissolved organic carbon (DOC) is the only significant parameter with an accuracy of 72%–75% to predict HC5s computed by the full BLMs. In 2-parameter models, Mg, Ca, or pH are selected by stepwise multiple regression for Ni, Cu, and Zn HC5, respectively, and increase the accuracy to 87%–94%. The accuracy is further increased by addition of a third parameter to 88%–97%. Three-parameter models have DOC and pH in common, the third parameter is Mg, Ca, or Na for HC5 of Ni, Cu, and Zn, respectively. Mechanisms of chemical speciation and competitive binding to the biotic ligand explain the selection of these parameters. User-defined requirements, such as desired level of reliability and the availability of measured data, determine the selection of functions to predict HC5. Integr Environ Assess Manag 2012;8:738–748.
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INTRODUCTION

Biotic ligand models (BLM) are risk assessment tools that compute no-effect concentrations for metals accounting for metal speciation and toxicity based on semimechanistic processes. The biotic ligand is a biological receptor that is used widely as the target site at which metals bind for uptake by the organism. It is assumed that binding to the biotic ligand has a proportional relationship with initial metal toxicological effects. Competitive interactions between metals and macroions (Ca, Na, Mg) for biotic ligand-binding and complexation of metals to dissolved organic carbon (DOC), OH, SO_4, and HCO_3 directly relate water composition to metal uptake. Biotic ligand models for Ni, Cu, and Zn are well studied. Over 500 articles have been published in scientific literature since 2000, and their value for regulatory frameworks is increasingly recognized (SCHER 2007, 2009a, 2009b; USEPA 2007).

Nevertheless, there are some major obstructions for a widespread, practical use and implementation of BLM. First, there is the conceptual complexity of the approach, requiring advanced chemical speciation calculations and normalization procedures with toxicity data. Second, BLM may require up to 10 measured input parameters, some of which are not readily available from standard monitoring programs. One way to overcome these obstructions is simplification of input parameters required for BLM modeling by estimation of these water chemistry input parameters from measured Ca concentrations (Peters et al. 2011). This introduces an uncertainty over several BLM input parameters that could be magnified if these parameters are combined in BLM calculations. Despite this uncertainty, Peters et al. (2011) found good agreement with measured data. Another way of model simplification is a metamodel. A metamodel is a look-up table, filled with results of full-model computations of many combinations of input parameters. This method was elaborated for HC5s of Ni, Cu, and Zn, and presented as a tool for regulatory use (Van Sprang et al. 2011).

Instead of input parameter estimation or meta models, a simplification can also be obtained by replacement of the BLM with 1 linear equation that contains only the most sensitive parameters. In this study we intend to derive reliable and empirical linear equations (also called transfer functions) with a maximum of 3 of the most sensitive input parameters to predict hazard concentrations to 5% of species in an aquatic ecosystem (HC5) for Cu, Ni, and Zn. A number of 3 parameters is set as a maximum for practical and financial reasons, to limit the chance of colinearity between input parameters and to facilitate interpretation of the model. These functions are based on a large toxicity data set and a broad range of water chemistry data covering almost all water types mentioned in the Water Framework Directive. Moreover, we will indicate which parameters are essential for refined risk assessment and should be included in monitoring programs, together with parameters that can be excluded without losing significant reliability. Outcomes of these transfer functions are compared to results from validated full BLMs. As validity is proven, it may replace complicated BLM procedures and increase the applicability for ''routine'' water-type specific risk assessment.

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METHODS

Water chemistry data

Monitoring data were collected from waterboard authorities, involving 209 052 water chemistry data over 2836 sites in the Netherlands in 2007. Annual average concentrations were computed for BLM parameters pH, DOC, Ca, Na, Mg, Cl, SO_4, and HCO_3. Sites that lacked 1 or more parameters as well as brackish surface waters ($Cl^- > 300$ mg/L) were removed from the data set, leaving 243 complete data sets. K (potassium) is required to maintain the charge balance in speciation calculations but does not interfere with chemical equilibria of potential ligands such as OH, SO_4 and HCO_3. Neither is K a competitive binder in BLM. As a result, the impact of the uncertainty in K^+ on calculated HC5s is negligible. K is selected from monitoring data when available. In case of missing K concentrations ($n = 32$), a default of 13.9 mg K/L was used, which is the mean of other K-records in the database. The annual mean temperature is set to 12° C in all water types.

The broadest applicability domain, described for Cu, Ni, and Zn-BLM, was used for sample selection. The applicability of BLM calculations is restricted to: pH = 5.5–8.8, hardness (i.e., [Ca + Mg]) = 10–500 mg $CaCO_3$/L, and alkalinity ([HCO_3]) = 0.01–453 mg $CaCO_3$/L. Some of the BLMs have narrower boundaries of applicability caused by a narrower range of experimental conditions. Two samples fell outside these applicability domains and were removed from the data set and excluded from analyses. Transfer functions were derived with annual average concentrations of 241 complete measured BLM parameter sets within the applicability domain. The database was subjected to a quality check, including an analysis of distributions (normality of data). The range of water chemistry data is provided in Table 1. To judge the representativity of our data for other regions, values for major BLM parameters pH, DOC, Ca, Mg, and Na in other European countries were also collected (ECI 2008). The selected sites cover a large number of water types mentioned under the Water Framework Directive (EU 2000): I, large rivers 14%; II, canals and lakes 6%; III, streams and brooks 48%; IV, ditches 25%; V, sandy springs 7%; VI, small acid ponds 0%.

