A new zinc bioavailability-based Environmental Quality Standard (EQS) for compliance assessment under the European Union-Water Framework Directive

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Data availability statement – All data (ecotoxicity database, data used for bioavailability modelling and local HC5 for all considered sites) are available in Supplemental Information. The bioavailability tool and associated files encoding the updated Zn EQS derivation approach can be accessed via https://figshare.com/s/bd848a4455311d9eab47

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ABSTRACT

Many countries list zinc (Zn) as a River Basin Specific Pollutant (RBSP) under the European Union's Water Framework Directive (WFD). Given the dependency of Zn toxicity to aquatic organisms on local water body conditions, it is essential that the derivation of Environmental Quality Standards (EQS) for Zn incorporates bioavailability. We have updated the Zn EQS by revising and updating the existing ecotoxicity dataset and by including optimized bioavailability models. Adhering to the existing EQS derivation guidance, a new bioavailability-based EQS ($EQS_{bioavailable}$) was derived for Europe. The updated chronic Zn freshwater ecotoxicity dataset contains high-quality data covering 40 species and covers the minimum data requirements for species sensitivity distribution-extrapolation set out in the WFD-guidance. The existing chronic Zn bioavailability models for invertebrates, vertebrates and algae were revised by optimizing their structure and by using the most recent version of WHAM (version VII) as the underlying speciation software. The updated models were validated with independent data for ten aquatic species. Zinc toxicity at regulatory relevant effect concentrations (e.g., 10% effect concentrations) were predicted within 2-fold error for most species, for one species within 3-fold error. Hence, the updated bioavailability models were used to predict Zn toxicity reliably across different species. The updated ecotoxicity dataset and chronic bioavailability models were combined with species sensitivity distribution techniques resulting in the updated Zn EQS derivation approach. This approach was used to derive a scientifically robust bioavailable EQS for Zn in European freshwaters based on the latest available technical guidance. The resulting $EQS_{bioavailable}$ of 11.2 µg dissolved Zn/L refers to conservative conditions and can serve as a first-tier screening level in compliance assessments under the WFD. In addition, the updated Zn EQS derivation approach developed in the current study will increase the ecological and ecotoxicological relevancy of higher screening levels by allowing incorporation of local bioavailability considerations.

Key words: Metal bioavailability, zinc, environmental quality standard, compliance assessment, aquatic risk assessment

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INTRODUCTION

Many member states of the European Union (EU) list Zn as a River Basin Specific Pollutant (RBSP) under the Water Framework Directive (WFD; European Commission [EC] 2000). This implies that member states may define their own specific Environmental Quality Standard (EQS) to monitor potential effects of Zn on freshwater ecosystems. Zinc is listed as a RBSP in 23 countries and is, therefore, measured frequently throughout Europe (European Environment Agency [EEA] 2026). Recently, the annual average-EQS (AA-EQS) has been reported to vary between 1.0 and 205 µg Zn/L among member states (EEA 2026). This variation in AA-EQS is primarily the result of differences in approaches for EQS derivation, underlying databases and models, dissimilar water conditions between countries, and different protection goals.

Although zinc is an essential element for life and functioning of many biological processes, it is important for risk assessments to identify the boundary of exposure which may cause toxic effects using a reliable and robust scientific methodology. Toxicity of zinc to aquatic organisms is dependent on the local physico-chemical composition of the water body. For Zn, dissolved organic carbon (DOC), pH, hardness (Ca^{2+} and Mg^{2+}) and sodium (Na^{+}) have been identified as the main toxicity modifying factors (TMF) (e.g., Heijerick et al. 2005, Stauber et al. 2023). To account for these TMFs, chronic bioavailability models are available to predict Zn toxicity under local conditions (e.g., Heijerick et al. 2002, 2005, De Schamphelaere et al. 2005). While an approach that integrates chronic bioavailability models to derive site-specific environmental thresholds has existed for more than a decade (Van Sprang et al. 2009), not all member states in the EU have integrated a bioavailability assessment in the derivation of their Zn EQS (Merrington et al. 2020). Ecotoxicity databases typically contain toxicity data for a large set of species obtained in media with various physico-chemical compositions. Bioavailability models are used to normalize the ecotoxicity database to a specific water chemistry from which the resulting EQS is ultimately derived (EU Water Directors 2018). Following the most recent EU technical guidance, compliance of metals should, in a first step when appropriate, be evaluated against a “bioavailable EQS” ($EQS_{bioavailable}$), an environmental threshold that considers high bioavailability conditions (Guidance Document No. 38, EU Water Directors 2021). In practice, the $EQS_{bioavailable}$ should be protective for the majority (usually 95%) of water conditions in the country (or region) with the highest bioavailability conditions in the EU (EU Water Directors 2018).

