Derivation of a Bioavailability-Based Environmental Quality Standard for Copper in European Freshwaters under the Water Framework Directive using an updated methodology.

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Derivation of a bioavailability-based environmental quality standard for copper in European freshwaters under the Water Framework Directive using an updated methodology

Abstract: Copper (Cu) is a river basin specific pollutant under the European Water Framework Directive (WFD). Compliance assessment under the WFD should be based on a tiered approach that considers a bioavailability correction integrating local physico-chemistry conditions. The existing procedure for deriving bioavailability-based, site-specific environmental quality standards (EQS) has remained largely unchanged for over 15 years. In this study, the methodology for deriving site-specific freshwater EQS for Cu was updated by integrating recent chronic ecotoxicity data. The updated database includes 376 high-quality data-entries covering 49 freshwater species. In addition, optimized Cu bioavailability models and an updated species sensitivity distribution (SSD) approach were also implemented in the Cu effects assessment methodology. Implementation of the revised methodology, in strict accordance with the WFD guidance, resulted in an updated European Union-wide bioavailable EQS ($EQS_{bioavailable}$) of 1.0 µg dissolved Cu/L, representative for high bioavailability conditions in Europe. Within the application range of the updated bioavailability-based EQS methodology (pH 5.5–8.6, calcium 2.4–124 mg/L, dissolved organic carbon ≤30 mg/L), this value is protective for the majority of European freshwaters (i.e., for at least 98% of the freshwaters in most database considered) and for 95% of sites in the region with the highest Cu bioavailability (i.e., Norway). The updated freshwater $EQS_{bioavailable}$ for Cu can serve as a conservative Tier 1 screening-level EQS for compliance assessment under the WFD. However, given its proximity to natural background concentrations (baseline European freshwaters report 0.88 µg dissolved Cu/L as median value) and

the influence of site-specific water chemistry on Cu toxicity, higher-tier compliance assessments may be needed. As such, in higher tiers, the developed updated EQS derivation methodology can serve as a scientifically robust framework to incorporate bioavailability. Finally, the proposed framework allows for the derivation of site, river basin or country-specific $EQS_{bioavailable}$ for Cu.

Keywords: bioavailability; water quality criteria, freshwater toxicology, ecological risk assessment, metal toxicity.

1. INTRODUCTION

Copper (Cu) bioavailability and toxicity to aquatic organisms is highly dependent on local water chemistry, with dissolved organic carbon (DOC), pH, water hardness (Calcium [Ca] & Magnesium [Mg]), and sodium [Na] reported to be the main factors influencing bioavailability (e.g., Brix et al. 2021; Nys et al. 2024). The Water Framework Directive (WFD) in the European Union (EU) acknowledges the essential role of bioavailability in assessing metal toxicity and supports the integration of bioavailability models in the derivation of environmental quality standards (EQS) and compliance evaluations (European Commission [EC], 2018; 2021). For metals with validated bioavailability models, a tiered compliance assessment approach is recommended. At the foundation of this approach lies the bioavailable EQS ($EQS_{bioavailable}$), which represents a protective threshold concentration. Specifically, it is defined as the concentration protective for 95% of surface waters in the European country or region exhibiting the highest Cu bioavailability (i.e., greatest sensitivity; EC 2018). While the $EQS_{bioavailable}$ serves as the screening-level concentration in the first tier of the bioavailability-based compliance assessment, in higher tiers bioavailability models may serve to correct for local bioavailability conditions (EC 2021, e.g., see Wilson et al. 2023).

The current methodology to implement bioavailability in EQS setting has been outlined under the Voluntary Risk Assessment of copper and copper compounds (European Copper Institute 2008; Cu VRAR, hereafter referred to as the “original EQS derivation methodology”). This methodology integrates the chronic copper ecotoxicity dataset with bioavailability models and applies it within a species sensitivity distribution (SSD) framework. Since its establishment, an extensive amount of additional chronic Cu freshwater toxicity data have become available, including data for several species that were not represented in the original EQS derivation methodology (e.g., Besser et al. 2020, Cremazy et al. 2018, Markich 2017). In addition, the chronic Cu bioavailability models for algae, invertebrates and vertebrates have recently also been updated by optimizing the model structure, while also integrating the most recent version of the Windermere Humic Aqueous Model as the underlying speciation model (WHAM VII; Nys et al. 2020, 2024). It has been demonstrated that the updated bioavailability models can be used to predict Cu toxicity across species and life-stages of all relevant trophic levels (i.e., vertebrates, invertebrates, and algae and higher plants) with reasonable accuracy (i.e., mostly within 2-fold error; Nys et al. 2020, 2024). As such, the updated chronic Cu bioavailability models represent a robust bioavailability model set that can be used in regulatory applications.

Under the Water Framework Directive, Cu is currently classified as a river basin-specific pollutant, allowing Member States to set their own EQS (EC 2008). Consequently, annual average-EQS (AA-EQS) values for Cu vary widely across Europe, ranging from 0.5 to 164 µg/L (23 countries, European Environment Agency [EEA] 2025). These standards aim to protect aquatic ecosystems from adverse effects of long-term exposure and are increasingly based on bioavailability corrections. Bioavailability-based AA-EQS ($EQS_{bioavailable}$) have been adopted in the Netherlands

(2.4 µg dissolved Cu/L; Rijksinstituut voor Volksgezondheid en Milieu [RIVM] 2025), France and the former EU-member state United Kingdom (for both countries 1.0 µg dissolved Cu/L; Journal Officiel de la République Française 2010; United Kingdom [UK] Environment Agency 2009), and by the International Commission for the Protection of the Rhine (2016; 2.8 µg dissolved Cu/L). However, not all member states account for bioavailability.

Given the significant variation in environmental quality standards for copper across EU Member States an ongoing effort aims to harmonize Cu EQS within the European Union (Joint Research Center, 2025). To support this harmonization effort, the present study aimed to update the bioavailability-based methodology for deriving long-term environmental quality standards of Cu under the Water Framework Directive (WFD) - hereafter referred to as the "updated EQS derivation methodology" or "updated EQS scenario". This refinement involved revising and expanding the underlying chronic ecotoxicity dataset and minor updates to the SSD methodology in alignment with the latest WFD guidelines (EC 2018, 2021), while also incorporating the most recent chronic Cu bioavailability models (Nys et al., 2024). In addition, using the updated EQS derivation methodology an EU-wide $EQS_{bioavailable}$ was derived. Finally, the updated EQS derivation methodology was compared to previous methodologies used in EU chemical regulation, and key similarities and differences with approaches from other regulatory jurisdictions were identified.

2. MATERIALS AND METHODS

2.1 Literature search and quality screening

An updated chronic Cu freshwater ecotoxicity database was collated starting from the database used for the update of the Predicted No-Effect Concentration (PNEC) under the Registration, Evaluation, Authorisation and Restriction of Chemicals

(REACH)-regulation in 2022 (Nys & Van Sprang 2022). This database includes the existing ecotoxicity database of the original EQS derivation methodology (ECI 2008, hereafter referred to as the "original Cu database"), which covers studies published until 2005 and is the result of a regulatory supported reliability and relevancy assessment. Within the 2022-update, this database was amended with studies identified from various library systems (Agricola, CAB, Chemical Abstracts, Embase, Medline, Proquest, PubAg, Web of Science, and Zoological Records). This literature search covered studies published until 2020. This database was extended with five unpublished study reports (e.g., Oregon State University [OSU] 2016a,b, 2017, OSU & University of Ghent 2022, Scymaris 2021). Five studies published prior to 2005 (Bossuyt & Janssen 2004, De Schamphelaere & Janssen 2004a, De Schamphelaere et al. 2003, Heijerick et al. 2002, 2005) but not considered in the original Cu database were also added to the list of studies taken forward for reliability and relevancy screening in the present study. These studies were previously not considered for EQS derivation because the data had been used for the development of the bioavailability models. Finally, all studies included in the original database were re-evaluated, and endpoints that had not been included in the original database were also added.

All data, both those resulting from the original ecotoxicity database as well as the newly identified studies were screened based on reliability and relevancy adhering to the fundamental principles outlined by Klimisch et al. (1997), as well as the more recent criteria outlined within the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) approach (Moermond et al. 2016). An overview of the specific criteria applied in the current study and their agreement with the CRED framework is given in online supplementary material, S1. The quality screening of toxicity data was largely the same as the one used for the database collation of the original EQS derivation methodology (Cu VRAR; ECI 2008, see online supplementary material, S1, Table S1.1 & S1.2), although for the current update some refinements were applied. These included additional clarifications related to the acceptability of biological endpoints, relevancy of the test medium, test acceptability and concentration-effect relationships. The most important refinements included I) the preference of 10% lethal or effect concentrations (L(E)C10s) over no-observable effect concentrations (NOECs; Azimonti et al. 2015), while within the original Cu database, NOECs were preferred over the L(E)C10. In addition, all L(E)10 and NOEC values were screened for statistical robustness and only robust values were retained (see online supplementary material, Table S1.1); II) L(E)C10 or NOEC were only considered when derived from measured concentrations. In the original Cu database the analytical verification of test concentrations was also set as a basic requirement, but a few nominal NOECs were included when measured data were lacking for a specific taxonomical group; III) while in the original database, if not reported, DOC was estimated with default assumptions, for the literature identified in the 2022-update the only studies considered for inclusion in the ecotoxicity database were those in which measured DOC was reported or where DOC could be confirmed by personal communication by one of the authors. From the previous list, criterion I) and II) were applied on the entire database (i.e., both those resulting from the original database as well as those resulting from the literature update), criterion III) was only applied on the newly identified data. This discrepancy in data treatment stems from the original database having been established through regulatory consensus, a choice that also prevents the loss of critical data from the Cu ecotoxicity database. The implications of this decision are assessed in the sensitivity analysis (Sensitivity Scenario 1).

For bioavailability normalization, it is crucial that data about the physico-chemical conditions of the test medium (pH, DOC, Ca, Mg, Na, K; SO_4, Cl, alkalinity and temperature) are available. For toxicity data originating from the original database the associated water chemistry was taken over as reported. For additional toxicity data resulting from the literature update, as a minimum, temperature, pH, DOC (but see previous paragraph) and Ca concentrations (or hardness) should have been reported. If measured concentrations for Mg, Na, K, SO_4, Cl and alkalinity were not reported in the original publication, these were estimated based on nominal added concentrations in synthetic medium or taken over from publications of the same research group using the same type of test medium.

Based on the relevancy and reliability screening all datapoints were categorized according to following scores: K1 (reliable and relevant standardized studies strictly following standard guidelines), K2 (reliable and relevant non-standard tests) or K3/4 (unreliable, non-relevant studies and/or not assignable; adapted from Klimisch et al. 1997). Only data that were rated as K1 or K2 were considered for Cu environmental threshold derivation (European Chemical Agency [ECHA] 2008a,b, EC 2018). The compiled database, incorporating both K1 and K2 data from the original database and the literature update, is hereafter referred to as the “updated ecotoxicity database”.

2.2 Development of the updated EQS derivation methodology

The actual methodology for calculating site-specific thresholds for Cu using the updated EQS derivation scenario consists of different steps combining the updated toxicity database with the updated bioavailability models (Nys et al. 2024) and applying an SSD-technique (see Figure 1). For simplicity, for the remainder of this publication, L(E)C10 is used to refer to LC10, EC10 and NOEC values.

In a first step, all L(E)C10 in the chronic database - expressed as dissolved concentrations in exposure medium *x* ($L(E)C10_{Cudiss,x}$) - were converted to free Cu^{2+} activities ($L(E)C10_{Cu2+,x}$) using WHAM VII and implementing the speciation assumptions taken in Nys et al. (2024). In a next step, the updated bioavailability model set was used to calculate an intrinsic sensitivity parameter (Q) from the $L(E)C10_{Cu2+,x}$. This intrinsic sensitivity parameter is specific for a given species and endpoint. Using the intrinsic sensitivity (Q), the corresponding L(E)C10 in target water y - expressed at free Cu^{2+} level (i.e., $L(E)C10_{Cu2+,y}$) - was predicted using the appropriate bioavailability model based on the physico-chemistry of target water y. As a last step of the bioavailability modelling the normalized species and endpoint specific $L(E)C10_{Cu2+,y}$ for target water y were converted to $L(E)C10_{Cudiss,y}$ using WHAM VII.

The updated EQS derivation methodology incorporated the latest version of the chronic Cu bioavailability models - referred to as the updated bioavailability model set. This set includes models for three trophic levels: invertebrates, algae, and fish (Nys et al., 2020, 2024). As part of the bioavailability normalization process, the updated algae model was applied to all algal and plant species, the updated invertebrate model to all invertebrate species, and the updated fish model to all vertebrate species. An in-depth description of these bioavailability models including model equations has been provided elsewhere (Nys et al. 2024). For sake of completeness, an overview of the model parameters and speciation assumptions used in the present study has been added to the online supplementary material, S2.

In a final step, the normalized $L(E)C10_{Cudiss,y}$ were used in the SSD approach to derive the site-specific environmental threshold. The SSD approach includes the species-averaging of toxicity data (using the geometric mean), the actual fitting of an SSD to the normalized ecotoxicity values and the derivation of the 5% hazardous concentration (HC5). Within the current study, the SSD-approach used in the original EQS derivation methodology was updated related to the following two aspects: I) Selection of most sensitive endpoint and II) the distributions considered for SSD-fitting. If for one species several chronic $L(E)C10_{Cudiss,y}$ values for different toxicological endpoints (e.g., mortality, reproduction, growth) were available, the most sensitive endpoint were selected (ECHA 2008a). For the current update, all reliable and relevant endpoints reported in the screened studies were considered at the database collation step (see Section *Literature search and quality screening*), and the selection of most sensitive endpoint occurred after normalisation and species-averaging. In addition, when for a specific endpoint data for different life-stages were reported, the geometric mean of the most sensitive life-stage was selected after normalisation. For this, the following life-stages were considered: embryonal, larval, juvenile and adult stages. A different approach was taken in the original EQS derivation methodology, where life-stage differences were considered during database collation (prior to normalisation): if toxicity values for different life-stages were available in a specific publication, only the most sensitive life-stage was selected to be included in the original ecotoxicity database and the species-averaging did not consider life-stage differences. A comparison between both approaches is given in online supplementary material, Table S1.4.