Toxicity data

A toxicity database was composed containing chronic toxicity test results (no observed effect concentration [NOEC] and 10% effect concentration [EC10] values, further denoted as NOEC) of Cu, Ni, and Zn, including the corresponding water chemistry data of the test media. Only toxicity data adopted in EU risk assessment reports were included in the database (Denmark 2008; ECI 2008; the Netherlands 2008). The database contained 136 chronic Cu toxicity test results of 27 aquatic species, 132 chronic Zn toxic test results of 19 species, and 126 chronic Ni test results over 22 species. The species in the databases were tested for various toxic endpoints, for example, reproduction, mortality, and growth. The most sensitive endpoint was used for the SSD and replicate toxicity data for the same endpoint are averaged by calculation of the geometric mean, according European guidelines (EC 2011). Fish, invertebrates, algae, insects, mollusks, amphibians, rotifers, bivalves, and plants were present in the database. An overview of species and taxa in the toxicity database is given by Verschoor et al. (2011).

Full biotic ligand modeling

The full BLM procedure requires 6 distinctive, sequential steps:

- Calculation of free ion activities of test media, using WHAM (or equal software);

Table 1. Major BLM parameters used for derivation of transfer functions (data of the Netherlands) compared with water chemistry data in other European countries OKE (ECI 2008)

	pH	DOC	Ca	Mg	Na
NL (this study)	5.7–8.7	1.5–33	10.7–175	1.94–42.7	7.15–153
Belgium	5.8–8.5	1.1–19.7	8.6–165	2.0–26.8	12–199
Germany	7.0–8.5	1.6–7.5	39–112	7.6–19.8	14–111
UK	5.9–8.5	0.5–18	0.5–182	0.1–63	0.1–546
Sweden	5.5–8.4	0.45–20.2	0.3–89.7	0.1–12.8	0.3–59.9
Spain	7.6–8.7	2.7–11.5	45–234	4.9–49.5	10.5–36.7
France	7.6–8.5	0.6–5.34	10.1–542	3.6–15.4	4.3–118
Austria	6.9–8.5	0.5–9.3	4.7–223	0.95–45.1	n.a.
Denmark	6.1–7.8	6.7–17.1	8.7–74.3	2.1–4.0	9.0–16.8
Finland	6.1–7.7	4.3–19.6	2.7–14.2	0.8–4.2	0.9–7.4
Ireland	5.6–8.0	6.1–16.9	2.8–157	0.9–10.8	3.7–134
Portugal	5.7–8.4	1.6–16.2	1.8–116	1.0–104	4.0–283

NL = The Netherlands; BLM = biotic ligand models; DOC = dissolved organic carbon; n.a., not available. Concentration of Ca, Na and Mg in mg/L, DOC in mg C/L.

- Calculation of intrinsic sensitivity of specific species = normalization of NOEC (BLM);
- Calculation of free ion activities of water sample, using WHAM (or equal software);
- Extrapolation of normalized NOEC to water type specific NOEC (BLM);
- Derivation of HC5 from a species sensitivity distribution curve (SSD), composed of NOEC from the most sensitive toxic endpoints;
- Transformation of HC5 to dissolved metal concentration, using WHAM (or equal software).

A modeling framework was developed to compute water-type specific chronic NOECs and HC5s for Cu, Ni, and Zn BLM in statistical software package R (version 2.12.0). Details were given by Verschoor et al. (2011). Chemical speciation and the effect of competition of cations on biotic binding sites was used to normalize and extrapolate the measured NOEC in toxicity tests. BLM for algae, crustacean, and fish were implemented. The biotic ligand binding constants are summarized in Table 2. HC5 values were derived from modeled cumulative frequency distributions of log(NOEC) at a probability of 0.05. Chemical speciation in surface water was computed WHAM VI (Tipping 1998) for all metals simultaneously, treating DOC as an active fulvic acid fraction of 50%. Databases for chemical equilibria were updated according NIST database (NIST 2004). Binding constants ($\log K_{MA}$) for Cu to fulvic acids was 2.1 (WHAM-default), 1.8 for Zn (Cheng et al. 2005), and 1.75 for Ni (Deleebeeck et al. 2007).

The distribution of taxa in the SSD was shown in a previous study (Verschoor et al. 2011). It appeared that algae are the most sensitive species for Zn, mollusks are the most sensitive species for Ni and Cu. Because BLMs for mollusks are not available, their NOECs are normalized using the crustacean-BLM according to the principles of read-across (ECI 2008). It is obvious that the most sensitive taxon accompanied by its specific BLM will dominate NOECs in the lower regions of the SSD. Regressions of HC5s with chemical monitoring parameters will be explained in the light of the dominant BLM.

Model simplification

The full BLM is a set of nonlinear equations involving chemical speciation and biotic ligand binding reactions, using 8 key parameters: pH, DOC, Ca, Na, Mg, Cl, SO_4, and HCO_3. The first step in model simplification is to transform the set of nonlinear equations into 1 linear equation. Multiple regression analyses were carried out to select the most relevant monitoring parameters to include in transfer functions for HC5. The general formula of multiple regression models (also called transfer function) is:

$$\mathrm{HC5} = a + (b \times \mathrm{par}_1) + (c \times \mathrm{par}_2) + (c \times \mathrm{par}_3) + \ldots + (I \times \mathrm{par}_8). \tag{1}$$

Parameters are subsequently removed (=backwise analyses) or included (=stepwise analyses) from the model equation, in order of significance (t value) to the independent variable HC5, using the step function in R (R Foundation for Statistical Computing 2011). Whether the model is significantly improved by addition of a parameter is determined by the Akaike information criterion (AIC) (Akaike 1981).

$$\mathrm{AIC} = P \times k - 2 \times \ln(L), \tag{2}$$

Table 2. Overview biotic ligand binding constants used for normalization of NOECs[a]

	Algae			Crustacea			Fish		
BLM	**Cu[b]**	**Ni[c]**	**Zn[d]**	**Cu[e]**	**Ni[f]**	**Zn[g]**	**Cu[h]**	**Ni[i]**	**Zn[j]**
logK BL-Me				8.02		5.3	8.02		5.5
logK BL-MeOH				8.02			7.32		
logK BL-$MeCO_3$				7.44			7.01		
logK BL-H				6.67		5.8	5.4		6.3
logK BL-Ca					3.53	3.2	3.47	3.6	3.6
logK BL-Mg		3.3			3.57	2.7	3.58	3.6	3.1
logK BL-Na				2.91		1.9	3.19		2.4
Slope	−1.140	0.143	−0.754		0.1987			0.324	
Intercept	−0.812		−1.294						

BLM = biotic ligand models; Me = metal; NOEC = no observed effect concentration.

