# Accounting for bioavailability of aluminum in Dutch surface waters;

## towards environmental quality standards for ecotoxicological effects

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# Abstract

Aluminum (Al) is the most abundant metal on earth, usually tightly captured in the mineral structure of rocks and clay minerals. Caused by soil acidification (by air pollution), mining and anthropogenic uses, aluminum is mobilized and can enter surface waters. Effects of aluminum on aquatic ecosystems show huge differences between water types, as dependent on three factors which jointly determine the bioavailability of Al: pH, dissolved organic carbon concentrations and hardness. In the past five years calculation methods have been developed in the US to calculate critical values for the protection of ecosystems that account for these differences in bioavailability. The goal of this report is to evaluate the compliance of these methodologies with EU-guidance and (if possible) to propose an environmental quality standard for Dutch fresh surface waters.

The US-EPA methodology and data have been well described, but the existing EPA-tools do not comply with the European Technical guidance for deriving environmental quality standards. However, with the existing data it was possible to adjust the US-EPA methodology to comply with EU-guidance. The adjustments were implemented in an Excel tool and an R-script was generated to compute water type-specific $HC_5$ values (Hazard Concentration for 5% of the species) according to European guidelines. An assessment factor of 5, reflecting remaining uncertainties in the toxicity data, is recommended on the $HC_5$.

Environmental quality standards (EQS) are values set by policy makers, ideally based on a sound scientific assessment of potential environmental impacts. In the case of aluminum however, calculated $HC_5$ values for Dutch fresh surface waters differed by six orders of magnitude depending on the water type (range: <0.003-3000 µg/L). The US-EPA observed a similar variation, and has therefore decided not to set a specific EQS, but always demand a site-specific risk assessment by the use of a calculation tool. It is not in the competence of the contractor to decide upon the desired level of protection and the practical implications of certain choices. In order to offer options for Dutch policy makers, different EQS values are presented, obviously with different levels of protection.

For future compliance testing it is important that the analytical method for aluminum in water samples quantifies a bioavailable fraction of aluminum that is representative for the bioavailability of aluminum in the toxicity experiments that were used to derive the EQS. This is currently not the case. Instead of the common strong acidification of water samples a mild acidification to pH 4 is recommended.

# Contents

Abstract ..... 1
1 Introduction ..... 5
2 Sources, behaviour and ecotoxicology of aluminum ..... 7
2.1 Sources of aluminum ..... 7
2.2 Ecotoxicology of aluminum ..... 7
2.3 Aluminum speciation and analysis ..... 9
3 Differences between US-EPA and EU approach ..... 11
3.1 Species sensitivity distribution ..... 11
3.2 Calculation of water type-specific quality criteria ..... 12
4 Multiple linear regression models (MLRs) ..... 13
4.1 Toxicity data for MLR development ..... 13
4.2 Single versus multiple regression and interactions ..... 14
4.3 Derivation of MLRs ..... 15
4.4 Validation and cross-species extrapolation of the MLRs ..... 16
5 Application of MLRs for Dutch EQS derivation ..... 17
5.1 Data requirements and selection ..... 17
5.2 Chronic toxicity data ..... 18
5.3 Acute toxicity data ..... 19
5.4 Creation of tools ..... 20
6 Calculation of water type-specific $HC_5$ ..... 21
6.1 General approach ..... 21
6.2 Selection of water types ..... 22
6.3 Representativity of selected samples ..... 23
6.4 Water chemistry ..... 24
6.5 $HC_5$ of water types in Waterkwaliteitsportaal 2020 ..... 27
6.6 $HC_5$ of a specific set of water chemistry conditions ..... 29
6.7 $HC_5$ of water types previously used for the copper-EQS ..... 31
7 Options for aquatic environmental quality standards of aluminum ..... 32
7.1 Assessment factor ..... 32
7.2 Summary of options ..... 33
7.3 Tentative implications for the risk assessment ..... 35
8 Summary ..... 40
Acknowledgements ..... 42
References ..... 43

Appendix 1 Method to analyze bioavailable aluminum in natural surface water ..... 45
Appendix 2 Chronic toxicity data for MLR development ..... 46
Appendix 3: MLR-models ..... 49
Appendix 4: Chronic toxicity database for calculation of water type- specific aluminum criteria. ..... 51
Appendix 5. Acute toxicity database for calculation of water type-specific Al-criteria ..... 54
Appendix 6 R-script for calculation of Aluminum $HC_5$ ..... 59
Appendix 7 Number of selected sampling sites per WFD water type ..... 61
Appendix 8 Sampling authorities ..... 63
Appendix 9 Normal distributions of water characteristics ..... 64
Appendix 10 Normal distributions calculated HC5 ..... 67
Appendix 11 Statistical data of water types used for copper EQS ..... 69
Appendix 12 Aluminum concentrations in filtered and unfiltered samples. ..... 70

# 1 Introduction

In the context of the project "Inhaalslag bezien watervergunningen", Rijkswaterstaat has commissioned RIVM to draft a proposal for revised environmental quality standards (EQS) for aluminum (Al) in surface water. It should be noted that the responsibility for standard setting lies with the Ministry for Infrastructure and Water Management. The present report serves as scientific background document.

The current Dutch environmental quality standard for aluminum in Dutch surface waters is in place since 2002. It concerns an indicative maximum permissible addition (MPA) of 12 µg/L. This concentration may be corrected for the natural background concentration to yield a maximum permissible concentration (MPC). The Dutch natural background concentration in fresh water has been set at 36 µg/L (van de Plassche, 2002; RIVM, 2022). It should be noted that this background concentration was derived in a different way than more recently used for other metals by Osté (2013).

Various comments were made about the derived MPA. The main points were that the behaviour, bioavailability and ecotoxicity of aluminum are highly dependent on pH and that the MPA was derived from studies that are not representative for the Dutch situation. In more recent years, water quality standards have been derived for aluminum in a number of countries, that could be useful for substantiating a revised Dutch Al-EQS. In Table 1 fresh water quality criteria from several countries are listed. The actual legal aquatic EQS values are often difficult to find, and the status of some values is uncertain. Some values may be outdated, whereas others may still have the status of a proposed value. Several derivation approaches have been employed in different countries. These include approaches that focus on different forms of bioavailable aluminum ($Al^{3+}$, monomeric aluminum or dissolved aluminum) and pH-dependent approaches.

*Table 1 Overview of existing water quality criteria for aluminum in freshwater in different countries.*

| Country | year | Al-form | Standard | µg/L | condition | Ref. |
|---|---|---|---|---|---|---|
| Canada | 1987 | Total Al | AQL | 5[1] | pH<6.5 | Canada 2007 |
| | | | | 100[2] | pH≥6.5 | |
| | 2021 | Total Al | FWQG | variable | Site -specific | Canada 2021 |
| Germany | 2010 | $Al^{3+}$ | AA-EQS | 50[2] | | LAWA, 2010 |
| | | | MAC-EQS | 250[3] | | |
| The Netherlands | 2002 | Dissolved Al | MPA (indicative) | 12 | | RIVM, 2022<br>Van de Plassche, 2002 |
| United Kingdom | 2007 | Monomeric Al | PNEC (chronic) | 0.05[3] | | EA, 2007 |
| | | | PNEC (acute) | 0.25[4] | | |
| | unknown | Total Al | MAC-EQS | 1000[4] | pH≥6 | UK, 2018 |
| | | Dissolved Al | | 100[4] | pH<6, soft | |
| | | Dissolved Al | | 1000[4] | pH<6, hard | |
| USA | 1988 | Total Al | AWQC (acute) | 750[2] | | EPA, 1988 |
| | | | AWQC (chronic) | 87[2] | | |
| | 2018 | Total Al | AWQC (acute) | 1-4800 | Site-specific | EPA, 2018a |
| | | | AWQC (chronic) | 0.8-3200 | | |

AQL=Acceptable Quality Limit; FWQG=Federal Water Quality Guideline; AA-EQS= Annual Average – Environmental Quality Standard; MAC-EQS=Maximum Acceptable Concentration; MPA = Maximum Permissible Addition; PNEC=Predicted No Effect Concentration; AWQC=Ambient Water Quality Criteria

[1] Outdated

[2] Proposal, legal reference not found.

[3] Not implemented due to absence of suitable analytical method.

[4] Indirect reference (mentioned in document as EQS surface water to derive discharge limits for Al), legal source not found.

The most promising approach seems to be available from the United States Environmental Protection Agency. The US-EPA published an ecotoxicologically based guideline for aluminum in 2018 that used several Multiple Linear Regression models (MLR) to describe the bioavailability of aluminum based on pH, hardness and DOC for freshwater species of different trophic levels (EPA, 2018). The US-EPA did not set fixed risk limits but provided a tool for site-specific risk assessment. The US-EPA 's way of deriving EQS values differs from the European one, but the dataset and normalization show potential to be adjusted to comply with European guidance. Canada simplified the US-EPA method, and used one overall equation for all the aquatic species instead of several bioavailability equations for species from different trophic levels.

Commissioned by RIVM, this report assesses the usefulness of the US-EPA methodology for the Dutch situation. Questions were:

1) Is the US-EPA methodology well described and properly executed?
2) Are the MLR equations and the underlying toxicity data useful for derivation of an AA-EQS[1] and MAC-EQS[2] according to European guidelines? If yes, which EQS-value options could be derived?

This study assesses the possibility of inclusion of Al-MLRs in the site-specific risk assessment for metals in the Netherlands. A site-specific risk assessment tool called PNEC-pro is authorized to be used to account for differences in metal bioavailability when assessing the chemical quality of a waterbody (Rijkswaterstaat, 2020). MLRs for Zn, Cu, Ni and Pb already have been included in PNEC-pro.

[1] AA-EQS is an annual average concentration for the risk assessment of annual average concentrations of sites.
[2] MAC-EQS is a maximum acceptable concentration for the risk assessment of individual samples.

# 2 Sources, behaviour and ecotoxicology of aluminum

## 2.1 Sources of aluminum

Aluminum (Al) is a natural metal in the earth's crust, where it is tightly bound by oxygen and silicon. Aluminum is extremely abundant; after oxygen and silicon it is the third most abundant element in the earth's crust and the most abundant metal. Aluminum exists naturally in ores like bauxite and in common clay and sand. The estimated crustal (lithospheric) abundance of aluminum is 82.3 gram/kg (Pogue and Lukiw, 2014). Despite its high abundance in rocks and soils, the aluminum concentrations in surface water are low, generally in the microgram per liter range. Approximately $1.1 \times 10^{12}$ tons of metallic aluminum have been extracted from geological deposits and exported into the biosphere since aluminum production began to grow in the beginning of the 20$^{th}$ century, and about two-thirds of this amount is estimated to be still in production, through recycling, re-use and intrinsic longevity (Pogue and Lukiw, 2014).

According to the Dutch national Pollutant Release and Transfer Register (PRTR), the annual release of aluminum to surface water between 2010 and 2020 was approximately 40-49 tonnes, caused by sewage treatment plants, traffic and industry (Rijksoverheid, 2022). Not included in the data of the PRTR is the natural weathering and leaching from soil, that also contribute to aluminum in surface water. This process of natural mobilization of aluminum is enhanced by soil acidification through air pollution (Lawrence et al., 2007, Li et al.2022). Due to atmospheric deposition of sulfur and nitrogen-compounds (acid rain), aluminum is mobilized in areas where the soil has a low buffer capacity (such as sandy soils). Under natural conditions the solubility of aluminum at low pH is especially relevant. At neutral pH, the solubility is very low.

## 2.2 Ecotoxicology of aluminum

Aluminum has no biologically important functions or beneficial properties to aquatic life, and is therefore considered a non-essential metal (EPA 2018a and reference therein). Elevated levels of aluminum can affect the regulation of ions (like salts) and inhibit respiratory functions, like breathing (EPA, 2018b). Aluminum can accumulate on the surface of a fish's gill, leading to respiratory dysfunction and possibly death. In plants reduced root growth and reduced yields in acidic soils are associated with the presence of free aluminum (Rahman and Upadhyaya, 2021). In algae, aluminum interferes with intracellular phosphorus and glucose metabolism (EA, 2007).

The bioavailability of aluminum to aquatic organisms depends on several water chemistry parameters. For example, pH determines both aluminum solubility and speciation, and the level of competition between $Al^{3+}$ and protons ($H^+$) and other cations, such as calcium ($Ca^{2+}$) for the uptake by aquatic biota. Organic aluminum complexes, such as those formed with humic and fulvic acids, tend to increase total aluminum concentrations in solution, but reduce the bioavailability of aluminum to aquatic organisms.

An ecotoxicological effect value such as $EC_{10}$, (or $EC_{20}$ or $EC_{50}$) of a substance is supposed to be an intrinsic value for a biological species. Particularly for metals, it was shown however, that effects of total (dissolved) metal concentrations in various ecotoxicity tests could not always be explained by differences in metal concentrations. It was observed that species-$EC_{xx}$ values can be highly variable, depending on the type of water that was used in the ecotoxicity test. Parameters like pH, DOC and hardness often appeared to have a substantial effect on the ecotoxicity. This is because these parameters determine chemical speciation reactions. Chemical speciation implies that metals can occur in different ionic forms that are not equally toxic, that metals can form complexes and

precipitates, and that hardness, for example, can mitigate the uptake of metals. Bioavailability has been widely studied, and the concept of bioavailability is translated into equations that reflect processes and reactions that play a role in predicting toxic effect levels. The most sophisticated models are the biotic ligand models (BLMs). An important aspect of the BLM-concept is competitive binding of the toxicant to ligands such as dissolved organic carbon and biological membranes (see Figure 1). Based on these concepts, tools for the prediction of bioavailability and ecotoxicity under various water chemistry variables are available for several metals (Zn, Cu, Ni, Pb, Cd) and authorized for regulatory use.

The concept of bioavailability is that toxicity only occurs when aluminum binds to or crosses biological membranes. There is a constant competition between the metal and other substances in the water for binding to the membrane. When the sites on the membrane are for example already occupied by Ca- or Mg-ions, binding of aluminum is reduced. When there is a high concentration of dissolved organic matter available, the binding of aluminum to the biological membranes is also reduced, and precipitated aluminum is not bioavailable either. The relative importance of these processes depends on the intrinsic binding coefficients, equilibrium partition coefficients and the actual chemical composition and concentrations of substances in the water.

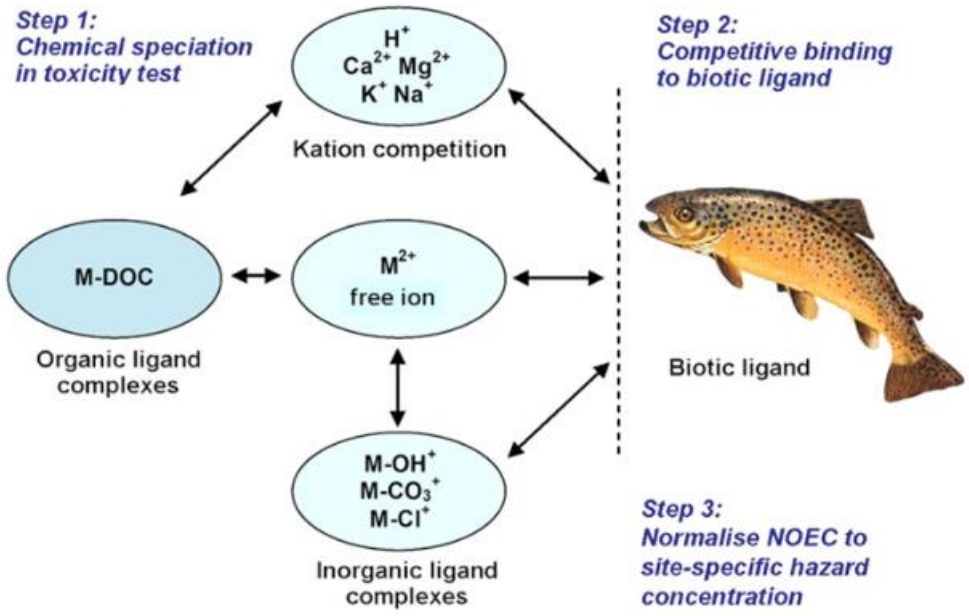


*Figure 1 Schematic overview of processes that determine the toxicity of metals (www.pnec-pro.nl).*

Theoretically, BLMs are species-specific and metal-specific and different BLMs exist for acute and chronic effects. For pragmatic reasons, BLMs for species of three different trophic levels including algae, invertebrates and fish are generally considered sufficient to normalize the toxicity data of all test organisms or a whole ecosystem (EC, 2018). Using read-across, the species-specific BLMs are assigned to species within the same trophic level for which no BLM has been developed. This is justified by the assumption that there is a large similarity between species on the cellular level.

Normalization (Step 3 in Figure 1) means that effect concentrations obtained in an ecotoxicity test with certain water characteristics ($EC_{xx,test}$) are adjusted to reflect the bioavailability in a particular water body of interest; so the $EC_{xx,test}$ is adjusted to a concentration that in the field situation would lead to similar occupation of the biotic ligand (membrane) and thus to similar effects. Normalized values are also called water type-specific values.

BLMs are also sometimes referred to as 'full' BLMs to emphasize that advanced iterative chemical speciation calculations are involved. Besides the complexity of the full BLM calculations, a large number of monitoring parameters (pH, DOC, Na, Ca, Mg, K, Fe, carbonates, sulphates and chloride) are required, which are not always available and it would raise extensive costs for water authorities to collect these. In order to overcome these drawbacks, a simplified procedure is often employed, using Multiple Linear Regression models (MLR). MLRs are straightforward equations, that enable calculation of the normalized $EC_{xx}$ with a common spreadsheet program. MLRs provide satisfactory results amongst others for Zn, Cu, Ni (Verschoor et al., 2012) and Pb (Vink and Broers, 2016) and have been included in the PNEC-pro tool that is offered by Deltares for site-specific water quality assessment (www.pnec-pro.nl).

## 2.3 Aluminum speciation and analysis

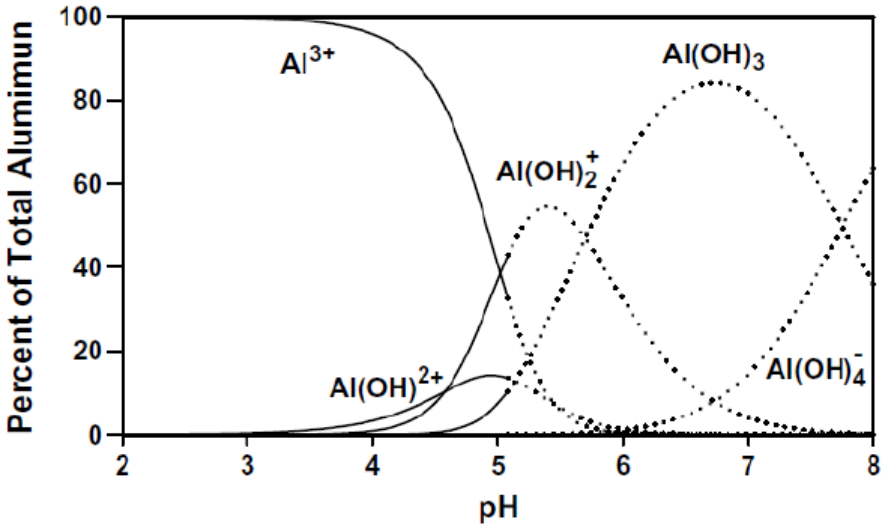


*Figure 2 Results of aluminum speciation calculations at a total of 65 µM aluminum (example) in the absence of ligands (Zhou et al., 2008).*

The chemistry of aluminum in surface water is complex (see example in Figure 2) because of the following properties:

- aluminum is amphoteric, meaning that it can act as an acid or a base depending on the pH in the environment
- aluminum is more soluble in both acidic solutions and in basic solutions than in circumneutral solutions. The solubility limit of aluminum varies from 20 µg/L at pH 6.5 to > 1000 µg/L at pH 5 (Santore et al. 2018).
- specific ions such as chloride, fluoride, nitrate, phosphate and sulfate form soluble complexes with aluminum;
- $Al^{3+}$ can form strong complexes with fulvic and humic acids;
- hydroxide ions can connect aluminum ions to form soluble and insoluble polymers (e.g. gibbsite, corundum);
- under at least some conditions, solutions of aluminum in water approach chemical equilibrium rather slowly, with monomeric species of aluminum transforming into insoluble polymers which precipitate out of solution over time;

For aluminum, the $EC_{xx}$ is a function of pH, DOC and hardness**.** The MLRs have been derived for total recoverable aluminum in reconstituted water of laboratory tests, because in several chronic studies ecotoxic effects increased with increasing total aluminum concentrations, while measured concentrations of dissolved and monomeric aluminum changed very little with increasing total aluminum concentrations (Gensemer et al., 2018; Cardwell et al., 2018). It appeared that although the toxicity of aluminum could be explained by the dissolved $Al^{3+}$ at pH 5.0 or lower, the toxicity of aluminum in circumneutral waters did not correlate with dissolved aluminum. Rodriguez et al.(2019) elaborated on this issue and explained this phenomenon by the two toxic mechanisms of aluminum: 1) at acidic pH the toxicity of aluminum appears to be attributable to iono-regulatory effects and 2) at neutral or alkaline pH conditions physical effects are often attributable to the coating of the respiratory membranes with Al-hydroxide precipitates.

Unlike laboratory test water, natural surface waters typically contain suspended solids that include aluminum in the forms of oxides or silicates. Common analytical methods using strong acid will dissolve most or all of the inert non-toxic aluminum present in solid particles, and will report these concentrations as "total or total recoverable" aluminum In natural surface waters with suspended solids, the aluminum mobilized from the suspended solids during analysis can result in "false" exceedances of the environmental quality criteria. Therefore, Rodriguez et al.(2019) recommended to use a method that could appropriately measure aluminum for regulatory purposes, concerning aluminum that is not associated with suspended solids, and including Al-hydroxide particulates that are present at circumneutral pH. This preferred method measures the fraction of aluminum responsible for toxicity and is described in Appendix 1 (see also paragraph 7.2).

# 3 Differences between US-EPA and EU approach

In this chapter it is explained to what extent the EPA derivation method of the Al-EQS deviates from the EU Technical guidance for deriving environmental quality standards (further referred to as EU-guidance no. 27) (EC, 2018).

## 3.1 Species sensitivity distribution

Water quality criteria are derived from a species sensitivity distribution (SSD) if there are sufficient ecotoxicity data available. From a distribution of ecotoxicity data a hazard concentration that is protective for aquatic ecosystems ($HC_5$) can be statistically derived. An assessment factor is eventually also applied to the $HC_5$, amongst others depending on the reliability of the statistical outcome, the presence of particularly sensitive groups of species, the number and relevance of species in the distribution and the type of experiments (field or lab).

Key differences between the US and EU approaches include:

1) use of chronic NOEC or $EC_{10}$ in the EU versus chronic $EC_{20}$ in the US;
2) use of species-mean endpoints in an SSD by the EU versus genus-mean endpoints in Genus Sensitivity Distribution (GSD) the US;
3) inclusion of algae and higher plant data in the EU SSD versus exclusion of algae and plant data in the US GSD, see Figure 3;
4) use of different statistical models for calculating the $HC_5$; the US-EPA derives the $HC_5$ from four most sensitive species, in the EU the complete SSD is used.

In the US-EPA guidance, acute and chronic criteria were derived that are allowed to be exceeded only once in 3 years, whereas in Europe annual average concentrations need to comply with an Annual Average - EQS (AA-EQS) and individual samples may not exceed the maximum acceptable EQS (MAC-EQS).

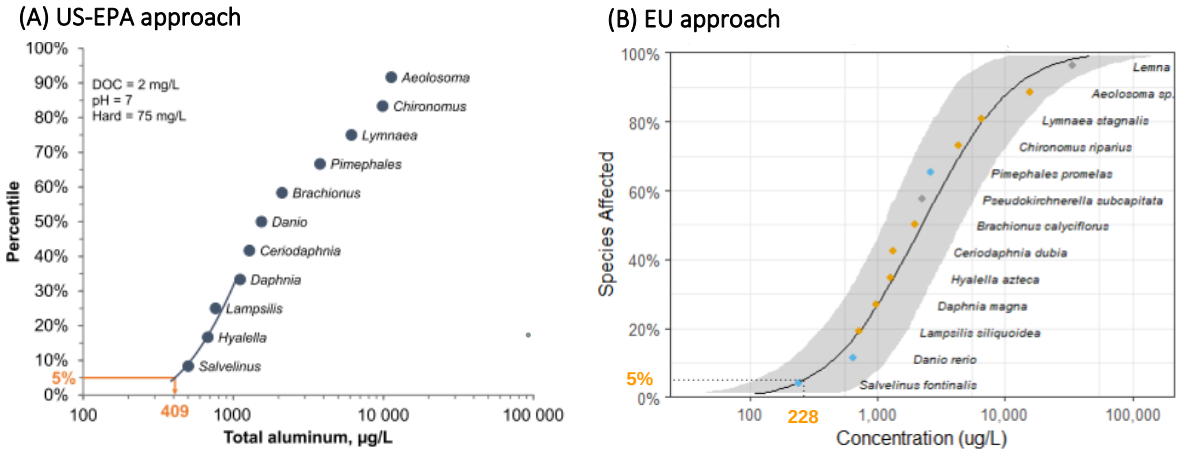


*Figure 3 Difference between US-EPA and EU approach for calculation of site-specific HC5. A. Genus sensitivity distribution for invertebrates and fish based on chronic total aluminum (Al) effect concentrations, 20% (EC20s; US Environmental Protection Agency [USEPA] approach) and (B) species sensitivity distributions for algae/plants, invertebrates, and fish based on chronic total aluminum EC10s (European Union [EU] approach) adjusted to a dissolved organic carbon (DOC) of 2 mg/L, a pH of 7, and hardness of 75 mg/. Figure B is generated with ssdtools in R using the dataset and models selected in this report.*

Based on the differences mentioned above, it is concluded that the US-EPA tool (results visualized in Figure 3A) is not useful for application within the European and Dutch regulatory frameworks. However, Figure 3B shows an assessment following European guidelines, so $EC_{10}$ for algae and plants

are available and the statistical derivation method can be adjusted to comply with the European guidelines.