The most sensitive endpoint per species, that is, "species-mean $L(E)C10_{dissolved,y}$" was further used as input in the SSD fitting and environmental threshold derivation, expressed as the HC5 (see Figure 1). Using this approach, a site-specific HC5 can be derived for each considered target water. The original Cu EQS derivation methodology considered a set of 12 different distributions (i.e., Erlang, Gamma, Normal, Beta, Logistic, Inverse Gaussian, Gumbel, Weibull, Pearson V, Pareto, Triangular and Pearson VI). For the current study, this selection was revised based on the distributions commonly used in SSD approaches by regulatory authorities across multiple jurisdictions worldwide (Fox et al. 2021). Within the updated Cu EQS derivation methodology, the site-specific HC5 is calculated by identifying, for each considered target water, the best-fitting SSD (fitted to $\log_{10}$-transformed, normalised toxicity data) from a set of six candidate distributions, i.e., gamma, log-normal, logistic, normal, Weibull and Gumbel. The best-fitting distribution is determined with the Anderson-Darling goodness-of-fit statistic (ECHA 2008a) which puts the emphasis on the tails of the distributions, being the region of interest for the threshold derivation. The final HC5 for a given target water is expressed as the median HC5 value (i.e. HC5–50) derived from the best-fitting distribution. To account for sampling uncertainty, a 90% confidence interval - represented by the HC5-5 and HC5-95 – is calculated using parametric bootstrap simulation with 2,000 samples. These simulations are performed with replacement of the normalized species-mean $L(E)C10_{Cudiss,y}$ (Verdonck et al. 2001). Unless otherwise specified, “HC5” refers to the median HC5 and is synonymous to the site-specific environmental threshold or EQS.

The entire site-specific EQS derivation method, including the updated ecotoxicity database, the optimized bioavailability models (coupled with WHAM VII) and SSD, has been implemented in CuBioM v2.0 software. The CuBioM v2.0 tool is freely available online (https://www.ceh.ac.uk/data/software-models/copper-bioavailability-modelling-tool) that allows the derivation of site-specific environmental thresholds (expressed as HC5). All calculations included in the present study were conducted using CuBioM v2.0.

2.3 Freshwater bioavailability datasets

The distribution of site-specific EQS in European surface waters under the updated EQS scenario was assessed using two physico-chemical datasets. The first dataset includes seven regulatory-defined freshwater bodies, known as the “ecoregion” dataset (ECHA 2008b), and is hereafter referred to as the “regulatory freshwater set” (see online supplementary material, Table S3.1 for a full description of the physico-chemical parameters). This selection of reference waterbodies has historically served as the basis for deriving the PNEC under the existing substances regulation using the original EQS derivation methodology (Cu VRAR; ECI 2008). The second physico-chemical dataset, the dataset collected under the auspices of the Forum of European Geological Surveys (FOREGS; Salminen et al. 2005) was selected to evaluate the distribution of site-specific EQS across a broader range of bioavailability conditions in Europe. The FOREGS database contains physico-chemical data from over 800 freshwater bodies sampled for 25 European countries, among which 22 European Union member-states. Within the FOREGS database, the following member states are not represented: Bulgaria, Cyprus, Luxembourg, Malta, Romania. For this assessment, only those sites with physico-chemical conditions falling within the application ranges of the updated Cu bioavailability models (pH 5.5–8.6, Ca 2.4–124 mg/L [Nys et al. 2024], and DOC≤30 mg/L [EC 2021]) were used to assess the distribution of Cu environmental thresholds in European surface waters (*n*=632, see online supplementary material, Figure S3.1). Temperature may influence Cu speciation as well as biological processes. However, for Cu the effect of temperature on chronic toxicity to *Daphnia magna* has been mainly related to effects on speciation (Pereira et al. 2017). Moreover, the reported effect of temperature on chronic Cu reproductive toxicity was limited (1.4-fold), and in general smaller than other toxicity modifying factors included in the biotic ligand model. Given the small influence of temperature, within the EQS derivation a default target water temperature of 15°C was used for all normalizations. The European Environment Agency reports that in large rivers in the central and southern region average annual water temperature varies between 11 and 18°C (EEA, 1995). Furthermore, 15°C is also within the range of more recent water temperatures of large European rivers and lakes (EEA, 2012). Based on this range, 15°C was selected as a "reference average temperature" for European freshwaters.

2.4 Scenario analysis

The updated EQS derivation methodology was developed in accordance with the relevant WFD guidance (EC, 2018), and metal-specific consideration (ECHA, 2008b). It is acknowledged, however, that alternative choices during database collation and implementation of the SSD-approach could have been made. To assess the robustness of the site-specific EQS derivation for Cu, a scenario analysis was conducted. In addition to the updated EQS derivation methodology, three different scenarios were developed for sensitivity analysis.

Dissolved Organic Carbon is one of the main physico-chemistry parameters influencing Cu toxicity. Nys et al. (2024) showed that uncertainties in measured DOC can have a large impact on Cu toxicity predictions. Within the database update, newly identified data for which DOC was not available were not considered. However, this criterion was not applied on the data originating from the Cu VRAR. Sensitivity scenario 1 evaluated what the influence of the inclusion of Cu VRAR data with uncertain DOC estimates (estimated based on default concentration) is on environmental threshold derivations for Cu. Under Sensitivity scenario 1, only data for which measured DOC was either reported in the original study or could be reliably extrapolated were included for bioavailability normalization.

The updated EQS scenario includes all species, regardless of their geographical distribution—encompassing both species occurring in Europe and those occurring elsewhere. Sensitivity scenario 2 assessed the impact of including geographically non-relevant species on the derivation of environmental thresholds. To this end, the geographical relevance of all species in the updated ecotoxicity database was evaluated based on their occurrence in European freshwater ecosystems. Species- and genus-level occurrence data were obtained from the Global Biodiversity Information Facility (GBIF; www.gbif.org, consulted on December 16th, 2021). Based on this information, species were grouped into four categories: Group I - Species frequently reported in European freshwaters; Group II - species not occurring in Europe, belonging to a genus that occurs in Europe and the genus is not already represented in the dataset by a species occurring in Europe; Group III - species not occurring in Europe, and from a genus occurring in Europe but the genus is already represented in the dataset by at least one European species; Group IV - species not occurring in Europe and with no representatives at genus-level occurring in Europe. Under Sensitivity scenario 2, only species from Groups I and II were considered geographically relevant and included in the threshold derivation.

Sensitivity scenario 3 examined the impact of differentiating between life stages in the species-averaging. In the updated EQS scenario, species-averaging selects the most sensitive endpoint of the most sensitive life stage for each species (based on the geometric mean). In contrast, under Sensitivity scenario 3, only endpoints were considered in the species-averaging process—that is the most sensitive endpoint was selected across all available life stages (based on the geometric mean), without distinguishing among them (see online supplementary material, Table S1.4). A detailed description of all sensitivity scenarios and their comparison with the updated EQS scenario is provided in online supplementary material, Table S1.3. The sensitivity scenarios were evaluated for the regulatory surface water set and the European surface water set FOREGS. In addition, to evaluate the robustness of the final derived $EQS_{bioavailable}$ the three scenarios were also evaluated for the country-specific dataset based on which the $EQS_{bioavailable}$ has been derived (i.e., Norway).

2.5 Derivation of the $EQS_{bioavailable}$ for Cu

Based on the updated EQS scenario, an updated $EQS_{bioavailable}$ for Cu was derived following the stepwise procedure outlined in the EU guidance on EQS derivation (EC 2018). In the first step, the European baseline monitoring database, FOREGS (Salminen et al. 2005, see section *Freshwater bioavailability datasets*), was used to identify the countries with the highest bioavailability conditions for Cu across Europe, based on the lower 5th percentile of the site-specific HC5 distribution of the countries represented within the FOREGS database.

In the second step, additional country-specific datasets were gathered from various sources for the countries identified in Step 1 as having the highest Cu bioavailability conditions. The relevant EU guidance (EC, 2018) specifies that only for the most sensitive region additional databases should be consulted to derive the bioavailable EQS. The FOREGS database mainly focusses on pristine waters, often sampled in upstream regions, which may have created a bias compared to actual bioavailability conditions in waters that are relevant for evaluating compliance under the WFD. Therefore, it was decided to consider the 7 countries with the highest bioavailability conditions for Cu in Step 2 of the bioavailable EQS derivation (i.e., representing the 30% most sensitive countries in the FOREGS database; *n*=25), rather than only the most sensitive country. Site-specific HC5 values were calculated for all monitoring data where at least pH, DOC, and Ca were reported. If not available Mg, Cl, K, and SO_4 were estimated using standard Ca-based regression models (Peters et al., 2011). If multiple samples for a single site were available, the resulting HC5 values were averaged to produce a site-specific HC5. Country-specific $EQS_{bioavailable}$ were calculated as the lower 5th percentile of the distribution of site-specific HC5 values within the country-specific database. The final $EQS_{bioavailable}$ was then determined as the lowest country-specific $EQS_{bioavailable}$ across the seven selected countries in Step 2.

For both datasets, when temperature data were unavailable, a default value of 15 °C was applied (see rationale in section *Freshwater bioavailability datasets*). In addition, only waters where pH, Ca and DOC fell within the applicability range of the chronic Cu bioavailability models were included in the assessment (pH 5.5–8.6, Ca 2.4–124 mg/L, DOC≤30 mg/L).

3. RESULTS

3.1 Chronic Cu ecotoxicity database update

Of the 139 datapoints included in the original database (the database considered in the original EQS derivation methodology, i.e., Cu VRAR, ECI 2008), 20 were not retained in the updated database, due to the use of nominal concentrations (*n*=3) or non-relevant endpoints (*n*=17), such as filtration rate for molluscs and biomass for algae (see online supplementary material, S4). For 20 datapoints retained from the original database, the NOEC was updated to a robust LC10 or EC10. Additionally, 12 new biological endpoints or life-stages were added to the updated database from eight studies already included in the original Cu database. From all studies included in the comprehensive literature review, 244 datapoints from 36 studies were deemed reliable and relevant (all K2 scores). The newly added data covered 30 species, including 24 species that were previously not represented in the original Cu database.

The updated Cu ecotoxicity database includes 376 high-quality chronic datapoints covering 49 species across 19 taxonomic groups (see Figure 2). Within the updated dataset, non-normalized L(E)C10 values range from 0.5 µg dissolved Cu/L (*Chlorella* sp.; DOC = 0.1 mg/L, pH 7.5, Ca = 7 mg/L) to 510 µg dissolved Cu/L (*Chlorella vulgaris*; DOC = 15.2 mg/L, pH 6.1, Ca = 128 mg/L), both growth rate endpoints. A detailed overview of the full ecotoxicity database, including data from the original database, new additions from the current update and non-retained toxicity datapoint (Klimisch K3), is provided in the supplemental information (see online supplementary material, Table S4).

3.2 Distribution of site-specific environmental thresholds in European surface waters

Site-specific environmental thresholds, expressed as the HC5, for the regulatory surface water set ranged from 4.7 to 26.1 µg dissolved Cu/L (Table 1). The corresponding normalized species-mean L(E)C10$_{Cudiss}$ for the seven regulatory surface waters are reported in online supplementary material, Table S5.1. Species sensitivity distributions for two physico-chemical conditions - Swedish Lake and the river Ebro - are visualized in Figure 3. The SSDs for the other waters of the regulatory surface water set are visualized in online supplementary material, Figure

S5.1. In the Swedish Lake scenario, the three most sensitive species in the SSD were the sturgeon *Acipenser transmontanus* (L(E)C10=3.8 µg/L, larval growth endpoint), the molluscs *Fluminicola sp.* (L(E)C10=5.5 µg/L, mortality) and *Lampsilis siliquoidea* (L(E)C10=5.7 µg/L, juvenile growth), while *Chlorella vulgaris* (L(E)C10=106 µg/L, growth rate), *Noemacheilus barbatulus* (L(E)C10=108 µg/L, adult mortality) and *Asellus aquaticus* (L(E)C10=109 µg/L, adult mortality) represented the least sensitive species. In the River Ebro scenario, the order of sensitivity among species shifted due to difference in physico-chemistry (see also Figure 4). In this high pH and moderate calcium scenario, the three most sensitive species in the SSD were the algae *Chlorella sp. (isolate 12*; L(E)C10=3.7 µg/L, growth rate), the sturgeon *A. transmontanus* (L(E)C10=9.0 µg/L, larval growth) and the higher plant *Lemna minor* (L(E)C10=9.7 µg/L, growth), while *Chironomus riparius* (L(E)C10=197 µg/L, larval growth), *Hyalella azteca* (L(E)C10=214 µg/L, juvenile mortality) and *Asellus aquaticus* (L(E)C10=310 µg/L, adult mortality) represented the least sensitive species.

An overview of the distribution of site-specific environmental thresholds under the updated EQS derivation scenario in European surface waters (based on the FOREGS dataset) is given in Figure 5. Site-specific HC5 across Europe ranged generally between 2.0 and 37.8 µg dissolved Cu/L (5th–95th percentile of site-specific HC5). Among the evaluated physico-chemical parameters, DOC emerged as the primary driver of site-specific HC5 values (Figure 5 and 6), with the highest sensitivity (i.e., lowest HC5 values) observed at low DOC concentrations. At the country-level physico-chemistry, including DOC concentrations, may vary substantially, and as such the range in HC5 can also span different orders of magnitude within a country. For instance, in Italy 5th to 95th percentile of site-specific HC5 differed 42-fold (Table 2), with lowest HC5 observed in the Italian Alps and highest HC5 in central Italy and Sardinia (Figure 5).

Although there was no clear relationship between the site-specific HC5 and pH or Ca within the European surface water dataset (see online supplementary material, Figure S5.2), the relationship is obscured by intercorrelations among DOC, pH, and calcium concentrations (see online supplementary material, Figure S3.1). As such, additional calculations of site-specific HC5 were made for a series of hypothetical freshwater conditions in which DOC, pH and Ca were univariately varied (Figure 4). An increase in site-specific HC5 (decreasing Cu sensitivity) was observed with increasing DOC and pH. For Ca, the relationship was less clear and depended on the considered pH-level. The latter is mainly due to different taxonomic groups that are identified as most sensitive species depending on the considered pH level. Al low pH, the fish *A. transmontanus* is identified as most sensitive species, while at high pH this is the tropical chlorophyte *Chlorella sp* isolated in Papoea New Guinea. Furthermore, invertebrates and fish show a trend of decreasing Cu toxicity when Ca increases, which is in line with the Ca competition effect included in their corresponding bioavailability model (Nys et al., 2020, 2024). In contrast, for algae a trend of increasing Cu toxicity when Ca increases was observed, which can be explained based on speciation considerations, as increased competition between Cu and Ca for binding sites of natural organic matter occurs with increasing Ca concentrations.