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Currently, the EQS implemented in the United Kingdom (10.9 µg dissolved Zn/L) has been taken up by some member states, such as Germany (Table S1.1). This UK-based $EQS_{bioavailable}$ was developed using a bioavailability-based approach and is protective for 95% of the waters of the UK's most sensitive region (Environment Agency 2012). More recently, a country specific $EQS_{bioavailable}$ was developed for France, equal to 11.3 µg dissolved Zn/L (Merrington et al. 2020).

The currently available approach for the derivation of an $EQS_{bioavailable}$ has existed for more than a decade (Van Sprang et al. 2009, hereafter referred to as the 'original Zn EQS derivation approach'). Van Regenmortel et al. (2017a) implemented some minor modifications to this methodology. However, even with these modifications, only ecotoxicity data published up to 2010 have been considered, while many more studies published after this date have reported on the aquatic toxicity of Zn to aquatic organisms. In addition, recently published optimisations in bioavailability models related to model structure and underlying speciation software have only partly been considered.

The original Zn EQS derivation approach includes bioavailability models for three trophic levels: invertebrates (based on *Daphnia magna;* Heijerick et al. 2005), algae (based on *Raphidocellis subcapitata*; De Schamphelaere et al. 2005) and fish (based on *Oncorhynchus mykiss*; De Schamphelaere & Janssen 2004; De Schamphelaere et al. 2005). The original invertebrate and fish model follow a traditional biotic ligand model (BLM) structure, where the effect of pH is modelled via a linear relationship between H^+ and free Zn^{2+} toxicity (parameterized via the biotic ligand stability constant; $K_{H\text{-}BL}$). The original algae model, on the other hand, follows the structure of a generalized bioavailability model (gBAM, Van Regenmortel et al. 2017b), in which the effect of pH on metal toxicity is incorporated as a log-linear relation between pH and free Zn^{2+} toxicity (parameterized via the pH-slope, S_{pH}). Due to their model structure, gBAMs may account for other factors that determine the effect of pH on Zn^{2+} toxicity in addition to the competitive effect of H^+ at the biotic ligand site. As such, they may deal more realistically with the non-linearity observed between H^+ and free Zn^{2+} toxicity which, for example, has been observed for *Daphnia magna* (Heijerick et al. 2005, Van Regenmortel et al. 2017b). Optimizations of the existing bioavailability model for invertebrates have been proposed through the use of the gBAM-structure as an alternative to the original BLM-type models (Van Regenmortel et al. 2017b).

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All existing bioavailability models for Zn have been set up in combination with Windermere Humic Aqueous Model V (WHAM V) as the speciation program, while the most recent version of this speciation software (WHAM VII) includes optimizations in expression of the heterogeneity of NOM binding sites and allows a better description of metal binding at higher pH compared with older versions (Tipping et al., 2011). For instance, Lofts & Tipping (2011) reported that free Zn^{2+} activity in 85% of the considered measurements were predicted with WHAM VII within 1-order of magnitude, highlighting the accuracy of WHAM VII for predicting free Zn^{2+} activity in field waters.

Given the large variability of Zn EQS in Europe, the aim of the present study was to derive an EU-wide $EQS_{bioavailable}$ for Zn following the most recent European technical guidance (Guidance Document No. 27, EU Water Directors 2018), while also integrating optimisations in the Zn EQS derivation approach (hereafter referred to as the "updated Zn EQS derivation approach"). To this end, the existing chronic Zn freshwater toxicity database (Van Sprang et al. 2009, Van Regenmortel et al. 2017a) was reassessed based on updated reliability and relevancy criteria and amended with recent high-quality data from peer-reviewed literature. In addition, the existing chronic Zn bioavailability models were reviewed and updated to include the most optimal model structure (gBAM) and speciation model (WHAM VII).