## 3.2 Calculation of water type-specific quality criteria

The EPA developed a spreadsheet-tool called "Al-criteria calculator v2" for the calculation of site-specific water quality standards (further referred to as the EPA-tool[3]). The EPA-tool provides an $EC_{20}$-based chronic EQS and an $EC_{50}$-based acute EQS. The EPA-tool is based on publications of DeForest et al. (2018 and 2020). Slightly different spreadsheet-tools are also provided by these authors in the supplemental information to their publications, further referred to as DeForest-tools. DeForest et al. implemented both US and European approaches for the calculation of chronic water type-specific EQS. These tools use separate equations for algae, crustaceans and fish. Canada derived one overall equation based on the work of DeForest et al. (2018 and 2020). The Canadian work was outside the scope of this assignment.

Thus, the considered calculation tools for water type-specific aluminum criteria are:

1) EPA Al-criteria calculator v2 (2018)
2) DeForest et al. 2018, supplemental information
3) DeForest et al. 2020, supplemental information

The DeForest 2020 version is an update of the 2018 version with an applicability for a broader range of water types and a larger toxicity database. All the tools run in Microsoft Excel.

The following steps were taken by DeForest et al. (2018 and 2020) to create an aluminum calculation tool for chronic toxicity according to European methodology:

1) Chronic toxicity data of algae *Pseudokirchneriella subcapitata* were added to the SSD. Data are available from Gensemer et al. (2018), supplemental information.
2) $EC_{10}$ values were selected instead of $EC_{20}$;
3) An algae MLR was added to the present crustacean and fish MLRs. So, three MLRs are present to normalize all species mean $EC_{10}$ values in the toxicity database;
4) An $HC_5$ calculation according to the EU statistical approach, i.e. the calculation of the $5^{th}$ percentile of log-transformed (normalized) $EC_{10}$ values by a Gaussian distribution function, including the 90% confidence interval around the $5^{th}$ percentile. The $HC_5$ is the median of a distribution of SSD-curves, also referred to as $HC_5$-50[4], with the $HC_5$-5 and $HC_5$-95 as lower and upper limit of the 90% confidence interval.

EPA also offers an R-script for normalization of toxicity data. Probably this tool is not suitable for the average water manager, but it can be used for research purposes. In order to make the R-script compliant with EU guidance no. 27 the same adjustments need to be made as in the US-spreadsheet.

PNEC-pro is a Dutch tool to calculate water type-specific quality criteria for Zn, Cu, Ni and Pb. PNEC-pro followed the European approach. Implementation of the DeForest-tool in PNEC-pro is feasible. The following chapters will go into more detail to the selected MLRs, the toxicity database and will show preliminary results for the Dutch situation.

[3] https://www.epa.gov/sites/default/files/2018-12/aluminum-criteria-calculator-v20.xlsm
[4] The $HC_5$-50 is generally slightly lower than a single-fit point estimate of the $HC_5$ (Aldenberg et al., 2002)

# 4 Multiple linear regression models (MLRs)

This chapter describes the $EC_{10}$-MLR models as presented in the most recent publication of DeForest et al. (2018 and 2020).

In paragraph 4.2 the differences between (simple) linear regression and multiple linear regression are briefly explained. In paragraph 4.3 it is described for which species the MLRs were developed and how they were derived. In paragraph 4.4 the level of validation is described.

The development of MLRs for regulatory purposes requires the collection or generation of many ecotoxicity data, and involves the following steps:

1) Performance of laboratory experiments to determine dose-response relationships for algae, crustaceans and fish at a variety of water chemistries (different pH, DOC and hardness);
2) Derivation of empirical relationships between $EC_{xx}$ and pH, DOC and hardness for each of the three species;
3) Validation of the MLRs with tests in natural waters.

These steps were executed and described by DeForest et al. (2018; 2020); Gensemer et al. (2018); and EPA (2018).

The application of MLRs to derive EQS values requires data of a larger variety of species in order to create a SSD. This is described in Chapter 5.

## 4.1 Toxicity data for MLR development

Three sets of toxicity data were used for the derivation of Al-MLRs for the following species: the algae *Pseudokirchneriella subcapitata*[5], the crustacean *Ceriodaphnia dubia* and the fish *Pimephales promelas (*see Appendix 2). The dataset provided by DeForest et al. (2020) is the most recent and most complete existing dataset. For MLR development DeForest et al. only used chronic toxicity data. Application to acute toxicity data assumes that the same toxic mechanisms are present.

The toxicity tests for MLR development were performed with reconstituted water, that means that the pH, DOC and hardness are adjusted to obtain a controlled range of water chemistry conditions. A combination of three DOC, three hardness and three (roughly) pH values was tested.

Chronic toxicity tests with *P. subcapitata* were performed according to OECD test guideline 201, a 72h static test. Short-term (7d), static renewal tests were done with *C. dubia* and *P. promelas* according to US guidelines. For *C. dubia*, which has a short life-cycle, a 7-days test duration represents chronic exposure, while for *P. promelas* this is an extended acute test. Reported endpoints were $EC_{10}$ and $EC_{20}$, as well as pH, DOC and hardness.

DeForest et al. (2020) added results of nine toxicity tests to the *C. dubia* dataset of 2018 and also nine additional test results to the *P. promelas* dataset, in order to extend the range of the water chemistry (see Table 2).

[5] Currently the formal name is *Raphidocelis subcapitata,* but in this report we keep using the name *Pseudokirchneriella subcapitata* to avoid confusion because this name is used in all the underlying studies and databases as well.

*Table 2 Water characteristics of the toxicity data which determines the applicability domain of the MLRs.*

| MLR parameter | test range |
|---|---|
| pH | 6.0 – 8.7 |
| Hardness | 9.8 – 428 |
| DOC (mg/L) | 0.08 – 12.3 |

## 4.2 Single versus multiple regression and interactions

A variety of statistical regression options were employed by DeForest et al. (2018 and 2020). The main approaches are described in this paragraph.

A simple linear regression equation expresses the influence of one parameter *x* (for instance DOC) on a variable *y* (for instance an $EC_{10}$), which is mathematically described by the general equation:

$y = a \cdot x + b$, where *a* is the slope of the line and *b* is the intercept with the y-axis. The equation is by definition visualized by a straight line in a x-y graph.

A multiple (log-)linear regression model contains more parameters, for instance in the case of bioavailability besides DOC also pH and hardness. This formula looks like:

$lnEC_{xx} = a \cdot \ln(DOC) + a_2 \cdot pH \; + \cdot a_3 \cdot \ln(Hardness) + \cdots b$ where each parameter has its own slope $a_1$, $a_2$, $a_3$ which determines how sensitive the $EC_{xx}$ is for changes in that particular parameter.

A MLR does not lead to a simple graph with one parameter on the x-axis, because there are several (ideally independent) parameters that determine the outcome; in fact it is a multidimensional case. The parameters DOC and hardness are transformed to the natural logarithms, because their values are usually not normally but log-normally distributed. The pH is not transformed because it is already expressed as the logarithm of the $H^+$-concentration.

Besides the effect of each individual parameter, interactions between parameters may exert an effect on the $EC_{xx}$. By the introduction of interaction terms, the model loses its linear character. Because the significance of the interaction terms can be tested by multiple linear regression techniques we still refer to these models as MLRs. The following four interactions were tested as potential extra terms in the development of the MLR (DeForest et al., 2018 and 2020):

$ln(DOC) \times pH$: a negative term would characterize the mitigating effect of DOC on aluminum bioavailability, which tends to decrease as pH increases;

$\ln(Hard) \times pH$: a negative term would reflect that the mitigating effect of hardness on aluminum bioavailability tends to decrease as pH increases;

$ln(Hard) \times \ln(DOC)$: a negative term would reflect that the mitigating effect of DOC on aluminum bioavailability tends to decrease as hardness increases;

$pH^2$ : a negative term would help account for decreasing aluminum bioavailability as pH increases from 6 to 7 and then increasing aluminum bioavailability as pH increases from pH 7 to pH 8.

In general, including more terms in the regression improves the accuracy of the model, but one has to be careful for over-parameterization. Some parameters may be correlated; including them both in the MLR introduces a pseudo-reliability. The selection of significant parameters and interactions by DeForest et al. (2018 and 2020) followed a stepwise approach. They tested the additional gain in accuracy after addition of an extra parameter or an extra interaction term. The accuracy was

evaluated based on adjusted $R^2$, predicted $R^2$, AIC and BIC[6], similar to the procedures used to derive the MLRs for Cu, Ni and Zn in PNEC-pro (Verschoor et al., 2012). A substantially lower predicted $R^2$ is an indication that the model may be overfitted and/or is reliant on individual data points.

## 4.3 Derivation of MLRs

MLRs were derived from correlation of experimental $EC_{10}$ values with pH, DOC and hardness of the test media. DeForest et al. (2018) derived MLRs based on chronic toxicity tests for the algae *P. subcapitata,* which is required according to European guidelines. DeForest et al. (2020) updated the MLRs of *C. dubia* and *P. promelas* to expand the applicability range of the MLRs over a broader range of test conditions. In DeForest et al. (2020), different mathematical ways of MLR derivation were compared:

1) Development of MLRs for *C. dubia* and *P. promelas*; each MLR had its own intercept and slope;
2) Development of pooled MLRs for *C. dubia* and *P. promelas*. The MLRs of *C. dubia* and *P. promelas* had a common slope derived from the combined dataset, but species-specific intercepts[7].

The first approach has the advantage of being more species-specific and fits better to the data of the individual species. The second approach has the advantage that the pooled dataset is larger, so the statistical power is enhanced. A pooled model also warrants that the normalization of $EC_{10}$ is identical for all taxa, and so a shift in the ranking of species in the SSD cannot occur.

An overview of all of the $EC_{10}$ and some $EC_{20}$ models, and in which tools they were implemented, is given in Appendix 3. In Table 3 an overview of some MLRs for normalization of $EC_{10}$ values is given. Three options are presented that illustrate the main differences between: 1) models without interactions, 2) species-specific models with statistically significant interactions, and 3) pooled models with statistically significant interactions.

In developing its final tool, the US-EPA decided to use the species-specific *C. dubia* and *P. promelas* models rather than the pooled *C. dubia* and *P. promelas* $EC_{20}$-models. This decision was based on patterns in the residuals of the pooled model that were not observed in the individual species models. For example, the US-EPA commented that the *C. dubia* pooled MLR model was over-predicting the $EC_{20}$ (predicted $EC_{20}$ higher than observed values) as pH increased, and under-predicting $EC_{20}$ as DOC and total hardness increased (lower predicted $EC_{20}$ than observed values). Conversely, the *C. dubia* individual-species MLR model showed no trends in the residuals over any of the test parameters. Likewise, there were similar trends in the residuals for the pooled *P. promelas* MLR model. Following this reasoning and previous approaches in application of BLMs in the PNEC-pro tool, we selected the species-specific MLRs for calculation of water type-specific quality criteria in the Netherlands.

[6] The Akaike information criterion (AIC) and the Bayesian Information Criterion (BIC) are estimators of prediction error and thereby of the relative quality of statistical models for a given set of data. They provide a means for model selection. BIC and AIC differ in the “penalty terms” they use for additional parameters.
[7] Note that intercepts are not used in the normalization procedure.

*Table 3 MLR-models for normalization of chronic EC10. For the Dutch approach models with the highest $R^2$ were selected.*

| | *a1* *ln* (DOC) | *a2* pH | *a3* *ln* (Hard) | *a4* *ln* (Hardness) x pH | *a5* *ln* (DOC) x pH | *a6* $pH^2$ | Adj. $R^2$ | Reference |
|---|---|---|---|---|---|---|---|---|
| **P. subcapitata** | | | | | | | | |
| No interactions | +0.349 | +0.346 | - | - | - | - | 0.312 | De Forest et al 2018 |
| With interactions | +2.342 | +20.923 | +4.560 | -0.628 | -0.288 | -1.274 | 0.940 | De Forest et al 2018 |
| ***C. dubia*** | | | | | | | | |
| No interactions | +0.536 | +0.871 | +0.375 | - | - | - | 0.658 | De Forest et al 2018 |
| With interactions | +0.700 | +2.368 | +3.030 | -0.375 | - | - | 0.925 | De Forest et al 2020 |
| Pooled | +0.660 | +1.969 | +2.216 | -0.278 | - | - | 0.916 | De Forest et al 2020 |
| **P. promelas** | | | | | | | | |
| No interactions | +0.495 | +0.966 | +0.354 | - | - | - | 0.822 | De Forest et al 2018 |
| With interactions | +1.828 | +1.932 | +1.914 | -0.248 | -0.193 | - | 0.913 | De Forest et al 2020 |
| Pooled | +0.660 | +1.969 | +2.216 | -0.278 | - | - | 0.875 | De Forest et al 2020 |

Note that $R^2$ of the pooled models for *C.dubia* and *P. promelas* are different because they describe the correlation between the model prediction and the observed $EC_{10}$ for each species separately. The intercept *b* is not included in the table because it is eliminated during the normalization process.

The EPA-tool was used to address all waters within a pH range of 5.0 to 10.5. This exceeds the boundaries of the applicability domain of the MLRs (see Table 2). The EPA took this approach so that the recommended criteria can be calculated for a broader range of natural waters found in the US. In the Netherlands pH values down to a value of 4 are sometimes encountered in natural waters and DOC values larger than 12.3 mg/L are quite common. In Chapter 0 the chemistry of Dutch waters is described in more detail. $HC_5$ values generated for water types outside of the applicability range should be considered carefully and used with caution. Also in PNEC-pro, it is possible to calculate a $HC_5$ for waters outside the applicability domain. The outcomes are flagged to draw attention on the fact that the $HC_5$ is more uncertain.

## 4.4 Validation and cross-species extrapolation of the MLRs

The validation of models is usually done by taking a new set of toxicity tests (so data not used for model development) preferably carried out with natural water samples to check if the predicted $EC_{10}$ for those tests is within a factor of two of the observed value. The nine additional toxicity tests with *C. dubia* and *P. promelas* can be considered as a validation step. In this case, validation resulted in a slight adjustment of the MLR. This approach was similar to the validation of Cu-BLMs (De Schamphelaere and Jansen, 2004).

The MLR of *C. dubia* is used for a variety of other invertebrate species: snails, annelids, rotifers, insects, and bivalves. Likewise the *P. promelas* MLR is used for other fish and frog species and the *P. subcapitata* MLR is used for aquatic plants *(Lemna minor)*. For Cu, Ni and Zn such a cross-species extrapolation is justified by additional studies. For Al, such studies were conducted with the rotifer and great pond snail at nominal pH 6.3, with various hardness and DOC levels. However, the results of this validation-study were not published, all $EC_{20}$ values are in the EPA-report, but only one (the lowest) $EC_{10}$ value of each species is available in the database of DeForest et al. (2020).

As a conclusion, one can state that the validation and justification for cross-species extrapolation of the available aluminum MLRs needs improvement.

# 5 Application of MLRs for Dutch EQS derivation

## 5.1 Data requirements and selection

Three MLRs were developed using ecotoxicity data of three species representing three different trophic levels. The **implementation of MLRs** requires additional toxicity data in order to create a species sensitivity distribution, from which an $HC_5$ can be derived.

According to the EU guidance no. 27, the $HC_5$ of an SSD is considered reliable if the database contains preferably more than 15, but at least 10 NOECs/$EC_{10}$ values, from different species covering at least eight specific taxonomic groups. An overview of data abundance is given in paragraphs 5.2 and 5.3.

Because quality criteria for data selection are approximately identical between the US-EPA (Stephan, 1986) and EU (EC, 2018) and many of the underlying test reports are not publicly available, we pragmatically adopted all studies that have been selected by US-EPA in their 2018 report. The chronic data in the EPA-tool are not directly suitable because they are expressed as $EC_{20}$-values. DeForest et al. collected $EC_{10}$-values from the same studies and extended the toxicity database with new studies. This database fulfils the requirements needed to derive water type-specific quality criteria according to the European methodology.

US-EPA (2018a) has set-up a list of criteria for quality control of toxicity data to decide upon including or not including particular studies in a database for aluminum EQS-derivation:

- US-EPA mentioned the importance of aging of the stock solution and pH control of the test as important factors for reliability checking;
- Only data from toxicity tests conducted using chloride, nitrate and sulfate salts (either anhydrous or hydrated) are used in this effects assessment;
- The assessment endpoints for aquatic life criteria are based on survival, growth and reproduction;
- A pH of the water tested of less than 5 was deemed too low to allow for the toxicity data to be used quantitatively;
- Because the chemical speciation and reactivity in seawater is very different from freshwater, only freshwater data for metals were selected;
- There were insufficient data with sea water to derive separate salt water MLRs.

The complete chronic and acute toxicity databases that may be used for implementation in a Dutch aluminum calculation tool are presented in Appendix 4 and Appendix 5. Each $EC_{xx}$ value is normalized to water type-specific conditions with either the algae MLR, the crustacean MLR or the fish MLR. The assignment of a specific MLR to other species is also indicated in Table 4 and Table 5.

## 5.2 Chronic toxicity data

In Table 4, a comparison is made between the number of toxicity data in the EPA Aluminum criteria calculator v2, the calculation tools provided by DeForest (2020) and the database we derived from those sources. 'The most prominent difference between our selection and those of DeForest is that we do not consider the 7-days fish tests as true chronic tests and removed them from the database.

*Table 4 Overview of chronic data in EPA document, the DeForest (2020) and our database. Three MLRs were assigned to normalize the toxicity data: 1) the P.promelas MLR, 2) the C. dubia MLR and 3) the P. subcapitata MLR.*

| Requirement EU guidance | EPA ($EC_{20}$) | DeForest 2020 | Our selection | | |
|---|---|---|---|---|---|
| **Taxonomic group:** | **# $EC_{20}$** | **# $EC_{10}$** | **# $EC_{10}$** | **species** | **MLR no.** |
| Fish (species frequently tested include salmonids, minnows, bluegill sunfish, channel catfish, etc.); | 2 | 31[1] | 1 | Fathead minnow, *Pimephales promelas* | 1 |
| | 1 | - | - | Atlantic salmon, *Salmo salar*[2] | 1 |
| A second family in the phylum Chordata (e.g. fish, amphibian, etc.); | 2 | 1 | 1 | Brook trout, *Salvelinus fontinalis*[3] | 1 |
| | 1 | 1 | 1 | Zebrafish, *Danio rerio* | 1 |
| | 1 | - | - | Wood frog, *Rana sylvatica*[4] | 1 |
| A crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish, etc.); | 33 | 30 | 31 | Cladoceran, *Ceriodaphnia dubia** | 1 |
| | - | 1 | - | Cladoceran, *Ceriodaphnia sp.* | |
| | 1 | 2 | 1 | Cladoceran, *Daphnia magna*[6] | 1 |
| | 2 | 1 | 2 | Amphipod, *Hyalella azteca*[7] | 1 |
| An insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.); | 3 | 1 | 1 | Midge, *Chironomus riparius*[8] | 2 |
| A phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.); | 6 | 1 | 1 | Rotifer, *Brachionus calyciflorus*[9] | 2 |
| | 1 | 1 | 1 | Fatmucket, *Lampsilis siliquoidea* | 2 |
| | 4 | 1 | 1 | Great pond snail, *Lymnaea stagnalis*[10] | 2 |
| An order of insect or any phylum not already represented; | 1 | 1 | 1 | Oligochaete, *Aeolosoma* sp. | 2 |
| Algae or Cyanobacteria; | - | 27 | 27 | *Pseudokirchnerielle subcapitata**[11] | 3 |
| Higher plants. | - | 1 | 1 | *Lemna minor*[11] | 3 |
| **Total data** | **58** | **100** | **70** | | |
| **#species** | **13** | **14** | **13** | | |

[1] This included tests used for BLM development. Because it concerned 7d (subchronic) test and not real chronic tests, these additional data were not selected in the "Dutch" database. We selected the 33-d study used by EPA also (Cardwell et al., 2018), that contained an $EC_{10}$. The other EPA datapoint (Kimball, 1978) was not available and an $EC_{10}$ of this study was not reported elsewhere.

[2] EPA-data concern tests with Al-sulphate, those were not selected by DeForest, a reason is not given. An $EC_{10}$ is not available.

[3] EPA-data concern tests with aluminum sulphate, the study with the lowest $EC_{10}$ was selected by DeForest. Since EPA did not provide $EC_{10}$, we adopted the $EC_{10}$ used by DeForest et al.2020.

[4] EPA considered test with *Rana sylvatica* not acceptable because test pH was too low. Still it was included in the aluminum criteria calculator.

[5] EPA and DeForest have 28 tests in common. DeForest deselected four tests with aluminum chloride (ENSR 1992b) and one test of McCauley 1986 also with Al-chloride. They added two new records (CIMM 2009) that were not rejected by EPA, but not included by EPA either. These two data were part of the dataset for BLM development. We took the dataset of DeForest, and added one test of McCauly, but the four test $EC_{10}$ of ENSR 1992 were not available to be included. Because there is no reason to believe that *Ceriodaphnia* sp. is different from *Ceriodaphnia dubia*, we combined the data.

[6] DeForest included a *Daphnia magna* test of Kimball 1978 (with Al-sulphate) that was disqualified by EPA because the survival in the control was too low. We removed this datapoint.
[7] DeForest removed the data of Wang et al.(2018); it is not clear why. EPA accepted the study. We included the data point of Wang et al.2018.
[8] Two tests with Al- sulphate (Palawski et al.1989) were not selected from the EPA database by DeForest. It is not clear why. $EC_{10}$ for these tests are not available, so we could not add them to our database.
[9] DeForest used the lowest EC20 of 431 µg/L from the EPA dataset, and the $EC_{10}$ from the same test. The other 5 $EC_{20}$-values in the EPA set were in the range 1604-4670 µg/L. $EC_{10}$ values for these missing tests are not available because the study (OSU 2018e) is not public.
[10] DeForest claims to have selected the test with the lowest $EC_{20}$ (out of 4 tests), This is true for the "raw" $EC_{20}$, but not for the normalized $EC_{20}$. The normalized $EC_{20}$ is the highest of aluminum 4 tests. Because $EC_{10}$ values are not available for the missing tests, we adopted the $EC_{10}$ that goes along with the high normalized $EC_{20}$.
[11] EPA did not use algae and plants in their Aluminum criteria calculator. So we adopted them from DeForest et al.2020.

## 5.3 Acute toxicity data

Acute toxicity tests were also collected by EPA in their EPA-tool. The EPA-tool contains $EC_{50}$ values for 22 species. The tools of DeForest (2018 and 2020) do not contain acute data. The dataset included in the EPA-tool does not contain algae or plants, so it is incomplete according to European methodology. An overview of the database composition is given in Table 5. Algae data are present to supplement the EPA-database.

*Table 5 Overview of acute data requirements in EPA document and adopted in our database. Three MLRs were assigned to normalize the toxicity data: 1) the P.promelas MLR, 2) the C. dubia MLR and 3) the P. subcapitata MLR.*

| Requirement EU guidance | | | | |
|---|---|---|---|---|
| **Taxonomic group:** | **# EPA data** | **# Our selection** | **species** | **MLR No.** |
| Fish (species frequently tested include salmonids, minnows, bluegill sunfish, channel catfish, etc.); | 2 | 2 | Atlantic salmon, *Salmo salar* | 1 |
| | 3 | 3 | Brook trout, *Salvelinus fontinalis* | 1 |
| | 8 | 8 | Fathead minnow, *Pimephales promelas* | 1 |
| | 1 | 1 | Green sunfish, *Lepomis cyanellus* | 1 |
| | 1 | 1 | Guppy, *Poecilia reticulata* | 1 |
| | 14 | 14 | Rainbow trout, *Oncorhynchus mykiss* | 1 |
| | 1 | 1 | Rio Grande silvery minnow, *Hybognathus amarus* | 1 |
| | 3 | 3 | Smallmouth bass, *Micropterus dolomieui* | 1 |
| A second family in the phylum Chordata (e.g. fish, amphibian, etc.); | 1 | 1 | Green tree frog, *Hyla cinerea* | 1 |
| A crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish, etc.); | 1 | 1 | Amphipod, *Crangonyx pseudogracilis* | 2 |
| | 1 | 1 | Amphipod, *Hyalella azteca* | 2 |
| | 54 | 54 | Cladoceran, *Ceriodaphnia dubia* | 2 |
| | 3 | 3 | Cladoceran, *Ceriodaphnia reticulata* | 2 |
| | 9 | 9 | Cladoceran, *Daphnia magna* | 2 |
| | 1 | 1 | Cladoceran, *Daphnia pulex* | 2 |
| An insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.); | 1 | 1 | Midge, *Paratanytarsus dissimilis* | 2 |
| | 1 | 1 | Midge, *Chironomus plumosus* | 2 |
| A phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.); | 2 | 2 | Fatmucket, *Lampsilis siliquoidea* | 2 |
| | 1 | 1 | Snail, *Melanoides tuberculata* | 2 |
| | 4 | 4 | Snail, *Physa sp.* | 2 |
| An order of insect or any phylum not already represented; | 1 | 1 | Ostracod, *Stenocypris major* | 2 |
| | 1 | 1 | Worm, *Nais elinguis* | 2 |
| Algae or Cyanobacteria; | - | 27 | *Pseudokirchneriella subcapitata* | 3 |
| Higher plants. | - | - | | |
| **# Total data** | **114** | **141** | | |
| **# Species** | **22** | **23** | | |

## 5.4 Creation of tools

MRLs were implemented in Excel as well as in R-scripts. Different versions of the tools were used to cross-check the calculations. The latest versions of the tools were finally checked for 10 randomly selected samples. Resulting $HC_5$ values were identical, which implies that both tools (which are quite different in how they function) have identical underlying toxicity databases, and identical calculation procedures. The R-script for the calculation of $HC_5$ is included in Appendix 6.