3.3 Scenario analysis

Normalized site-specific thresholds calculated under the different sensitivity scenarios are visualized in Figure 7 for the regulatory surface waters set. Compared with the updated EQS scenario, the DOC refinement considered in Sensitivity scenario 1 resulted in on average 0.9-fold (median value; range between 0.8- and

1.0-fold) lower HC5 across the different considered water types (see online supplementary material, Table S6.1). The consideration of only geographically relevant species in Sensitivity scenario 2, resulted in on average 1.2-fold (median-value; range between 1.1- and 1.3-fold) higher HC5 across the different considered water types. The ignorance of potential sensitivity difference between life stages in Sensitivity scenario 3 resulted in on average 1.1-fold (median-value; range between 1.0- and 1.1-fold) higher HC5 across the different considered water types. Scenarios with fewer species showed wider 90% confidence intervals than the updated EQS scenario (error bars in Figure 7). This was most apparent for Sensitivity Scenario 2 (24 species) and to a lesser extent for Scenario 1 (43 species). Overall, median differences between the updated EQS scenario and the three sensitivity scenarios were similar when compared between the regulatory surface water and the European surface water set (see online supplementary material, Table S6.2, Figure S6.1).

3.4 EQS derivation

Step 1 – identification of countries with high bioavailability conditions for Cu

A summary of normalized HC5 for the monitoring sites included in the FOREGS database is provided in Figure 5.A and Table 2. Based on this assessment, the countries exhibiting the highest Cu bioavailability conditions—and thus the greatest sensitivity, as indicated by the lowest 5th percentiles of the site-specific HC5 distributions—were identified as Switzerland, Italy, Norway, Austria, Slovenia, Germany and France.

For some of the waters classified among those with the highest bioavailability conditions, measured DOC concentrations were reported to be below the detection limit (DL = 0.50 mg/L, see online supplementary material, Figure S7.1). In these cases, a value of DL/2 (i.e., 0.25 mg/L) was used in calculations. This assumption introduces uncertainty, as the actual DOC may fall anywhere between near-zero and 0.50 mg/L. To assess the impact of this assumption, additional bioavailability calculations were performed for the 19 sites with DOC below detection limits. In these calculations, DOC was varied between 0.10 and 0.50 mg/L. This fivefold variation in DOC yielded, on average, a 2.9-fold change in site-specific HC5 values (see online supplementary material, Figure S7.2). Nonetheless, the influence of this assumption on the overall ranking of countries based on Cu bioavailability conditions was limited (see online supplementary material, Table S7.1). Therefore, all seven countries were retained for the second step of the $EQS_{bioavailable}$ derivation.

Step 2 – Derivation of an $EQS_{bioavailable}$ using country-specific databases

Country-specific datasets were collected from various sources for these seven countries (Table 2). Distribution of site-specific HC5 and variability between sample-specific HC5 for the different sites for all databases are visualized in the online supplementary material, Figure S7.3. The country-specific $EQS_{bioavailable}$ for all considered countries is given in Table 3. Among the six European countries evaluated in Step 2, Norway had the lowest country-specific $EQS_{bioavailable}$, that is, 1.0 µg dissolved Cu/L. Germany, by contrast, exhibited the highest country-specific $EQS_{bioavailable}$, that is, 4.1 µg dissolved Cu/L. The lowest country-specific $EQS_{bioavailable}$—1.0 µg dissolved Cu/L from Norway—is proposed to be carried forward as EU-wide $EQS_{bioavailable}$. All site-specific physico-chemistry and corresponding HC5 used for deriving the $EQS_{bioavailable}$ in the current study are available in the online supplementary material, S8.

To evaluate the robustness of the proposed $EQS_{bioavailable}$, a scenario analysis was performed using the underlying country-specific dataset (i.e., Norway) and assessing the three defined sensitivity scenarios. Overall, the corresponding threshold (lower 5th percentile of HC5 distribution) varied only limitedly over the different considered sensitivity scenarios, i.e., ranging between 1.0 (Sensitivity scenario 1 and 2) and 1.1 (Sensitivity scenario 3) µg dissolved Cu/L (see online supplementary material, Figure S7.4, Table S7.2). As such, the proposed $EQS_{bioavailable}$ —1.0 µg dissolved Cu/L - can be considered a robust environmental threshold.

For Slovenia—ranked as the fifth most sensitive country in Step 1—no country-specific monitoring datasets containing relevant physico-chemical data could be identified. Despite the small number of sites for Slovenia in the FOREGS database, the variability in normalized HC5 values across Slovenian samples is relatively low (Table 2), suggesting consistently high bioavailability conditions, especially due to the low DOC concentrations (<1.3 mg/L). However, based on the fact that all FOREGS-Slovenian site-specific HC5 exceed the $EQS_{bioavailable}$, our analysis suggests that the proposed threshold is also applicable to achieve freshwater ecosystem protection in Slovenia, although additional data would be needed to fully confirm this conclusion.

DISCUSSION

Updated EQS derivation scenario

The updated EQS derivation scenario is meant to be a step forward in the risk assessment of Cu compared to the original EQS derivation methodology, as this scenario considered more recent ecotoxicity data and an improved reliability and relevancy screening, leading to a database that includes only high-quality data representing endpoints relevant for effects of Cu at the population level. The database considers several species that have been reported to be particularly sensitive to chronic copper exposure (i.e., in the lower µg/L region), such as the sturgeon *Acipenser transmontanus* (e.g., Ingersoll & Mebane 2014), and the pond snail *Lymnaea stagnalis*, including juvenile growth data (Cremazy et al. 2018). The species richness of the updated database (covering 49 species) is significantly larger compared to the original database, which contained 27 species (ECI, 2008). Moreover, the species richness is comparable with recent datasets collated for Cu environmental threshold derivation in Australia and New Zealand (59 species; Australian and New Zealand Governments [ANZG] 2023), and with datasets from other data-rich metals gathered within the European regulatory-context (e.g., 53 species for nickel; Peters et al., 2023).

The species included in the updated database represent 19 different taxonomic groups (Figure 2). As such, the minimum data requirements for the use of an SSD in environmental threshold derivation have been more than fulfilled – that is, at least 15 L(E)C10 values across 8 taxonomic groups as specified by EC (2018). Among the taxonomic groups considered in the database, mollusc species are disproportionally represented: 18 out of the 49 species are mollusc species (Figure 2). The focus on Cu sensitivity to gastropods and bivalves in literature has been guided because molluscs have been reported to be among the most threatened faunal groups in freshwater ecosystems worldwide (Böhm et al., 2021).

The updated EQS derivation methodology implements the recently updated Cu bioavailability models (see Nys et al., 2024). The update of the bioavailability models included an update of the most recent version of the speciation software WHAM (WHAM VII) and adoption of a generalized bioavailability model (gBAM) structure. In this optimized model structure, the log-linear pH term describing the effect of pH on Cu toxicity alongside cationic competition, provides a reduced model complexity compared to the original Cu bioavailability models (Nys et al., 2020, 2024). The updated invertebrate model also incorporated the competition effects of Ca^{2+} and Mg^{2+} at the biotic ligand, which have been shown to enhance model performance for invertebrates (Van Regenmortel et al., 2015).

The SSD fitting and corresponding site-specific threshold derivation in the updated EQS derivation methodology employed a best-fit method, selecting the most appropriate distribution from six options commonly used in environmental threshold derivation across various jurisdictions worldwide (Fox et al., 2021). Next to the classic distributions used for SSD fitting (i.e., (log)-normal and logistic), the selection also includes the Gamma, Weibull, and Gumbel distribution, which can model asymmetrical tails in SSDs (Zajdlik, 2005). This characteristic can be considered as relevant for SSD fitting, as the lower tail is the region where the HC5 is derived. Although originally developed to describe extrema (minimum or maximum) in environmental science, such as wave heights, droughts (Gumbel), or for time-to-failure data (Weibull and Gamma; Zajdlik, 2005), several jurisdictions (e.g., Canada and the United States) have adopted these distributions as default models for SSD fitting because of their characteristic shape (Fox et al., 2021). The implementation of a best-fit approach is particularly relevant given that different environmental conditions—and associated shifts in species sensitivity—can influence the shape of the SSD.

Finally, the application of the updated methodology to European surface waters highlights the important role of bioavailability in driving site-specific Cu thresholds. Conditions associated with low dissolved organic carbon (Figure 6), low pH, and/or low Ca (or low hardness; see online supplementary material, Figure S5.3, Figure 4) correspond to increased bioavailability and greater ecological hazard. These conditions mainly prevail in the alpine region (Switzerland, Italy, Austria, Slovenia, Germany and France) and Norway (Figure 5.A), while other regions are characterized with lower bioavailability conditions. This variability in ecosystem sensitivity emphasizes the need of incorporating bioavailability explicitly into EQS derivation frameworks.

Scenario analysis

The scenario analysis demonstrates that the updated EQS derivation methodology is robust to alternative assumptions in both data selection and SSD construction. Across all scenarios, only minor variation in site-specific HC5 values was observed, indicating limited sensitivity to these assumptions.

First, restricting the dataset to studies with measured dissolved organic carbon (DOC) concentrations resulted in only negligible changes in derived thresholds. Dissolved Organic Carbon is an important parameter for determining Cu toxicity (see Figure 6, but also Brix et al., 2021; De Schamphelaere & Janssen, 2004a) and uncertainties associated with using default DOC values instead of measured concentrations can significantly affect predicted copper toxicity (see, for example, Nys et al., 2024). However, our analysis shows that at the level of deriving a site-specific local HC5 the influence of restricting the dataset to studies with measured DOC concentration, while excluding legacy data without measured DOC is minimal.

Second, limiting the dataset to geographically relevant species led to a modest increase in HC5 values. According to the EQS derivation guidance of the WFD (EC, 2018), all available high-quality data for any taxonomic group should be considered for EQS derivation, as it is assumed that species geographically not present in

European surface waters might be surrogate species for species in European surface waters but not represented in the database (EC, 2018). The use of species that are not representative of the ecosystem to be protected has, however, been criticized in the literature (Banks et al., 2010; Forbes and Calow, 2002). As a refinement, several jurisdictions have considered the geographical relevance of a species for a specific region as a criterion to collate SSDs (e.g., United States, Canada, Australia and New-Zealand; van Dam et al., 2019). In Sensitivity scenario 2, we evaluated the influence of the consideration of non-geographically relevant species on the environmental threshold derivation for Cu. Of the 49 species included in the updated ecotoxicity database, only 24 are geographically relevant for Europe (see section *Scenario analysis* for definition, and also online supplementary material, Table S6.4). Non-geographically relevant species include predominantly molluscs (16 species) and fish (6 species), along with a chlorophyte, an amphipod, and an insect. Several of these, including *Chlorella* sp., *Fluminicola* sp., *Lampsilis siliquoidea*, and *Villosa iris*, rank among the five most sensitive species across target waters in the regulatory surface water set (see online supplementary material, Table S5.1). Nonetheless, sensitivity scenario 2 indicated that the local HC5 is not strongly dependent on species biogeography.

In this context, especially the *Chlorella sp.* data is interesting, as this is a tropical species isolated from Papua New Guinea. This species is approximately 10-fold more sensitive compared to the European *Chlorella vulgaris* (see online supplementary material, Figure S6.2). Given the large sensitivity difference between these two Chlorella species and the fact that the *Chlorella sp.* data drives the SSD at high pH, this dataset may need some additional exploration in future research. Moreover, the species driving the HC5 are not always ecologically representative for the considered physico-chemistry and/or geographical location. A clear example is the American sturgeon *A. transmontanus,* which is the most sensitive species in freshwaters with low pH, conditions mainly occurring in Scandinavian countries (see online supplementary material, Figure S5.3) or freshwaters in the alpine region. While this species is not occurring in EU surface waters, other sturgeon species, for example, *Acipenser sturio,* are native to Europe. Sturgeons, which are anadromous, breed during spring and summer mainly in slow flowing large river systems. After the larval stage, juvenile migrate to the open sea to grow into adults. According to literature, the early life stages of European sturgeons (fertilized eggs and yolk sac larvae) inhabit neutral to slightly alkaline large river systems, with temperature of ±20° in spring/summer (Brevé et al. 2024; Delage et al. 2019) and therefore it is unlikely that this species occurs in Norwegian freshwaters or in Alpine fast flowing rivers. A potential further refinement of the updated EQS derivation methodology could be to link the habitat preference (e.g., flow rate) and/or physiological tolerance of species (e.g., temperature, pH) of the species considered in the Cu SSD to the abiotic conditions occurring in a particular region or freshwater system.

Third, ignoring differences in sensitivity between life stages had only a limited impact on local HC5 values. Although accounting for life-stage variability aligns with current guidance and is ecologically justified—given the importance of sensitive early life stages for population-level effects (e.g., Vlaeminck et al., 2019)—its quantitative influence on the finally derived thresholds is small. This indicates that the current approach, which selects the most sensitive endpoint and life stage, is appropriately precautionary without substantially altering outcomes.

Overall, the limited variation across all sensitivity scenarios demonstrates that the updated EQS derivation methodology is robust to reasonable alternative assumptions. In contrast, variability in site-specific HC5 values driven by differences in water chemistry is substantially larger. This highlights that it is essential that any freshwater environmental threshold derivation approach for Cu considers bioavailability, which is also supported by the relevant European guidance's regarding environmental risk assessment (ECHA, 2008a,b; EFSA, 2021) and compliance assessment under the Water Framework Directive (EC, 2018, 2021).

4.3 Derivation of the $EQS_{bioavailable}$

The EU-wide $EQS_{bioavailable}$ for Cu derived in the present study (1.0 µg dissolved Cu/L) can be considered to be protective for the majority of surface waters in Europe. This follows from the observation, that for 98.5% of sites in the FOREGS database, the site-specific HC5 is higher than the $EQS_{bioavailable}$ (only considering the waters within the defined applicability range). The FOREGS dataset was specifically designed to represent baseline geochemical conditions across Europe, using harmonized sampling (Salminen et al., 2005), and therefore gives a broad overview of water chemistry within Europe. While our analysis indicates that the $EQS_{bioavailble}$ is generally protective, this level of protectiveness should be interpreted as an approximation, particularly given the limited number of samples available for some regions or countries or geographical areas. However, the general protectiveness of the $EQS_{bioavailable}$ was confirmed based on the data the country-specific databases (>99% sites, Table 3). Norway by definition represents an exception, reflecting the prevalence of water chemistry conditions associated with higher Cu bioavailability (Hoppe et al., 2015). This highlights that regional differences in water chemistry are indeed relevant, and may occasionally lead to lower site-specific HC5 compared to the $EQS_{bioavailable}$. More specifically, combinations of low DOC concentrations (≤0.7 mg/L), Ca concentrations ≤46 mg/L and pH up to 7.9 may not be fully protected by $EQS_{bioavailble}$. These conditions may sporadically occur in specific regions such as parts of Scandinavia (Norway) and in mountainous areas (e.g., alps in Austria, Italy,…).