MATERIALS AND METHODS

Ecotoxicity database update

The update of the chronic Zn freshwater ecotoxicity database started from the database published by Van Regenmortel et al. (2017a), who added data for three species to the database of Van Sprang et al. (2009). To complement these, recent literature data published after the collation of the database of Van Sprang et al. (2009), so, published between 2006 and 2019 were collected. The quality of all ecotoxicity data, both derived from the original and modified Zn freshwater ecotoxicity database and data identified in the literature search, was carefully screened against a set of reliability and relevance criteria. These criteria were based on the Criteria for Reporting and Evaluating Ecotoxicity Data (Moermond et al. 2016) and followed the relevant EU guidance for environmental threshold derivation (ECHA 2008a, EU Water Directors 2018), including metal specific considerations (ECHA 2008b, OECD 2016). Compared to the reliability and relevancy screening performed by Van Sprang et al. (2009) and

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Van Regenmortel et al. (2017a), the assessment included significant adjustments, mainly related to the exclusion of studies that reported only nominal concentrations, which were excluded in the present study, and the preference of EC10s over NOECs. An overview of the applied CRED-criteria is given in Supplemental Information S2.

Bioavailability modeling

The existing chronic Zn bioavailability models for algae, invertebrates and vertebrates (De Schamphelaere et al. 2005, Heijerick et al. 2005, De Schamphelaere & Janssen 2004) were updated both in terms of model structure (i.e., all models updated to a generalized BioAvailability Model structure; gBAM) and underlying speciation software (WHAM VII). A more in-depth description of the methodology used for the bioavailability model update, including speciation assumptions and model equations is given in supplemental information (S3).

The prediction performance of the updated chronic Zn bioavailability models was evaluated using independent data not included in model development. Two validation approaches were considered: independent validation, using independent data for species included in model calibration, and cross-species validation, using data for species not used to calibrate the model parameters. In the main paper, prediction performance of the updated chronic Zn bioavailability models was assessed using the Factor of Agreement (FoA), defined as the proportion of datapoints predicted within a two-fold error. The evaluation focused on L(E)C10 values because of their regulatory relevance. In supplemental information (S3), a more in-depth evaluation of all effect levels using the multi-metric model performance score (MPS) proposed by Besser et al. (2021) is available.

Development of the updated Zn EQS derivation approach

The updated Zn EQS derivation approach combines the updated chronic Zn ecotoxicity dataset with the updated bioavailability models and a species-sensitivity distribution (SSD-) approach (Figure 1). The SSD-approach includes both species-averaging of endpoint concentrations and the fitting of a distribution to the normalised species-averaged toxicity data. Species-averaging was used to avoid overrepresentation of ecotoxicity data of data-rich species (ECHA 2008a), and to account for sensitivity

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variation unrelated to physico-chemical conditions (e.g. intra-laboratory and inter-laboratory variation). When multiple endpoints were available for a species, the most sensitive endpoint was selected.

Averaging was performed using the arithmetic mean at the level of intrinsic sensitivity (Q10), which is independent of water chemistry and represents metal binding to the biotic ligand at the EC10. This approach assumes one Q10 value per species and differs from Van Sprang et al. (2009), where averaging was performed on normalized dissolved Zn toxicity values. However, it is consistent with the bioavailability normalization approach used for Ni (Peters et al. 2023a) and improves computational efficiency by requiring normalization of only one value per species rather than the full Zn toxicity database.

Final SSD-fitting and derivation of the site-specific environmental threshold, or local HC5, were performed using the normalised Zn effect concentrations. For each target water, six distributions - gamma, log-normal, logistic, normal, Weibull and Gumbel - were fitted to the log_{10}-transformed, normalised dissolved concentrations in R (v4.6.0) using the 'fitdistrplus'-package (Delignette-Muller et al. 2022). The best-fitting distribution was selected based on the Anderson-Darling statistic (ECHA 2008a), and the median HC5, or HC5-50, was derived from this distribution using parametric bootstrap of the normalised geomean EC10 (Verdonck et al. 2001). using parametric bootstrap of the normalised geomean EC10 (Verdonck et al. 2001). From the bootstrapping, the HC5-5 and HC5-95 were calculated to quantify the 90% confidence interval on the HC5-50 estimate.