In PNEC-pro, single transfer functions for Zn, Cu and Ni are used for the calculation of $QS_{bioavailable.}$. This is an approach that can be considered for aluminum also. Such a simplification introduces however additional uncertainties because the transfer function is not an exact simulation of the normalization procedure but an approximation of it. Transfer functions for acute and chronic $HC_5$ are derived by regression of predicted site-specific $HC_5$ values with DOC, pH and hardness of the water types.

The following transfer functions resulted in the best fits for chronic and acute $HC_5$:

$\log_{10}(HC_5) = \text{Intercept} + a_1 \cdot \log_{10}(\text{DOC}) + a_2 \cdot \text{pH} + a_3 \cdot \log_{10}(\text{Hardness}) + a_4 \cdot \text{pH} \cdot \log_{10}(\text{Hardness}) \cdot$

Introduction of other interaction terms such as $\text{pH} \times \log_{10}(\text{DOC}) \cdot$ and pH×pH did not significantly improve the predictive power of the transfer functions. The parameters in the transfer functions are listed in Table 6, the graphs showing the statistical fit are shown in Figure 4.

*Table 6 Parameters of the simplified equations for calculations of site-specific $HC_5$.*

| | Intercept | $a_1$ | $a_2$ | $a_3$ | $a_4$ | Adjusted $r^2$ |
|---|---|---|---|---|---|---|
| chronic | -11.32348 | 0.40637 | 1.82928 | 6.74640 | -0.89380 | 0.9210 |
| acute | -9.13133 | 0.40176 | 1.61797 | 5.92374 | -0.77501 | 0.8985 |

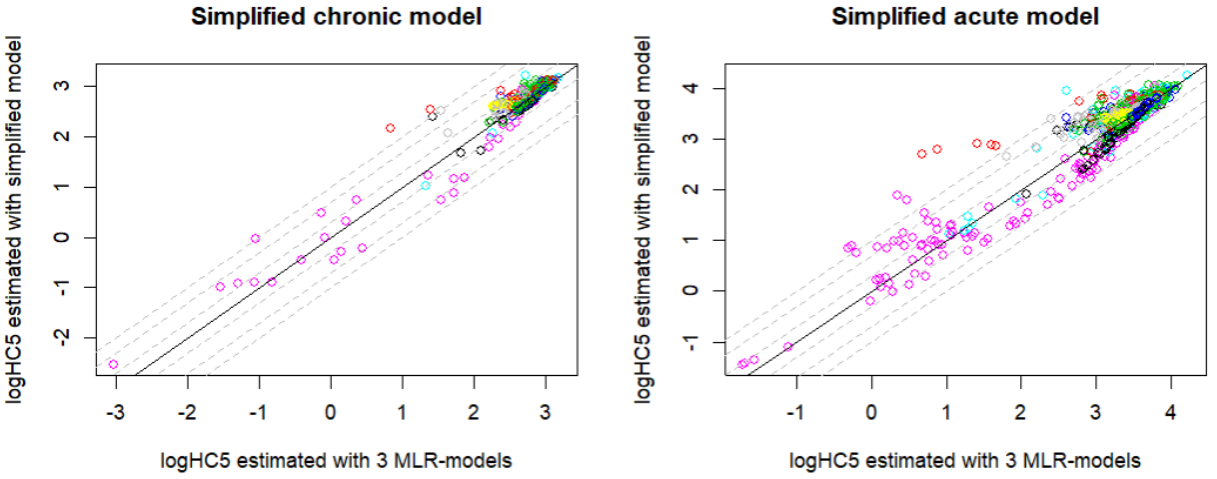


*Figure 4 Goodness-of-fit between site-specific $HC_5$ values predicted with the normalization procedure with 3 MLRs and the $HC_5$ values estimated with the simplified transfer functions. Colored dots represent different water types, the pink dots refer to fens. The grey dotted lines represent uncertainty range of a factor 2, 5 and 10 above and below the best fitted line.*

The simplified model is not used in the following chapters.

# 6 Calculation of water type-specific $HC_5$

## 6.1 General approach

For the Dutch water quality evaluation two consecutive steps may be applied; 1) generic and 2) site-specific risk assessment (Rijkswaterstaat, 2020). To obtain insight in water types, the geographical distribution and surface areas involved, monitoring data were retrieved from Waterkwaliteitsportaal.nl, the most recent data were from 2020 (see paragraph 6.2, 6.3 and 6.4). Three approaches are described in the following paragraphs; a summary is provided in Chapter 7.

### 1) Assessment with monitoring data of Waterkwaliteitsportaal 2020

The EU guidance no. 27 states that: "The $HC_5$ selected should be protective of 95% of waters in the region (country) shown to have the highest bioavailability of that particular metal." Therefore normalizations are performed for as many natural samples that could be retrieved from Waterkwaliteitsportaal.nl for the most recent year (2020). When $HC_5$ endpoints are aggregated to water type, $5^{th}$ percentiles are reported.

Two sets of $HC_5$ values are calculated (see paragraph 6.5) :

a) $HC_5$ for all samples, regardless of the DOC, pH and hardness. This approach covers a maximum number of water types although it may contain less reliable $HC_5$ values if they were derived for samples that were outside the applicability domain of the MLRs.
b) $HC_5$ only for samples within the applicability domain of the MLRs.

### 2) Assessment with a specific set of water chemistry conditions

In the EU guidance document no. 27 on Derivation of environmental quality standards it is stated that: "The $EQS_{bioavailable}$ is a total dissolved metal concentration which is highly bioavailable and which does not make any allowance for background in its derivation. It is derived, initially, as the normalized, estimate of the $HC_5$ for a specific set of water chemistry conditions - one that is reflective of high bioavailability conditions.

An a priori selection of one specific set of parameter values that reflects a high bioavailability is not obvious, because the normalization of the toxicity data is affected by pH, DOC and hardness simultaneously and by interactions between these parameters. Different combinations of pH, DOC and hardness can lead to the same $HC_5$. The most vulnerable specific water chemistry conditions are determined from $HC_5$ values calculated with samples from Waterkwaliteitsportaal.nl (see paragraph 6.6).

### 3) Assessment with water types previously used for derivation of a EQS for copper

In 2012, a bioavailability based EQS for copper was adopted, based on the work of Vijver et al. (2008). This work included the establishment of six water types. For reasons of comparison, Al- $HC_5$ values are also calculated for these water types (see paragraph 6.7).

## 6.2 Selection of water types

Waterkwaliteitsportaal.nl contains more than 2.7 million chemical monitoring data of 2020. The database contains concentrations of 1046 chemical substances and more than 50 physico-chemical characteristics of the samples. The presence of monitoring data on pH, DOC and hardness is required to be able to normalize toxicity data of aluminum. The presence of Al-concentrations in the monitoring data is not required for derivation of EQS, but it would be nice to have them for a preliminary assessment of the consequences of a proposed $HC_5$ or EQS (see paragraph 7.2).

Following the Water Framework Directive, The Netherlands distinguishes approximately 50 water types, with different salinity, flow velocity, alkalinity, depth, width, surface area and soil type. For each water type biotic and abiotic characteristics have been identified and ecological targets described. The MLR models can only be applied to freshwaters; MLRs are not valid for saline water. Therefore samples from marine, coastal and transitional waters were not selected[8]. This concerns approximately 79% of the total surface water area of the Netherlands (Van Puijenbroek and Clement, 2010). The remaining 21% concerns freshwater types (including brackish waters[9]). Freshwater samples were grouped into 12 water types (see Table 7). A distribution of selected samples over WFD water types and a description of the WFD water types in Dutch is given in Appendix 7.

Because pH, DOC and hardness were not always determined in samples taken on exactly the same date, monthly average values were calculated in order to construct complete records for the calculation of water type-specific quality criteria. Sites with incomplete data or with missing water type were removed. On average approximately 7 monthly records could be retrieved per site. In this way, 5544 complete monthly records were obtained for 779 sites for the calculation of a site-specific $HC_5$.

*Table 7 Overview of Dutch water types in the selected dataset. Surface area figures were obtained from Van Puijenbroek and Clement (2010). Monthly data concerns complete datasets with mean concentrations of DOC, pH and hardness.*

| Water type | Area | | WFD types in selected dataset | Selected sites | | Monthly data |
|---|---|---|---|---|---|---|
| | ha | % | | # | % | # |
| Brackish waters | 27 205 | 8 | M30, M31, M32 | 19 | 2 | 161 |
| Brooks, fast flowing | 172 | <0.1 | R13, R14, R15, R17, R18 | 35 | 4 | 273 |
| Brooks, slow flowing | 2 097 | 1 | R2, R3, R4, R5, R12,R19, R20 | 275 | 35 | 1926 |
| Canals | 18 733 | 5 | M3, M6, M7, M10 | 138 | 18 | 1003 |
| Ditches | 15 630 | 4 | M1, M2, M8 | 111 | 14 | 597 |
| Fens | 2 407 | 1 | M12, M13, M26 | 34 | 4 | 140 |
| Lakes, large | 182 866 | 51 | M21 | 11 | 1 | 118 |
| Lakes, medium | 47 195 | 13 | M14, M20, M27 | 57 | 7 | 525 |
| Lakes, small, peat | 6 697 | 2 | M25 | 3 | 0.4 | 13 |
| Lakes, small, sand clay | 19 803 | 6 | M11, M16, M22 | 23 | 3 | 159 |
| Rivers | 23 369 | 7 | R6, R7, R8, R16 | 71 | 9 | 623 |
| Water in riverine area | 10 569 | 3 | M5 | 2 | 0.3 | 6 |
| Total | 329 538 | | | 779 | | 5544 |

[8] This concerned WFD water-types with codes K (coastal), O (transitional)

[9] Brackish waters concern WFD water types M30 (0.3 to 3 g Cl/L), M31 (3-10 g Cl/L) and M32 (>10 g Cl/L).

## 6.3 Representativity of selected samples

The geographical distribution of the selected samples is given in Figure 5, which shows that the selected samples do not have a homogeneous coverage of The Netherlands. The main reason is that not all waterboards measured the hardness. A distribution of available data over sampling authorities is given in Appendix 8.

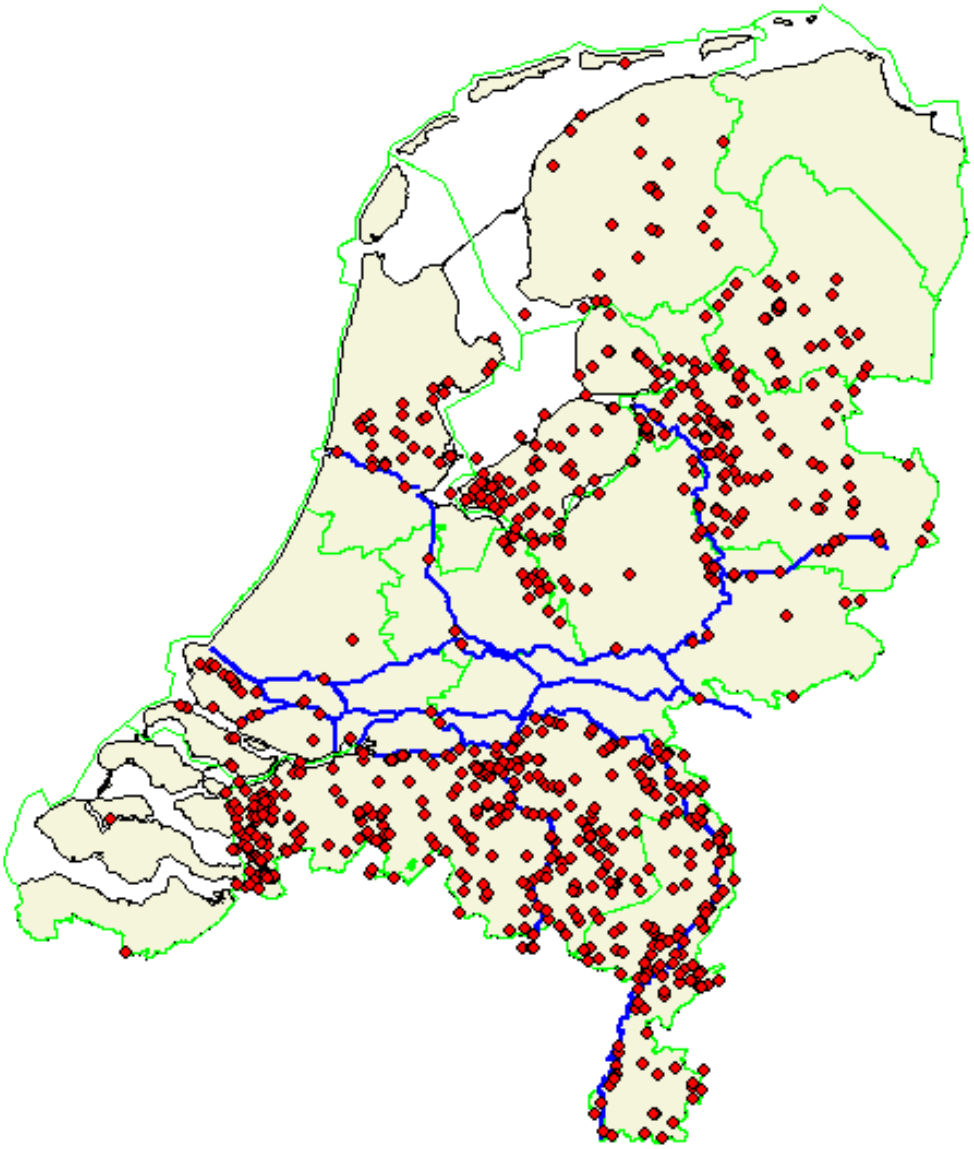

*Figure 5 Geographical distribution of selected data. Provincial borders are indicated green.*

Table 7 shows that the selected freshwater records represent all the major types of fresh waters: rivers, canals, ditches, fens and several types of lakes and brooks. However, the number of samples for each water type is not correlated with the relative surface area of the water types in The Netherlands. It appears that especially brooks are overrepresented in the data selection, whereas large lakes are underrepresented in the selected dataset. An overrepresentation of vulnerable, or highly diverse water types is however justifiable from an ecological point of view.

## 6.4 Water chemistry

The variation of water chemistry parameters used for the calculation of water type-specific quality criteria is shown in Box-Whisker plots in Figure 6. Probability distribution plots are provided in Appendix 8. The boxes represent the 25 to 75$^{th}$ percentile of the data and the bold lines represent the median values. The whisker represents the values within 3x the standard deviation, that is the 99.7 percentile interval assuming a Gaussian distribution. Data outside the whiskers are considered as outliers. The 5$^{th}$ and 95$^{th}$ percentiles of the water chemistry parameters, as well as the percentage of sites and samples with DOC, pH or hardness outside the applicability domain of the MLRs, are summarized in Table 8.

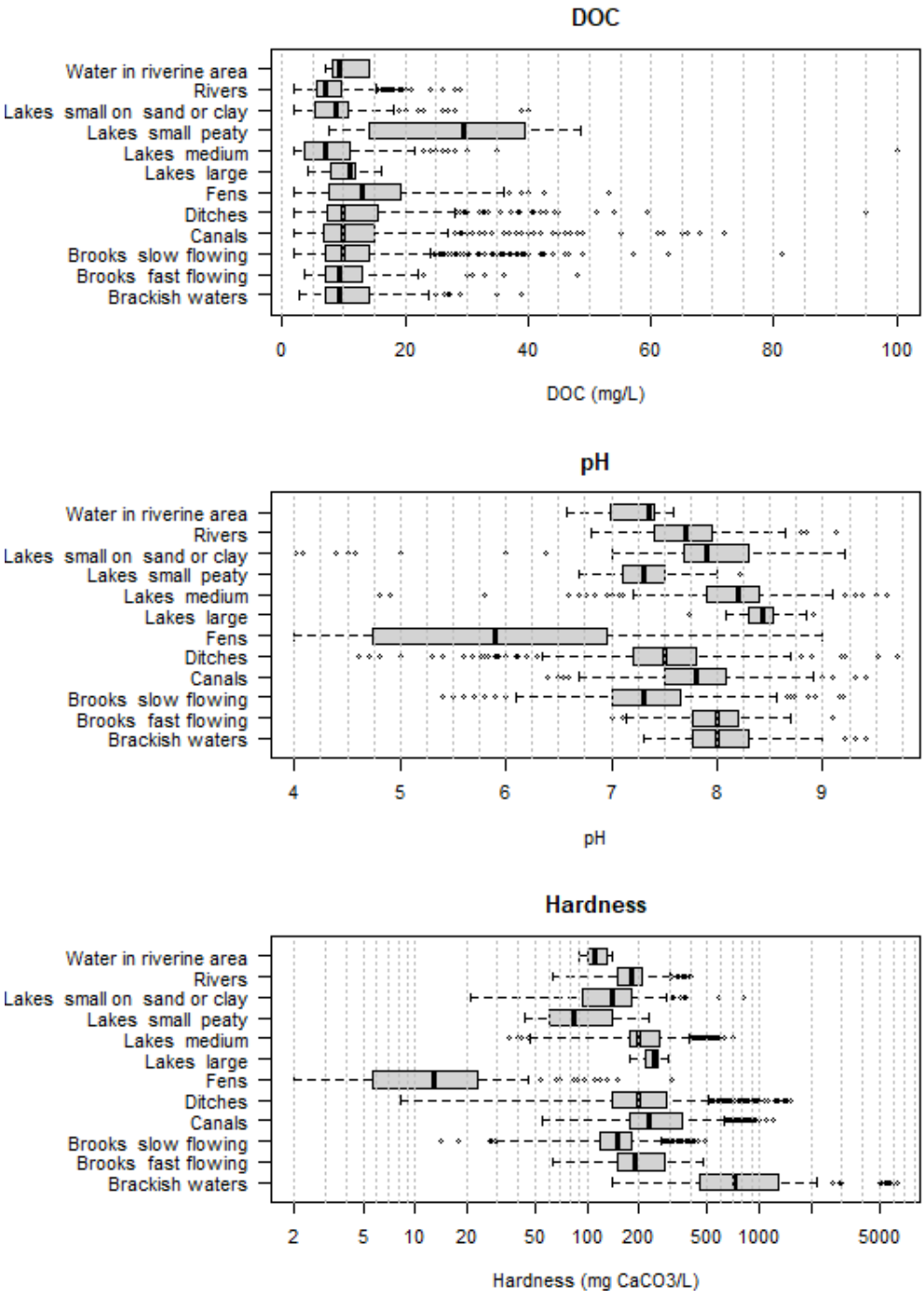


*Figure 6 Box-Whisker plots that show the variation of water chemistry parameters used for calculation of water type-specific quality criteria of aluminum.*

Figure 6 shows a few water types that are clearly distinct from the other water types, which implies that calculated $HC_5$ values for these waters will also likely be different from other water types. These concern:

- fens, which can be characterized by relatively low pH and low hardness;
- small peaty lakes, with relatively high DOC;
- brackish water, with relatively high hardness.

These water types also have a large proportion of samples with pH, DOC and/or hardness values outside the applicability domain of the MLRs. The boundaries of the applicability domain are most frequently exceeded by DOC (30% of the samples), followed by hardness and pH (each 4% of the samples). Overall 37% of the samples are outside the applicability domain of the MLRs (see Table 8).

*Table 8 .Overview of water chemistry conditions for different water types. (OAD = Outside of Applicability Domain of the MLRs). Parameter values are derived from annual mean values per sampling site.*

| **Water type** | **DOC (mg/L)** | | | | | **pH** | | | | | **Hardness (mg $CaCO_3$/L)** | | | | | **Overall** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50p | 5p | 95p | Sites OAD | Samples OAD | 50p | 5p | 95p | Sites OAD | Samples OAD | Median, | 5p | 95p | Sites OAD | Samples OAD | Sites OAD | Samples OAD |
| Brackish waters | 9.2 | 5.2 | 22.3 | 21% | 33% | 8.0 | 7.8 | 8.5 | 5% | 7% | 552 | 360 | 3099 | 89% | 75% | 100% | 89% |
| Brooks fast flowing | 10.4 | 6.2 | 30.3 | 23% | 26% | 8.0 | 7.6 | 8.3 | 3% | 0% | 187 | 90 | 323 | 0% | 1% | 26% | 28% |
| Brooks slow flowing | 10.5 | 5.1 | 11.6 | 32% | 32% | 7.4 | 6.6 | 7.7 | 0% | 1% | 155 | 103 | 198 | 0% | 0% | 32% | 33% |
| Canals | 11.1 | 3.8 | 26.0 | 41% | 37% | 7.8 | 7.3 | 8.2 | 0% | 1% | 206 | 121 | 563 | 11% | 17% | 48% | 48% |
| Ditches | 11.0 | 5.1 | 28.1 | 44% | 39% | 7.6 | 6.7 | 7.1 | 2% | 4% | 209 | 103 | 836 | 19% | 16% | 53% | 49% |
| Fens | 14.7 | 4.6 | 31.1 | 65% | 54% | 5.8 | 4.3 | 7.8 | 59% | 53% | 13.2 | 4.0 | 112 | 44% | 41% | 85% | 85% |
| Lakes large | 11.7 | 6.0 | 12.7 | 27% | 19% | 8.3 | 8.3 | 8.6 | 0% | 5% | 204 | 204 | 262 | 0% | 0% | 27% | 23% |
| Lakes medium | 8.1 | 2.6 | 17.5 | 30% | 19% | 8.2 | 7.4 | 8.5 | 4% | 9% | 198 | 95 | 384 | 4% | 7% | 35% | 32% |
| Lakes small peaty | 19.2 | 8.7 | 37.8 | 67% | 85% | 7.9 | 7.2 | 8.1 | 0% | 0% | 194 | 75 | 226 | 0% | 0% | 67% | 85% |
| Lakes small on sand or clay | 9.1 | 5.3 | 19.9 | 17% | 16% | 7.8 | 7.2 | 8.4 | 4% | 6% | 166 | 69 | 281 | 4% | 1% | 26% | 21% |
| Rivers | 8.3 | 4.8 | 14.3 | 10% | 12% | 7.7 | 7.2 | 8.2 | 0% | 1% | 187 | 127 | 237 | 0% | 0% | 10% | 13% |
| Water in riverine area | 10.6 | 9.8 | 11.4 | 0% | 33% | 7.2 | 7.1 | 7.3 | 0% | 0% | 115 | 110 | 119 | 0% | 0% | 0% | 33% |
| Overall (emperical) | 10.2 | 4.1 | 23.2 | 33% | 30% | 7.6 | 6.5 | 8.3 | 4% | 4% | 181 | 49 | 525 | 9% | 4% | 40% | 37% |
| Overall (area-weighted) | 11.1 | 2.0 | 27.0 | | | 8.2 | 6.7 | 8.6 | | | 200 | 70 | 1615 | | | | |

## 6.5 $HC_5$ of water types in Waterkwaliteitsportaal 2020

An overview of the variation of acute and chronic $HC_5$ values is shown in Figure 7. In Appendix 10 the probability distributions of the $HC_5$ values are provided.