Importantly, the applicability of both the $EQS_{bioavailable}$ and the associated methodology to derive site-specific HC5-values is constrained to a defined application range (i.e., pH 5.5–8.6, Ca 2.4–124 mg/L [Nys et al., 2024]; DOC≤30 mg/L [EC, 2021]). While these boundaries encompass the majority of European surface waters (i.e., 78% of the waters within the FOREGS database), a subset of systems falls outside this range. In most cases, exceedance of the upper boundaries (e.g., high DOC, high Ca, or high pH) is unlikely to correspond to high bioavailability conditions, and the $EQS_{bioavailable}$ can also serve as a protective screening threshold under these conditions. However, approximately 9% of the surface waters in Europe represent conditions below the lower boundaries for Ca and/or pH. These conditions may represent high bioavailability conditions, and hence, the $EQS_{bioavailable}$ is potentially not protective under these conditions. Such conditions occur in specific regions, including parts of the Iberian Peninsula, the UK, and Scandinavia, and are particularly prevalent in Norwegian freshwaters which are typically characterized by a combination of (extremely) low Ca and low pH conditions (Hoppe et al., 2015, see also online supplementary material, Figure S3.2). Limited evidence suggests that the relationship between hardness and metal toxicity is not consistent over the entire hardness range (e.g., for Cu & Ni; Deleebeeck et al., 2007; Long et al., 2004, Peters et al., 2018). Based on this, it can be concluded that the updated EQS derivation methodology cannot be simply extrapolated to low Ca and/or low pH conditions.

Hence, there remains a need to further investigate Cu toxicity to aquatic organisms at low hardness and low pH conditions, preferably with species adapted to these conditions. Furthermore, in this context it should be acknowledged that Arctic soft waters are very specific environments which generally have lower species richness than hardwater lakes because the lower nutrient content and higher environmental stress in cold soft water systems limits the variety of species that can survive and thrive (Lento et al., 2019).

4.4 Comparison with existing environmental thresholds of Cu for the aquatic compartment

Europe – WFD The $EQS_{bioavailable}$ of 1.0 µg dissolved Cu/L derived in the present study is in line with existing $EQS_{bioavailable}$ that were based on the original EQS derivation methodology. Earlier assessments, including those by the UK Environment Agency (2009) and more recent EU-wide evaluations (Peters et al., 2019, Wilson et al., 2023), reported similar thresholds (≈1.0–1.1 µg/L), indicating that updates to the derivation framework have not substantially altered the overall level of protection.

Despite this consistency in the final value, the environmental conditions driving the EQS have shifted. Whereas earlier assessments were driven by conditions in the UK or Austria, the current derivation identifies Norwegian conditions as most critical. This shift likely reflects updates to the bioavailability modelling, particularly the inclusion of Ca^{2+} and Mg^{2+} competition in the invertebrate model (Nys et al., 2024). As a result, while the overall threshold remains stable, differences in site-specific HC5 values can arise under specific physicochemical conditions depending on the modelling approach.

Within the defined application range (pH 5.5–8.6, Ca 2.4–124 mg/L, DOC≤30 mg/L; EC, 2021; Nys et al., 2024), the $EQS_{bioavailable}$ of 1.0 µg dissolved Cu/L, can be used as the screening-level threshold in lower tier compliance assessments under the Water Framework Directive (Tier 1 under EC, 2021). However, this value is relatively close to natural background concentrations of Cu in European freshwaters. For instance, the median Cu concentration in the FOREGS database (Salminen et al., 2005), a database representing mainly baseline conditions which can be used for determining European background concentrations (EC, 2021), is equal to 0.88 µg dissolved Cu/L (10th–90th percentile ranging between 0.3 and 2.4 µg dissolved Cu/L). In combination with the dependency of Cu sensitivity on local water body conditions, compliance of Cu is thus best assessed using higher-tier bioavailability corrections (EC, 2021). The updated EQS derivation methodology presented in the current study provides a robust and up-to-date basis for incorporating bioavailability in regulatory assessments. Moreover, given that Cu is regulated as a river-basin-specific pollutant under the Water Framework Directive (EC, 2008), the approach offers flexibility to derive regionally adapted thresholds, or country/region-specific $EQS_{bioavailable}$. Such refinement could improve the ecological relevance and efficiency of compliance assessments by better reflecting local water chemistry conditions.

Europe – REACH While the WFD aims to protect European freshwater systems by ensuring good ecological and chemical status (EC, 2008), the EU REACH framework focuses more broadly on protecting human health and the environment against exposure to potentially harmful substances such as Cu (EC, 2006). These differing objectives are reflected in their respective protection goals and derivation approaches for environmental thresholds.

Under the WFD, the $EQS_{bioavailable}$ is designed to protect 95% of the surface waters under worst-case bioavailability conditions, whereas REACH derives thresholds based on a set of seven recognized standard water bodies considered in the regulatory surface water dataset, that is, the ecoregions (ECHA, 2008). The regulatory surface water dataset was previously also used for derivation of the Cu PNEC in the Cu Voluntary Risk Assessment Report (ECI, 2008). The resulting PNEC of 7.8 µg dissolved Cu/L, derived from the River Otter ecoregion under the original environmental threshold derivation methodology (ECI, 2008; Table 1), has served as the reference value under the European Union's REACH framework for over a decade. More recently, the PNEC was revised to 6.3 µg dissolved Cu/L following updates to the ecotoxicity database and bioavailability models, with the Swedish lake ecoregion now identified as the critical scenario. In the present study, a lower site-specific HC5 is derived for this same scenario (4.7 µg dissolved Cu/L), which can be attributed to differences in methodological choices related to the reliability and relevancy assessment, to species selection and data aggregation (see online supplementary material, Table S1.3 and S4). This highlights the importance of transparency in methodological decisions.

Across the globe – While European Cu environmental threshold derivation has evolved since its initial development (ECI, 2008), the underlying principle—incorporating bioavailability through (semi-)mechanistic models describing Cu binding at the biotic ligand—has remained consistent (Nys et al., 2024). In contrast, approaches used globally vary substantially in how bioavailability is accounted for.

Some jurisdictions have until now largely ignored the influence of bioavailability on Cu sensitivity and fixed thresholds are applied across all surface waters (e.g. China

& Brazil, Liao et al., 2023 & Conselho Nacional do Meio Ambiente, 2005). Notably, recent scientific developments in these regions increasingly recognize the importance of bioavailability (Duarte et al., 2024, Zhang et al., 2017).

In other regions, the approaches historically used for incorporating bioavailability in environmental threshold derivations for Cu include hardness-corrections (United States of America [USA], Australia & New-Zealand), Water Effect Ratios (USA), and biotic ligand models (BLMs; USA, Canada & Europe; reviewed in more detail by Adams et al., 2020).

More recently, motivated by the perceived complexity of BLM-based methods, alternative approaches based on regression techniques have gained attention. These alternative approaches include DOC-based corrections (Australia & New-Zealand; ANZG, 2023) The results of the present study support the central role of DOC as a primary driver of Cu bioavailability, consistent with these simplified approaches. However, they also demonstrate that other factors, particularly pH and Ca, can exert a substantial influence on derived thresholds. This suggests that approaches relying on a limited set of predictors may not fully capture variability in Cu toxicity across environmental conditions and could lead to both over and under protection of freshwater systems (see also Figure 4).

An alternative approach are multiple linear regression models (MLRs), which have been proposed to incorporate bioavailability in Cu water quality criteria derivation in the United States (Mebane, 2023). Multiple linear regression models allow to integrate the combined influence of different toxicity modifying factors using multiple-factor equations (Brix et al., 2021). The Cu MLR relates dissolved Cu toxicity directly to DOC, hardness, and pH, and therefore does not include the computationally more intensive speciation-step that is inherent to a speciation-based bioavailability modelling approach such as used in the current study.

This comparison shows that although there are clear differences in how bioavailability is integrated between jurisdictions and as such also in site-specific threshold derivation (see Figure 4), there are also similarities as all jurisdictions identify DOC as the main toxicity modifying factor for Cu. While the bioavailability approach under the European regulation may appear the most complex and data-hungry approach, a (user-friendly) tool to derive site-specific HC5 is available, that is, CuBioM v2.0 (available on https://www.ceh.ac.uk/data/software-models/copper-bioavailability-modelling-tool). The CuBioM tool allows the derivation of site-specific freshwater environmental threshold values for Cu under the updated EQS scenario discussed in the current study for a multitude of sites with as little as three needed physico-chemistry parameters (i.e., pH, DOC and Ca). As such, this tool facilitates the derivation of site-specific EQS values and supports more ecologically relevant risk assessments, including compliance evaluations under the WFD.

Conclusion

This study presents an updated methodology for deriving site-specific environmental quality standards for Cu under the European Union’s Water Framework Directive (WFD), integrating recent ecotoxicity data, applying a stricter data screening, updated bioavailability models, and a refined species sensitivity distribution approach. Together, these developments provide a robust and scientifically advanced framework for incorporating bioavailability into environmental threshold derivation for copper in the aquatic compartment.

Application of this methodology results in an EU-wide $EQS_{bioavailable}$ of 1.0 µg dissolved Cu/L, derived in full accordance with the corresponding WFD guidance and consistent with existing thresholds. The threshold is protective for the vast majority of European surface waters within the defined applicability range (pH 5.5–8.6, Ca 2.4–124 mg/L, DOC≤30 mg/L) and can be used as a conservative screening value in lower-tier compliance assessments. However, given the proximity of this value to natural background concentrations and the strong influence of local water chemistry on Cu toxicity, higher-tier, site-specific bioavailability corrections remain essential for accurate compliance assessment. In this context, the updated framework provides a practical and robust basis for implementing Cu bioavailability within regulatory assessments.

Finally, the updated EQS derivation methodology allows for the derivation of regionally adapted thresholds, which may improve both the ecological relevance and efficiency of compliance assessments. This is particularly relevant for Cu as a river-basin-specific pollutant under the WFD, where region-specific thresholds can reduce unnecessary monitoring efforts.

References

Adams, W., Blust, R., Dwyer, R., Mount, D., Nordheim, E., Rodriguez, P. H., & Spry, D. (2020). Bioavailability assessment of metals in freshwater environments: A historical review. Environmental Toxicology and Chemistry, 39, 48–59.

Azimonti, G., Galimberti, F., Marchetto, F., Menaballi, L., Ullicci, S., Pellicioli, F., Alessandra, C., Lidia, C., Akkesio, I., Angelo, M., de Boer, W., & van der Voet, H. (2015). Comparison of NOEC values to EC10/EC20 values, including confidence intervals, in aquatic and terrestrial ecotoxicological risk assessment. EFSA Supporting Publication, 2015(EN-906), Article EN-906, 274 pp.

Australian and New Zealand Governments. (2023). Toxicant default guideline values for aquatic ecosystem protection: Dissolved copper in freshwater. In Australian and New Zealand Guidelines for Fresh and Marine Water Quality (Draft).

Banks, J. E., Ackleh, A. S., & Stark, J. D. (2010). The use of surrogate species in risk assessment: Using life history data to safeguard against false negatives. Risk Analysis, 30, 175–182.

Besser, J. M., Dorman, R., Ivey, C. D., Cleveland, D., & Steevens, J. A. (2020). Sensitivity of warm water fishes and rainbow trout to selected contaminants. Bulletin of Environmental Contamination and Toxicology, 104, 321–326.

Böhm, M., Dewhurst-Richman, N. I., Seddon, M., et al. (2021). The conservation status of the world’s freshwater molluscs. Hydrobiologia, 848, 3231–3254.

Bossuyt, B. T. A., & Janssen, C. R. (2004). Influence of multigeneration acclimation to copper on tolerance, energy reserves, and homeostasis of Daphnia magna. Environmental Toxicology and Chemistry, 23(8), 2029–2037.

Brevé, N., van Dieren, D., Weeber, M., Mosselman, E., Nagelkerke, L., Murk, A., & Buijse, A. (2024). Assessing potential spawning and nursery habitat availability in the River Rhine for the critically endangered European sturgeon. Aquatic Conservation: Marine and Freshwater Ecosystems, 34, Article e70016.

Brix, K. V., DeForest, D. K., Tear, L., Grosell, M., & Adams, W. J. (2021). Use of multiple linear regression models for setting water quality criteria for copper: A complementary approach to the biotic ligand model. Environmental Science & Technology, 51, 5182–5192.

Conselho Nacional do Meio Ambiente. (2005, March 17). Resolução no. 357, de 17 de março de 2005: Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para seu enquadramento. Diário Oficial da República Federativa do Brasil.

Cremazy, A., Brix, K. V., & Wood, C. M. (2018). Chronic toxicity of binary mixtures of six metals (Ag, Cd, Cu, Ni, Pb, and Zn) to the great pond snail (Lymnaea stagnalis). Environmental Science & Technology, 52, 5979–5988.

CuBioM v2.0. (2026). Copper bioavailability modelling tool. Available on: https://www.ceh.ac.uk/data/software-models/copper-bioavailability-modelling-tool.

Delage, N., Couturier, B., Jatteau, P., Larcher, T., Ledevin, M., Goubin, H., Cachot, J., & Rochard, E. (2019). Oxythermal window drastically constrains the survival and development of European sturgeon early life phases. Environmental Science and Pollution Research International, 1, 3651–3660.

Deleebeeck, N. M. E., Muyssen, B. T. A., De Laender, F., Janssen, C. R., & De Schamphelaere, K. A. C. (2007). Comparison of nickel toxicity to cladocerans in soft versus hard surface waters. Aquatic Toxicology, 84, 223–235.

De Schamphelaere, K. A. C., Vasconcelos, F. M., Heijerick, D. G., Allen, H. E., Tack, F., & Janssen, C. R. (2003). The effects of dissolved organic matter, pH, and water hardness on copper toxicity to the green alga Pseudokirchneriella subcapitata: Development and field validation of a predictive model. Environmental Toxicology and Chemistry, 22, 2454–2465.

De Schamphelaere, K., & Janssen, C. R. (2004a). Effects of dissolved organic carbon concentration and source, pH, and water hardness on chronic toxicity of copper to Daphnia magna. Environmental Toxicology and Chemistry, 23(5), 1115–1122.

Duarte, R. M., Cremazy, A., Wood, C. M., Almeida-Val, V. M. F., & Val, A. L. (2024). The biotic ligand model as a promising tool to predict Cu toxicity in Amazon blackwaters. Environmental Pollution, 341, Article 122988.