[a] In some cases the NOECs are normalized with a nonlinear function containing pH as only variable and described by a slope and intercept.

[b] De Schamphelaere et al. 2003.

[c] Deleebeeck et al. 2009.

[d] De Schamphelaere et al. 2005.

[e] De Schamphelaere and Janssen 2004a.

[f] Deleebeeck et al. 2008.

[g] Heijerick et al. 2005.

[h] De Schamphelaere and Janssen 2002.

[i] Deleebeeck et al. 2007.

[j] De Schamphelaere and Janssen 2004b.

where P is a penalty factor for the number of parameters in the model, k is the number of parameters in the statistical model, and L is the maximized value of the likelihood function for the estimated model. AIC accounts for goodness of fit, but discourages overfitting, by penalizing the number of parameters included in the model. Default P is 2, however by increasing p, parameter selection becomes more stringent. If the stepwise procedure results in more than 3 significant parameters, P was elevated to higher values to limit the number of model parameters to 3, 2, or 1. The model with the lowest AIC is the preferred one, from a statistical point of view.

Comparison of models

The AIC, residual standard error, and adjusted r^2 give an indication of reduction of model performance when parameters are removed. The overlap and correlation between statistically selected model parameters is taken as a basis for further pragmatic model development. The method aims at finding models that are able to calculate watertype-specific HC5 with the same set of parameters, to optimize the use of monitoring databases and minimize future monitoring efforts. Moreover, the performance of DOC + pH + Ca models was tested, as they are intended to be included in the user-friendly tool Bio-met (Van Sprang 2011). The performance of the most abundant parameters in monitoring databases (pH, Cl, and SO_4) is also tested.

The performance of the simplified models to predict HC5 values was compared with full BLM calculations, using the adjusted r^2 and residual standard error (RSE) and AIC. All statistics were computed by R2.12.0. Using the statistical uncertainty of derivation of site-specific risk limits, the probability of annual average concentrations exceeding the risk limit was computed. Using the residual standard error, 95% prediction intervals are computed and samples are assigned to 3 different risk classes:

- ''No risk'': probability that the measured concentration exceeds the site-specific HC5 <0.025 (the lower boundary of the 95% prediction interval);
- ''Potential risk'': probability that the measured concentration exceeds the site-specific HC5 is between 0.025 and 0.975 (=95% prediction interval); and
- ''At risk'': probability that the measured concentration exceeds the site-specific HC5 > 0.975 (the upper boundary of the 95% prediction interval)

The risk class boundaries in this study are set according to common statistical practice, in which 5% false predictions is considered acceptable. However, the desired level of protection and consequently the risk class boundaries are ultimately policy decisions, based on environmental as well as socio-economical considerations.

RESULTS

The distribution of 241 water chemistry data and calculated HC5 values is given in Figure 1. It shows that Cu and Zn HC5 values are more sensitive to changes in water chemistry than Ni, which is relatively insensitive, indicative for a narrow frequency band. The median values and 90th percentile interval for HC5s are 14 (8.2–31) μg Ni/L, 31 (6.5–80) μg Cu/L, and 22 (12–62) μg Zn/L.

Water characteristics may be positively or negatively correlated (Table 3), and significant correlations occur for a fair amount of BLM parameters. The BLM input parameters, Na and Mg are both strongly correlated with Cl, whereas Ca is correlated with HCO_3. This implies that parameters can be either substituted or neglected in the general equations, as described in the method section. DOC is the only parameter that correlates strongly with HC5s of Ni, Cu, and Zn.

Multiple regression analyses were applied to further identify the relation between multiple independent parameters (i.e., water characteristics). In Table 4, transfer functions resulting from the stepwise statistical selection procedure are listed. Models with 5–7 parameters are a good reflection of full BLM. With explained variations of 97.0% (Ni-HC5), 89.5% (Cu-HC5), and 96.3% (Zn-HC5) the transformation of nonlinear full BLM equations to 1 linear transfer function is very good. Further simplification is justified because the influence of some parameters could be replaced or compensated by others. Moreover, for pragmatic reasons (limitation of monitoring efforts and optimal use of existing monitoring databases) we aim for a maximum of 3 parameters in the transfer function.

Three-parameter models

Limiting the transfer function to 3 parameters leads to a slight reduction of 0.4%–1.3% of the explained variation (see Table 4). Adjusted r^2 are 96.6% (Ni-HC5), 88.2% (Cu-HC5), and 95.4% (Zn-HC5). BLM parameters that are highly correlated no longer coexist in the transfer function. DOC and pH are significant descriptive parameters in 3-parameter transfer functions for all metals, the third parameter differs. Mg is a significant descriptive parameter for Ni-HC5, Ca is significant for Cu, and Na is significant for Zn. The predictive capacity of the optimal 3-parameter models is presented in Figure 2, showing excellent agreement between HC5s computed with full BLM and HC5s computed with the transfer functions (r^2 0.882–0.966).