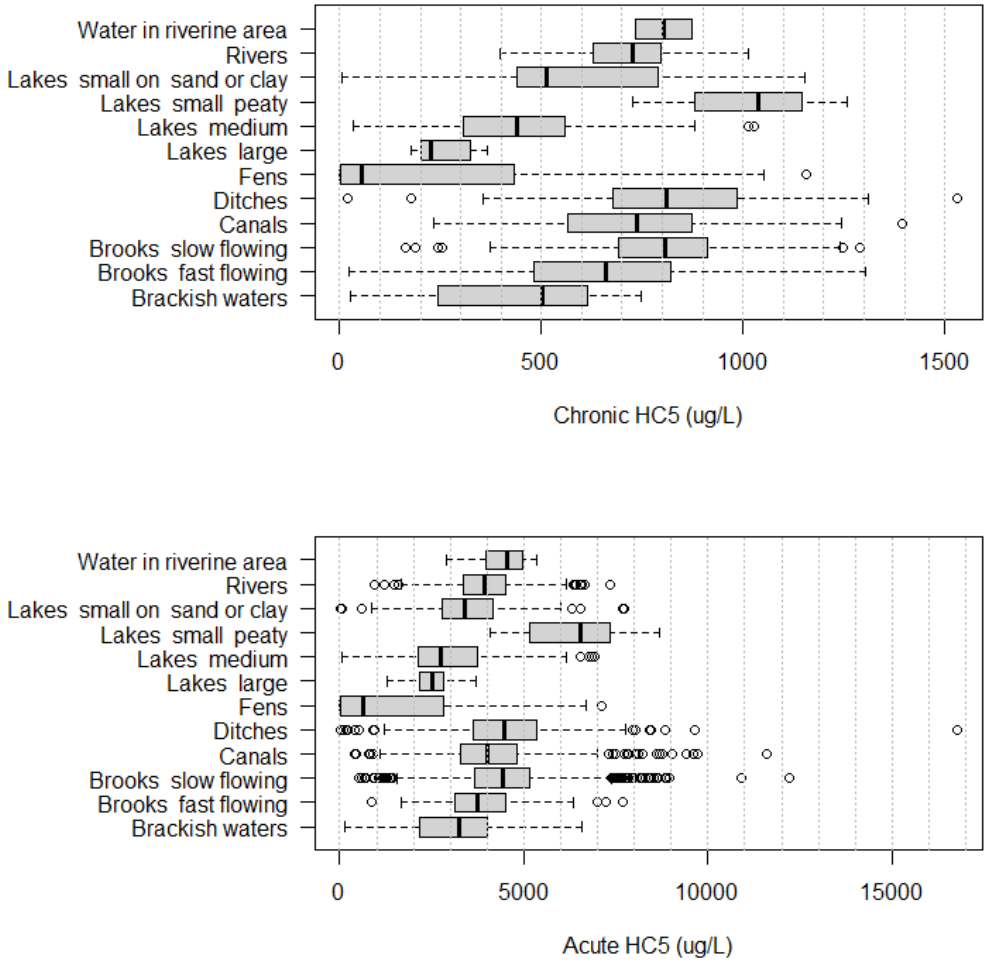


*Figure 7 Variation of chronic and acute water type-specific HC5 values (µg/L).*

Although the distributions of DOC and hardness tend to be log-normally distributed (see Appendix 9), the calculated $HC_5$ appears to be normally distributed in most of the water types. Therefore, the 5$^{th}$ percentile $HC_5$ for each water type was derived from the normal distribution curve, except for fens. In fens the distribution is skewed towards higher $HC_5$ values. Because neither a log-normal distribution nor a normal distribution seemed to be appropriate (see Appendix 10), an empirical 5$^{th}$ percentile was derived for fens.

In Table 9, the 5$^{th}$-percentile $HC_5$ for each water type is listed. In Table 9A, the 5$^{th}$ percentile is derived from all data (from all water samples), whereas in Table 9B the 5$^{th}$ percentile is derived from samples with pH, DOC and hardness within the applicability domain of the MLRs.

*Table 9 $HC_5$ values (fitted 5th percentile for each watertype) compared with the cumulative area the HC5 would protect. The $HC_5$ that protects 95% of the surface water area is estimated by interpolation of the two HC5 values that are closest to the 95% area.*

Table A: Based on all samples

| Chronic | 5p-$HC_5$ (µg Al/L) | area% | Acute | 5p-$HC_5$ (µg Al/L) | area% |
|---|---|---|---|---|---|
| Fens | 0.04 | 100.0% | Fens | 0.9 | 100% |
| Brackish waters | 53 | 99% | Brackish waters | 792 | 99% |
| Lakes, medium | 92 | 92% | Lakes, medium | 929 | 92% |
| Lakes, large | 126 | 78% | Lakes, large | 1234 | 78% |
| Lakes, small, sand clay | 139 | 27% | Lakes, small, sand clay | 1585 | 27% |
| Brooks, fast flowing | 214 | 22% | Brooks, fast flowing | 1854 | 22% |
| Canals | 331 | 22% | Canals | 1880 | 22% |
| Ditches | 388 | 16% | Ditches | 1921 | 16% |
| Rivers | 452 | 12% | Rivers | 2332 | 12% |
| Brooks, slow flowing | 464 | 5% | Brooks, slow flowing | 2324 | 5% |
| Lakes, small, peat | 534 | 5% | Lakes, small, peat | 2876 | 5% |
| Water in riverine area | 605 | 3% | Water in riverine area | 3874 | 3% |
| Area weighted 95th percentile | 75 | | Area weighted 95th percentile | 870 | |

Table B: Based on samples within the applicability domain of the MLRs

| Chronic | 5p-$HC_5$ (µg Al/L) | area% | Acute | 5p-$HC_5$ (µg Al/L) | area% |
|---|---|---|---|---|---|
| Brackish waters | -[1] | 100% | Fens | 1005 | 100% |
| Lakes, medium | 122 | 92% | Lakes, medium | 1341 | 99% |
| Lakes, large | 125 | 79% | Brackish waters | 1548 | 86% |
| Fens | 151 | 28% | Lakes, large | 1753 | 78% |
| Lakes, small, sand clay | 221 | 27% | Lakes, small, sand clay | 1935 | 27% |
| Brooks, fast flowing | 295 | 22% | Brooks, fast flowing | 2062 | 22% |
| Canals | 395 | 22% | Canals | 2233 | 22% |
| Ditches | 414 | 16% | Ditches | 2470 | 16% |
| Rivers | 446 | 12% | Brooks, slow flowing | 2494 | 12% |
| Brooks, slow flowing | 457 | 5% | Rivers | 2503 | 11% |
| Water in riverine area | 534 | 5% | Water in riverine area | 2631 | 5% |
| Lakes, small, peat | 605 | 3% | Lakes, small, peat | 3283 | 2% |
| Area weighted 95th percentile | 98 | | Area weighted 95th percentile | 1408 | |

[1] Not enough data within the applicability domain, HC5 was adopted from Table A (All data).

Most striking are the low $HC_5$ values computed for fens (see Table 9). Note that the majority of the samples of these water types were outside the applicability domain of the MLR. The low pH in fens (53% of the samples has a pH<6) results in a high bioavailability of $Al^{3+}$ and thus a higher predicted toxicity of aluminum in this water type. Because the MLRs were not validated to pH<6 the $HC_5$ values are more uncertain than $HC_5$ values at pH>6.

The relatively low $HC_5$ in brackish water is surprising at a first glance, because on average the samples have a high hardness and pH, which is thought to be a mitigating factor for metal toxicity. The low $HC_5$ values appear to correspond with those samples in the brackish water type with relatively low hardness values (<20 mg $CaCO_3$/L) and a pH<6.

Small peaty lakes also have a high percentage of samples outside the applicability domain, in this water type it concerns high DOC, leading to relatively high $HC_5$ values.

Removal of samples outside the applicability domain of the MLRs results in elimination of extremely low and extremely high $HC_5$ values. For Brackish water and Water in riverine area, the remaining dataset was too small to derive a $5^{th}$ percentile. In those case the $5^{th}$ percentile of the whole dataset (including samples outside the applicability domain was tentatively adopted).

## 6.6 $HC_5$ of a specific set of water chemistry conditions

The MLRs describe a combined effect of DOC, pH and hardness on the $HC_5$. For the establishment of a specific set of water chemistry conditions the overall relation between each individual parameter and the $HC_5$ is visualized in Figure 8.

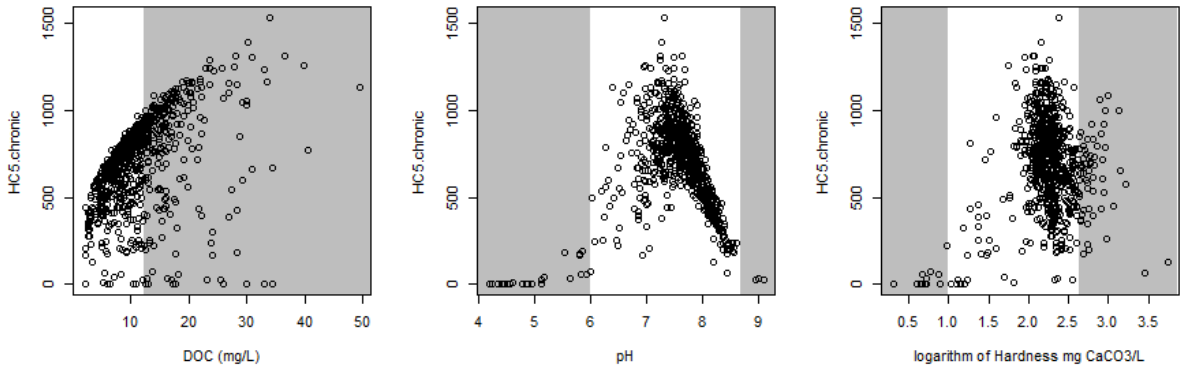


*Figure 8 Relation between pH, DOC and hardness with the HC5. The range outside the applicability domain is shaded grey.*

High toxicity (low $HC_5$) is observed particularly at relatively low and relatively high pH; at pH values outside the applicability domain. There is a general tendency of increasing $HC_5$ with increasing DOC, but occasionally low $HC_5$ also occur at high DOC. The effect of hardness is not very dominant, however, the lowest $HC_5$ values always coincide with lower hardness. These patterns do not necessarily imply a causal relationship, because a low hardness often coincides with a low pH (see Figure 9).

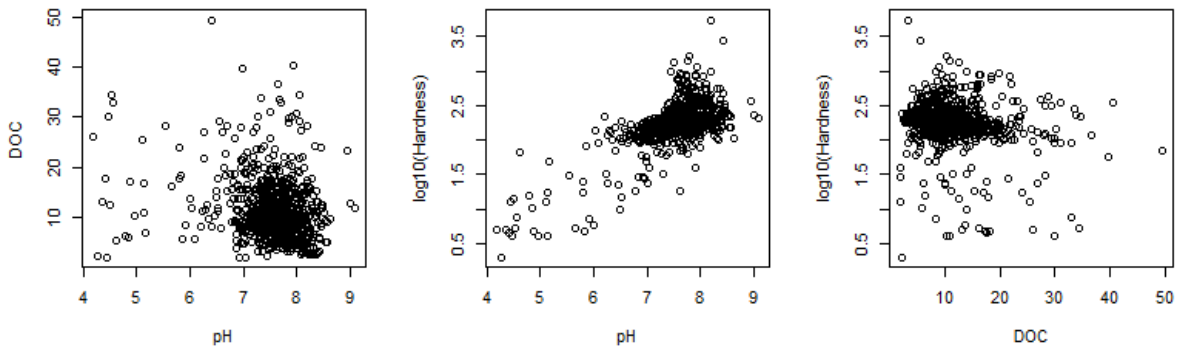


*Figure 9 Correlation between pH, DOC and hardness.*

A specific set of water chemistry conditions - one that is reflective of high bioavailability conditions, as EU guideline no. 27 suggests, could therefore be one with either a low or a high pH and relative low DOC and hardness. Several options are described:

a) Worst case, based on the 5th percentile DOC and hardness and the $5^{th}$ and $95^{th}$ percentile pH of the most vulnerable water type (fens);
b) Area-weighted 5th percentile DOC and hardness and the $5^{th}$ and $95^{th}$ percentile pH off all water types;
c) Boundaries of the applicability domain.

In Table 10 the options are applied and corresponding $HC_5$ values are shown. It appears that at the upper boundaries of the pH-range, higher $HC_5$ values were calculated than at the lower boundaries. This implies that there is no need to define an upper boundary for the pH, because the low $HC_5$ values at the lower pH also protect water types with a higher pH.

*Table 10 Options for choosing a specific set of water chemistry parameters for derivation of an aluminum EQS.*

| Option | DOC (mg/L) | hardness (mg $CaCO_3$/L) | pH | $HC_5$ (µg/L) | |
|---|---|---|---|---|---|
| | | | | chronic | acute |
| $5^{th}$ percentile of the most vulnerable water type | 4.6 | 4.0 | 4.3<br>7.8 | 0.002<br>478 | 0.07<br>3393 |
| Area weighted $5^{th}$ percentile of all water types | 2.6 | 70 | 6.7<br>8.6 | 202<br>281 | 1455<br>2186 |
| Applicability domain | 0.08 | 9.8 | 6.0<br>8.7 | 3<br>131 | 17<br>722 |

## 6.7 $HC_5$ of water types previously used for the copper-EQS

EQS values for copper were set in 2012, based on bioavailability calculations in 6 Dutch water types: 1) large rivers, 2) canals, large and small lakes, 3) sandy springs, 4) streams and brooks, 5) ditches and 6) small acidic ponds. For each water type a range of pH and DOC and other parameters (Ca, Mg, Na, K, alkalinity, sulphate and chloride) were available. The data were assumed to be normally distributed, which enabled the calculation of a range of $HC_5$ values for each water type (Vijver et al., 2008). The $5^{th}$ percentile $HC_5$ of the most sensitive national water type was adopted as first tier EQS in The Netherlands (RIVM, 2012). In the case of Cu, sandy springs appeared to be the most sensitive water type.

Monitoring data for the copper assessment originate from approximately 2006 or previous years. Since then, monitoring frequency and parameters have changed, DOC and hardness for example are nowadays more frequently measured and on more places than 15 years ago. Sets of simultaneously measured parameters can now be selected, which will provide a more precise insight in the variability of the corresponding $HC_5$ values. Moreover, the assignment of water types is nowadays recorded well for each individual sampling point in Waterkwaliteitsportaal.nl, which facilitates the selection and characterization of a larger variety of water types. Nevertheless, the water types used for copper, were used as a reference.

Average water chemistry of these water types were published and could be used to compute average $HC_5$ values for aluminum also. Vijver et al. (2008) determined that the data were normally distributed, so the $5^{th}$ percentile for DOC and pH could be derived from the mean and the standard deviation (see Appendix 11). The $5^{th}$ percentile hardness for these water types was not available and was estimated from data in Waterkwaliteitsportaal 2020. The results are shown in Table 11.

*Table 11 Calculated HC5 for water types previously used for the derivation of the Cu-EQS. The $5^{th}$ percentiles DOC and pH were calculated from normally distributed DOC and pH data (Vijver et al., 2008), the $5^{th}$ percentiles hardness was estimated from the data in Waterkwaliteitsportaal.*

| $5^{th}$ percentile: | 5p-DOC | 5p-pH | 5p-Hardness | $HC_5$ (µg/L) | |
|---|---|---|---|---|---|
| | mg/L | - | mg $CaCO_3$/L | chronic | acute |
| Large rivers | 1.6 | 7.4 | 129 | 310 | 2002 |
| Canals, large lakes, small lakes | 1.2 | 7.4 | 95 | 256 | 1691 |
| Streams, brooks | 11.1 | 7.2 | 89 | 746 | 4486 |
| Ditches | 7.4 | 5.6 | 103 | 188 | 1074 |
| Sandy springs | 0.6 | 6.5 | 65 | 61 | 471 |
| Small acid ponds | 10.1 | 3.8 | 4 | 0.0003 | 0.02 |

# 7 Options for aquatic environmental quality standards of aluminum

## 7.1 Assessment factor

For the translation of a $HC_5$ to a QS an assessment factor must be applied (see paragraph 3.1). A default assessment factor (AF) of 5 is recommended if $HC_5$-values are derived from an SSD (EU technical guidance No. 27).

The environmental quality standard is then: QS= $HC_5$/AF

An AF of 5 is used by default, but may be reduced where substantial evidence removes residual uncertainty. The exact value of the AF depends on an evaluation of the uncertainties around the derivation of the $HC_5$. As a minimum, the following points have to be considered when determining the size of the assessment factor (ECHA, 2008):

1. the overall quality of the database and the endpoints covered, e.g., if all the data are generated from "true" chronic studies (e.g., covering all sensitive life stages);
2. the diversity and representativity of the taxonomic groups covered by the database, and the extent to which differences in the life forms, feeding strategies and trophic levels of the organisms are represented;
3. the knowledge on presumed mode of action of the chemical (covering also long-term exposure); Details on justification could be referenced from structurally similar substances with established mode of action;
4. statistical uncertainties around the $HC_5$ estimate, e.g. reflected in the goodness of fit or the size of the confidence interval around the $5^{th}$ percentile, and consideration of different levels of confidence (e.g., by a comparison between the median estimate of the $HC_5$ with the lower estimate (90% confidence interval) of the $HC_5$);
5. the comparisons between field and mesocosm studies, where available, and the $HC_5$ and mesocosm/field studies to evaluate the level of agreement between laboratory and field evidence.

The aluminum SSD contains plenty of data from eight different taxonomic groups for at least ten different species. However, many species are only present with one experimental data. Also, still there are some residual uncertainties that need to be accounted for, these uncertainties are described in Table 12. Based on the evaluation of the assessment factor criteria we recommend to keep the default assessment factor of 5.

*Table 12 Evaluation of HC5 assessment factor criteria for aluminum.*

| | |
|---|---|
| 1 True chronic studies were used for the SSD | No |
| 2 High diversity of species and taxonomic groups is present | Reasonable number of species are available, but the cross-species extrapolation is not validated. |
| 3 Mode of action of the aluminum toxicity | Potential toxicity of Al-hydroxide suspended particles not fully understood. |
| 4 Uncertainty around $HC_5$ | Relatively high uncertainty in normalized $EC_{10}$, because many samples were outside applicability domain of MLR |
| 5 Presence of mesocosms or field studies | No |

## 7.2 Summary of options

The previous paragraphs offered an assessment of the variability of $HC_5$ values in Dutch fresh surface waters. The ultimate establishment of regulatory EQS values for aluminum should be made by policy makers. To support the decision making process this paragraph offers four options (see Table 13), based on choices and recommendations in other projects:

1) In the EU guidance document no 27 on Derivation of environmental quality standards it is stated that: "The $HC_5$ selected should be protective of 95% of waters in the region (country) shown to have the highest bioavailability of that particular metal. By definition, it will also be protective of almost all other waters in Europe within the validated range of the BLMs." Based on 5544 samples in Waterkwaliteitsportaal.nl, the highest bioavailability was found in fens.
2) In the EU guidance document no 27 on Derivation of environmental quality standards it is stated that: "The $EQS_{bioavailable}$ is a total dissolved metal concentration which is highly bioavailable and which does not make any allowance for background in its derivation. It is derived, initially, as the normalized, estimate of the $HC_5$ for a specific set of water chemistry conditions - one that is reflective of high bioavailability conditions." Several potential reference sets are presented (see Table 13). Note that in the case of aluminum also a fraction of suspended Al-hydroxide particles are included in the EQS.
3) For comparison purposes the water types previously defined for derivation of a copper $HC_5$ in 2012 were used. The most vulnerable water type for copper was "sandy springs". For aluminum the most vulnerable water type is "small acid ponds" which is synonymous to "fens."
4) The US-EPA has recommended to always compute water type-specific quality standards for aluminum, that account for differences in bioavailability. Therefore they do not provide a QS. Given the high frequency of false exceedances when conservative Al-QS are used, the EPA-recommendation of always applying a bioavailability correction could be considered in The Netherlands as well.

The four options for $QS_{bioavailable}$ are presented in Table 13.

*Table 13 Options for Environmental Quality standards of aluminum in freshwater, using an assessment factor of 5. Rounding of the $QS_{bioavailable}$ is recommended, but not applied in this table. (DOC expressed in mg/L, hardness in mg $CaCO_3$/L). $HC_5$ are accompanied by the 90% confidence intervals (90% CI).*

| | Options for selection $HC_5$: | Chronic HC5 (µg/L) | | Acute HC5 (µg/L) | | $QS_{bioavailable}$ (µg/L) | |
|---|---|---|---|---|---|---|---|
| | | $HC_5$ | (90% CI) | $HC_5$ | (90% CI) | AA | MAC |
| **1** | **most vulnerable water type (Waterkwaliteitsportaal, 2020)** | | | | | | |
| a | 5th percentile of all (Fen) samples | 0.04 | (0.002-0.2) | 0.9 | (0.1-3.4) | 0.008 | 0.18 |
| b | 5th percentile of (Lakes, medium) samples within applicability domain | 122 | (18-394) | 1005 | (440-1799) | 24 | 201 |
| **2** | **specific set of water chemistry conditions (Waterkwaliteitsportaal, 2020)** | | | | | | |
| a | 5th percentiles of chemistry of most vulnerable water type (valid for DOC≥4.6, pH≥4.3, hardness≥4) | 0.008 | (0.005-0.05) | 0.2 | (0.03-0.7) | 0.002 | 0.04 |
| b | Area weighted 5th percentiles of chemistry of all water-types (valid for DOC≥2.6, pH≥6.7, hardness≥70) | 202 | (54-457) | 1455 | (638-2599) | 40 | 291 |
| c | Boundaries of the applicability domain (valid for DOC>0.08, pH between 6-8.7, hardness>9.8 ) | 2.8 | (0.9-5.8) | 17 | (7.4-32) | 0.6 | 3.5 |
| **3** | **water type from copper assessment, 2012** | | | | | | |
| | Most vulnerable water type for aluminum: small acid ponds | 0.0003 | (0.00001-0.03) | 0.02 | (0.002-0.1) | 0.00006 | 0.004 |
| **4** | **case-by-case bioavailability correction** | Use tools | | Use tools | | Use tools | |

AA=Annual Average; MAC=Maximum Acceptable Concentration

## 7.3 Tentative implications for the risk assessment

### 7.3.1 A fixed $QS_{bioavailable}$ for all watertypes (options 1, 2 and 3)

QS values were compared with total aluminum in filtered and unfiltered samples present in the database of Waterkwaliteitsportaal.nl. The assessment just provides a first impression of ranges of potential risks.

Aluminum concentrations were either determined in samples after filtration, or in unfiltered samples, that include suspended matter. The first method would underestimate potential risks, because bioavailable Al-hydroxide particulates are removed, whereas the second method could overestimate potential risks because tightly bound aluminum in natural suspended matter is included. The preferred method of analysis is described in Appendix 1. A summary of sample numbers, frequency of concentrations below the limit of detection (LOD) and concentration ranges is provided in Appendix 12. For range finding purposes, the best (filtered) and worst (unfiltered) case concentrations are compared with potential EQS-values. The AA-EQS values of option 1a, 2a and 3 are far below the lowest measured concentrations and could therefore not be shown in Figure 10. The EQS values of options 1b, 2b and 2c (all derived for samples within the applicability domain of the MLRs) are within the range of measured concentrations, drawn in Figure 10. Similar results are found for the preliminary assessment of exceedances of a potential MAC-EQS (see Figure 11).

Usually the LOD of aluminum in natural surface waters was 50 µg Al/L, but LOD values of 1, 2, 10 or 20 µg Al/L were also encountered. Concentrations <LOD were divided by 2, to combine these data in calculation with samples with concentrations larger than the LOD. Brooks (fast and slow flowing), canals, ditches, small peaty lakes and water in riverine have a median concentration below the LOD of 50 µg/L. Some options for the QS are lower that the LOD, which means that analytical methods need improvement.