European Commission. (2006). Regulation (EC) No. 1907/2006 (REACH).

European Commission. (2008). Directive 2008/105/EC on environmental quality standards in the field of water policy (amending Directives 82/176/EEC, et al.). Official Journal of the European Union.

European Commission. (2018). Technical guidance for deriving environmental quality standards (Guidance Document No. 27). Common Implementation Strategy for the Water Framework Directive.

European Commission. (2021). Technical guidance for implementing environmental quality standards (EQS) for metals (Guidance Document No. 38). Common Implementation Strategy for the Water Framework Directive.

European Chemicals Agency. (2008a). Appendix R.10: Characterisation of dose (concentration)-response for environment. In Guidance on information requirements and chemical safety assessment.

European Chemicals Agency. (2008b). Appendix R.7.13-2: Environmental risk assessment for metals and metal compounds.

European Copper Institute. (2008). European Union risk assessment report: Voluntary risk assessment of copper and its compounds.

European Environment Agency. (1995). Inland waters: Annual summary report 1995. Retrieved December 4, 2025, from https://www.eea.europa.eu/en/analysis/publications/92-9167-005-7

European Environment Agency. (2025). Surface-water methodology: Standard types and threshold values for river basin specific pollutants. Retrieved August 4, 2025, from https://water.europa.eu/freshwater/resources/metadata/wfd-dashboards/surface-water-methodology-standard-types-and-threshold-values-for-river-basin-specific-pollutants-table

European Environment Agency. (2012). Climate change, impacts and vulnerability in Europe 2012: An indicator-based report.

European Food Safety Authority PPR Panel. (2021). Statement of the PPR Panel on a framework for conducting environmental exposure and risk assessment for transition metals as active substances in plant protection products. EFSA Journal, 19(3), Article 6498.

Forbes, V. E., & Calow, P. (2002). Species sensitivity distributions revisited: A critical appraisal. Human and Ecological Risk Assessment, 8(3), 473–492.

Fox, D. R., van Dam, R. A., Fisher, R., Batley, G. E., Tillmanns, A. R., Thorley, J., Schwarz, C. J., Spy, D. J., & McTavish, J. (2021). Recent developments in species sensitivity distribution modeling. Environmental Toxicology and Chemistry, 40, 293–308.

Global Biodiversity Information Facility. (2021). GBIF. Retrieved December 16, 2021, from https://gbif.org

Heijerick, D., Bossuyt, B., & Janssen, C. (2002). Euro ECOLE: Bioavailability and potential ecological effects of copper in European surface waters (Subproject 4). European Copper Institute.

Heijerick, D. G., Bossuyt, B. T. A., De Schamphelaere, K. A. C., Indeherberg, M., Mingazinni, M., & Janssen, C. R. (2005). Effects of varying physico-chemistry of European surface waters on the copper toxicity to the green alga Pseudokirchneriella subcapitata. Ecotoxicology, 14, 661–670.

Hoppe, S., Garno, O. A., Leppänen, M. T., Borg, H., & Ndungu, K. (2015). Soft and sour: The challenge of setting environmental quality standards for bioavailable metal concentration in Fennoscandinavian freshwaters. Environmental Science & Policy, 54, 210–217.

Ingersoll, C. G., & Mebane, C. A. (Eds.). (2014). Acute and chronic sensitivity of white sturgeon (Acipenser transmontanus) and rainbow trout (Oncorhynchus mykiss) to cadmium, copper, lead, or zinc in laboratory water-only exposures (U.S. Geological Survey Scientific Investigations Report 2013-5204). U.S. Geological Survey.

International Commission for the Protection of the Rhine. (2016). Substance data sheet: Copper (Report No. 234). Retrieved December 4, 2025, from https://www.iksr.org/fileadmin/user_upload/DKDM/Dokumente/Fachberichte/EN/rp_En_0234.pdf

Joint Research Centre. (2025). Copper EQS dossier: Discussion of comments on draft. 31st Meeting of the WFD CIS Working Group Chemicals. Retrieved March 18, 2026, from https://circabc.europa.eu/ui/group/9ab5926d-bed4-4322-9aa7-9964bbe8312d/library/1eeea779-6157-4502-aed6-5e60c5bdfd43/details

Journal Officiel de la République Française. (2010). Arrêté relatif aux méthodes et critères d'évaluation des eaux de surface (articles R.212-10, R.212-11 et R.212-18 du code de l'environnement). Retrieved March 13, 2025, from https://www.legifrance.gouv.fr/

Klimisch, H. J., Andreae, M., & Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology, 25, 1–5.

Lento, J., Goedkoop, W., Culp, J., Christoffersen, K. S., Lárusson, K. F., Fefilova, E., Guðbergsson, G., Liljaniemi, P., Ólafsson, J. S., Sandøy, S., Zimmerman, C., Christensen, T., Chambers, P., Heino, J., Hellsten, S., Kahlert, M., Keck, F., Laske, S., Lau, D. C. P., Lavoie, I., Levenstein, B., Mariash, H., Rühland, K., Saulnier-Talbot, E., Schartau, A. K., & Svenning, M. (2019). State of the Arctic freshwater biodiversity. Conservation of Arctic Flora and Fauna International Secretariat.

Liao, W., Zheng, Y., Feng, C., Zhong, W., Zhu, Z., Xie, H., Li, W., Jin, X., & Giesy, J. P. (2023). An often overestimated ecological risk of copper in Chinese surface water: Bioavailable fraction determined by multiple linear regression of water quality parameters. Environmental Sciences Europe, 35, Article 84.

Long, K. E., Van Genderen, E. J., & Klaine, S. J. (2004). The effects of low hardness and pH on copper toxicity to Daphnia magna. Environmental Toxicology and Chemistry, 23, 72–75.

Markich, S. J. (2017). Sensitivity of the glochidia (larvae) of freshwater mussels (Bivalvia: Unionida: Hyriidae) to cadmium, cobalt, copper, lead, nickel, and zinc: Differences between metals, species, and exposure time. Science of the Total Environment, 601–602, 1427–1436.

Mebane, C. A. (2023). Bioavailability and toxicity models of copper to freshwater life: The state of regulatory science. Environmental Toxicology and Chemistry, 42, 2529–2563.

Metals Environmental Research Association. (2026). Metals Environmental Research Association database. Retrieved December 6, 2025, from https://mera-rapps.shinyapps.io/EU_PhysChem_DB_Tool/

Moermond, C. T. A., Kase, R., Korkaric, M., & Ågerstrand, M. (2016). CRED: Criteria for reporting and evaluating ecotoxicity data. Environmental Toxicology and Chemistry, 35(5), 1297–1309. https://doi.org/10.1002/etc.3259

Nationale Dauerüberwachung Fliessgewässer. (2024). Swiss database of the National River Monitoring and Survey Program. Retrieved November 25, 2024, from https://www.bafu.admin.ch/

Nys, C., & Van Sprang, P. (2022). Environmental effects assessment of copper in the freshwater environment [Final report]. European Copper Institute.

Nys, C., Van Sprang, P., Lofts, S., Baken, S., Delbeke, K., & De Schamphelaere, K. (2024). Updated chronic copper bioavailability models for invertebrates and algae. Environmental Toxicology and Chemistry, 43, 450–467.

Nys, C., Vlaeminck, K., Van Sprang, P., & De Schamphelaere, K. A. C. (2020). A generalized bioavailability model (gBAM) for predicting chronic copper toxicity to freshwater fish. Environmental Toxicology and Chemistry, 39, 2424–2436.

Oregon State University Aquatic Toxicology Laboratory. (2016a). Early lifestage toxicity of copper to the rainbow trout (Oncorhynchus mykiss) under varying pH conditions [Final report]. European Copper Institute.

Oregon State University Aquatic Toxicology Laboratory. (2016b). Short-term chronic toxicity of copper to the fathead minnow, Pimephales promelas, under varying pH conditions [Final report]. European Copper Institute.

Oregon State University Aquatic Toxicology Laboratory. (2017). Short-term chronic toxicity of copper to the fathead minnow, Pimephales promelas, at a pH of 6 [Final report]. European Copper Institute.

Oregon State University and Ghent University. (2022). Chronic toxicity of copper, under varying pH conditions, to two laboratory clones of the cladoceran Ceriodaphnia dubia [Final report]. European Copper Institute.

Pereira, C. M. S., Deruytter, D., Blust, R., & De Schamphelaere, K. A. C. (2017). Effect of temperature on chronic toxicity of copper, zinc, and nickel to Daphnia magna. Environmental Toxicology and Chemistry, 36(7), 1909–1916.

Peters, A., Merrington, G., De Schamphelaere, K., & Delbeke, K. (2011). Regulatory consideration of bioavailability for metals: Simplification of input parameters for the chronic copper biotic ligand model. Integrated Environmental Assessment and Management, 7, 437–444.

Peters, A., Merrington, G., Schlekat, C., De Schamphelaere, K., Stauber, J., Batley, G., Harford, A., van Dam, R., Pease, C., Mooney, T., Warne, M., Hickey, C., Glazebrook, P., Chapman, J., Smith, R., & Krassoi, R. (2018). Validation of the nickel biotic ligand model for locally relevant species in Australian freshwaters. Environmental Toxicology and Chemistry, 37(9), 2566–2574.

Peters, A., Wilson, I., Merrington, G., Heijerick, D., & Baken, S. (2019). Assessing compliance of European freshwaters for copper: Accounting for bioavailability. Bulletin of Environmental Contamination and Toxicology, 102(2), 153–159.

Peters, A., Nys, C., Leverett, D., Wilson, I., Van Sprang, P., Merrington, G., Middleton, E., Garman, E., & Schlekat, C. (2023). Updating the chronic freshwater ecotoxicity database and biotic ligand model for nickel for regulatory applications in Europe. Environmental Toxicology and Chemistry, 42, 566–580.

Rijksinstituut voor Volksgezondheid en Milieu. (2025). Koper: Stofgegevens. Retrieved August 4, 2025, from https://rvszoeksysteem.rivm.nl/stof/detail/861

Salminen, R., Batista, J., Bidovec, M., Demetriades, A., De Vivo, B., De Vos, W., Duris, M., Gilucis, A., Gregorauskiene, V., Halamic, J., Heitzmann, P., Lima, A., Jordan, G., Klaver, G., Klein, P., Lis, J., Locutura, J., Marsina, K., Mazreku, A., O'Connor, P. J., Olsson, S. Å., Ottesen, R.-T., Petersell, V., Plant, J. A., Reeder, S., Salpeteur, I., Sandström, H., Siewers, U., Steenfelt, A., & Tarvainen, T. (2005). Part 1: Background information, methodology and maps. In R. Salminen (Ed.), Geochemical atlas of Europe. EuroGeoSurveys. Retrieved August 20, 2025, from https://weppi.gtk.fi/publ/foregsatlas/

Scymaris. (2021). Copper: Determination of effects on reproduction in Lymnaea stagnalis (Study No. 1113.00101, OECD Guideline 243) [Report to the European Copper Institute].

UK Environment Agency. (2009). Using biotic ligand models to help implement environmental quality standards for metals under the Water Framework Directive (Science Report SC080021/SR7b).

van Dam, R. A., Hogan, A. C., Harford, A. J., & Humphrey, C. L. (2019). How specific is site-specific? A review and guidance for selecting and evaluating approaches for deriving local water quality benchmarks. Integrated Environmental Assessment and Management, 15, 683–702.

Van Regenmortel, T., Janssen, C. R., & De Schamphelaere, K. A. C. (2015). Comparison of the capacity of two biotic ligand models to predict chronic copper toxicity to two Daphnia magna clones and formulation of a generalized bioavailability model. Environmental Toxicology and Chemistry, 34, 1597–1608.

Verdonck, F. A. M., Jaworska, J., Thas, O., & Vanrolleghem, P. A. (2001). Determining environmental standards using bootstrapping, Bayesian, and maximum likelihood techniques: A comparative study. Environmental Science & Technology.

Vlaeminck, K., Viaene, K. P., Van Sprang, P., Baken, S., & De Schamphelaere, K. A. (2019). The use of mechanistic population models in metal risk assessment: Combined effects of copper and food source on Lymnaea stagnalis populations. Environmental Toxicology and Chemistry, 38(5), 1104–1119.

Wilson, I., Peters, A., Merrington, G., & Baken, S. (2023). Following the evidence and using the appropriate regulatory tools: A European-wide risk assessment of copper in freshwaters. Integrated Environmental Assessment and Management, 19, 1570–1580.

Zajdlik, B. (2005). Statistical analysis of the SSD approach for development of Canadian water quality guidelines (CCME Project No. 354 2005). Retrieved August 4, 2025, from https://ccme.ca/en/res/2005-statistical-analysis-of-the-ssd-approach-for-development-of-canadian-water-quality-guidelines-en.pdf

Zhang, Y., Zang, W., Qin, L., Zheng, L., Cao, Y., Yan, Z., Yi, X., Zeng, H., & Liu, Z. (2017). Water quality criteria for copper based on the BLM approach in freshwater in China. PLOS ONE, 12(2), Article e0170105.

Figure 1 Schematic overview of the procedure for bioavailability model-based normalization of ecotoxicity data (L(E)C10; 10% lethal or effect concentrations) obtained in exposure medium *x* to the physico-chemistry of target water *y* and derivation of the site-specific threshold (HC5) using the species sensitivity distribution approach. See text for full description of the methodology.

Alt text-Figure 1: Flow diagram showing the normalization of a chronic copper toxicity value from the original exposure medium to a target water. The process converts dissolved copper to free copper ion activity, applies a bioavailability model, converts the result back to dissolved copper, and uses a species sensitivity distribution to derive the site-specific 5% hazardous concentration.

Figure 2. Distribution of species over the main taxonomic groups represented in the updated copper ecotoxicity database. Taxonomic groups are divided based on the divisions outlined for taxonomic groups for species sensitivity distributions in the guidance for the environmental quality standard derivation (EC 2018).

Alt text- Figure 2: Sunburst graph showing the distribution of 49 freshwater species across 19 taxonomic groups in the updated chronic copper ecotoxicity database. Molluscs are the most strongly represented taxonomic group.

Figure 3. Species sensitivity distribution for a low calcium scenario (i.e., Swedish lake, upper panel) and a moderate calcium scenario (river Ebro; lower panel) showing normalised species geomean 10% lethal or effect concentrations (symbols; expressed as µg dissolved Cu/L) for the updated environmental quality standard derivation methodology. The fit of the best-fitting distribution is also shown in addition with its 90% confidence interval.