Striking is the minus-sign for the Ca effect on Cu-HC5, implying that Ca concentrations lead to lower Cu HC5. This Ca effect on Cu-HC5 is rather unexpected, because Ca is generally considered as a risk mitigating agent. In its role as competitive binder on the biotic ligand, increasing Ca-concentrations would result in more protection of organisms against metal exposure, and as a consequence in higher HC5 values. To explain the effects of competitive cations it is important to know which taxonomic groups are the most sensitive, i.e., which BLM determine the HC5.

In Dutch waters, Cu-HC5 is mostly dictated by the crustacean BLM. The Cu-BLM for crustacean describes a competition between Cu-species and Na and H, but no competition with Ca (see Table 2). A direct effect of Ca on Cu-binding in crustacean is therefore not to be expected. The reason for the rather unexpected Ca effect must be a shift on chemical equilibria between Cu, Ca, and the ligands (L) HCO_3 and DOC. $Cu + L \leftrightarrow CuL$ and $Ca + L \leftrightarrow CaL$. With the presence of more Ca, equilibria shift toward CaL, at the expense of CuL. As a result, Cu-species shift to Cu, which has a higher affinity for biotic ligands than CuL. Ca therefore increases the bioavailability of Cu, resulting in a lower HC5.

Na was selected as the most significant third parameter for Zn-HC5. This is in contrast to the mechanistic BLM model

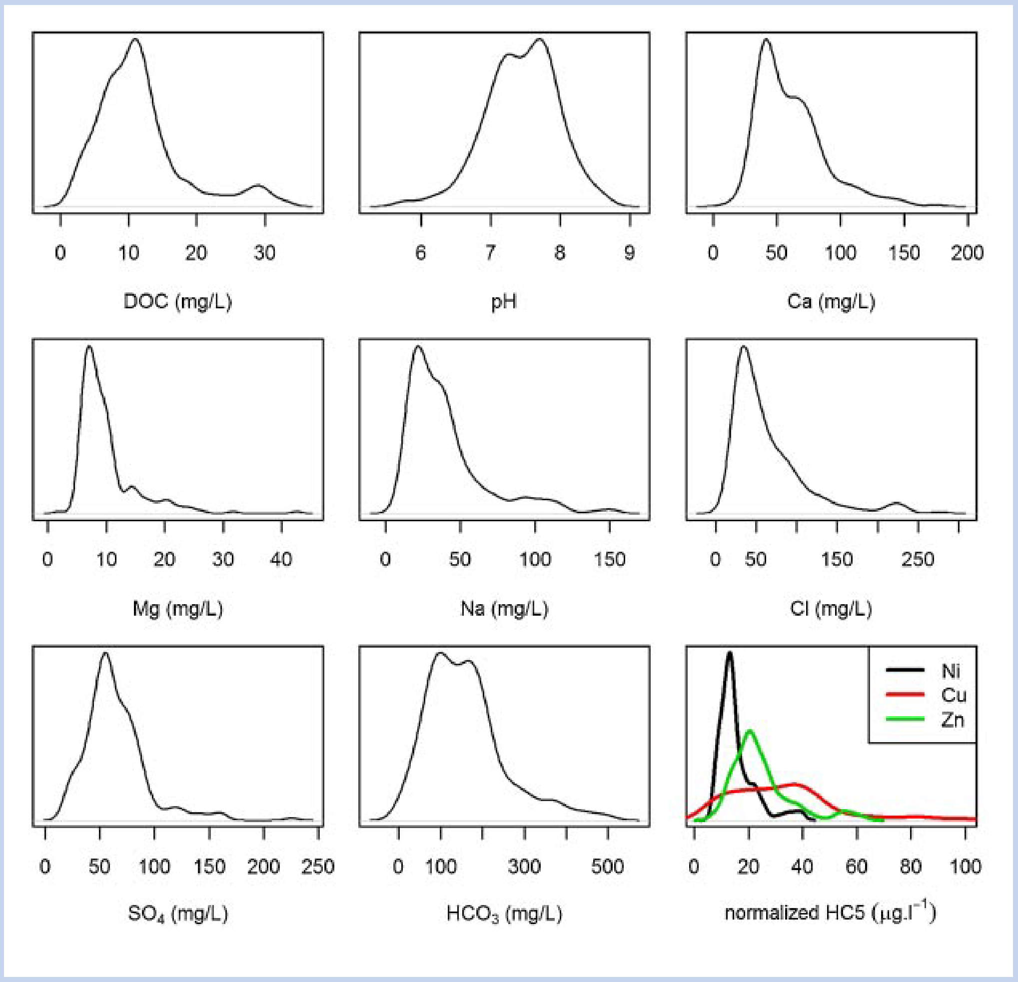

Figure 1. Frequency distributions of major monitoring parameters and normalized HC5 values of Cu, Ni, and Zn, computed with full BLM on 241 sites in The Netherlands.

Table 3. Range of monitoring parameters and Pearson's correlation with HC5 of Ni, Cu, and Zn