The entire approach is outlined in Figure 1. The bioavailability normalization procedure starts with translating the chronic Zn ecotoxicity database, with Zn toxicity expressed as dissolved or total concentration, to free ion activities in the Windermere Humic Aqueous Model (WHAM) version VII. The free Zn^{2+} toxicity and Ca^{2+}, Mg^{2+} and Na^{+} activity resulting from these calculations are used to calculate an intrinsic sensitivity (Q10) for each toxicity data entry separately using the appropriate taxon-specific bioavailability model selected from the updated chronic Zn bioavailability models. At this level, the intrinsic sensitivities are averaged for each species and endpoint, and the average Q10 of the most sensitive endpoint is selected. In the next step, the species-specific average intrinsic sensitivities, $Q10_{species\ j}$, is used for predicting Zn^{2+} toxicity in a target water using the taxon-specific bioavailability model and WHAM VII-

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calculated free Ca^{2+}, Mg^{2+} and Na^{+} activities and the pH of the target water. This results in a list of normalized Zn^{2+} toxicities: one for each species per target water, expressed at the free ion activity level. These Zn^{2+} toxicities are then translated back to dissolved Zn concentrations using WHAM VII and the target water-specific physico-chemistry. Finally, a site- specific environmental threshold, the HC5-50 (i.e., median concentration resulting in at least 10% effect for 5% of the species in the SSD) is calculated, and represents the local HC5 which forms the basis of the bioavailable EQS derivation and can be used to incorporate bioavailability in compliance assessment. For simplicity, HC5 refers to HC5-50 throughout this study.

Bioavailable EQS derivation

The $EQS_{bioavailable}$ for Zn was derived using the stepwise approach outlined in the EU EQS derivation guidance (Guidance Document No. 27, EU Water Directors 2018). In the first step, the European baseline monitoring database, FOREGS (Salminen et al. 2005), was used to identify the countries characterized by the highest bioavailability conditions. The FOREGS database contains physico-chemical data for monitoring sites across 25 European countries. Site-specific HC5s were calculated for all FOREGS sites with physico-chemical conditions within the applicability range of the updated Zn EQS derivation approach: pH 5.7–8.4, Ca 2.0–160 mg/L, and DOC 0.1–30 mg/L. In the second step, the $EQS_{bioavailable}$ was derived from additional country-specific databases for the eight countries with the highest Zn bioavailability conditions, representing one third of the countries in the FOREGS database. Countries were selected based on the lower 5^{th} percentile of their site-specific HC5 distribution In Step 2, only datasets from EU member states or countries that have formally implemented the WFD have been considered. For instance, Norway was considered because it implemented the WFD via its European Economic Area (EEA) membership, whereas Switzerland - being neither an EU nor EEA member - was not considered. Site-specific HC5 values were calculated for all monitoring data within the applicability range of the updated Zn EQS derivation approach. To account for within-site variability, including seasonal variation in physico-chemical conditions, sample-specific HC5 values were first calculated and then aggregated at site level using the median. This resulted in one median site-specific HC5 per site, consistent with implementation of the $EQS_{bioavailable}$ as an annual average EQS under the WFD (EU Water Directors 2021). The final $EQS_{bioavailable}$ was derived from

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these calculations and represents the lowest 5th percentile of the HC5 across all sites of the countries taken forward in Step 2 of the calculations (EU Water Directors 2018).

RESULTS & DISCUSSION

Effects database update

The chronic Zn freshwater toxicity dataset combined high-quality data from the existing Zn freshwater toxicity datasets (Van Sprang et al. 2009, Van Regenmortel et al. 2017a) with more recent high-quality data identified in the literature screening.

From the 128 datapoints considered in the database of Van Regenmortel et al. (2017a), 16 datapoints covering three species (*Ceriodaphnia dubia, Danio rerio, and Chlorella sp.)* were excluded due to nominal concentrations, non-relevant effect sizes or sub-optimal test conditions (see description in supplemental information S2-non retained data). In addition, for 40 datapoints included in the original Zn ecotoxicity database NOECs or minimum detectable effect concentrations were replaced by EC10s. For three species considered in the database of Van Sprang et al. (2009) and/or Van Regenmortel et al. (2017a) additional endpoints from the same study were considered (see supplemental information S2).

Within the literature search (considering literature up to 2019), 57 studies reporting on chronic toxicity of Zn to aquatic organisms were identified. From these, 81 toxicity datapoints from 27 studies were considered as being reliable and relevant and were added to the updated Zn ecotoxicity databases. The newly identified data cover 26 species, 20 of which were not yet represented in the existing chronic Zn databases. As such, the updated chronic freshwater ecotoxicity database contains 208 high-quality datapoints covering 40 species.