Alt text- Figure 3: Two species sensitivity distributions comparing normalized chronic copper toxicity under the low-calcium conditions of Swedish Lake and the moderate-calcium conditions of the River Ebro. Species sensitivities and the best-fitting distribution differ between the two waters, illustrating the influence of water chemistry on copper toxicity.

Figure 4 Normalized effect values, either median 5% hazardous concentration (HC5, lines and black circles) or geomean 10% lethal or effect concentration (L(E)C10) under hypothetical bioavailability conditions under the updated environmental quality standard derivation methodology. The geomean L(E)C10 represents the most sensitive species for each of the trophic levels, i.e. *Acipenser transmontanus* for fish (blue squares), *Flumincola sp.* for invertebrates (orange crosses) and *Chlorella sp.* for algae and higher plants (green triangles).

Alt text- Figure 4: *Plots showing how the site-specific 5% hazardous concentration for copper and the normalized toxicity values of the most sensitive fish, invertebrate, and algal species change under hypothetical variations in water chemistry. Copper sensitivity generally decreases with increasing dissolved organic carbon and pH, while the effect of calcium varies among taxonomic groups and pH conditions.*

Figure 5 Overview the distribution of (A) site-specific 5% hazardous concentrations (HC5; in µg dissolved Cu/L) and (B) dissolved organic carbon concentration (DOC; in mg/L) in the European freshwater set. Site-specific HC5 were calculated with the Updated environmental quality standard derivation methodology. Physico-chemistry for site-specific HC5 derivation was taken from the FOREGS database (Salminen et al. 2005), only waters for which the physico-chemistry is within the application ranges of the updated Cu bioavailability model are plotted (*n*=624).

Alt text- Figure 5: Maps of Europe showing the spatial distribution of site-specific 5% hazardous concentrations for copper and dissolved organic carbon concentrations in freshwater monitoring sites from the FOREGS database. Lower copper thresholds, indicating higher bioavailability and sensitivity, generally occur at sites with low dissolved organic carbon.

Figure 6. Site-specific water quality criteria (in µg dissolved Cu/L) using different bioavailability corrections as a function of dissolved organic carbon concentration (DOC; in mg/L) for the European freshwater set. For the Updated European Union (EU) environmental quality standard (EQS) scenario water quality criteria represent site-specific 5% hazardous concentrations were calculated with the Updated environmental quality standard derivation methodology (present study). For the United States (US) Multiple Linear Regression (MLR) calculation, the chronic water quality criteria calculated with the chronic Cu MLR (Brix et al. 2021) is depicted. For Australia & New Zealand, the depicted water quality criteria represent the DOC adjusted default guideline value (DGV) at the 95% protection level (Australian and New Zealand Governments [ANZG] 2023). Physico-chemistry for site-specific HC5 derivation was taken from the FOREGS database (Salminen et al. 2005), only waters for which the physico-chemistry is within the application ranges of the updated Cu bioavailability model are plotted (*n*=624).

Alt text- Figure 6: Scatter plots comparing copper water-quality thresholds as a function of dissolved organic carbon concentration using three regulatory approaches: the updated European environmental quality standard methodology, the United States multiple linear regression model, and the Australian and New Zealand dissolved-organic-carbon-adjusted guideline. All approaches show increasing copper *thresholds with increasing dissolved organic carbon, although the predicted values differ among jurisdictions.*

Figure 7 Comparison of the site-specific environmental threshold (calculated as median 5% hazardous concentration; HC5) under the updated environmental quality standard scenario and the different sensitivity scenarios for the regulatory surface water set. Error bars denote 90th percentile confidence intervals on derived HC5 (representing HC5-5 to HC5-95).

Alt text- Figure 7: Comparison of site-specific 5% hazardous concentrations for seven regulatory surface waters under the updated copper environmental quality standard methodology and three sensitivity scenarios. Median threshold values differ only slightly among scenarios.

TABLES

Table 1 Overview of normalized 5% hazardous concentration (HC5)[a] and best fitting distribution for the regulatory surface waters set under different environmental quality standard (EQS) derivation scenarios.

Water ID (country)	pH	DOC (mg/L)	Ca (mg/L)	Original EQS scenario[b]	Updated EQS scenario
Swedish Lake (SW)	6.7	3.8	8.7	11.5 (11.1-12.0) Beta	4.7 (3.6-5.9) Weibull
Lake Monate (IT)	7.7	2.5	13.6	10.6 (7.0-14.4) Normal	6.5 (5.4-7.7) Log-normal
River Rhine (NL)	7.8	2.8	68.9	8.2 (4.7-12.1) Normal	7.2 (5.7-8.8) Log-normal
River Otter (UK)	8.1	3.2	46.9	7.8 (4.4-11.7) Normal	8.5 (6.7-10.5) Log-normal
River Ebro (SP)	8.2	3.7	72.9	9.3 (8.6-10.0) Beta	8.9 (6.4-12.5) Logistic
River Teme (UK)	7.6	8.0	49.9	17.6 (15.9-19.2) Beta	18.9 (15.1-22.9) Log-normal
Ditches adjusted DOC + pH (NL)	6.9	12.0	88.2	22.1 (19.8-24.2) Beta	26.1 (20.5-31.9) Log-normal

[a] reported as median HC5 (µg dissolved Cu/L) derived from the best-fitting model. HC5-5 and HC5-95 reported between parentheses. All distributions are fitted to log_{10}-transformed L(E)C10

[b] Representing site-specific HC5 resulting from the original EQS derivation methodology. Values were taken from the Cu Voluntary Risk Assessment Report (European Copper Institute 2008)

DOC=Dissolved organic carbon, SW=Sweden; IT=Italy; NL=The Netherlands; UK=United Kingdom; SP=Spain

Table 2. Overview of ranges in physico-chemistry[a] and site-specific 5% hazardous concentrations (HC5)[b] in European freshwaters based on the FOREGS database (Salminen et al. 2005)

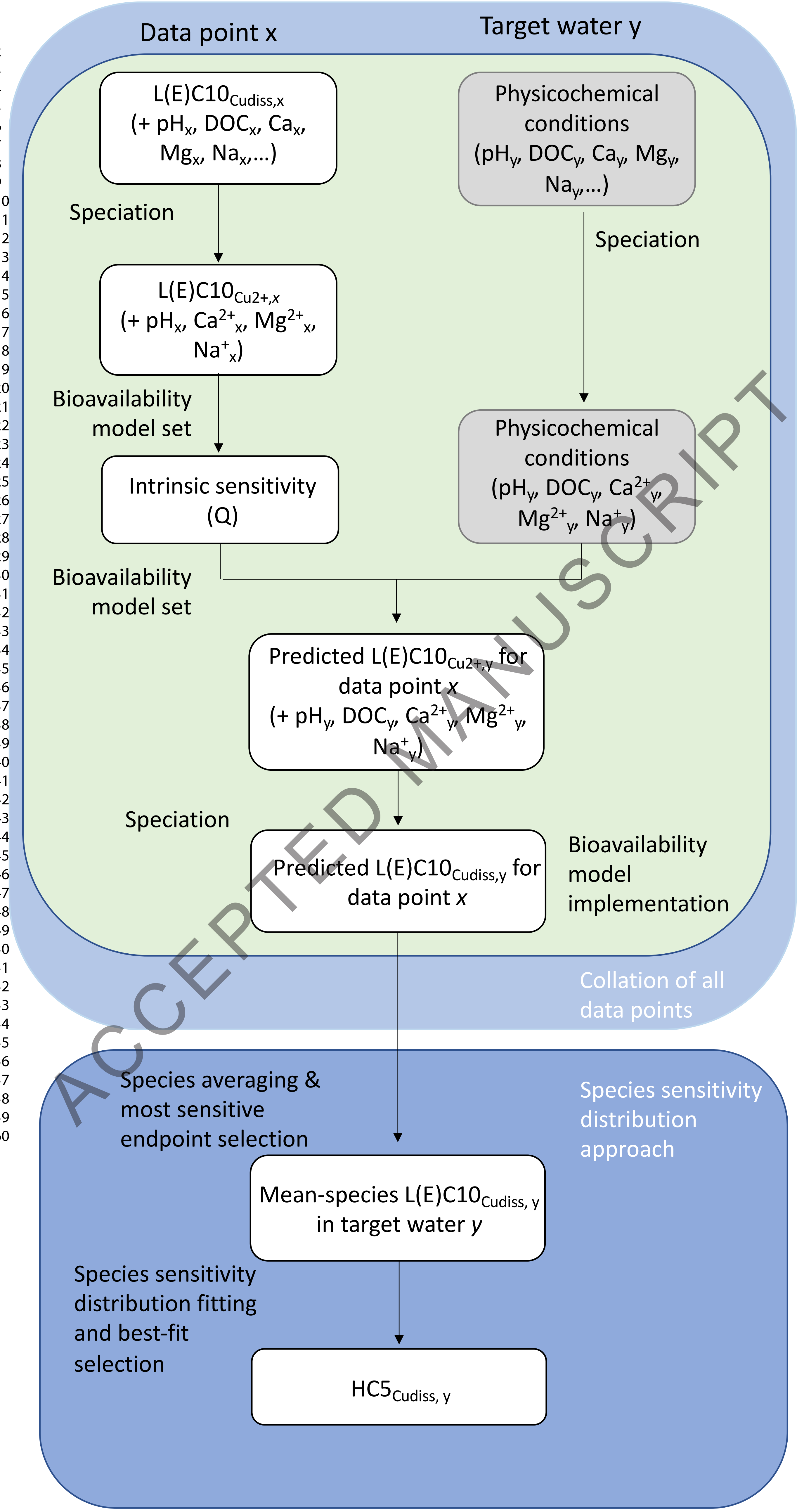
Country/ Region[c]	Number of sites[d]	pH	DOC (mg/L)	Ca (mg/L)	HC5[b] (µg dissolved Cu/L)
Europe	623/795	7.7 (6.3-8.4)	4.9 (0.6-19.7)	43.8 (3.3-114.8)	10.7 (2.0-37.8)
Switzerland*	9/10	7.9 (6.5-8.4)	1.9 (0.4-4.9)	57.0 (5.0-92.3)	6.2 (0.7-13.1)
Italy*	37/44	8.1 (7.2-8.5)	1.3 (0.3-12.9)	52.7 (5.6-117.5)	4.3 (0.8-32.7)
Norway*	18/53	6.5 (6.2-8.2)	3.6 (0.5-12.1)	6.7 (2.6-21.9)	3.8 (1.2-23.8)
Austria*	20/20	8.1 (7.3-8.5)	1.3 (0.5-5.3)	38.1 (14.1-101.0)	4.2 (1.6-13.8)
Slovenia*	4/4	7.7 (7.5-7.8)	0.9 (0.5-1.3)	62.8 (43.0-66.8)	2.6 (1.7-4.1)
Germany*	60/74	7.6 (6.5-8.1)	2.7 (0.7-21.3)	59.7 (8.9-108.6)	7.0 (1.8-44.6)
France*	102/119	7.8 (6.7-8.4)	2.9 (0.7-9.1)	43.5 (4.5-118.7)	7.0 (2.1-20.6)
Belgium	4/5	6.3 (5.8-6.6)	4.5 (3.0-5.4)	37.9 (15.4-110.6)	4.3 (2.3-6.9)
Greece	25/27	8.2 (7.6-8.5)	1.4 (0.3-3.6)	56.8 (32.6-90.5)	5.0 (2.5-10.6)
Portugal	15/19	6.8 (6.2-8.3)	3.4 (1.7-12.8)	15.7 (3.9-108.2)	4.9 (2.6-32.1)
Spain	61/87	8.2 (7.3-8.5)	4.4 (1.0-9.8)	43.8 (5.1-116.3)	12.5 (2.9-26.1)
Czech Republic	9/10	7.5 (6.8-8.2)	3.5 (1.6-7.5)	28.7 (12.0-71.4)	8.6 (3.4-15.2)
United Kingdom	52/60	7.9 (7.1-8.3)	7.0 (1.4-15.5)	20.3 (2.9-114.6)	17.4 (3.9-41.0)
Ireland	6/11	6.6 (5.6-7.9)	13.1 (6.5-19.3)	11.7 (3.3-79.2)	10.2 (4.0-41.7)
Slovakia	14/15	7.8 (7.4-8.1)	4.4 (1.6-9.4)	50.9 (13.4-100.8)	13.0 (4.2-23.5)
Poland	48/56	7.7 (7.1-8.1)	7.9 (1.8-16.1)	79.2 (41.0-113.5)	18.6 (4.8-35.5)
Denmark	5/5	6.4 (6.1-7.6)	9.0 (6.7-25.5)	13.7 (9.0-65.3)	11.2 (5.8-26.0)
Sweden	43/50	6.7 (5.7-7.5)	13.4 (7.1-23.3)	5.5 (2.6-20.2)	18.3 (6.2-36.2)
Hungary	8/10	7.9 (7.2-8.3)	3.9 (1.8-13.8)	93.2 (33.8-122.3)	10.6 (6.6-35.3)
Finland	36/65	6.8 (6.2-7.4)	14.2 (5.7-27.0)	4.4 (2.9-12.0)	19.7 (8.3-42.2)
Estonia	10/11	7.6 (7.3-8.0)	11.4 (3.4-16.7)	88.9 (55.0-110.8)	26.3 (8.5-38.8)
Croatia	10/10	8.1 (7.2-8.3)	4.4 (2.5-11.5)	94.6 (28.3-117.1)	14.6 (8.9-24.6)
Lithuania	12/14	7.7 (7.1-8.0)	10.2 (5.5-14.4)	96.6 (59.5-119.9)	24.2 (12.5-33.3)
The Netherlands	8/9	7.4 (7.2-7.7)	11.5 (6.1-21.4)	67.0 (47.9-103.7)	26.5 (14.8-48.9)
Latvia	7/7	7.7 (7.5-7.9)	16.5 (7.8-24.0)	76.1 (57.8-97.9)	38.3 (19.4-56.3)