		Correlation										
	Range	DOC	pH	Ca	Mg	Na	Cl	SO$_4$	HCO$_3$	Ni.HC5	Cu.HC5	Zn.HC5
DOC	1.55–33.0 mg C/L		−0.10	0.04	0.19	0.12	0.11	−0.09	0.08	0.85	0.86	0.86
pH	5.7–8.7	−0.10		0.59	0.45	0.45	0.50	0.03	0.60	0.29	−0.40	0.35
Ca	10.7–175 mg/L	0.04	0.59		0.67	0.47	0.57	0.26	0.92	0.40	−0.34	0.39
Mg	1.94–42.7 mg/L	0.19	0.45	0.67		0.71	0.82	0.52	0.61	0.61	−0.12	0.48
Na	7.15–153 mg/L	0.12	0.45	0.47	0.71		0.92	0.41	0.55	0.43	−0.04	0.42
Cl	8.4–277 mg/L	0.11	0.50	0.57	0.82	0.92		0.37	0.60	0.47	−0.10	0.42
SO$_4$	17.6–226 mg/L	−0.09	0.03	0.26	0.52	0.41	0.37		0.09	0.14	−0.15	0.03
HCO$_3$	6.9–499 mg/L	0.08	0.60	0.92	0.61	0.55	0.60	0.09		0.42	−0.28	0.43
Ni.HC5	6.7–33.0 μg/L	0.85	0.29	0.40	0.61	0.43	0.47	0.14	0.42		0.51	0.97
Cu.HC5	5.1–116 μg/L	0.86	−0.40	−0.34	−0.12	−0.04	−0.10	−0.15	−0.28	0.51		0.56
Zn.HC5	8.0–81.7 μg/L	0.86	0.35	0.39	0.48	0.42	0.42	0.03	0.43	0.97	0.56	

Table 4. Transfer functions with decreasing number of monitoring parameters selected by stepwise statistical procedure[a]

HC5 (μg/L)	*n*	Transfer function	P	RSE	AIC	Adj. r^2
Ni	7	$\sim DOC + pH + Ca + Mg + Na + SO_4 + HCO_3$	2	1.2	77	0.970
	3	$-21.0 + 0.86 \times DOC + 2.98 \times pH + 0.43 \times Mg$	14	1.2	105	0.966
	2	$0.25 + 0.81 \times DOC + 0.58 \times Mg$	180	1.8	293	0.926
	1	$5.06 + 0.90 \times DOC$	n.d.	3.4	592	0.743
Cu	6	$\sim DOC + pH + Ca + Mg + Cl + HCO_3$	2	6.8	929	0.895
	3	$62.6 + 2.74 \cdot DOC - 6.38 \cdot pH - 0.23 \cdot Ca$	22	7.2	953	0.882
	2	$18.8 + 2.80 \cdot DOC - 0.30 \cdot Ca$	31	7.6	982	0.867
	1	$1.05 + 2.75 \cdot DOC$	n.d.	11.0	1159	0.721
Zn	5	$\sim DOC + pH + Na + SO_4 + HCO_3$	2	2.1	368	0.963
	3	$-53.6 + 1.51 \cdot DOC - 7.79 \cdot pH + 0.06 \cdot Na$	48	2.4	419	0.954
	2	$-62.7 + 1.55 \cdot DOC + 9.28 \cdot pH$	64	2.8	493	0.937
	1	$7.30 + 1.48 \cdot DOC$	n.d.	5.5	825	0.750

AIC = Aikaike information criterion; Adj. r^2 = explained variance; DOC = dissolved organic carbon; N= number of monitoring parameters; n.d.= not decisive; P = penalty factor for model selection; RSE = residual standard error.
The default P = 2, was gradually increased to eliminate monitoring parameters. Single parameter model of Ni and Zn was selected based on highest *t* value in the 2-parameter model, because the stepwise procedure was not decisive.
[a]Concentrations of Ca, Mg and Na in mg/L, DOC in mg C/L.

that indicates that Na is not the main competitor for binding to the biotic ligand. Table 2 shows that Ca and Mg are also defined as competitors for biotic ligand binding, with higher affinities for the biotic ligand (logK respectively 3.2, 2.7 versus 1.9 for Na). The selection of Ca or Mg as the most significant third parameter would have been more obvious. In any case, the contribution of the third parameter to accuracy of Zn-HC5 prediction is very small, as the adjusted r^2 is reduced by only 0.017 when the third parameter is eliminated from the regression. This leads to the conclusion that DOC and pH are the dominant parameters for Zn-HC5, which is obvious as algae are the most dominant species in the lower regions of the Zn-SSD.

As 3-parameter models have DOC and pH in common, and only differ in the third parameter (Mg, Ca, or Na), we investigated the possibility of harmonization of the third parameter; i.e., we computed the loss of accuracy if either Mg, Ca, or Na is used as the third parameter in the transfer function. The fitted transfer functions are shown in Table 5.

The statistics of the fits indicate that a transfer function with Ca or Na reduces the accuracy of the Ni-HC5 transfer function with approximately 5%, compared with the DOC + pH + Mg model obtained by stepwise parameter selection. For Cu-HC5, Na was eliminated from the transfer function, as it was not a significant parameter. Replacement of Ca by Mg in Cu-HC5 model resulted in a reduction of 3% in explained variance. For Zn-HC5, models with Na are statistically the best, but Mg or Ca seem to be almost equally good.

Regressions of HC5 with the most abundant parameters in monitoring databases (pH, Cl and SO_4) resulted in unreliable transfer functions, indicated by explained variances of 20%–21% and AIC of 866, 1412, and 1107 for HC5 of Ni, Cu, and Zn, respectively.

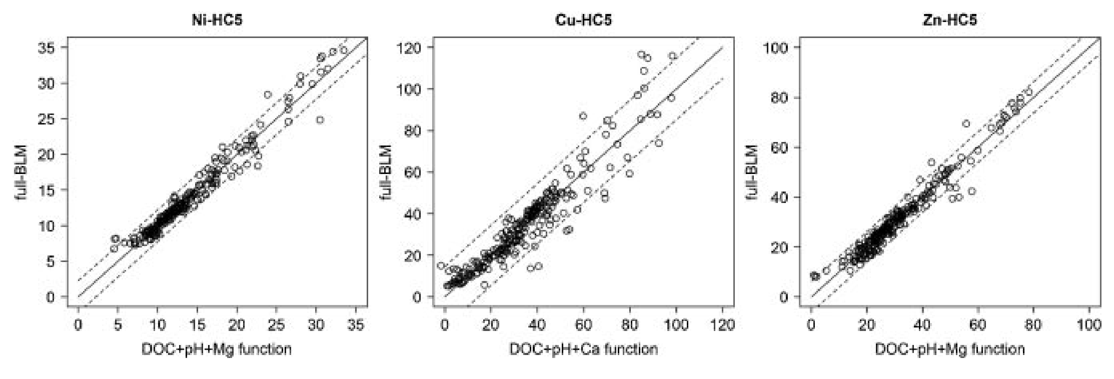

Figure 2. Agreement between HC5 computed with full-BLM and HC5 computed with simplified 3-parameter transfer functions.