Non-normalised effect concentrations in the updated ecotoxicity database range by 3 orders of magnitude, with toxicity values (either EC10, LC10 or NOECs) ranging between 4.9 µg dissolved Zn/L (72-h growth rate for the algae *Raphidocellis subcapitata)* and 1,629 µg dissolved Zn/L (28-d growth inhibition for the mollusc *Lymnaea stagnalis*). The entire database with retained ecotoxicity data, including detailed information of physicochemical parameters used for bioavailability modeling, is given in Supplemental

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Information (Supplemental Information S2), where in addition, argumentation for non-retained data is provided.

Bioavailability model update

The existing chronic Zn bioavailability models were updated to reflect the most recent optimizations in model structure (gBAM structure) and speciation software (WHAM VII) (see Supplemental Information S3). Such a relationship is more accurately described by the log-linear relationship between H^+ and Zn^{2+} toxicity integrated in the gBAM structure compared to the linear relationship in a classic BLM-type model (Van Regenmortel et al. 2017b). The current update aligns all bioavailability models for Zn in terms of model structure, which now only differ in the value of pH slopes and of biotic ligand constants for cationic competition of Ca^{2+}, Mg^{2+} and Na^+ (Table 1). In addition, the update also follows the approaches taken in the most recent bioavailability model sets for Ni (Peters et al. 2023a) and Cu (Nys et al. 2024).

The derived pH slope, S_{pH}, for the different taxa at EC10-level ranged between -0.262 (for *D. magna*) and -0.625 (for *R. subcapitata*) (see Figure S3.1). For those species for which a gBAM type model was available in the original Zn EQS derivation approach (*D. magna* & *R. subcapitata*), S_{pH} values were comparable with those derived in the present study (Table 1). This indicates that updating bioavailability models from WHAM V to WHAM VII has only limited effect on derived bioavailability parameters for Zn when the same data for calibration is considered.

The log-linear S_{pH}-slope integrated in the gBAM can account for other possible mechanisms by which pH may affect Zn^{2+} toxicity besides the competition of H^+ at a unidentate biotic ligand site of Zn. These may include changes in algal-cell wall deprotonation and associated increases in Zn adsorption capacity at high pH (Crist et al. 1988), contribution of metal complexes (e.g., $MeOH^+$ and $MeCO_3$) to metal toxicity (Grosell et al., 2004), effects on membrane permeability (Lavoie et al. 2012)) and membrane potential (Kinraide 2006) or dissociation of metal complexes near uptake sites (e.g., Tao et al. 2002).

The prediction performance of the updated bioavailability models across datasets, endpoints and effect levels is detailed in the Supplemental Information (S3). Given the relevance of low effect levels (EC10, LC10 or NOEC) for deriving annual average EQS in Europe (EU Water Directors 2018), the discussion here focuses on these values rather than EC50s. For invertebrates, Zn toxicity at these effect levels was

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predicted by the updated invertebrate model within a two-fold error for 89% of the datapoints (n=38) across six species (i.e., *D. magna*, *L. stagnalis, B. calyciflorus, C. dubia, D. thompsoni, and N. triangulifer; Figure 2*). The Factor of Agreement ranged between 0.8 and 1.0 across datasets (Table 2), with one exception.

This demonstrates that the updated invertebrate model reliably predicts Zn toxicity at the EC10 level across different invertebrate species, with predictions generally falling within a two-fold error. It should also be acknowledged that for one dataset substantial bias in predictions was observed, i.e., the *C. dubia* dataset of Nys et al. (2017; FoA=0.5; Table 2). This bias may reflect species-specific differences in the effect of toxicity modifying factors, particularly pH, as previously reported by Nys et al. (2017) or the influence of factors not included in the gBAM (e.g., bicarbonate). The cross-species evaluation of the invertebrate model included mollusc, rotifer, insect, and crustacean data, thereby covering all major invertebrate groups in the updated Zn ecotoxicity database. Therefore, the updated invertebrate model can be used to normalize toxicity data across the invertebrate species included in the Zn ecotoxicity database, although with some uncertainty for *C. dubia*.