Chronic risk assessment

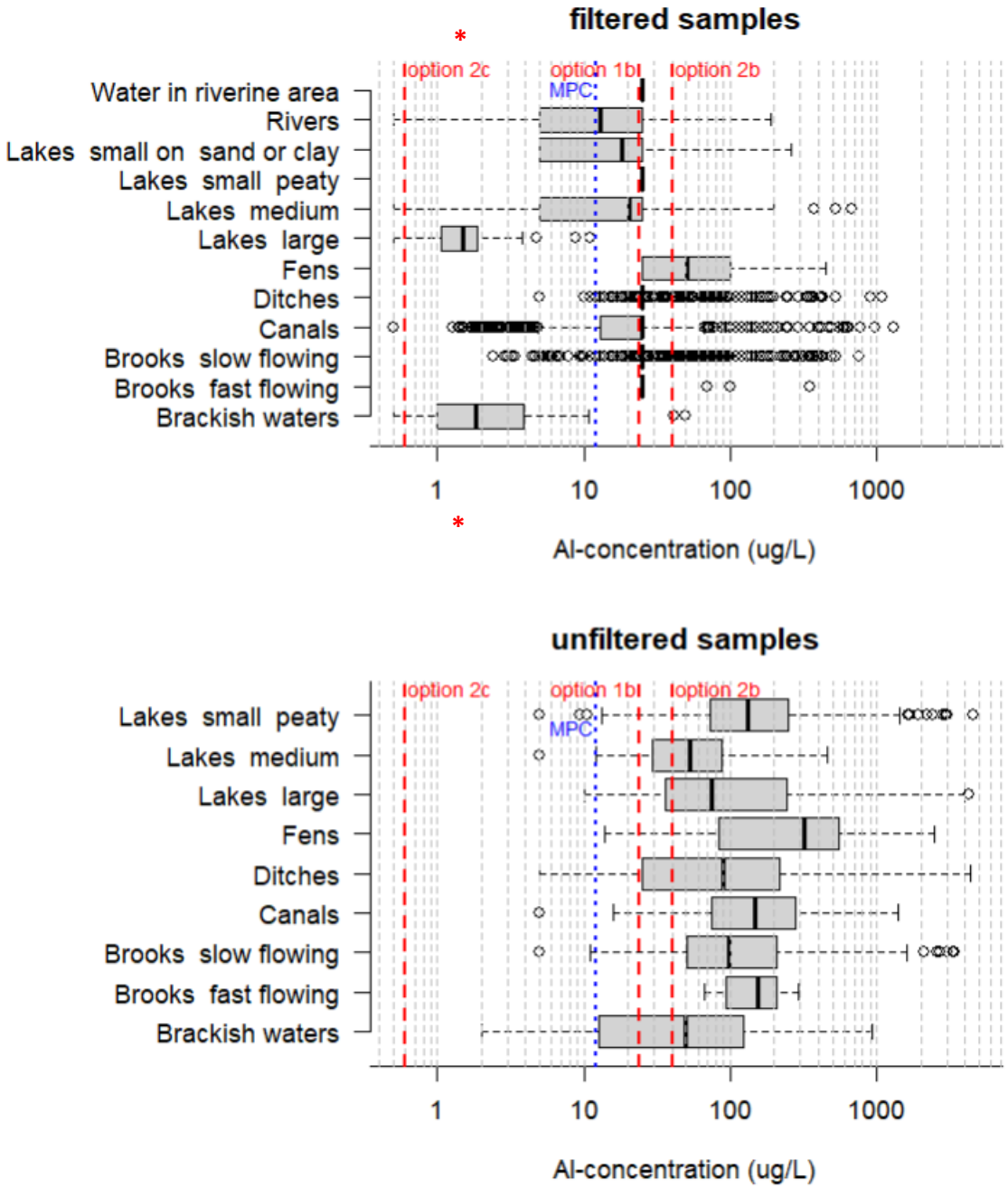


*Figure 10 Variation of the aluminum concentration in filtered and unfiltered samples, compared with some options of* ***chronic*** *$QS_{bioavailable}$ values. The red lines are tentatively drawn as a preliminary comparison of current Al-concentrations with some options for $QS_{bioavailable}$-values, the blue line shows the current MPC. MPC Options 1a, 2a and 3 are too small to show in the graphs. See text in this chapter for comments about the method for sampling and analysis of aluminum.*

Acute risk assessment

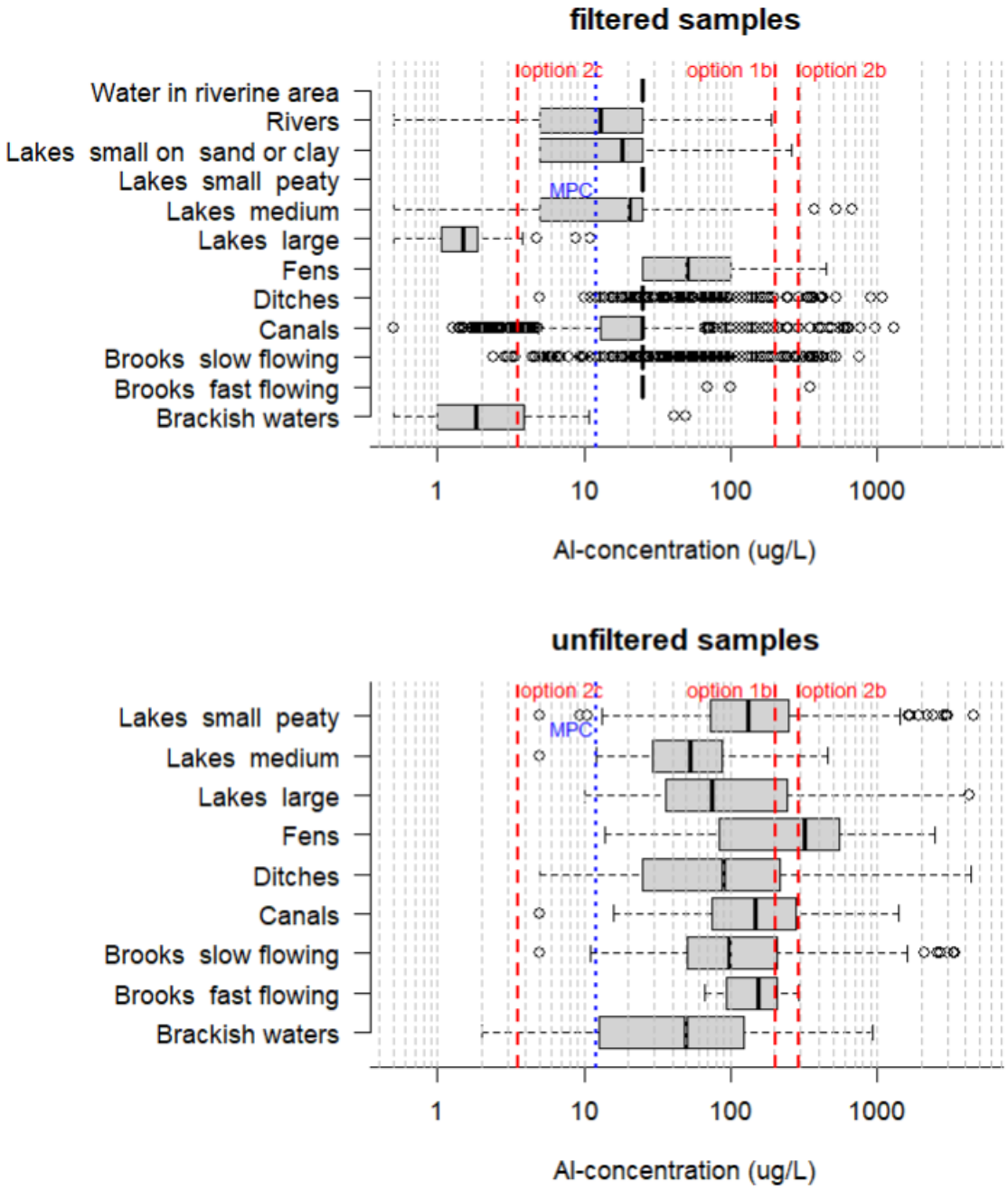


*Figure 11 Variation of the aluminum concentration in filtered and unfiltered samples, compared with some options of* ***acute*** *$QS_{bioavailable}$ values. The red lines are tentatively drawn as a preliminary comparison of current Al-concentrations with some options for $QS_{bioavailable}$-values, the blue line shows the current MPC. MPC Options 1a, 2a and 3 are too small to show in the graphs. See text in this chapter for comments about the method for sampling and analysis of aluminum.*

### 7.3.2 A site-specific $QS_{bioavailable}$ (option 4)

In Figure 12 and Figure 13 the results of respectively a chronic and acute site-specific risk assessment are shown. Exact percentages of samples exceeding the QS are not reported because the current measurements are not done according to the preferred method of analysis, as mentioned above. The comparison is only presented as a preliminary impression of the range of predicted impact.

Application of a site-specific risk assessment on the currently available measurements confirms that mainly fens seem to be at risk; chronic as well as acute $QS_{bioavailable}$ are exceeded in the filtered samples. Not all watertypes have aluminum measurements in unfiltered samples. For the nine watertypes which do have unfiltered samples, the chronic risk ratio≈1, except for large lakes, where the risk seems to be a factor 10 higher. Acute risks for unfiltered samples are generally below 1, although data for the most vulnerable water type (fens) are missing.

<u>Chronic risk ratios:</u>

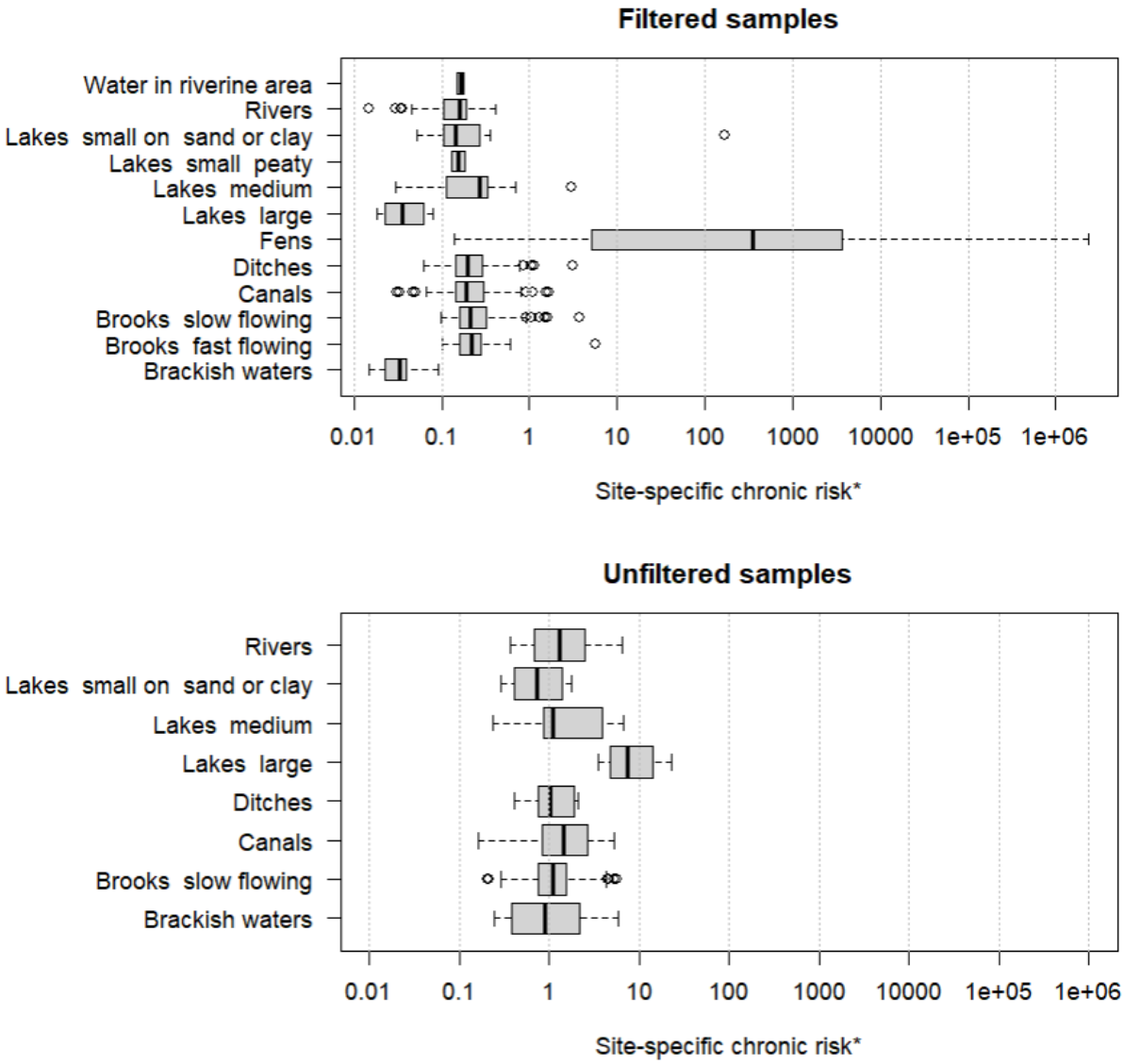


*Figure 12 Chronic risk ratios for aluminum in different water types based on measurements in filtered and unfiltered samples. * emphasizes the uncertainties related to the analytical method.*

Acute risk ratios:

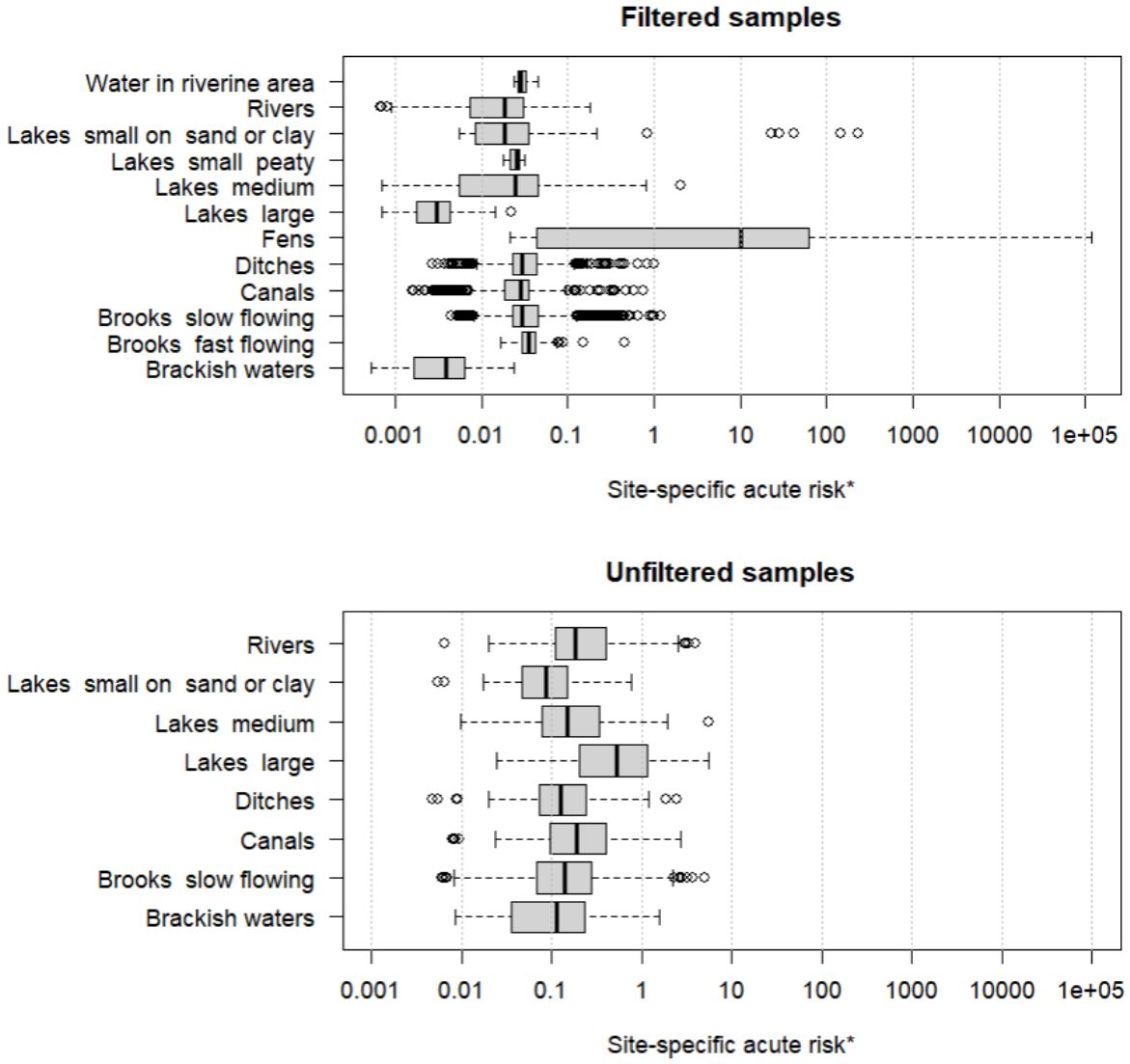


*Figure 13 Acute risk ratios for aluminum in different water types based on measurements in filtered and unfiltered samples. * emphasizes the uncertainties related to the analytical method.*

# 8 Summary

Although aluminum is the most abundant metal on earth, their concentrations in surface water are usually low because aluminum is tightly captured in the mineral structure of rocks and clay minerals. Caused by soil acidification (by air pollution), mining activity and anthropogenic uses, aluminum is mobilized and can enter surface waters. Effects of aluminum on aquatic ecosystems show huge differences between water types. Differences in pH, dissolved organic carbon (DOC) and hardness determine whether aluminum occurs as toxic and bioavailable $Al^{3+}$ or less toxic and less bioavailable Al-complexes or Al-hydroxide particulates. In the past five years, calculation tools have been developed in the US to calculate $HC_5$ values that account for differences of Al-toxicity amongst water types. The tools use multiple linear regression equations (MLR) derived from toxicity experiments with water types with pH between 6-8.7, DOC between 0.08 and 12.3 mg/L and hardness between 9.8 and 430 mg $CaCO_3$/L. The range in pH, DOC and hardness in the toxicity experiments determines the applicability domain of the MLRs. The goal of this report was to evaluate the compliance of the methodology with EU-guidance and if possible to calculate EQS for Dutch water types.

The US-EPA methodology and data (based on work of DeForest et al., 2018 and 2020) have been well described, but the existing EPA-tools do not comply with European methodology. The US-EPA chronic assessment is based on $EC_{20}$ values, whereas in Europe $EC_{10}$ or NOEC values are required. Furthermore, data and MLRs for algae are not included in the USE-EPA tool and the statistical methods to derive an $HC_5$ value from a species sensitivity distribution (SSD) are different. However, the $EC_{10}$ values and algal data necessary to adjust the methodology in accordance with EU-guidance are available. We pragmatically relied on the EPA quality standards for the evaluation of toxicity studies; these are more or less compliant with European quality standards. An additional literature search to check the completeness of the data described in the EPA report was outside the scope of this study.

MLRs were used to extrapolate (normalize) the $EC_{10}$ values obtained in test media with specific pH, DOC and hardness to conditions of an outdoor sample or water type of interest. MLRs are available for the algae *Pseudokirchneriella subcapitata,* crustacean *Ceriodaphnia dubia* and fish *Pimephales promelas.* Toxicity data for other 10 species are available to construct an SSD. These toxicity data were normalized with either one of the three MLRs depending of the trophic level of the test organism.

The adjustments were implemented in an Excel tool and an R-script to compute water type-specific $HC_5$ values according to European guidelines. The tools flag samples that fall outside the applicability domain of the MLRs. $HC_5$ values estimated for samples outside the applicability domain are not eliminated, but are considered less reliable.

Environmental quality criteria are values set by policy makers, ideally based on a sound scientific assessment of environmental vulnerabilities and risks. In the EU-guidance no. 27 it is stated that: “The $HC_5$ selected should be protective of 95% of waters in the region (country) shown to have the highest bioavailability of that particular metal.” Often (but not necessarily) a specific set of water chemistry conditions reflecting a high bioavailability is used to derive $HC_5$ values. In this report $HC_5$ values were derived based on: 1) monitoring data of 5544 samples of 779 sites retrieved from archives of Waterkwaliteitsportaal.nl 2020, 2) a specific set of water chemistry conditions, and 3) water types previously used for derivation of a copper $HC_5$ in 2012.

In the case of aluminum, water type-specific $HC_5$ values may differ six orders of magnitude, and therefore, the US have decided not to set a specific EQS, but always demand a site-specific risk assessment by the use of their calculation tool. This report does not decide upon the desired level of protection and the feasibility of certain choices. In order to offer options for Dutch policy makers,

different EQS values are presented, obviously with different levels of protection. An assessment factor, reflecting remaining uncertainties in the toxicity data, needs to be considered when $HC_5$ is translated to EQS. There was no reason to lower the default assessment factor of 5 (recommended by EU guidance no 27), because: 1) some species only are represented by one experimental measurement; 2) there are no field or mesocosm studies available; 3) no validation to natural waters was described; 4) no cross-species validation of the used MLRs was described and 5) many water samples are outside the applicability domain of the MLRs.

For future compliance testing it is important that the analytical method for aluminum in the samples matches the bioavailability of aluminum that is reflected by the EQS. A mild acidification to pH4 prior to filtration over a 0.45 μm filter is recommended. However, currently samples are acidified to pH1-2, either prior to or after filtration. The first method overestimates bioavailable Al, because it releases aluminum from natural suspended matter, whereas the second method removes Al-hydroxide particulates that are assumed to contribute to Al-toxicity. Despite these shortcomings, it is clear that $HC_5$ values derived for the most vulnerable water type (fens), as recommended by EU-guidance no.27 are exceeded by Al-concentrations on all water types, regardless of the analytical method. Application of a site-specific risk assessment tentatively shows that fens are at risk, due to a combination of relatively high Al-concentrations and low $HC_5$ values. The risks for other water types seem to be limited.

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# Appendix 1 Method to analyze bioavailable aluminum in natural surface water

The method of Rodriguez et al.(2019) consists of the preparation of a 1M sodium acetate buffer (2.449 g sodium acetate and 4.7 mL acetic acid, completed to 100 mL with deionized water in a volumetric flask) to achieve a pH of 4 in the buffer. If the prepared buffer is outside a range of 4.0 to 4.2, add acetic acid in µL aliquots to bring the pH within the target range. Polypropylene cups with 25 mL of the test solution are titrated to pH 4 (pH 4.0–4.2) by addition of 1M buffer and poured into 50 - mL conical polypropylene tubes. The tubes are capped, agitated by hand, and mixed for 3 h at 22 °C at 100 rpm in an orbital shaker. After incubation, tubes are agitated again by hand, and 10 mL of the supernatant is filtered through a 0.45 - µm PVDF membrane (Millex - HV; Merck - Millipore). Samples are acidified to 1% nitric acid (Merck Suprapur) and stored at 4 °C until analysis (by ICP - MS). Buffer reagents were Merck, pro analysis grade.

# Appendix 2 Chronic toxicity data for MLR development

Table S1. Chronic aluminum toxicity data for *C. dubia. (DeForest et al.2020)*

| Dataset[1] | Endpoint | Duration | DOC (mg/L) | pH | Hardness (mg/L) | EC10 (µg/L) | (95% CI) | EC20 (µg/L) | (95% CI) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Previous | Reproduction | 7 d | 0.1 | 6.92 | 9.8 | 66 | (4-1211) | 124 | (12-1259) | CIMM 2009 |
| Previous | Reproduction | 7 d | 0.1 | 7.84 | 9.8 | 312 | (83-1174) | 379 | (141-1020) | CIMM 2009 |
| Previous | Reproduction | 7 d | 0.1 | 6.34 | 25 | 20 | (11-37) | 37 | (22-62) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 0.1 | 6.4 | 60 | 104 | (74 - 146) | 160 | (123 - 209) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 0.1 | 6.38 | 121 | 143 | (52 - 393) | 222 | (105 - 466) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 2 | 6.34 | 25 | 284 | (93 - 868) | 377 | (159 - 895) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 2 | 6.38 | 61 | 504 | (226 - 1126) | 631 | (362 - 1101) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 2 | 6.37 | 121 | 924 | (548 - 1558) | 1012 | (692 - 1479) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 4 | 6.33 | 25 | 494 | (396 - 616) | 623 | (532 - 729) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 4 | 6.3 | 61 | 550 | (436 - 694) | 693 | (618 - 777) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 4 | 6.38 | 121 | 671 | (603 - 747) | 841 | (773 - 914) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 0.3 | 7.15 | 50 | 1659 | (na) | 1780 | (na) | McCauley et al. 1986[2] |
| Previous | Reproduction | 7 d | 0.3 | 7.61 | 51 | 306 | (111-844) | 426 | (249-727) | McCauley et al. 1986[2] |
| Previous | Reproduction | 7 d | 2 | 6.37 | 25 | 281 | (191 - 414) | 353 | (268 - 465) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 2 | 6.34 | 25 | 411 | (357 - 472) | 452 | (401 - 511) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 2 | 6.35 | 25 | 348 | (200 - 495) | 440 | (357 - 523) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 0.5 | 6.34 | 26 | 210 | (80 - 250) | 260 | (170 - 310) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 0.5 | 6.36 | 122 | 300 | (60 - 360) | 390 | (170 - 450) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 0.5 | 7 | 26 | 190 | (90 - 320) | 250 | (150 - 340) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 0.5 | 7.1 | 123 | 620 | (120 - 860) | 860 | (590 - 1090) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 0.5 | 8 | 25 | 630 | (270 - 690) | 700 | (510 - 830) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 0.5 | 8 | 62 | 770 | (450 - 1030) | 1010 | (740 - 1180) | Gensemer et al. 2018 |
| Previous | Reproduction | 7 d | 0.5 | 8.1 | 123 | 680 | (600 - 1020) | 870 | (710 - 1130) | Gensemer et al. 2018 |
| New | Reproduction | 7 d | 1.87 | 6.42 | 64 | 679 | (299-1542)) | 829 | (437-1572) | Supplemental Data 1 |
| New | Reproduction | 7 d | 8.71 | 6.33 | 133 | 3494 | (na) | 3829 | (na) | Supplemental Data 1 |
| New | Reproduction | 7 d | 12.30 | 6.40 | 138 | 5628 | (3206-9880) | 6224 | (3866-10022) | Supplemental Data 1 |
| New | Reproduction | 7 d | 1.64 | 6.30 | 428 | 1467 | (969-2222) | 2011 | (1539-2628) | Supplemental Data 1 |
| New | Reproduction | 7 d | 6.57 | 7.21 | 125 | 4909 | (2695-8941) | 6401 | (4274-9588) | Supplemental Data 1 |
| New | Reproduction | 7 d | 12.01 | 7.19 | 127 | 6106 | (na) | 6612 | (na) | Supplemental Data 1 |
| New | Reproduction | 7 d | 1.30 | 8.17 | 263 | 2297 | (1575-3350) | 3749 | (2904-4838) | Supplemental Data 1 |
| New | Reproduction | 7 d | 1.20 | 8.21 | 425 | 2045 | (946-4422) | 2852 | (1647-4939) | Supplemental Data 1 |
| New | Reproduction | 7 d | 1.04 | 8.70 | 125 | 1514 | (na) | 1693 | (na) | Supplemental Data 1 |

CI = confidence interval; na = indeterminable [1] "Previous" data were used to develop the *C. dubia* MLR models described in DeForest et al. (2018).. [2] "Previous" and "New" data were combined to develop the *P. promelas* MLR models described in DeForest et al.(2020)..