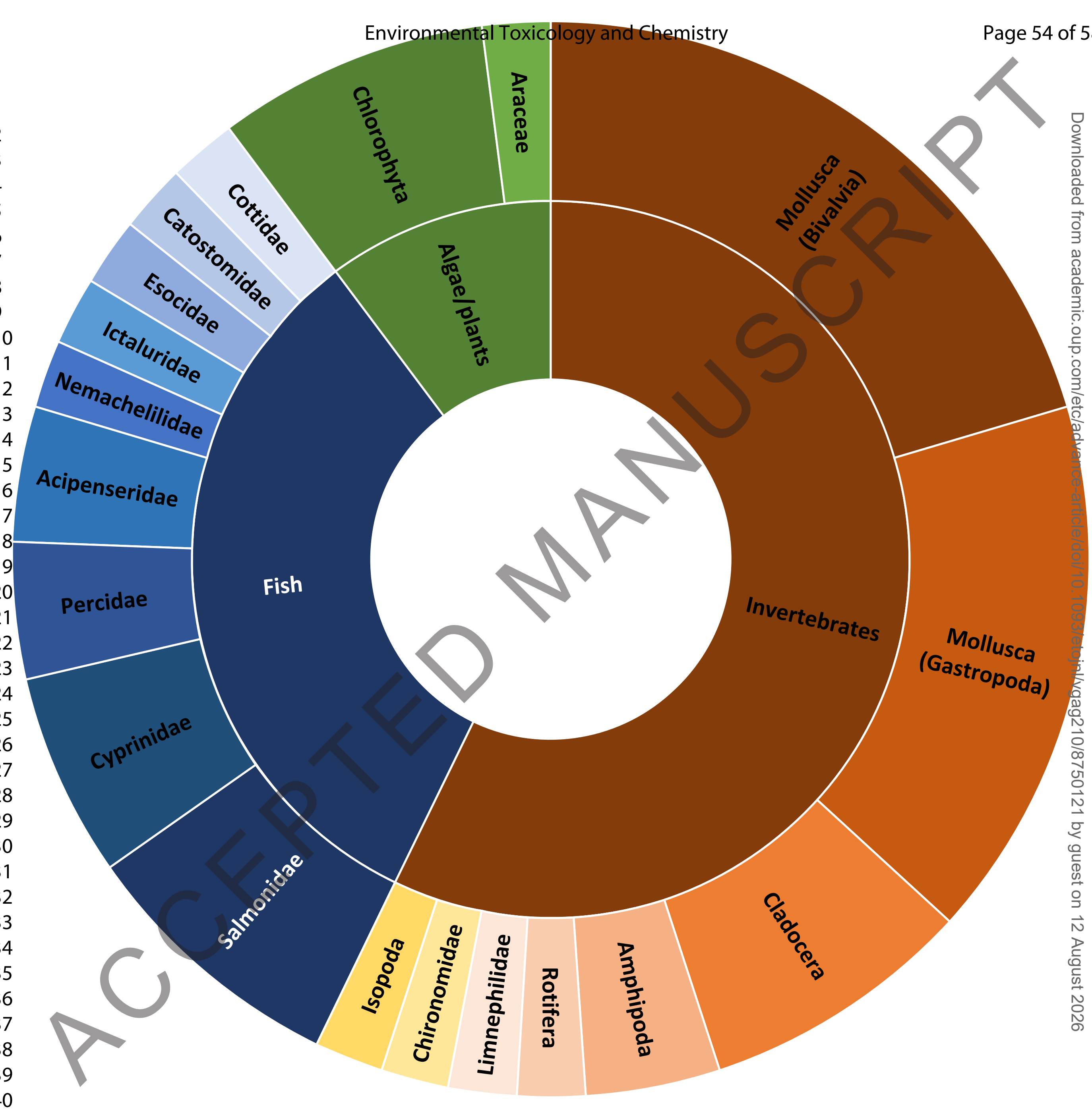
[a] For the physico-chemistry parameters, median (5^{th}-95^{th} percentile) of the waters within the applicability range are reported.
[b] For each country and Europe, median site-specific median HC5 (HC5-50, in µg dissolved Cu/L) of the waters within the applicability range are reported. Between brackets 5^{th}-95^{th} percentile of site-specific HC5 per country are reported. HC5 represent the site-specific HC5 calculated with the updated EQS derivation methodology.
[c] Countries are ordered from lowest to highest lower 5^{th} percentile of HC5 distribution. Countries indicated with asterisks are considered in Step 2 of the bioavailable environmental quality standard derivation, i.e., representing the 30% most sensitive countries of countries represented in the FOREGS database.
[d] The values represent the number of datapoints within the applicability ranges of the Cu bioavailability normalization approach (pH 5.5-8.6, Ca 2.4-124 mg/L, DOC≤30 mg/L)/ total number of datapoints.
DOC=dissolved organic carbon; HC5= 5% hazardous concentration

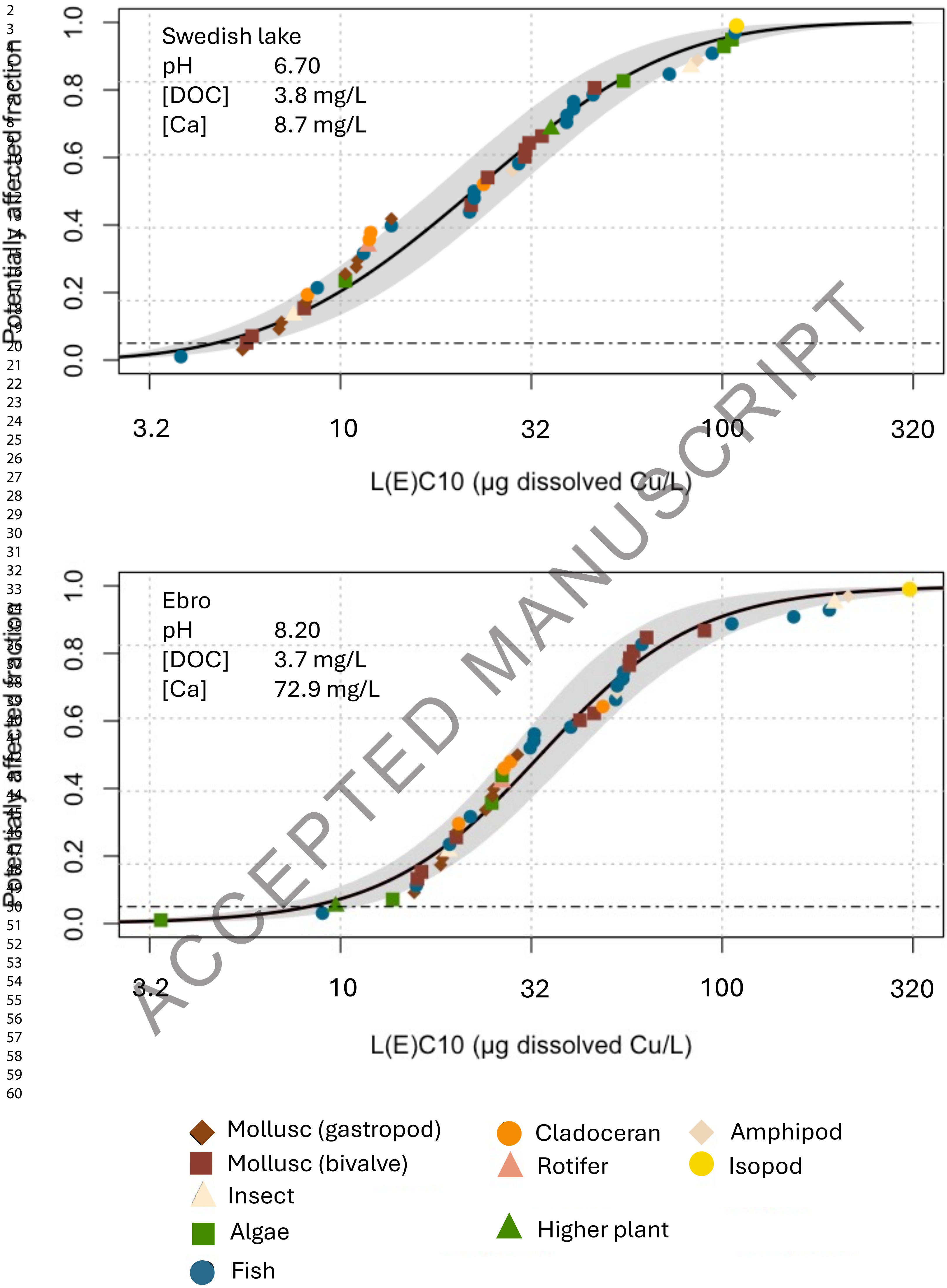
Table 3. Overview of ranges in site-specific 5% hazardous concentrations (HC5) in European freshwaters based on FOREGS ('Europe') and the country-specific monitoring databases for the seven countries identified as the countries with highest bioavailability conditions for Cu.

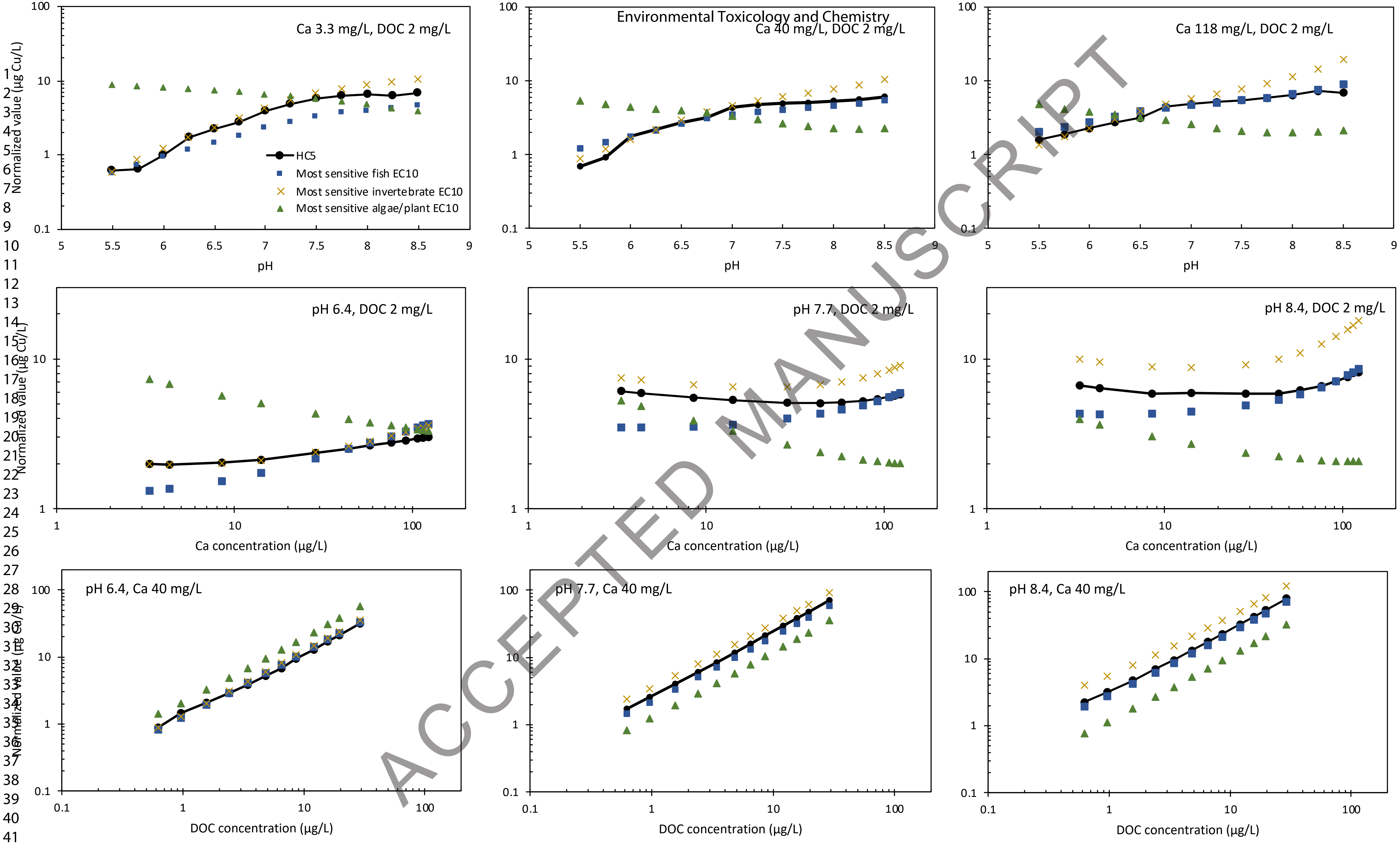
Country	Source	Monitoring period	Number of samples[a]	Number of sites[b]	Country-specific $EQS_{bioavailable}$[c] (µg dissolved Cu/L)	Percentage of waters protected by $EQS_{bioavailable}$[d] (%)	
						Samples	Sites
Europe	FOREGS[e]	-	623/795	623/795	-	98.5	98.5
Switzerland	NADUF[f]	2014-2023	6019/6021	22/22	1.9	99.8	100
Italy	MERA[g]	2017-2020	5975/6518	675/684	1.9	99.2	99.7
Norway	MERA[g]	2019-2022	3773/7341	564/1320	1.0	96.1	95.0
Austria	MERA[g]	2016-2020	4752/4970	236/240	1.9	99.7	100
Slovenia	No data						
Germany	MERA[g]	2021	9825/11998	1239/1253	4.1	99.9	99.9
France	MERA[g]	2017	6425/7458	2191/2345	2.7	99.8	99.8