For algae, independent validation data were available for two species, *R. subcapitata* and *Chlorella sp.* For *R. subcapitata*, Zn toxicity at regulatory relevant effect sizes was predicted within a two-fold error for 84% of the considered datapoints (n=44, Figure 2, middle panel), with FoA ranging between 0.6 and 0.9 across datasets (Table 2). For *Chlorella sp.,* previous work has reported higher variability between replicated experiments, and suggested that a three-fold error may be more appropriate than the conventional two-fold criterion (Price et al. 2023b). For *Chlorella sp., 65%* of the datapoints were predicted within two-fold error, whereas the Factor of Agreement increased to 88% when considering a 3-fold error margin (n=49; Table 2). The latter shows at least that the *Chlorella sp*. data does not contradict the updated algae model, and as such it is assumed that the updated algae model can be extrapolated to other algae species. For the primary producers, the Zn ecotoxicity database also contains data on a plant species, *Lemna gibba*. To our knowledge, no datasets are available on the effects of toxicity modifying factors on chronic toxicity of Zn to aquatic plants. However, until additional data become available, the updated algae model is considered suitable to predict chronic Zn toxicity to algae and macrophyte taxa. This approach is

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supported by evidence for other metals, such as Cu (Nys et al. 2024), for which the algae models have been shown to predict metal toxicity across algae and plants species.

For fish, the updated fish model accurately predicted Zn toxicity in natural waters for *O. mykiss*, with all datapoints predicted within a two-fold error (n=5, Figure 2, lower panel). For *S. trutta*, three out four datapoints were predicted within a two-fold error (Table 2). Although independent vertebrate data were limited, the updated fish model was considered suitable for predicting chronic Zn toxicity to vertebrates. This is consistent with evidence for other metals showing that fish bioavailability models can be extrapolated between vertebrate species (Peters et al. 2023a, Nys et al. 2024). Based on these conclusions, the updated Zn EQS derivation approach applied the updated invertebrate model to all invertebrates, the updated algae model to all algae and macrophytes, and the updated fish model to all fish.

Each bioavailability model was developed and validated within a specific physico-chemistry applicability range (see Table 1). The models should only be applied within this range, as predictions outside these ranges are uncertain. The applicability range of the updated Zn EQS approach corresponds to the narrowest pH and Ca validation ranges covered by all three bioavailability models: pH 5.7-8.4 and Ca 2.0-160 mg/L. Applicability ranges for Mg and Na were not defined because these parameters are not consistently measured within monitoring programs and generally covary with Ca (Peters et al. 2011). For DOC, all models have been validated at least between 0.3 to 22.3 mg/L, while bioavailability models are generally considered applicable over a broader DOC range of 0.1 to 30 mg/L (He et al. 2023, Nys et al. 2024).

Site-specific threshold for Zn in Europe

The updated Zn ecotoxicity database and the updated bioavailability models were combined with species sensitivity distribution (SSD) techniques into the updated Zn EQS derivation approach (Figure 1). This bioavailability normalisation procedure was applied to the FOREGS database (Salminen et al. 2005), considering only waters within the model applicability range, to evaluate the distribution of site-specific HC5 values across Europe. Site-specific HC5 varied by almost two-orders of magnitude (Figure 3, Figure S4.1), but generally ranged between 14.5 and 91.0 µg dissolved Zn/L, corresponding to the 5th to 95th percentile

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of the European HC5 distribution (Table 3). An overview of normalized HC5 for all sites is given in Supplemental Information S7.

The influence of the main toxicity modifying factors - DOC, pH and Ca - on local HC5 values was further assessed using hypothetical bioavailability conditions (Figure S4.1). This analysis shows that low DOC generally resulted in the highest Zn bioavailability conditions, and thus lowest HC5. The HC5 values were further influenced by pH and calcium, with the lowest values typically occurring at low to moderate Ca and/or high pH. Low Ca represented sensitive conditions for invertebrates and fish, whereas high pH increased Zn toxicity to plants and algae (Figure S4.1). These high bioavailability conditions characterized by low DOC, low Ca and/or high pH are primarily situated in (sub-)mountainous regions (Figure 3, red and pink squares).

Derivation of the $EQS_{bioavailable}$ for Zinc

Step 1: Identification of countries with the highest bioavailability - Countries with the highest Zn bioavailability conditions were identified using the 5th percentile of country-specific HC5 distributions in the FOREGS database (Table 3). The selected countries were Norway, Italy, Switzerland, Austria, the United Kingdom, France, the Czech Republic, and Germany (Figure 3; Table 3). Only EU Member States and Norway, as an EEA member, were taken forward to Step 2 of the Zn $EQS_{bioavailable}$ derivation; the United Kingdom and Switzerland were excluded.