Table 5. Transfer functions with a pragmatic selection of 3 monitoring parameters[a]

HC5 (µg/L)	Transfer function	RSE	AIC	Adj. r^2	Best fit
	DOC + pH + Ca models				
Ni	$-23.2 + 0.91 \cdot DOC + 3.33 \cdot pH + 0.05 \cdot Ca$	1.9	321	0.917	
Cu	$62.6 + 2.74 \cdot DOC - 6.38 \cdot pH - 0.23 \cdot Ca$	7.2	953	0.882	[b]
Zn	$-52.2 + 1.53 \cdot DOC + 7.42 \cdot pH + 0.06 \cdot Ca$	2.4	435	0.951	
	DOC + pH + Na models				
Ni	$-25.7 + 0.90 \cdot DOC + 3.87 \cdot pH + 0.05 \cdot Na$	2.0	332	0.913	
Cu	$102 + 2.64 \cdot DOC - 13.4 \cdot pH$	8.7	1043	0.829	
Zn	$-53.6 + 1.51 \cdot DOC + 7.79 \cdot pH + 0.06 \cdot Na$	2.4	419	0.954	[b]
	DOC + pH + Mg models				
Ni	$-21.0 + 0.86 \cdot DOC + 2.98 \cdot pH + 0.43 \cdot Mg$	1.2	105	0.966	[b]
Cu	$81.8 + 2.78 \cdot DOC - 9.89 \cdot pH - 0.75 \cdot Mg$	8.0	1008	0.852	
Zn	$-53.9 + 1.49 \cdot DOC + 7.76 \cdot pH + 0.33 \cdot Mg$	2.4	421	0.953	

AIC = Aikaike information criterion; Adj. r^2 = explained variance; DOC = dissolved organic carbon; N = number of monitoring parameters, P = penalty factor for model selection, RSE = residual standard error.

[a] Concentrations of Ca, Mg and Na in mg/L, DOC in mg C/L.

[b] Best fit by stepwise parameter selection (taken from Table 1).

Two-parameter models

Further limitation to 2 BLM parameters resulted in selection of DOC + Mg for Ni-HC5, DOC + Ca for Cu-HC5, and DOC + pH for Zn-HC5 (see Table 5). Elimination of the third parameter reduced the explained variance with another 1.4%–4.0%. At the same time the residual standard error and AIC increased. To find out whether 1 DOC + pH model for Ni, Cu, and Zn would suffice, additional multiple regressions were done for Ni-HC5. The DOC + pH model for Cu-HC5 was already shown in Table 5. The transfer function for Ni is Ni-HC5 = −32.4 + 0.94 × DOC + 4.97 × pH, RSE2 = 0.2, AIC3 = 92, and adjusted $r^2 = 0.888$.

Striking is the opposite direction of the pH effect on normalized HC5 of Cu and Zn as shown by plus and minus-signs of regression constants in the transfer functions. Cu-HC5 is reduced by higher pH whereas Zn-HC5 is reduced by lower pH. Theoretically, the pH can affect the normalized HC5 in 2 different ways: 1) HC5 is reduced with increasing pH, because at higher pH the competitive binding of H^+ is reduced and the exposure of the biotic ligand to metal-cations is higher, or 2) HC5 is reduced with decreasing pH because at lower pH the chemical equilibria shifts toward more free metal-cations, so the metal exposure is higher, resulting in lower HC5.

It seems that pH-dependent chemical equilibria dominate Zn-HC5, whereas the competitive effect of H^+-ions at the biotic ligand dominate Cu-HC5. The pH effect on chemical Cu-species is not very relevant, because the toxic effect of Cu^{2+} (dominant at $pH < 7$) and $CuOH^+$ (dominant at $pH > 7$) are similar, indicated by the same affinity for the biotic ligand (logK = 8.02).

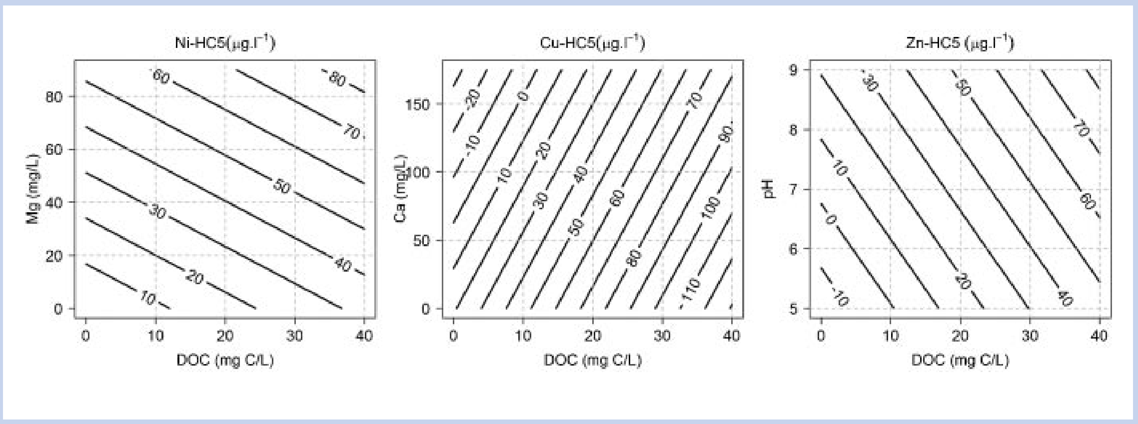

Figure 3. Effect of the 2 most significant BLM input parameters on HC5. Ni model (adj $r^2 = 0.923$), Cu model (adj $r^2 = 0.829$), and Zn model (adj $r^2 = 0.867$). Lines are iso-HC5 lines computed from the transfer functions; combinations of BLM parameters with similar HC5, the level of HC5 is indicated by values.