Table S2. Chronic aluminum toxicity data for *P. promelas*. (DeForest et al., 2020)

| Dataset[1] | Endpoint | Duration | DOC (mg/L) | pH | Hardness (mg/L) | EC10 (µg/L) | (95% CI) | EC20 (µg/L) | (95% CI) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Previous | Mean Dry Biomass | 7 d | 0.3 | 8 | 48 | 5117 | (274-95679) | 10,753 | (1458-79301) | Parametrix 2009 |
| Previous | Mean Dry Biomass | 7 d | 0.08 | 6 | 10.6 | 117 | (na) | 127 | (na) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 0.19 | 6.1 | 25.8 | 93 | (62-140) | 136 | (98-188) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 0.22 | 6 | 60.8 | 241 | (156-372) | 314 | (200-495) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 0.09 | 6 | 123.9 | 481 | (280-828) | 624 | (410-951) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 0.92 | 6.1 | 10.2 | 403 | (372-436) | 426 | (402-451) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 0.86 | 6.1 | 61 | 582 | (273-1240) | 634 | (338-1190) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 0.88 | 6.1 | 123.7 | 627 | (433-907) | 773 | (559-1070) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 1.73 | 6.1 | 10.6 | 589 | (436-797) | 633 | (497-805) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 1.74 | 6 | 59.9 | 1238 | (999-1535) | 1326 | (1119-1571) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 1.56 | 6 | 118.2 | 1388 | (997-1933) | 1494 | (1116-1999) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 3.35 | 6 | 11.8 | 726 | (548-963) | 829 | (691-995) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 3.51 | 6 | 64.8 | 2164 | (1841-2543) | 2523 | (1971-3230) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 3.27 | 6 | 119.6 | 2729 | (2048-3636) | 2938 | (2288-3772) | Gensemer et al. 2018 |
| Previous | Larval Survival | 33 d | 0.3 | 6 | 93.9 | 389 | (na) | 429 | (na) | Cardwell et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 0.7 | 6.1 | 25.9 | 562 | (269-1173) | 660 | (364-1197) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 0.9 | 6 | 116 | 627 | (228-1728) | 824 | (393-1729) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 2.9 | 6.1 | 122 | 1762 | (1195-2596) | 2210 | (1640-2978) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 0.8 | 7.1 | 26.5 | 985 | (516-1882) | 1534 | (932-2522) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 2.5 | 7 | 123 | 3979 | (1994-7940) | 5411 | (3144-9313) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 0.7 | 8 | 28.8 | 4662 | (2400-9057) | 7262 | (4714-11187) | Gensemer et al. 2018 |
| Previous | Mean Dry Biomass | 7 d | 5 | 7.9 | 127 | 4672 | (1552-14069) | 6795 | (3161-14607) | Gensemer et al. 2018 |
| New | Mean Dry Biomass | 7 d | 7.0 | 6.04 | 134 | 3611 | (2345-5560) | 4618 | (3281-6499) | Supplemental Data 2 |
| New | Mean Dry Biomass | 7 d | 11.5 | 6.04 | 131 | 8375 | (6074-11549) | 9511 | (7291-12408) | Supplemental Data 2 |
| New | Mean Dry Biomass | 7 d | 1.1 | 6.82 | 422 | 1648 | (947-2868) | 2969 | (2010-4386) | Supplemental Data 2 |
| New | Mean Dry Biomass | 7 d | 7.2 | 7 | 135 | 6164 | (4199-9049) | 8047 | (6273-10322) | Supplemental Data 2 |
| New | Mean Dry Biomass | 7 d | 11.6 | 6.96 | 125 | 11064 | (5057-24206) | 12542 | (6598-23842) | Supplemental Data 2 |
| New | Mean Dry Biomass | 7 d | 1.1 | 8.06 | 288 | 3031 | (642-14310) | 5634 | (1768-17957) | Supplemental Data 2 |
| New | Mean Dry Biomass | 7 d | 1.6 | 8.12 | 396 | 7363 | (2646-20489) | 13274 | (6674-26401) | Supplemental Data 2 |
| New | Mean Dry Biomass | 7 d | 0.8 | 6.1 | 49 | 560 | (322-974) | 885 | (574-1365) | Supplemental Data 3 |
| New | Mean Dry Biomass | 7 d | 1.6 | 6 | 94 | 1424 | (1028-1974) | 1817 | (1444-2287) | Supplemental Data 3 |

CI = confidence interval; na = indeterminable

[1] "Previous" data were used to develop the *P. promelas* MLR models described in DeForest et al. (2018). "Previous" and "New" data were combined to develop the *P. promelas* MLR models described in DeForest et al.(2020).

Table S3. Chronic aluminum toxicity data for *P. kirchneriella*. (Gensemer et al., 2017)

| Endpoint | $EC_{10}$ (µg Al/L) | (95% CI) | $EC_{20}$ (µg Al/L) | (95% CI) | $EC_{50}$ (µg Al/L) | (95% CI) | Test type | Test Duration | Temp °C | pH | Hardness (mg/L) | DOC (mg/L) | Measured Al |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Growth Rate | 146.0 | (92 - 231) | 285.0 | (224 - 362) | > 504 | | static | 72 h | 24 | 6.21 | 24.3 | 0.3 | X |
| Growth Rate | 206.7 | (121 - 353) | 455.3 | (298 - 696) | > 982 | | static | 72 h | 24 | 6.19 | 60 | 0.3 | X |
| Growth Rate | 407.5 | (194 - 856) | 657.3 | (381 - 1135) | 1697.3 | (1284 - 2243) | static | 72 h | 24 | 6.17 | 120 | 0.3 | X |
| Growth Rate | 1263.1 | (857 - 1862) | 1474.4 | (1213 - 1792) | 2004.0 | (1833 - 2191) | static | 72 h | 24 | 7.02 | 24.3 | 0.3 | X |
| Growth Rate | 1036.0 | (0.977 - 1.10) | 1342.4 | (1234 - 1460) | 2244.0 | (1857 - 2713) | static | 72 h | 24 | 6.99 | 60 | 0.3 | X |
| Growth Rate | 1194.5 | (944 - 1512) | 1577.4 | (1329 - 1873) | 2739.0 | (2463 - 3045) | static | 72 h | 24 | 6.97 | 120 | 0.3 | X |
| Growth Rate | 1083.0 | (631 - 1860) | 1593.4 | (1188 - 2138) | 3428.0 | (2456 - 4785) | static | 72 h | 24 | 8.05 | 24.3 | 0.3 | X |
| Growth Rate | 950.1 | (809 - 1115) | 1345.0 | (1190 - 1520) | 2681.0 | (2446 - 2938) | static | 72 h | 24 | 8.01 | 60 | 0.3 | X |
| Growth Rate | 533.1 | (331 - 860) | 858.3 | (613 - 1202) | 2208.0 | (1667 - 2924) | static | 72 h | 24 | 8.00 | 120 | 0.3 | X |
| Growth Rate | 291.7 | (78 - 1093) | 496.0 | (200 - 1229) | 1422.1 | (623 - 3248) | static | 72 h | 23 | 6.22 | 23 | 2 | |
| Growth Rate | 850.5 | (673 - 1075) | 1021.6 | (852 - 1225) | 1469.7 | (1253 - 1725) | static | 72 h | 23 | 6.18 | 60 | 2 | |
| Growth Rate | 1397.1 | (1118 - 1653) | 1611.6 | (1447 - 1795) | 2140.0 | (2002 - 2287) | static | 72 h | 23 | 6.14 | 120 | 2 | |
| Growth Rate | 2062.0 | (1721 - 2471) | 2336.0 | (2014 - 2708) | 2990.0 | (2376 - 3764) | static | 72 h | 23 | 7 | 22 | 2 | |
| Growth Rate | 1970.6 | (1765 - 2200 | 2459.0 | (2209 - 2739) | > 3200 | | static | 72 h | 23 | 7.01 | 60 | 2 | |
| Growth Rate | 2429.0 | (1833 - 3220) | 2805.0 | (2304 - 3417) | 3733.0 | (3524 - 3954) | static | 72 h | 23 | 7.03 | 120 | 2 | |
| Growth Rate | 1570.4 | (1358 - 1816) | 2308.0 | (2080 - 2560) | 4952.0 | (4561 - 5377) | static | 72 h | 23 | 7.89 | 22 | 2 | |
| Growth Rate | 773.9 | (582 - 1029) | 1094.2 | (909 - 1317) | 2175.0 | (1924 - 2460) | static | 72 h | 23 | 7.92 | 60 | 2 | |
| Growth Rate | 672.6 | (601 - 752) | 901.9 | (847 - 960) | 1614.4 | (1502 - 1736) | static | 72 h | 23 | 7.96 | 120 | 2 | |
| Growth Rate | 583.6 | (395 - 863) | 836.1 | (606 - 1154) | 1706.6 | (953 - 3057) | static | 72 h | 23 | 6.16 | 23 | 4 | |
| Growth Rate | 1100.7 | (927 - 1307) | 1356.6 | (1191 - 1545) | 2054.0 | (1699 - 2483) | static | 72 h | 23 | 6.16 | 60 | 4 | |
| Growth Rate | 1239.8 | (1119 - 1374) | 1532.4 | (1345 - 1746) | 2333.0 | (1852 - 2939) | static | 72 h | 23 | 6.18 | 120 | 4 | |
| Growth Rate | 2478 | (2011 - 3053) | 2865.0 | (2452 - 3348) | 3824.0 | (3469 - 4214) | static | 72 h | 23 | 7.05 | 22 | 4 | |
| Growth Rate | 2655 | (2277 - 3096) | 3119.0 | (2806 - 3468) | 4295.0 | (4123 - 4474) | static | 72 h | 23 | 7.03 | 60 | 4 | |
| Growth Rate | 3154 | (2953 - 3369) | 3633.0 | (3478 - 3795) | 4810.0 | (4603 - 5026) | static | 72 h | 23 | 7.05 | 120 | 4 | |
| Growth Rate | 1342.9 | (769 - 2344) | 2108.0 | (1453 - 3060) | > 5000 | | static | 72 h | 23 | 7.91 | 22 | 4 | |
| Growth Rate | 1508.7 | (1355 - 1680) | 1896.8 | (1668 - 2157) | 2987.0 | (2105 - 4238) | static | 72 h | 23 | 7.83 | 60 | 4 | |
| Growth Rate | 1085.8 | (1028 - 1147) | 1472.4 | (1256 - 1726) | 2694.0 | (1849 - 3927) | static | 72 h | 23 | 7.79 | 120 | 4 | |

CI = confidence interval; na = indeterminable

# Appendix 3: MLR-models

In bold: Models used in Dutch tool. Ref 1 and DF2018 refer to DeForest et al., 2018, Ref 2 and DF2020 refer to DeForest et al., 2020.

All EC10 models published

| Species | Endpoint Statistic | Model Variables | Model Statistic | Intercept | ln(DOC) | ln(Hard) | pH | $pH^2$ | ln(DOC)× pH | ln(Hard)× pH | ln(DOC)× ln(Hard) | Adj. R2 | Ref | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P. subcapitata | EC10 | No interactions | AIC | 4.372 | 0.349 | | 0.346 | | | | | 0.312 | 1 | |
| P. subcapitata | EC10 | | BIC | 4.372 | 0.349 | | 0.346 | | | | | 0.312 | 1 | |
| **P. subcapitata** | **EC10** | **With interactions** | **AIC** | **-77.283** | **2.342** | **4.560** | **20.923** | **-1.274** | **-0.288** | **-0.628** | | **0.940** | **1,2** | **DF2018,** |
| P. subcapitata | EC10 | | BIC | -77.283 | 2.342 | 4.560 | 20.923 | -1.274 | -0.288 | -0.628 | | 0.940 | 1 | |
| P. subcapitata | EC10 | Exclude DOC×pH | AIC | -72.032 | 0.313 | 4.435 | 19.564 | -1.187 | | -0.610 | | 0.830 | 1 | |
| P. subcapitata | EC10 | | BIC | -72.032 | 0.313 | 4.435 | 19.564 | -1.187 | | -0.610 | | 0.830 | 1 | |
| P. subcapitata | EC10 | pooled | not published | | | | | | | | | 0.354 | 2 | |
| | | | | | | | | | | | | | | |
| C. dubia | EC10 | No interactions | AIC | -1.311 | 0.536 | 0.375 | 0.871 | | | | | 0.658 | 1 | |
| C. dubia | EC10 | | BIC | -1.311 | 0.536 | 0.375 | 0.871 | | | | | 0.658 | 1 | |
| C. dubia | EC10 | With interactions | AIC | -31.740 | 5.891 | 0.441 | 9.889 | -0.674 | -0.716 | | -0.183 | 0.731 | 1 | |
| C. dubia | EC10 | | BIC | -31.740 | 5.891 | 0.441 | 9.889 | -0.674 | -0.716 | | -0.183 | 0.731 | 1 | |
| C. dubia | EC10 | Exclude DOC×pH | AIC | -51.420 | 1.208 | 3.639 | 13.338 | -0.737 | ex | -0.490 | -0.155 | 0.719 | 1 | DF2018 |
| C. dubia | EC10 | | BIC | -11.054 | 0.571 | 2.900 | 2.282 | | ex | -0.365 | | 0.685 | 1 | |
| **C. dubia (update)** | **EC10** | **With interactions** | **AIC** | **-32.273** | **0.697** | **2.768** | **6.089** | **-0.269** | | **-0.335** | | **0.929** | **2** | |
| C. dubia (update) | EC10 | | BIC | -11.824 | 0.700 | 3.030 | 2.368 | | | -0.375 | | 0.925 | 2 | |
| C. dubia (update) | EC20 | | | ? | 0.597 | 2.089 | 8.802 | -0.491 | | -0.230 | | ? | 3 | EPA |
| C. dubia (update) | EC10 | exclude $pH^2$ | AIC | -11.824 | 0.700 | 3.030 | 2.368 | ex | | -0.375 | | 0.925 | 2 | |
| C. dubia (update) | EC10 | | BIC | -11.824 | 0.700 | 3.030 | 2.368 | ex | | -0.375 | | 0.925 | 2 | |
| C. dubia (update) | EC10 | exclude $pH^2$, DOC×hardness | AIC | -11.824 | 0.700 | 3.030 | 2.368 | ex | | -0.375 | ex | 0.925 | 2 | |
| C. dubia (update) | EC10 | | BIC | -11.824 | 0.700 | 3.030 | 2.368 | ex | | -0.375 | ex | 0.925 | 2 | |
| C. dubia (update) | EC10 | pooled | - | -8.474 | 0.660 | 2.216 | 1.969 | | | -0.278 | | 0.895 | 2 | DF2020 |
| | | | | | | | | | | | | | | |
| P. promelas | EC10 | No interactions | AIC | -0.696 | 0.495 | 0.354 | 0.966 | | | | | 0.822 | 1 | |
| P. promelas | EC10 | | BIC | -0.696 | 0.495 | 0.354 | 0.966 | | | | | 0.822 | 1 | |
| P. promelas | EC10 | With interactions | AIC | -0.741 | 2.758 | 0.412 | 0.941 | | -0.360 | | | 0.889 | 1 | |
| P. promelas | EC10 | | BIC | -0.741 | 2.758 | 0.412 | 0.941 | | -0.360 | | | 0.889 | 1 | |
| P. promelas | EC10 | Exclude DOC×pH | AIC | -12.009 | 0.545 | 3.201 | 2.794 | | ex | -0.460 | | 0.850 | 1 | DF2018 |
| P. promelas | EC10 | | BIC | -12.009 | 0.545 | 3.201 | 2.794 | | ex | -0.460 | | 0.850 | 1 | |
| **P. promelas (update)** | **EC10** | **With interactions** | **AIC and BIC** | **-6.700** | **1.828** | **1.914** | **1.932** | | **-0.193** | **-0.248** | | **0.898** | **2** | |
| P. promelas (update) | EC20 | | AIC and BIC | -7.371 | 2.209 | 1.862 | 2.041 | | -0.261 | -0.232 | | 0.923 | 2 | EPA |
| P. promelas (update) | EC10 | pooled | - | -7.424 | 0.660 | 2.216 | 1.969 | | | -0.278 | | 0.833 | 2 | DF2020 |

Additional notes on model selection:

In order to prevent overparameterization of the models, DeForest et al.(2018 and 2020) compared which parameters were retained or rejected in the MLRs following different statistical approaches.

For *P. subcapitata* they compared models with and without interaction terms and concluded that interaction terms are necessary. By inclusion of all four interaction terms, the $pH^2$ term, hardness × pH and DOC × pH were retained and the adjusted $r^2$ increased from 0.312 to 0.940. No clear pattern in the residuals was observed and all predicted $EC_{10}$ values were within a factor 2 of observed (DeForest et al.2018).

For *C. dubia* inclusion of an interaction hardness × DOC is probably not necessary. It appeared that this interaction term was selected in the $EC_{20}$ models but not in the $EC_{10}$ models, whereas consistency is expected between $EC_{10}$ and $EC_{20}$ models. Also there were doubts about the necessity to include the $pH^2$ term, because it was not consistently selected in both AIC and BIC-models. The inconsistency sμggests that it was an unnecessary term in the models where it was retained. Also the variance inflation factors suggested that there was (not surprisingly) severe collinearity between pH and $pH^2$. So, the only interaction term in the *C. dubia* is hardness × pH.

Whereas the interaction DOC × pH is not included in the *C. dubia* model, they were retained in the *P. promelas* models. DeForest et al.(2020) questioned the need to include this term because it appeared to have no substantial influence on the model performance (adjusted $r^2$ dropped from 0.923 to 0.903 in the *P. promelas* $EC_{20}$ models. Still, the model, including the DOC × pH was put forward as the final selected model, which was adopted for the Dutch approach too.

# Appendix 4: Chronic toxicity database for calculation of water type-specific aluminum criteria.

| species | endpoint | test | DOC | pH | H | EC10 | Reference |
|---|---|---|---|---|---|---|---|
| **BLM: *P. subcapitata*_ With interactions** | | | | | | | |
| *Lemna minor* (duckweed) | Total Dry Weight | 7 d | 0.3 | 6.05 | 52 | 2175 | Cardwell et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 2.0 | 6.22 | 23 | 292 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 2.0 | 6.18 | 60 | 851 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 2.0 | 6.14 | 120 | 1397 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 2.0 | 7.00 | 22 | 2062 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 2.0 | 7.01 | 60 | 1971 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 2.0 | 7.03 | 120 | 2429 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 2.0 | 7.89 | 22 | 1570 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 2.0 | 7.92 | 60 | 774 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 2.0 | 7.96 | 120 | 673 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 4.0 | 6.16 | 23 | 584 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 4.0 | 6.16 | 60 | 1101 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 4.0 | 6.18 | 120 | 1240 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 4.0 | 7.05 | 22 | 2478 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 4.0 | 7.03 | 60 | 2655 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 4.0 | 7.05 | 120 | 3154 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 4.0 | 7.91 | 22 | 1343 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 4.0 | 7.83 | 60 | 1509 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 4.0 | 7.79 | 120 | 1086 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 0.3 | 6.21 | 24 | 146 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 0.3 | 6.19 | 61 | 207 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 0.3 | 6.17 | 121 | 408 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 0.3 | 7.00 | 22 | 1263 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 0.3 | 7.00 | 60 | 1036 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 0.3 | 7.00 | 120 | 1195 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 0.3 | 8.00 | 22.4 | 1083 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 0.3 | 8.00 | 60 | 950 | Gensemer et al. 2018 |
| *Pseudokirchnerella subcapitata* | Growth Rate | 72 h | 0.3 | 8.00 | 120 | 533 | Gensemer et al. 2018 |
| **BLM: *C. dubia* (update)_ With interactions** | | | | | | | |
| *Aeolosoma sp* | Population Size | 17 d | 0.3 | 6.00 | 50 | 988 | Cardwell et al. 2018 |
| *Brachionus calyciflorus* | # Individuals | 48 h | 0.3 | 6.30 | 100 | 304 | Cardwell et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 0.1 | 6.92 | 9.8 | 66 | CIMM 2009 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 0.1 | 7.84 | 9.8 | 312 | CIMM 2009 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 0.1 | 6.34 | 25 | 20 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 0.1 | 6.40 | 60 | 104 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 0.1 | 6.38 | 121 | 143 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 2.0 | 6.34 | 25 | 284 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 2.0 | 6.38 | 61 | 504 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 2.0 | 6.37 | 121 | 924 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 4.0 | 6.33 | 25 | 494 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 4.0 | 6.30 | 61 | 550 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 4.0 | 6.38 | 121 | 671 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 2.0 | 6.37 | 25 | 281 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 2.0 | 6.34 | 25 | 411 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 2.0 | 6.35 | 25 | 348 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 0.5 | 6.34 | 26 | 210 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 0.5 | 6.36 | 122 | 300 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 0.5 | 7.00 | 26 | 190 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 0.5 | 7.10 | 123 | 620 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 0.5 | 8.00 | 25 | 630 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 0.5 | 8.00 | 62 | 770 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 0.5 | 8.10 | 123 | 680 | Gensemer et al. 2018 |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 1.9 | 6.42 | 64 | 679 | OSU 2018a |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 8.7 | 6.33 | 133 | 3494 | OSU 2018a |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Ceriodaphnia dubia* | Reproduction | 7 d | 12.3 | 6.40 | 138 | 5628 | OSU 2018a |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 1.6 | 6.30 | 428 | 1467 | OSU 2018a |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 6.6 | 7.21 | 125 | 4909 | OSU 2018a |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 12.0 | 7.19 | 127 | 6106 | OSU 2018a |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 1.3 | 8.17 | 263 | 2297 | OSU 2018a |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 1.2 | 8.21 | 425 | 2045 | OSU 2018a |
| *Ceriodaphnia dubia* | Reproduction | 7 d | 1.0 | 8.70 | 125 | 1514 | OSU 2018a |
| *Ceriodaphnia sp.* | Reproduction | 8 d | 0.3 | 7.15 | 50 | 1659 | Call et al. 1984 |
| *Chironomus riparius* | Reproduction | 28 | 0.5 | 6.60 | 95 | 1271 | Cardwell et al. 2018 |
| *Daphnia magna* | Reproduction | 21 d | 2.0 | 6.30 | 141 | 709 | Gensemer et al. 2018 |
| *Daphnia magna* | Reproduction | 28 d | 0.5 | 6.40 | 95 | 171 | Kimball 1978 |
| *Hyalella azteca* | Reproduction | 42 d | 0.3 | 6.04 | 106 | 272 | Cardwell et al. 2018 |
| *Hyalella azteca* | Reproduction | 42 d | 0.4 | 6.10 | 105 | 109 | Wang et al. 2018 |
| *Lampsilis siliquoidea* | Mean Dry Weight | 28 d | 0.3 | 6.10 | 113 | 861 | Wang et al. In Review |
| *Lymnaea stagnalis* | Wet Weight | 30 d | 2.0 | 6.37 | 121 | 924 | Cardwell et al. 2018 |
| | | | | | | | |
| BLM: *P. promelas* (update)_ With interactions | | | | | | | |
| Danio rerio (zebrafish) | Biomass | 33 d | 0.3 | 6.100 | 77 | 98 | Cardwell et al. 2018 |
| Pimephales promelas | Larval Survival | 33 d | 0.3 | 600 | 93.9 | 389 | Cardwell et al. 2018 |
| Salvelinus fontinalis | Weight | 60 d | 1.9 | 6.5 | 14.7 | 100 | Cleveland et al. 1989 |

Comment:

1) It is arguable whether it was correct to treat *Ceriodaphnia dubia* (30 data) and *Ceriodaphnia sp.* (1 data) as separate species. We combined the two sets.

## References chronic toxicity database:


Call DJ, Brooke LT, Lindberg CA, Markee TP, McCauley DJ, Poirier SH. 1984. Toxicity of aluminum to freshwater organisms in water of pH 6.5 – 8.5. Testing laboratory: Center for Lake Superior Environmental Studies. University of Wisconsin-Superior, Superior, WI, USA. Owner company: Camp Dresser and McKee, Annandale, VA, USA. Technical Report: Project No. 549-238-RT-WRD.

Cardwell AS, Adams WJ, Gensemer RW, Nordheim E, Santore RC, Ryan AC, Stubblefield WA. 2018.Chronic toxicity of aluminum, at a pH of 6, to freshwater organisms: empirical data for the development of international regulatory standards/criteria. *Environ Toxicol Chem*. 37, 36-48. https://doi.org/10.1002/etc.3901

CIMM (Chilean Mining and Metallurgy Research Center). 2009. Draft of the final report: Systematic characterization of the relationship between BLM parameters and aluminium toxicity in *Daphnia magna*, *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata*. Santiago, Chile.

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Gensemer RW, Gondek J, Rodriguez P, Arbildua JJ, Stubblefield WA, Cardwell AS, Santore RC, Ryan AC, Adams WJ, Nordheim E. 2018. Evaluating the effects of pH, hardness, and dissolved organic carbon on the toxicity of aluminum to aquatic organisms under circumneutral conditions. *Environ Toxicol Chem*.37, 49-60. https://doi.org/10.1002/etc.3920

Kimball G. 1978. The Effects of Lesser Known Metals and One Organic to Fathead Minnows (Pimephales promelas) and Daphnia magna. Manuscript, Department of Entomology, Fisheries and Wildlife, University of Minnesota, Minneapolis, MN. 88 p.