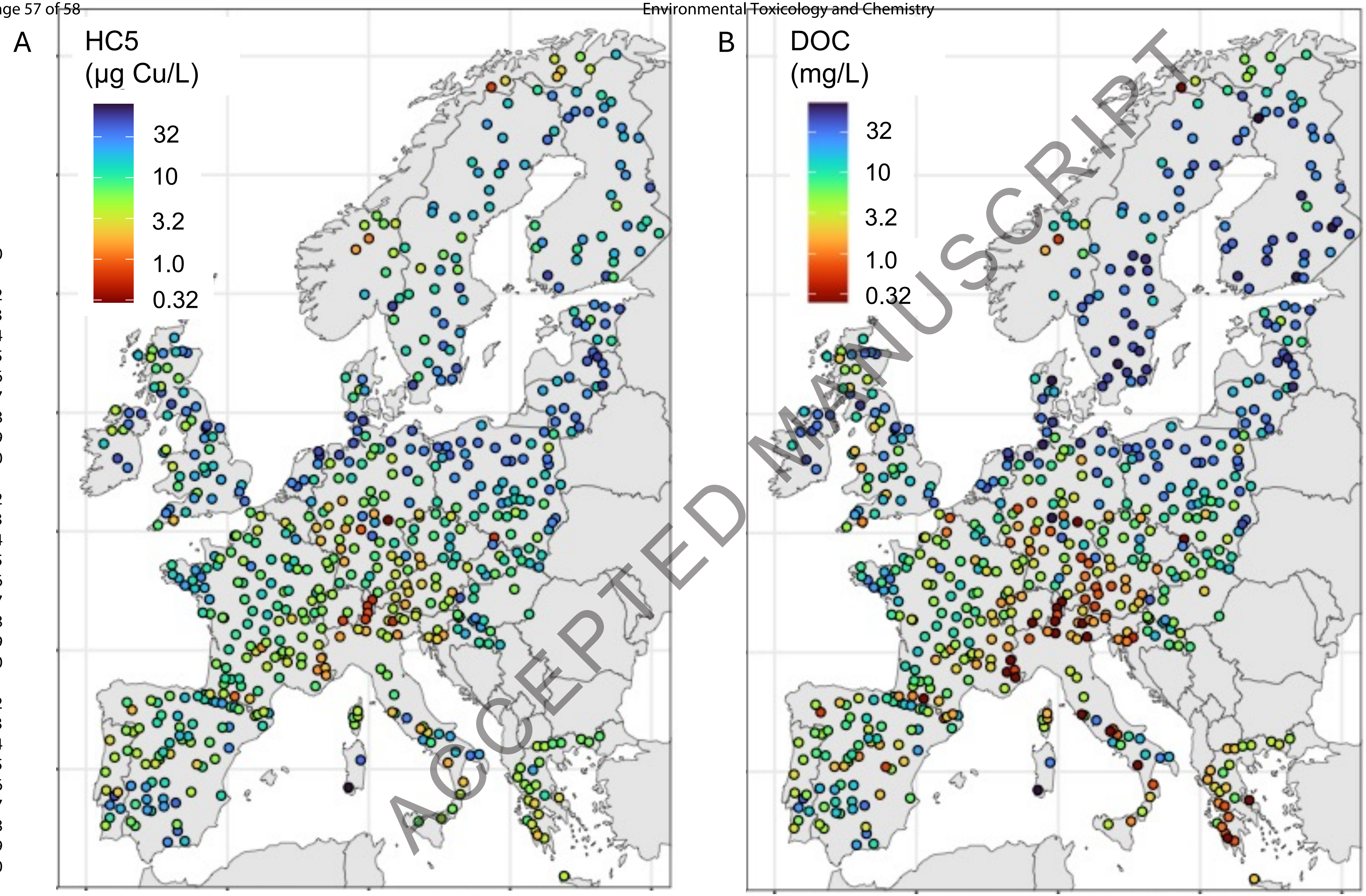
[a] Number of samples within the applicability ranges of the Cu EQS derivation methodology (pH 5.5-8.6, Ca 2.4-124 mg/L, DOC≤30 mg/L)/ total number of samples. Only samples with at least pH, DOC and Ca were retained.
[b] Number of sites with at least one sample within the applicability ranges of the Cu bioavailability normalization approach (pH 5.5-8.6, Ca 2.4-124 mg/L, DOC≤30 mg/L)/ total number of samples
[c] Representing the lower 5th percentile of the site-specific HC5 distribution in the country-specific database (see Figure S7.3 for the distribution of HC5 in the different databases).
[d] Based on $EQS_{bioavailable}$= 1.0 µg dissolved Cu/L; indicating the percentages of samples and sites with a site-specific HC5≥ $EQS_{bioavailable}$.
[e] Salminen et al (2005)
[f] Swiss database of the National River Monitoring and Survey Program (Nationale Dauerüberwachung Fliessgewässer [NADUF] 2024)
[g] Online database of the Metals Environmental Research Association (MERA 2026)
DOC=dissolved organic carbon; $EQS_{bioavailable}$= bioavailable environmental quality standard

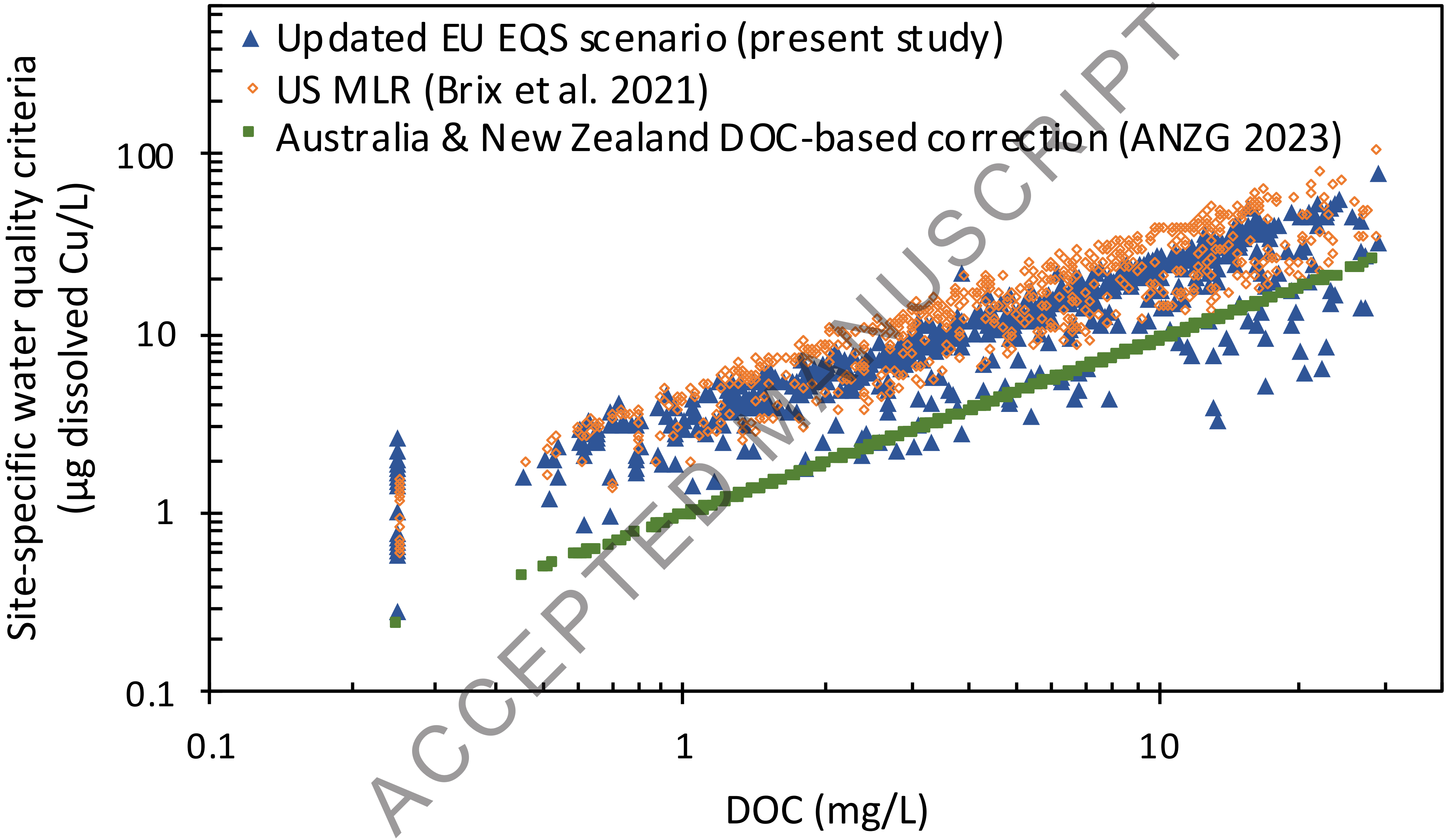

Data point x
Target water y
L(E)C10$_{Cudiss,x}$ (+ pH$_x$, DOC$_x$, Ca$_x$, Mg$_x$, Na$_x$,…)
Physicochemical conditions (pH$_y$, DOC$_y$, Ca$_y$, Mg$_y$, Na$_y$,…)
Speciation
Speciation
L(E)C10$_{Cu2+,x}$ (+ pH$_x$, Ca^{2+}$_x$, Mg^{2+}$_x$, Na$^+$$_x$)
Bioavailability model set
Physicochemical conditions (pH$_y$, DOC$_y$, Ca^{2+}$_y$, Mg^{2+}$_y$, Na$^+$$_y$)
Intrinsic sensitivity (Q)
Bioavailability model set
Predicted L(E)C10$_{Cu2+,y}$ for data point *x* (+ pH$_y$, DOC$_y$, Ca^{2+}$_y$, Mg^{2+}$_y$, Na$^+$$_y$)
Speciation
Predicted L(E)C10$_{Cudiss,y}$ for data point *x*
Bioavailability model implementation
Collation of all data points
Species averaging & most sensitive endpoint selection
Species sensitivity distribution approach
Mean-species L(E)C10$_{Cudiss, y}$ in target water *y*
Species sensitivity distribution fitting and best-fit selection
HC5$_{Cudiss, y}$

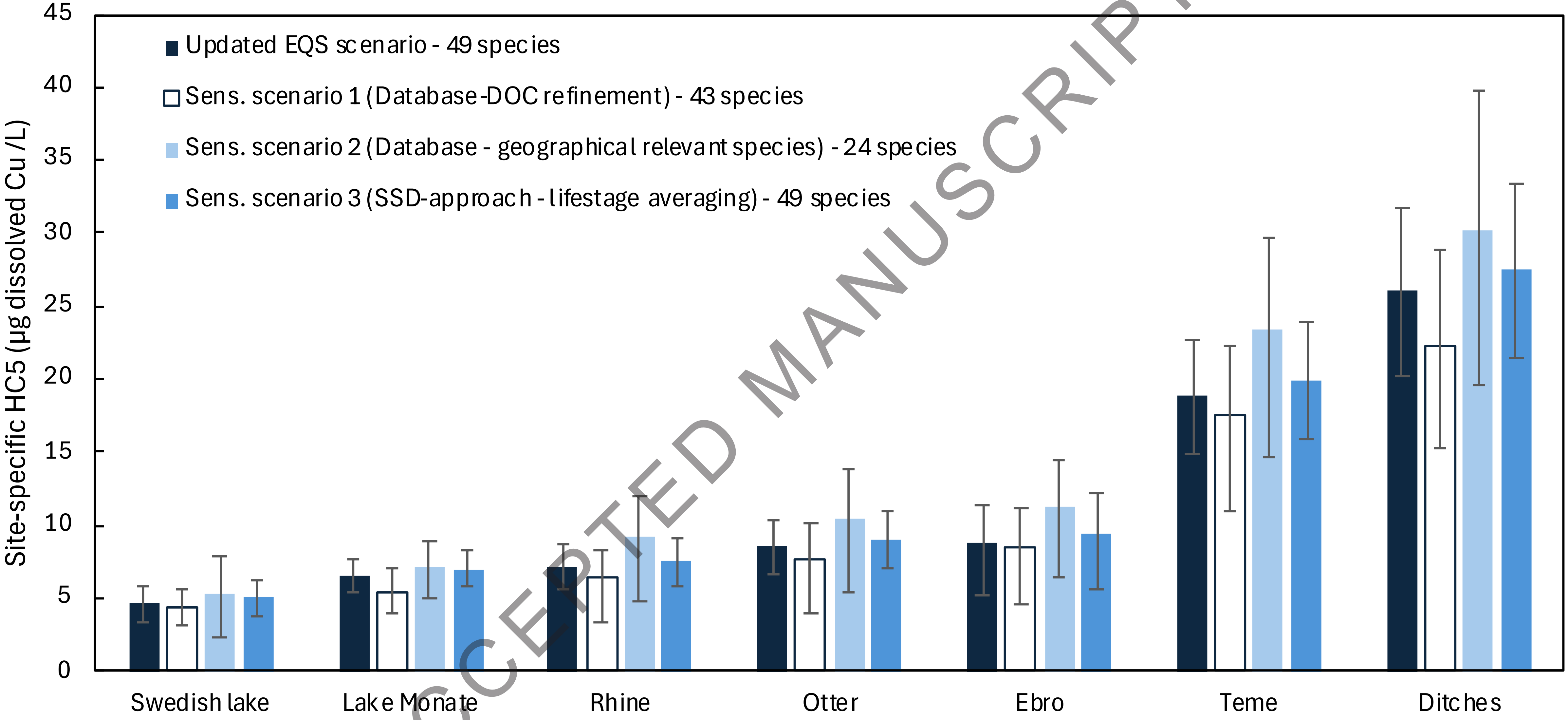

Araceae
Chlorophyta
Cottidae
Catostomidae
Esocidae
Ictaluridae
Nemachelilidae
Acipenseridae
Percidae
Cyprinidae
Salmonidae
Algae/plants
Fish
Invertebrates
Mollusca (Bivalvia)
Mollusca (Gastropoda)
Cladocera
Amphipoda
Rotifera
Limnephilidae
Chironomidae
Isopoda


Swedish lake
pH 6.70
[DOC] 3.8 mg/L
[Ca] 8.7 mg/L
Potentially affected fraction
0.0 0.2 0.4 0.6 0.8 1.0
3.2 10 32 100 320
L(E)C10 (µg dissolved Cu/L)
Ebro
pH 8.20
[DOC] 3.7 mg/L
[Ca] 72.9 mg/L
Potentially affected fraction
0.0 0.2 0.4 0.6 0.8 1.0
3.2 10 32 100 320
L(E)C10 (µg dissolved Cu/L)
Mollusc (gastropod)
Mollusc (bivalve)
Insect
Algae
Fish
Cladoceran
Rotifer
Higher plant
Amphipod
Isopod


Ca 3.3 mg/L, DOC 2 mg/L
Ca 40 mg/L, DOC 2 mg/L
Ca 118 mg/L, DOC 2 mg/L
pH 6.4, DOC 2 mg/L
pH 7.7, DOC 2 mg/L
pH 8.4, DOC 2 mg/L
pH 6.4, Ca 40 mg/L
pH 7.7, Ca 40 mg/L
pH 8.4, Ca 40 mg/L
HC5
Most sensitive fish EC10
Most sensitive invertebrate EC10
Most sensitive algae/plant EC10
Normalized value (µg Cu/L)
pH
Ca concentration (µg/L)
DOC concentration (µg/L)


A
HC5
(µg Cu/L)
32
10
3.2
1.0
0.32
B
DOC
(mg/L)
32
10
3.2
1.0
0.32


Updated EU EQS scenario (present study)
US MLR (Brix et al. 2021)
Australia & New Zealand DOC-based correction (ANZG 2023)
Site-specific water quality criteria
(µg dissolved Cu/L)
100
10
1
0.1
0.1
1
10
DOC (mg/L)


Site-specific HC5 (µg dissolved Cu /L)
45
40
35
30
25
20
15
10
5
0
Updated EQS scenario - 49 species
Sens. scenario 1 (Database-DOC refinement) - 43 species
Sens. scenario 2 (Database - geographical relevant species) - 24 species
Sens. scenario 3 (SSD-approach - lifestage averaging) - 49 species
Swedish lake
Lake Monate
Rhine
Otter
Ebro
Teme
Ditches