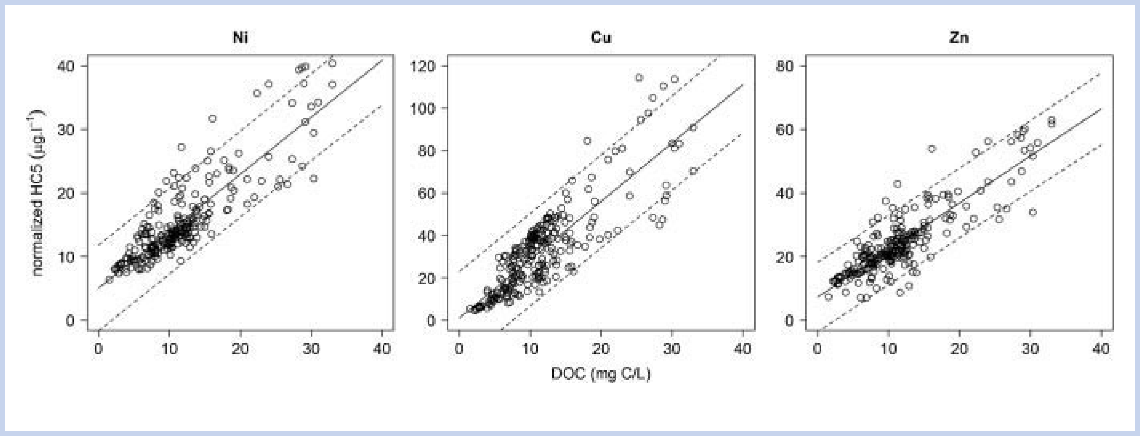

Figure 4. Relation between DOC and HC5 of Ni, Cu, and Zn. Lines fitted by single regression. HC5 $= a + b \times$ BLM parameter. Straight line, most likely relation; dashed line, 95% prediction interval.

The influence of DOC and the most significant second parameter are shown in Figure 3. For each combination of DOC and a second parameter, the HC5 can be read from the graph. The iso-HC5 lines indicate combinations of DOC and Mg (for Ni), pH for (for Cu and Zn), that give the same HC5. Iso-HC5 lines are drawn at intervals of 10 µg/L, values between the iso-lines can be estimated by linear interpolation. The graph however is only a visualization tool. Exact values can be calculated easily with the transfer functions.

One parameter models

The major parameter explaining HC5 is definitely DOC, indicated by the high correlation coefficients in Table 1. For Cu, DOC is selected as the only significant parameter by the stepwise procedure when the penalty factor for parameter selection is set to very restrictive values (see Table 4). For Ni and Zn the stepwise procedure could not decide between the 2 last remaining parameters. In that case, the parameter with the highest significance (highest *t* value) was chosen for the single parameter model. Transfer functions with only DOC resulted in a considerable reduction of explained variance: 74%, 72%, and 75% for HC5 of Ni, Cu, and Zn, respectively. Relations of DOC with HC5 are visualized in Figure 4.

Model uncertainty

In the regulatory use of models, uncertainties are generally not explicitly mentioned. Currently in Europe a discussion is on going on how to set predicted no effect concentrations (PNECs) for metals. In the REACH and the latest WFD guidance, PNECs are set by dividing the HC5 by appropriate assessment factors (AF) based on several considerations like the uncertainty of the HC5. Assessment factors for SSD-derived HC5s are set between 1 and 5 by expert judgment, depending on diversity and representativeness of the selected test species and taxonomic groups (i.e., with respect to sensitive life stages, feeding strategies, and trophic levels), the comparison of laboratory and field data, and the goodness of fit of the SSD (EC 2011).

The Water Framework Directive however, recommends to provide the probability that risk limits are exceeded (EU 2000). Our article indicates that this may be done based on the uncertainty around the HC5. The probabilities are used to classify sites in 3 risk categories: no risk, potential risk, and at risk, according to the boundaries of the 95% prediction interval. Table 6 shows that the majority of sites are in the no-risk category. A fair number of sites are potentially at risk. These sites need additional research or monitoring to assess the risk. The number of sites at risk is lower following the probabilistic approach, than the nonprobabilistic approach. With respect to predicting number of sites at risk, the nonprobabilistic approach of the full BLM is comparable with the transfer functions, except for Cu. For Cu, the transfer functions predict a higher number of sites at risk than the full-BLM. This reflects the broader prediction interval of the transfer function of Cu (see Figure 2 and Figure 4) in combination with the relatively high number Cu-concentration in close proximity of the predicted HC5. Incorporating the uncertainty in HC5 prediction is therefore highly relevant and enables one to distinguish between sites that need risk management or mitigation measures and sites that need additional monitoring.

Transfer functions with less parameters or lower accuracy lead to more sites with ''potential risk.'' Measuring additional BLM parameters is rewarded by narrower prediction limits, and more sites with a ''no risk'' or ''at risk'' classification. The probabilistic approach supports a tiered approach of risk assessment. The first tier of screening water quality on sites is based on total dissolved metal concentrations. Simplified BLMs could be used as a second tier, as it accounts for bioavailability using fewers input parameters. Samples with concentrations within the 95% prediction interval of the simplified BLM could be subjected to an additional higher tier assessment using full BLM, which reduces the uncertainty of the estimated site-specific HC5. This implies including additional monitoring parameters and advanced modeling before decisions on costly risk mitigating measures should be taken. In this way the tiered approach facilitates the optimization of monitoring programs and risk assessment complexity.