OSU 2018a, Chronic Toxicity of Aluminum to the cladoceran, *Ceriodaphnia dubia*: Expansion of the empirical database for bioavailability modeling. Oregon State University Aquatic Toxicology Laboratory. Prepared for the Aluminium REACH Consortium. June 2018. Available as Supplemental data 1 in DeForest et al.2020. https://doi.org/10.1002/etc.4796

OSU 2018b, Short-term chronic toxicity of Aluminum to the fathead minnow, *Pimephales promelas*: Expansion of the empirical database for bioavailability modeling. Oregon State University Aquatic Toxicology Laboratory. Prepared for the Aluminium REACH Consortium. Aμgust 2018**.** Available as Supplemental data 2 in DeForest et al.2020. https://doi.org/10.1002/etc.4796

OSU 2018c Short-term chronic toxicity of Aluminum to the fathead minnow, *Pimephales promelas*: Validation of Aluminum bioavailability models. Oregon State University Aquatic Toxicology Laboratory. Prepared for the Aluminium REACH Consortium. Aμgust 2018**.** Supplemental data 3 in DeForest et al.2020. https://doi.org/10.1002/etc.4796

Parametrix. 2009. Chronic toxicity of aluminium to the fathead minnow (*Pimephales promelas*) in filtered and unfiltered test solutions. Report No. 598-6012-001. Albany, Oregon, USA.

Wang N, Ivey CD, Brunson EL, Cleveland D, Ingersoll CG, Stubblefield WA, Cardwell AS.. Acute and chronic toxicity of aluminum to a unionid mussel (*Lampsilis siliquoidea*) and an amphipod (*Hyalella azteca*) in water-only exposures. *Environ Toxicol Chem*. 37:61-69. https://doi.org/10.1002/etc.3850

# Appendix 5. Acute toxicity database for calculation of water type-specific Al-criteria

| species | endpoint | test | DOC | pH | H | EC50 | Reference |
|---|---|---|---|---|---|---|---|
| **BLM: P. subcapitata_ With interactions** | | | | | | | |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 0.3 | 6.21 | 24.3 | > 504 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 0.3 | 6.19 | 60 | > 982 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 0.3 | 6.17 | 120 | 1697.3 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 0.3 | 7.02 | 24.3 | 2004 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 0.3 | 6.99 | 60 | 2244 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 0.3 | 6.97 | 120 | 2739 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 0.3 | 8.05 | 24.3 | 3428 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 0.3 | 8.01 | 60 | 2681 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 0.3 | 8 | 120 | 2208 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 2 | 6.22 | 23 | 1422.1 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 2 | 6.18 | 60 | 1469.7 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 2 | 6.14 | 120 | 2140 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 2 | 7 | 22 | 2990 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 2 | 7.01 | 60 | > 200 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 2 | 7.03 | 120 | 3733 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 2 | 7.89 | 22 | 4952 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 2 | 7.92 | 60 | 2175 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 2 | 7.96 | 120 | 1614.4 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 4 | 6.16 | 23 | 1706.6 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 4 | 6.16 | 60 | 2054 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 4 | 6.18 | 120 | 2333 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 4 | 7.05 | 22 | 3824 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 4 | 7.03 | 60 | 4295 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 4 | 7.05 | 120 | 4810 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 4 | 7.91 | 22 | > > 5000 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 4 | 7.83 | 60 | 2987 | Gensemer et al. 2018 |
| *P. subcapitata (alga)* | Growth Rate | 72 h | 4 | 7.79 | 120 | 2694 | Gensemer et al. 2018 |
| **BLM: C. dubia (update)_With interactions** | | | | | | | |
| *Nais elinguis* | | | 3.2 | 6.51 | 17.89 | 3,874 | Shuhaimi-Othman et al. 2012a, 2013 |
| *Snail, Physa sp.* | | | 1.1 | 6.59 | 47 | > 23400 | Call 1984; Call et al. 1984 |
| *Snail, Physa sp.* | | | 1.1 | 7.55 | 47 | 30600 | Call 1984; Call et al. 1984 |
| *Snail, Physa sp.* | | | 1.1 | 8.17 | 47 | > 24700 | Call 1984; Call et al. 1984 |
| *Snail, Physa sp.* | | | 1.1 | 7.46 | 47 | 55500 | Call 1984; Call et al. 1984 |
| *Melanoides tuberculata* | | | 3.2 | 6.68 | 18.72 | 68230 | Shuhaimi-Othman et al. 2012b, 2013 |
| *Lampsilis siliquoidea* | | | 0.5 | 8.19 | 107 | > 54300 | Ivey et al. 2014 |
| *Lampsilis siliquoidea* | | | 0.48 | 6.12 | 106 | > 6302 | Wang et al. 2016, 2018 |
| *Ceriodaphnia dubia* | | | 1.1 | 7.42 | 50 | 1900 | McCauley et al. 1986 |
| *Ceriodaphnia dubia* | | | 1.1 | 7.86 | 50.5 | 1500 | McCauley et al. 1986 |
| *Ceriodaphnia dubia* | | | 1.1 | 8.13 | 50 | 2560 | McCauley et al. 1986 |
| *Ceriodaphnia dubia* | | | 0.5 | 7.5 | 25 | 720 | ENSR 1992d |
| *Ceriodaphnia dubia* | | | 0.5 | 7.65 | 49 | 1880 | ENSR 1992d |
| *Ceriodaphnia dubia* | | | 0.5 | 7.9 | 95 | 2450 | ENSR 1992d |
| *Ceriodaphnia dubia* | | | 0.5 | 8.05 | 193 | > 99600 | ENSR 1992d |
| *Ceriodaphnia dubia* | | | 0.5 | 7.15 | 90 | 3727 | Fort and Stover 1995 |
| *Ceriodaphnia dubia* | | | 0.5 | 7.15 | 90 | 5673 | Fort and Stover 1995 |
| *Ceriodaphnia dubia* | | | 0.5 | 8.2 | 89 | 2880 | Soucek et al. 2001 |
| *Ceriodaphnia dubia* | | | 1.6 | 8.2 | 142 | 153440 | Griffitt et al. 2008 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.01 | 10.6 | 71.12 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 2 | 6.05 | 10.6 | 686.5 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 4 | 6.09 | 10.6 | 1558.1 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.01 | 10.6 | 68.1 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.03 | 10.6 | 163 | European aluminum Association 2009 |

| species | endpoint | test | DOC | pH | H | EC50 | Reference |
|---|---|---|---|---|---|---|---|
| *Ceriodaphnia dubia* | | | 0.5 | 5.97 | 10.6 | 193.5 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 0.5 | 5.92 | 10.6 | 141 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.99 | 10.6 | > 1300 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 0.5 | 7.85 | 10.6 | > 5000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 2 | 6.8 | 10.6 | > 10000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 2 | 7.82 | 10.6 | > 15000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 4 | 6.77 | 10.6 | > 10000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 4 | 7.66 | 10.6 | > 15000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 0.5 | 7.9 | 10.6 | > 2000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 0.5 | 7.89 | 10.6 | > 2000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.04 | 60 | 111 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 2 | 5.98 | 60 | 1137 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 4 | 5.73 | 60 | 8047 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.71 | 60 | > 10000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 0.5 | 7.83 | 60 | > 5000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 2 | 6.79 | 60 | > 10000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 2 | 7.67 | 60 | > 15000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 4 | 6.68 | 60 | > 15000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 4 | 7.62 | 60 | > 15000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 2 | 6.06 | 120 | 3387 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 4 | 5.6 | 120 | 10484 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.93 | 120 | > 5000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 0.5 | 7.88 | 120 | > 5000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 2 | 6.76 | 120 | > 15000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 2 | 7.71 | 120 | > 15000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 4 | 6.6 | 120 | > 15000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 4 | 7.6 | 120 | > 15000 | European aluminum Association 2009 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.03 | 10.6 | 120 | European aluminum Association 2010 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.03 | 10.6 | 275 | European aluminum Association 2010 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.03 | 10.6 | 119.98 | European aluminum Association 2010 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.07 | 10.6 | 92.495 | European aluminum Association 2010 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.09 | 10.6 | 313.37 | European aluminum Association 2010 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.1 | 10.6 | 332.35 | European aluminum Association 2010 |
| *Ceriodaphnia dubia* | | | 0.5 | 7.08 | 10.6 | > 886.4 | European aluminum Association 2010 |
| *Ceriodaphnia dubia* | | | 0.5 | 7.79 | 10.6 | > 4293.3 | European aluminum Association 2010 |
| *Ceriodaphnia dubia* | | | 0.5 | 7.53 | 10.6 | 132.04 | European aluminum Association 2010 |
| *Ceriodaphnia dubia* | | | 0.5 | 6.01 | 60 | 463 | European aluminum Association 2010 |
| *Ceriodaphnia dubia* | | | 0.5 | 5.99 | 60 | > 859 | European aluminum Association 2010 |
| *Ceriodaphnia reticulata* | | | 1.1 | 7.25 | 45.1 | 2800 | Shephard 1983 |
| *Ceriodaphnia reticulata* | | | 1.1 | 6 | 45.1 | 304 | Shephard 1983 |
| *Ceriodaphnia reticulata* | | | 1.1 | 5.5 | 4 | 362 | Shephard 1983 |
| *Daphnia magna* | | | 1.1 | 7.8 | 48.5 | 3900 | Biesinger and Christensen 1972 |
| *Daphnia magna* | | | 1.1 | 7.25 | 45.1 | 2800 | Shephard 1983 |
| *Daphnia magna* | | | 0.5 | 5.99 | 168 | > 500 | European aluminum Association 2009 |
| *Daphnia magna* | | | 0.5 | 6.98 | 168 | > 500 | European aluminum Association 2009 |
| *Daphnia magna* | | | 0.5 | 7.93 | 168 | > 500 | European aluminum Association 2009 |
| *Daphnia magna* | | | 0.5 | 7.92 | 168 | 795 | European aluminum Association 2009 |
| *Daphnia magna* | | | 2 | 7.95 | 168 | > 1200 | European aluminum Association 2009 |
| *Daphnia magna* | | | 3 | 7.93 | 168 | > 1200 | European aluminum Association 2009 |
| *Daphnia magna* | | | 1.6 | 7.6 | 220 | 38200 | Kimball 1978 |
| *Daphnia pulex* | | | 1.6 | 8.2 | 142 | 3650 | Griffitt et al. 2008 |
| *Stenocypris major* | | | 3.2 | 6.51 | 15.63 | 3102 | Shuhaimi-Othman et al. 2011a |
| *Crangonyx pseudogracilis* | | | 1.6 | 6.75 | 50 | 9190 | Martin and Holdich 1986 |
| *Hyalella azteca* | | | 0.48 | 6.13 | 105 | > 5997 | Wang et al. 2016, 2017 |
| *Paratanytarsus dissimilis* | | | 2.8 | 7.28 | 17.43 | > 77700 | Lamb and Bailey 1981, 1983 |
| *Chironomus plumosus* | | | 1.6 | 7 | 80 | 30000 | Fargasova 2001, 2003 |
| | | | | | | | |
| BLM: P. promelas (update)_ With interactions | | | | | | | |
| *Oncorhynchus mykiss* | | | 0.4 | 5 | 14.3 | 160 | Holtze 1983 |
| *Oncorhynchus mykiss* | | | 0.4 | 5.5 | 14.3 | 310 | Holtze 1983 |
| *Oncorhynchus mykiss* | | | 1.1 | 6.59 | 47.4 | 7400 | Call et al. 1984 |
| *Oncorhynchus mykiss* | | | 1.1 | 7.31 | 47.4 | 14600 | Call et al. 1984 |

| species | endpoint | test | DOC | pH | H | EC50 | Reference |
|---|---|---|---|---|---|---|---|
| *Oncorhynchus mykiss* | | | 1.1 | 8.17 | 47.4 | > 24700 | Call et al. 1984 |
| *Oncorhynchus mykiss* | | | 1.1 | 7.46 | 47.4 | 8600 | Call et al. 1984 |
| *Oncorhynchus mykiss* | | | 0.5 | 7.61 | 26.35 | > 9840 | Gundersen et al. 1994 |
| *Oncorhynchus mykiss* | | | 0.5 | 7.59 | 45.5 | > 8070 | Gundersen et al. 1994 |
| *Oncorhynchus mykiss* | | | 0.5 | 7.6 | 88.05 | > 8160 | Gundersen et al. 1994 |
| *Oncorhynchus mykiss* | | | 0.5 | 7.61 | 127.6 | > 8200 | Gundersen et al. 1994 |
| *Oncorhynchus mykiss* | | | 0.5 | 8.28 | 23.25 | 6170 | Gundersen et al. 1994 |
| *Oncorhynchus mykiss* | | | 0.5 | 8.3 | 35.4 | 6170 | Gundersen et al. 1994 |
| *Oncorhynchus mykiss* | | | 0.5 | 8.31 | 83.6 | 7670 | Gundersen et al. 1994 |
| *Oncorhynchus mykiss* | | | 0.5 | 8.31 | 128.5 | 6930 | Gundersen et al. 1994 |
| *Salmo salar* | | | 0.5 | 5.5 | 6.8 | 584 | Hamilton and Haines 1995 |
| *Salmo salar* | | | 0.5 | 6.5 | 6.8 | 599 | Hamilton and Haines 1995 |
| *Salvelinus fontinalis* | | | 1.6 | 5.6 | 40 | 6530 | Tandjung 1982 |
| *Salvelinus fontinalis* | | | 1.6 | 5.6 | 18 | 3400 | Tandjung 1982 |
| *Salvelinus fontinalis* | | | 1.6 | 5.6 | 2 | 370 | Tandjung 1982 |
| *Hybognathus amarus* | | | 0.5 | 8.1 | 140 | > 59100 | Buhl 2002 |
| *Pimephales promelas* | | | 1.1 | 7.61 | 47.4 | > 48200 | Call et al. 1984 |
| *Pimephales promelas* | | | 1.1 | 8.05 | 47.4 | > 49800 | Call et al. 1984 |
| *Pimephales promelas* | | | 0.9 | 6.5 | 21.6 | > 400 | Palmer et al. 1989 |
| *Pimephales promelas* | | | 0.9 | 7.5 | 21.6 | > 400 | Palmer et al. 1989 |
| *Pimephales promelas* | | | 0.9 | 7.5 | 21.6 | > 400 | Palmer et al. 1989 |
| *Pimephales promelas* | | | 0.9 | 6.5 | 21.6 | > 400 | Palmer et al. 1989 |
| *Pimephales promelas* | | | 0.9 | 7.5 | 21.6 | > 400 | Palmer et al. 1989 |
| *Pimephales promelas* | | | 0.5 | 8.1 | 140 | > 59100 | Buhl 2002 |
| *Micropterus dolomieui* | | | 1.6 | 5.05 | 12.15 | 130 | Kane 1984; Kane and Rabeni 1987 |
| *Micropterus dolomieui* | | | 1.6 | 6.25 | 12.4 | > 993.4 | Kane 1984; Kane and Rabeni 1987 |
| *Micropterus dolomieui* | | | 1.6 | 7.5 | 12 | > 216.8 | Kane 1984; Kane and Rabeni 1987 |
| *Hyla cinerea (frog)* | | | 0.5 | 5.49 | 4.55 | > 405.2 | Jung and Jagoe 1995 |
| *Poecilia reticulata* | | | 3.2 | 6.68 | 18.72 | 6760 | Shuhaimi-Othman et al. 2013 |
| *Lepomis cyanellus* | | | 1.1 | 7.55 | 47.4 | > 50000 | Call et al. 1984 |

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# Appendix 6 R-script for calculation of Aluminum $HC_5$

```
# Water types
Samples<-read.csv("Monitoringset2020.csv")
attach(Samples)

# Toxicity data
Chronic.data<-read.csv("chronic data.csv",sep=';')
Acute.data<-read.csv("acute data.csv",sep=";")

# Applicability domain
# Flag samples outside domain
OoD1<-which(DOC>12.3|DOC<0.08)
OoD2<-which(pH>8.7|pH<6)
OoD3<-which(Hardness>430|Hardness<0.01)
table(table(OoD<-c(OoD1,OoD2,OoD3)))
Samples$OoD<-NA
Samples$OoD[OoD]<-"OoD"

# MLR functions
Alg.ECxx_norm<-function(Test=Tox.data){
  log(Test$ECxx[i])-
    2.342*(log(Test$DOC[i])-log(DOC))-
    4.560*(log(Test$Hardness[i])-log(Hardness))-
   20.923*(Test$pH[i]-pH)-
   -1.274*(Test$pH[i]^2-pH^2)-
   -0.288*(log(Test$DOC[i])*Test$pH[i]-log(DOC)*pH)-
   -0.628*(log(Test$Hardness[i])*Test$pH[i]-log(Hardness)*pH)}

Crust.ECxx_norm<-function(Test=Tox.data){
  log(Test$ECxx[i])-
    0.697*(log(Test$DOC[i])-log(DOC))-
    2.768*(log(Test$Hardness[i])-log(Hardness))-
    6.089*(Test$pH[i]-pH)-
   -0.269*(Test$pH[i]^2-pH^2)-
    0*(log(Test$DOC[i])*Test$pH[i]-log(DOC)*pH)-
   -0.335*(log(Test$Hardness[i])*Test$pH[i]-log(Hardness)*pH)}

Fish.ECxx_norm<-function(Test=Tox.data){
  log(Test$ECxx[i])-
    1.828*(log(Test$DOC[i])-log(DOC))-
    1.914*(log(Test$Hardness[i])-log(Hardness))-
    1.932*(Test$pH[i]-pH)-
    0 *(Test$pH[i]^2-pH^2)-
   -0.193*(log(Test$DOC[i])*Test$pH[i]-log(DOC)*pH)-
   -0.248*(log(Test$Hardness[i])*Test$pH[i]-log(Hardness)*pH)}

# Execute MLR functions for all watertypes
logNorm.acute<-matrix(NA,nrow=dim(Acute.data)[1],ncol=dim(Samples)[1])
j<-which(Acute.data$MLR=="Algae");
       for (i in j){logNorm.acute[i,]<-Alg.ECxx_norm(Acute.data)}
j<-which(Acute.data$MLR=="Crustaceans");
       for (i in j){logNorm.acute[i,]<-Crust.ECxx_norm(Acute.data)}
j<-which(Acute.data$MLR=="Fish");
       for (i in j){logNorm.acute[i,]<-Fish.ECxx_norm(Acute.data)}

logNorm.chronic<-matrix(NA,nrow=dim(Chronic.data)[1],ncol=dim(Samples)[1])
j<-which(Chronic.data$MLR=="Algae");
       for (i in j){logNorm.chronic[i,]<-Alg.ECxx_norm(Chronic.data)}
j<-which(Chronic.data$MLR=="Crustaceans");
       for (i in j){logNorm.chronic[i,]<-Crust.ECxx_norm(Chronic.data)}
j<-which(Chronic.data$MLR=="Fish");
       for (i in j){logNorm.chronic[i,]<-Fish.ECxx_norm(Chronic.data)}

# Aggregate ECxx and calculate HC5
L<-dim(Samples)
Acute.summary<-as.data.frame(as.data.frame(logNorm.acute) %>%
      group_by(Acute.data$Species) %>%
      summarize(across(1:L[1],mean,na.rm=T)))
      logMeans<-sapply(Acute.summary[,-1],mean)
      logStdevs<-sapply(Acute.summary[,-1],sd)
      Samples$HC5.acute<-exp(qnorm(0.05,logMeans,logStdevs)) #point estimate
```

```
# Calculation of HC5-50 and its 90th percentile confidence interval:
# Constants k for n=23 (species) for calculation of 90th percentile around the HC5 are taken from
SSD-book, Posthuma, Suter and Traas, 2002)
      Samples$HC5.acute_low<-exp(logMeans-2.32832*logStdevs)
      Samples$HC5.acute_median<-exp(logMeans-1.66752*logStdevs)
      Samples$HC5.acute_high<-exp(logMeans-1.20181*logStdevs)

Chronic.summary<-as.data.frame(as.data.frame(logNorm.chronic) %>%
      group_by(Chronic.data$Species) %>%
      summarize(across(1:L[1],mean,na.rm=T)))
      logMeans<-sapply(Chronic.summary[,-1],mean)
      logStdevs<-sapply(Chronic.summary[,-1],sd)
      Samples$HC5.chronic<-exp(qnorm(0.05,logMeans,logStdevs)) # point estimate

# Calculation of HC5-50 and its 90th percentile confidence interval:
# Constants k for n=13 (species) for calculation of 90th percentile around the HC5
      Samples$HC5.chronic_median<-exp(logMeans-1.68700*logStdevs)
      Samples$HC5.chronic_low<-exp(logMeans-2.6705*logStdevs)
      Samples$HC5.chronic_high<-exp(logMeans-1.0814*logStdevs)
```

# Appendix 7 Number of selected sampling sites per WFD water type

| PBLcode and description (in Dutch) | | WFD code and description (in Dutch) | | # |
|---|---|---|---|---|
| MBR | Brakke wateren | M30 | Zwak brakke wateren | 14 |
| | | M31 | Kleine brakke tot zoute wateren | 1 |
| | | M32 | Grote brakke tot zoute wateren | 4 |
| MGD | Grote meren | M21 | Grote diepe gebufferde meren | 11 |
| MKD | Kleine diepe plassen | M16 | Diepe gebufferde meren | 3 |
| | | M17 | Diepe zwak gebufferde meren | - |
| | | M18 | Diepe zure meren | - |
| | | M24 | Diepe kalkrijke meren | - |
| | | M28 | Diepe laagveenmeren | - |
| MKO | Kleine ondiepe plassen (zand, kalk) | M11 | Kleine ondiepe gebufferde plassen | 18 |
| | | M22 | Kleine ondiepe kalkrijke plassen | 3 |
| MKV | Kleine ondiepe veenplassen | M25 | Ondiepe laagveenplassen | 3 |
| MMO | Matig grote ondiepe meren | M14 | Ondiepe gebufferde plassen | 24 |
| | | M15 | Ondiepe grote gebufferde plassen | - |
| | | M23 | Grote ondiepe kalkrijke plassen | - |
| | | M27 | Matig grote ondiepe laagveenplassen | 5 |
| MMD | Matig grote diepe meren | M20 | Matig grote diepe gebufferde meren | 28 |
| | | M29 | Matig grote diepe laagveenmeren | - |
| MVN | Vennen | M12 | Kleine, ondiepe zwak gebufferde plassen | 22 |
| | | M13 | Kleine, ondiepe zure plassen | 5 |
| | | M26 | Ondiepe, zwak gebufferde hoogveenplassen | 7 |
| MKA | Kanalen | M3 | Gebufferde (regionale) kanalen | 62 |
| | | M4 | Zwak gebufferde (regionale) kanalen | - |
| | | M6 | Grote ondiepe kanalen | 44 |
| | | M7 | Grote diepe kanalen | 16 |
| | | M10 | Laagveen vaarten en kanalen | 16 |
| MSL | Sloten | M1 | Gebufferde sloten (overgangssloten, sloten in rivierengebied) | 104 |
| | | M2 | Zwak gebufferde sloten (poldersloten) | 4 |
| | | M8 | Gebufferde laagveensloten | 3 |
| | | M9 | Zwak gebufferde hoogveen sloten | - |
| MWR | Wateren in het rivierengebied | M5 | Ondiep lijnvormig water, open verbinding met rivier/geïnundeerd | 2 |
| | | M19 | Diepe meren in open verbinding met rivier | - |
| RRV | Langzaam stromende rivier | R7 | Langzaam stromende rivier/nevengeul op zand/klei | 11 |
| | | R8 | Zoet getijdenwater (uitloper rivier) op zand/klei | 8 |
| RRS | Snel stromende rivier | R16 | Snelstromende rivier/nevengeul op zand of grind | 1 |
| RMB | Riviertje | R6 | Langzaam stromend riviertje op zand/klei | 51 |
| RBL | Langzaam stromende wateren | R1 | Droogvallende bron | - |
| | | R2 | Permanente bron | 3 |
| | | R3 | Droogvallende langzaam stromende bovenloop op zand | 4 |
| | | R4 | Permanent langzaam stromende bovenloop op zand | 113 |
| | | R5 | Langzaam stromende midden/benedenloop op zand | 112 |
| | | R11 | Langzaam stromende bovenloop op veen | - |
| | | R12 | Langzaam stromende midden/benedenloop op kalk | 2 |
| | | R19* | Doorstroommoeras | 9 |
| | | R20* | Moerasbeek | 34 |
| RSL | Snel stromende wateren | R9 | Langzaam stromende bovenloop op kalk | - |
| | | R10 | Langzaam stromende middenloop op kalk | - |
| | | R13 | Snelstromende bovenloop op zand | 5 |
| | | R14 | Snelstromende midden/benedenloop op zand | 4 |
| | | R15 | Snelstromende riviertje op kiezelhouden bodem | 2 |
| | | R17 | Snelstromende bovenloop op kalkhoudende bodem | 16 |
| | | R18 | Snelstromende midden/benedenloop op kalk | 8 |
| OTY | Overgangswater | O2 | Estuarium met matig getijverschil | - |
| ZEE | Zee | K1 | Polyhalien kustwater | - |
| | | K2 | Euhalien kustwater | - |
| KBS | Waddenzee, Oosterschelde | K3 | Beschut polyhalien kustwater | - |