CONCLUSIONS

Linear models were derived from full BLM and provide simple and reliable descriptions of HC5 based on a maximum of 3 relevant monitoring parameters. With these simple

Table 6. Number of sites in different risk categories[a]

	Probabilistic transfer function			At risk: nonprobabilistic	
	No risk	**Potential risk**	**At risk**	**Transfer function**	**Full BLM**
Ni					38
DOC + pH + Ca	186	25	30	39	
DOC + pH + Na	189	23	29	38	
DOC + pH + Mg[b]	194	14	33	38	
DOC + Mg[b]	185	28	28	39	
DOC + pH	186	28	27	37	
DOC	151	68	22	35	
Cu					0
DOC + pH + Ca[b]	191	50	0	5	
DOC + pH + Mg	186	55	0	8	
DOC + Ca[b]	193	48	0	7	
DOC + pH	188	53	0	8	
DOC	156	85	0	0	
Zn					96
DOC + pH + Ca	133	26	82	95	
DOC + pH + Na[b]	133	24	84	94	
DOC + pH + Mg	134	23	84	95	
DOC + pH[b]	130	31	80	95	
DOC	105	68	68	96	

BLM = biotic ligand models; DOC = dissolved organic carbon.

Not at risk (metal concentration < lowest value of the prediction interval; at risk (metal concentration > highest value of the prediction interval); and potential risk (metal concentration within the prediction interval).

Optimal 2- and 3-parameter models.

transfer functions, a major obstruction for implementation of biotic ligand models is removed. In general, the accuracy of the transfer functions increases when more parameters are included. However, due to correlation between monitoring parameters, including all 8 BLM parameters seems redundant. Overfitting is prevented by using the stepwise statistical procedure. As is shown in Table 4 and Table 5, different combinations of monitoring parameters perform similar as the full BLM. The selected parameter sets are all statistically significant, considering the high penalty factors applied in the stepwise selection procedure and low p values (<0.001) applied in pragmatic parameter selection. Which transfer functions should be used in practice depends on data availability, monitoring budget, and desired reliability.

Most optimal 3-parameter models were Ni-HC5 = *f*(DOC, pH, Mg), Cu-HC5 = *f*(DOC, pH, Ca,), and Zn-HC5 = *f*(DOC, pH, Na). From a pragmatic point of view aiming at optimization of monitoring effort, it would be efficient if HC5 of Cu, Ni, and Zn could be described with the same set of monitoring parameters. This is accomplished by either choosing Ca, Mg, or Na as a third parameter (see Table 5). Van Sprang et al. (2011) demonstrated the performance of a model based on DOC + pH + Ca for HC5 calculations of Cu, Ni, and Zn. Overall, the performance of DOC + pH + Mg models is slightly better than DOC + pH + Ca, mainly due to a better prediction of Ni-HC5.

Restricting the models to 2 parameters leads to DOC + Mg (for Ni) and DOC + Ca (for Cu) and DOC + pH (for Zn). For pragmatic reasons DOC + pH models were tested for Ni and Cu as well. DOC + pH models are approximately 5% less accurate than optimal 2-parameter transfer functions.

The presence of DOC in the transfer functions is beyond doubt, as it is the major complexing agent for dissolved Cu, Ni, and Zn. The presence of pH in transfer functions is also clear, as it has a great impact on chemical equilibria as well as on competitive binding at biotic ligands. Because Ca, Na, and Mg concentrations are correlated, it is understandable that they are able to predict HC5s even when they are considered mechanistically not relevant (i.e., Ca and Na in the algae Ni-BLM, and Ca and Mg in the crustacean Cu-BLM). This implies that the predictive capacity of 1 competitor includes, covers, or camouflages the predictive effect of the other competitors.

The transfer functions are valid between the boundaries of the tested data set, given in Table 2. The applicability range of the transfer functions covers most but not all samples in European fresh surface waters (Table 1). HC5 values outside the applicability domain are not false by definition but should be considered with care because they are less reliable. However, full BLM models also have limitations. The applicability range of water chemistry conditions is limited by the ability of standard test species to survive and reproduce under extreme conditions. Typically, low pH and high alkalinity and hardness cannot be tolerated by standard species.

From a scientific point of view, transfer functions with the highest reliability and parameters that are linked to the concepts of speciation and biotic ligand modeling are preferred. The parameters DOC, pH, and Mg or Ca appear to be the main descriptors and meet the requirements of monitoring efficiency, conceptual justification, and reliability best. The overall explained variance equals 92%, which is a very slight reduction compared to transfer models with 5–7 parameters. The overall explained variance of DOC + pH + Na models is slightly less ($r^2 = 0.89$). It will depend on data availability and required reliability which model is most suitable.

Predictions go along with uncertainty, indicated by the residual standard error. Dealing with explicit uncertainties in a regulatory context is a challenge. In full-BLM validation studies, typical uncertainty margins for NOEC are $\pm 10\,\mu g/L$, approximating a residual standard error of 5 (De Schamphelaere et al. 2003, 2005; De Schamphelaere and Janssen 2004a; Deleebeeck et al. 2009). Uncertainties of the transfer functions are in the same order and comparable with the uncertainties in full-BLM predictions. The relevance of the uncertainties in HC5 for risk assessment becomes evident when sites are assigned to risk categories. A fair amount of sites are at potential risk, implying that the probability of unjust risk qualification is relatively high. In these cases, we recommend the full BLM, before a final risk characterization is made.

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