*R19 en R20 komen in de PBL classificatie uit 2010 nog niet voor, toen werden deze wateren nog geclassificeerd als R4. In 2018 zijn ze toegevoegd als aparte KRW water types (Verdonschot, R en Verdonschot, P., 2018, Maatlatten voor doorstroommoerassen en moerasbeken, Notitie zoetwatersystemen, WUR, 84p., link

Further aggregation of water types in Table 7 was done as follows"

| Water type | PBL code |
|---|---|
| | |
| Brackish waters | MBR |
| Brooks, fast flowing | RBS |
| Brooks, slow flowing | RBL |
| Canals | MKA |
| Ditches | MSL |
| Fens | MVN |
| Lakes, large | MGD |
| Lakes, medium | MMD+MMO |
| Lakes, small, peat | MKV |
| Lakes, small, sand clay | MKD+MKO |
| Rivers | RRV+RRS+RMB |
| Water in riverine area | MWR |

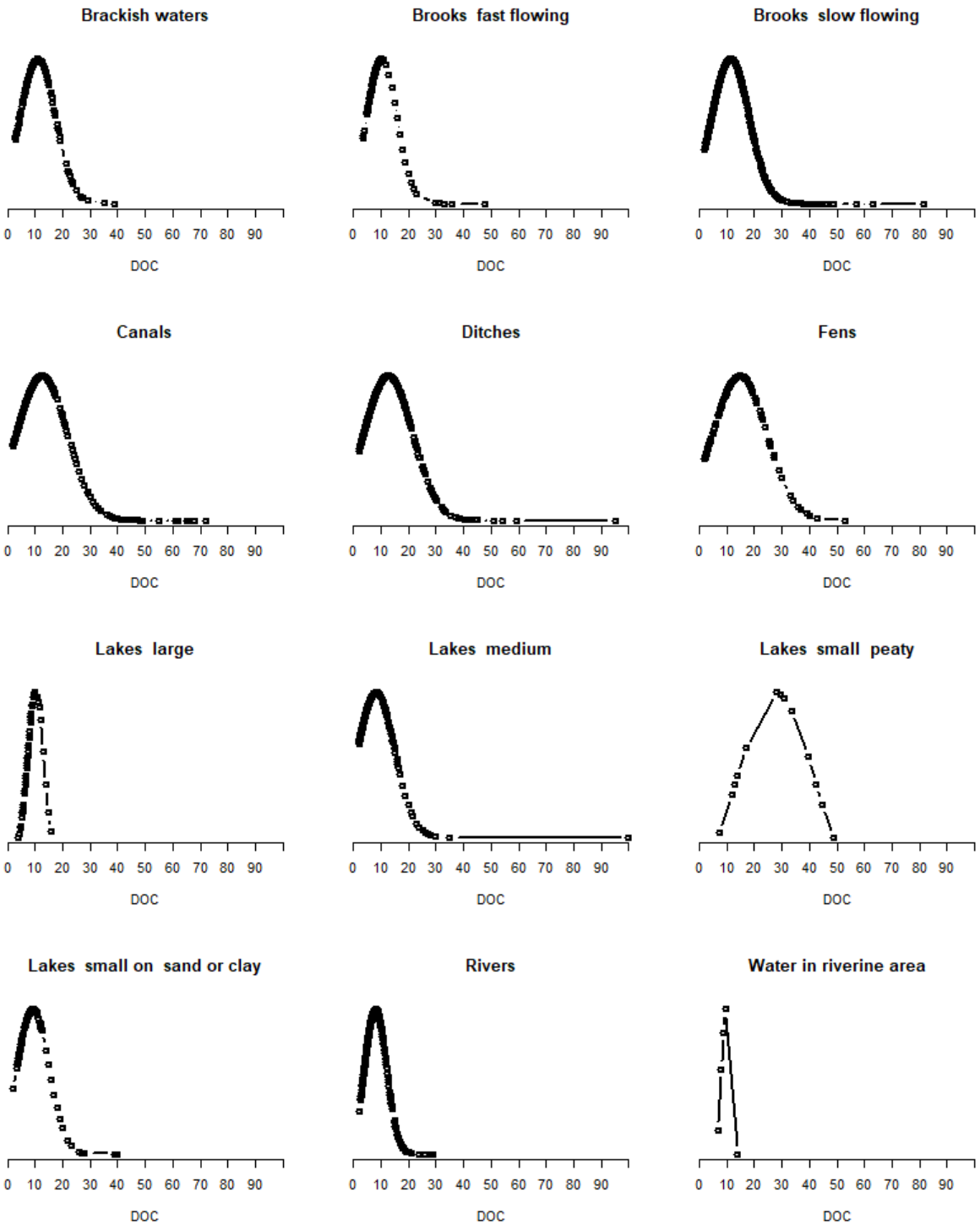
# Appendix 8 Sampling authorities

*Table 14 Authorities involved in collection of the selected data.*

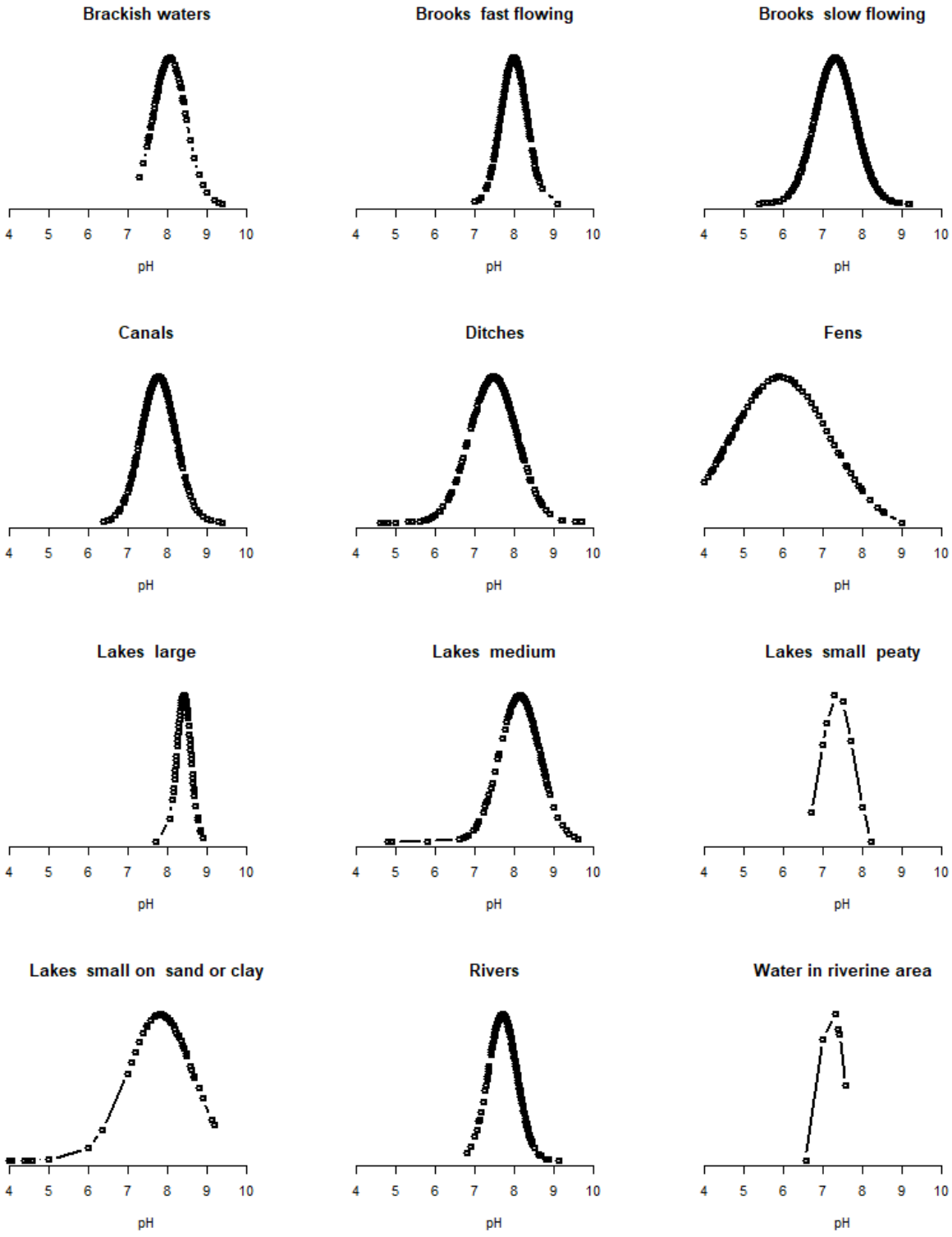
| | Compiled dataset | |
|---|---|---|
| | Monthly mean data (#samples) | Annual mean data (#sites) |
| HHRS De Stichtse Rijnlanden | 0 | 0 |
| HHRS Hollands Noorderkwartier | 283 | 24 |
| HHRS van Delfland | 0 | 0 |
| HHRS van Rijnland | 0 | 0 |
| HHRS van Schieland en Krinpenerwaard | 0 | 0 |
| Rijkswaterstaat | 505 | 44 |
| Wetterskip Fryslân | 82 | 20 |
| WS Aa en Maas | 595 | 99 |
| WS Amstel Gooi en Vecht | 0 | 0 |
| WS Brabantse Delta | 1058 | 121 |
| WS De Dommel | 875 | 82 |
| WS Drents Overijsselse Delta | 354 | 95 |
| WS Hollandse Delta | 237 | 27 |
| WS Hunze en AA's | 0 | 0 |
| WS Limburg | 971 | 132 |
| WS Noorderzijlvest | 0 | 0 |
| WS Rijn en IJssel | 37 | 9 |
| WS Rivierenland | 0 | 0 |
| WS Scheldestromen | 0 | 0 |
| WS Vallei en Veluwe | 52 | 39 |
| WS Vechtstromen | 156 | 31 |
| WS Zuiderzeeland | 339 | 56 |
| **Total** | **5544** | **779** |

## Appendix 9 Normal distributions of water characteristics

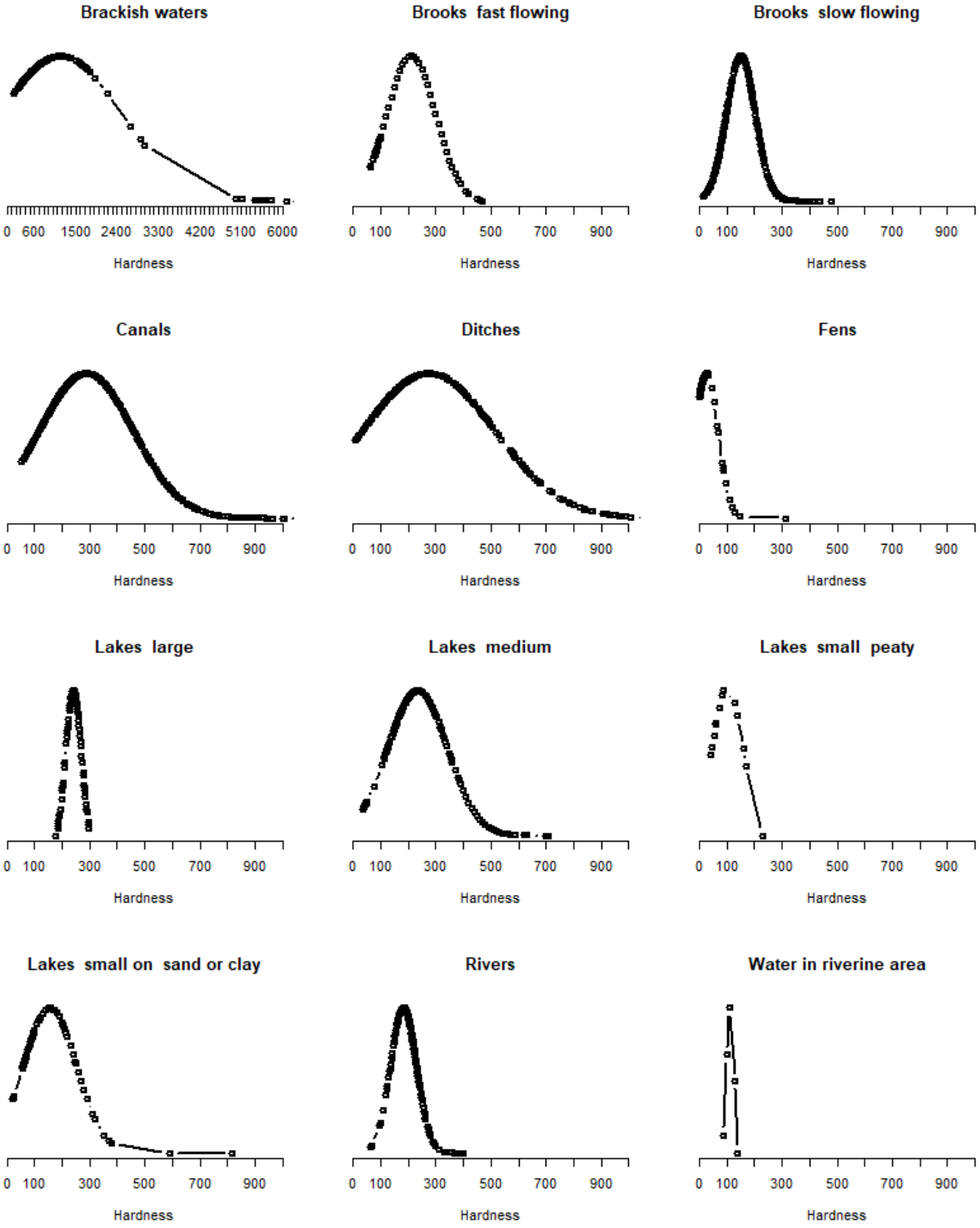
# Dissolved organic carbon (mg/L)



# pH

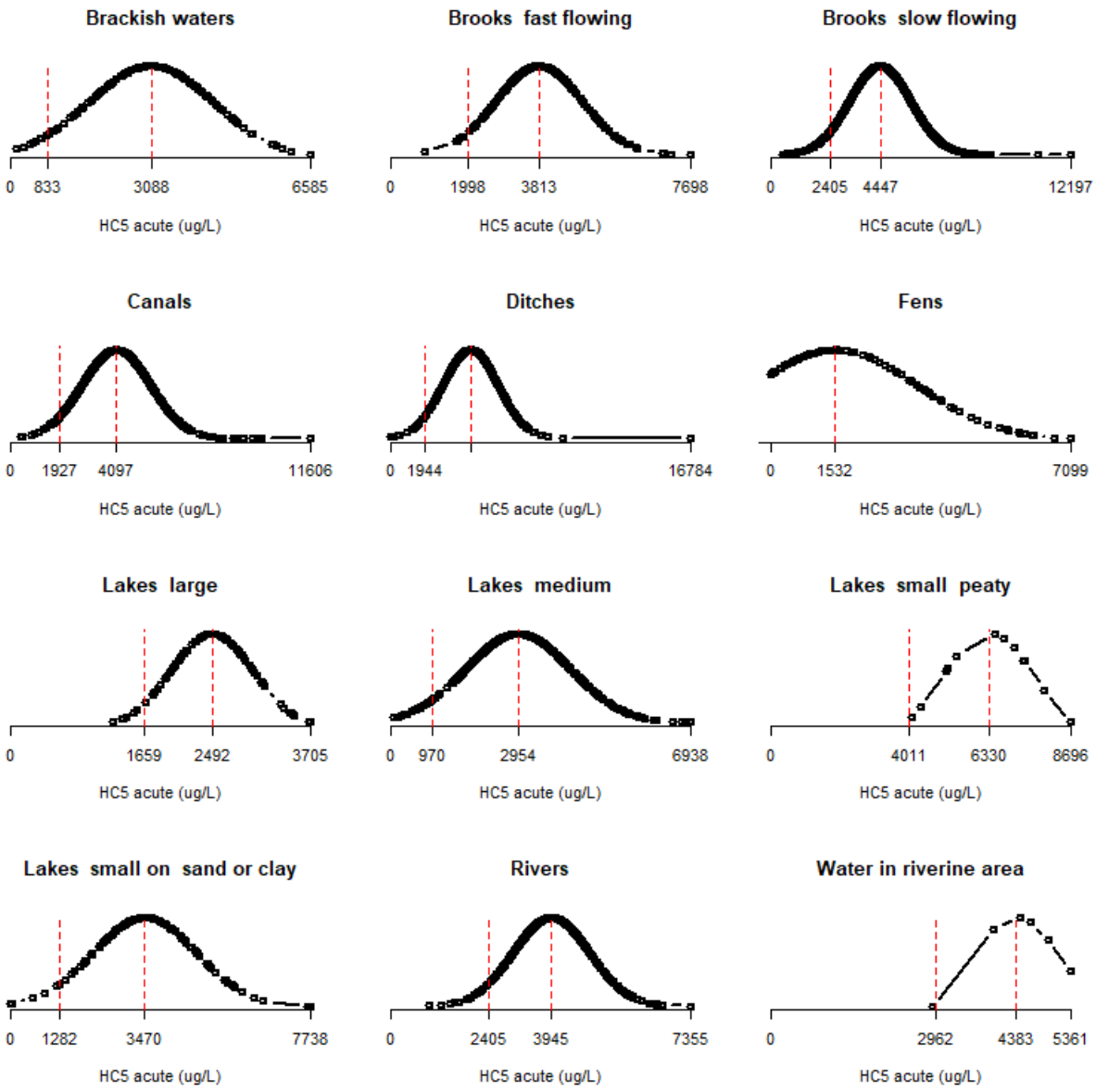

Brackish waters
Brooks fast flowing
Brooks slow flowing
Canals
Ditches
Fens
Lakes large
Lakes medium
Lakes small peaty
Lakes small on sand or clay
Rivers
Water in riverine area
4 5 6 7 8 9 10
pH

# Hardness (mf $CaCO_3$/L)



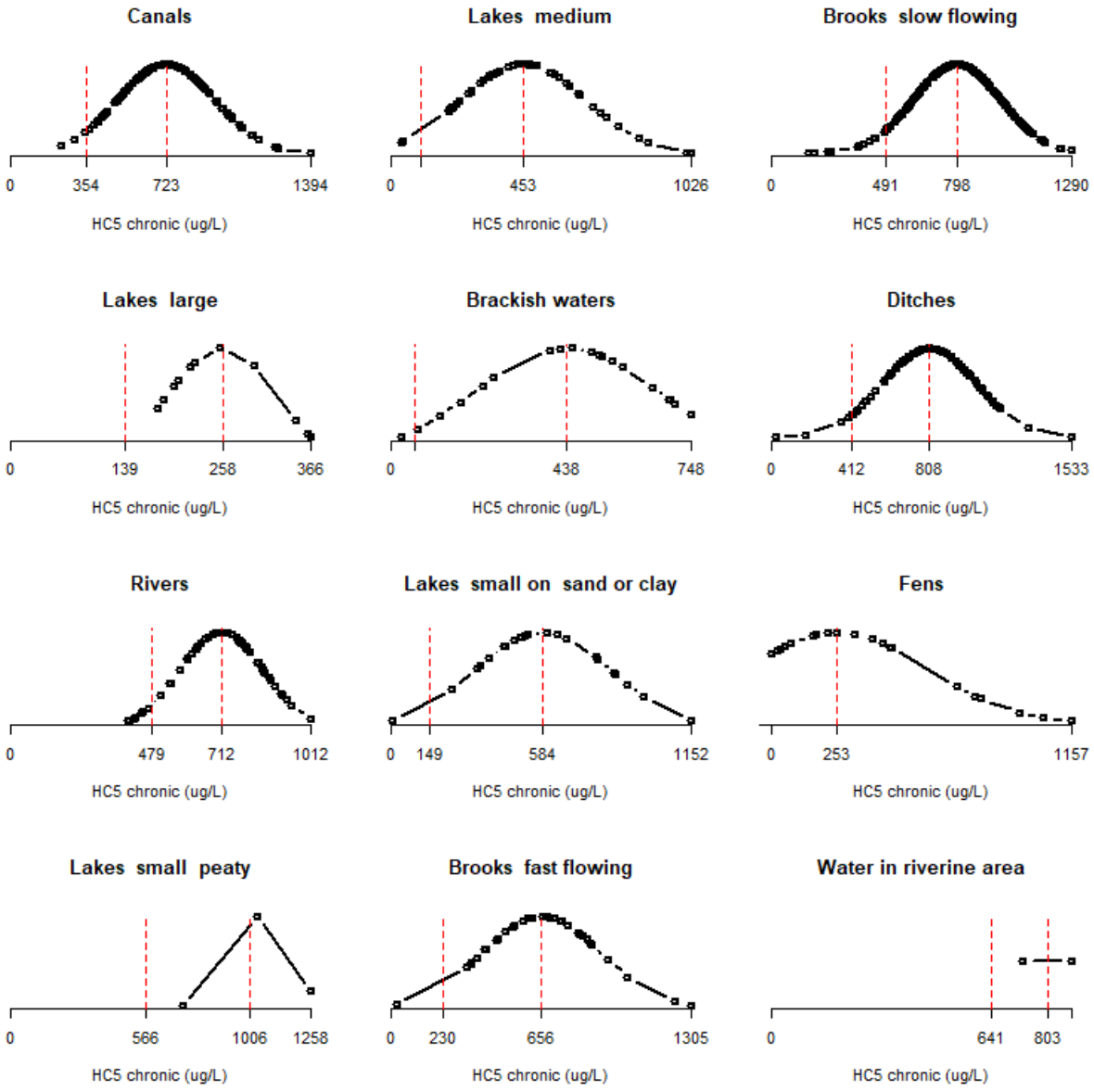
## Appendix 10 Normal distributions calculated HC5

# Acute HC5




*Distribution of acute HC5 values fitted. Dotted lines represent the fitted 5th percentile and 50th percentile of each watertype, assuming a normal distribution. Based on 5544 monthly records.*

# Chronic HC5




*Distribution of chronic HC5 values fitted. Dotted lines represent the fitted 5th percentile and 50th percentile of each watertype, assuming a normal distribution. Based on 779 annual records.*

# Appendix 11 Statistical data of water types used for copper EQS

Source: Vijver et al., 2008; RIVM,2012.

| | DOC | | | | pH | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | mean | ± | sd | #samples/ | #sites | mean | ± | sd | #samples | #sites |
| Large rivers | 3.1 | ± | 0.9 | 926 | 12 | 7.7 | ± | 0.2 | 4024 | 12 |
| Canals, large lakes, small lakes | 8.4 | ± | 4.4 | 425 | 16 | 8.1 | ± | 0.4 | 443 | 15 |
| Streams, brooks | 18.2 | ± | 4.3 | 12 | 3 | 7.4 | ± | 0.1 | 401 | 5 |
| Ditches | 27.5 | ± | 12.2 | 7 | 4 | 6.9 | ± | 0.8 | 7 | 4 |
| Sandy springs | 2.2 | ± | 1.0 | 4 | 1 | 6.7 | ± | 0.1 | 4 | 1 |
| Small acid ponds | 17.3 | ± | 4.4 | 22 | 7 | 5.1 | ± | 0.8 | 22 | 7 |

# Appendix 12 Aluminum concentrations in filtered and unfiltered samples.

| | Samples with aluminum (filtered) data | | | | Samples with aluminum (total) data | | | |
|---|---|---|---|---|---|---|---|---|
| | # | <LOD | Conc. range (µg/L) | Median (µg/L) | # | <LOD | Conc. range (µg/L) | Median (µg/L) |
| Brackish waters | 110 | 50% | <1 - 50 | 1.8 | 88 | 0% | 2.0 - 934 | 50 |
| Brooks fast flowing | 273 | 99% | <50 - 350 | 25* | 8 | 0% | 67 - 290 | 155 |
| Brooks slow flowing | 1692 | 62% | 2.4 - 760 | 25* | 715 | 5% | <10 - 3400 | 98 |
| Canals | 905 | 60% | <1 - 1300 | 25* | 167 | 7% | <10 - 1400 | 148 |
| Ditches | 673 | 71% | <10 - 1100 | 25* | 368 | 25% | <10 - 4400 | 90 |
| Fens | 61 | 49% | <50 - 450 | 51 | 0 | | | |
| Lakes large | 50 | 20% | <1 - 11 | 1.5 | 51 | 0% | 13.9 - 2480 | 319 |
| Lakes medium | 273 | 48% | <1 - 670 | 20 | 155 | 12% | <20 - 4300 | 74 |
| Lakes small peaty | 7 | 100% | <50 - 25 | 25* | 0 | | | |
| Lakes small on sand or clay | 133 | 83% | <10 - 260 | 18 | 76 | 4% | <10 - 460 | 53 |
| Rivers | 612 | 31% | <1 - 190 | 13 | 504 | 5% | <10 - 4600 | 133 |
| Water in riverine area | 6 | 100% | <50 | 25* | 0 | | | |
| Overall | 4795 | 60% | <1 - 1300 | | 2132 | 9% | 2.0 - 4600 | |

Concentration below the limit of detection were divided by 2 before they were combined with other data to calculate the median.

In most (81%) of the samples reported below the limit (LOD) of detection the LOD was 50 µg Al/L. In the other 19% of the cases the LOD was smaller (either, 1,2,10 or 20 µg Al/L).