Step 2 – Derivation of the $EQS_{bioavailable}$ – Following the EQS derivation guidance (EU Water Directors 2018), the $EQS_{bioavailable}$ was derived from site-specific HC5 calculations using country-specific databases for the countries with the highest bioavailability conditions in Step 1. Compared with the FOREGS database, these databases generally include more sites, better capture temporal variability and include waters that are influenced by human activity, which are most relevant for assessing 'Good Chemical Status' under the WFD. Typical ranges in site-specific HC5 for Norway, Italy, Austria, France, Czech Republic, and Germany are summarized in Table 4, with variability within datasets shown in Figure S5.1. An overview of normalized HC5 for all samples is given in Supplemental Information S7.

The $EQS_{bioavailable}$ is defined as the HC5 protective for 95% of the surface waters in the region with the highest bioavailability conditions (EU Water Directors 2018). At the 5th percentile of site-specific HC5 distributions, values varied 2.2-fold across the country-specific databases: from 11.2 (Norway) to 24.2

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(Czech Republic) µg dissolved Zn/L. The lowest value, 11.2 µg dissolved Zn/L, is therefore proposed to be taken forward as the updated $EQS_{bioavailable}$ for Zn in European freshwaters. This value is close to the EQS of 10.9 µg dissolved Zn/L derived by the UK Environment Agency (2012) using the original Zn EQS derivation approach, which is still used as $EQS_{bioavailable}$ in some of the EU member states, including Germany (Table S1.1).

Recently, Merrington et al. (2020) calculated an $EQS_{bioavailable}$ for France using the original Zn EQS derivation approach, proposing an $EQS_{bioavailable}$ of 11.3 µg dissolved Zn/L for the most sensitive region, Bretagne, and 13.7 µg dissolved Zn/L at the national level. In the current evaluation, the 5th percentile of French HC5s was 15.4 µg dissolved Zn/L. This small difference may reflect differences in the datasets used, including the broader set of sites and sampling occasions considered by Merrington et al. (2020), as well as differences in HC5 calculation. Merrington et al. (2020) accounted for seasonal variability before bioavailability calculations, which is computationally more efficient, but may not fully reflect the bioavailability conditions occurring at each site. In addition, they used the simplified bio-met tool, which includes additional conservativity compared with the full bioavailability normalisation approach used here.

Robustness of the proposed $EQS_{bioavailable}$ and site-specific threshold derivation approach

The environmental threshold of 11.2 µg dissolved Zn/L derived in the present study, is proposed as a basis for an EU-wide $EQS_{bioavailable}$. The final regulatory $EQS_{bioavailable}$ is derived through the application of an assessment factor to the selected HC5 to account for uncertainty (EU Water Directors 2018). According to the guidance, the assessment factor should range between 1 and 5, and is ultimately a regulatory decision. This decision is based on a robustness assessment that includes the following considerations: the quality and representativity of the underlying ecotoxicity database, statistical uncertainties around the HC5-estimate, and protectiveness of the EQS in relation to Zn toxicity in field and mesocosm studies.

Quality and representativity of the ecotoxicity database - The Zn EQS derivation is based on a high-quality chronic ecotoxicity database covering 40 species, including algae, plants, amphibians, amphipods, cladocerans, copepods, fish, insects, molluscs, and rotifers. Hence, this dataset covers the minimum data requirements for species sensitivity distribution-extrapolation in terms of both number of datapoints and

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taxonomic coverage (EU Water Directors 2018, Figure 4 and Table S7.1). Overall, unicellular algae and invertebrates were the most sensitive taxa in the zinc database.

Statistical uncertainties around the HC5-50-estimate - For each sample, the site-specific HC5 was derived using a best-fit approach, and represents the median HC5-estimate, or HC5-50. The candidate distributions were selected based on SSD approaches recommended across several jurisdictions (Fox et al. 2021). The Anderson-Darling statistic was used to select the best-fitting distribution for each sample as it gives greater weight to the tails of the distribution, which are most relevant for EQS derivation (EU Water Directors 2018). Therefore, deriving the HC5-50 from the best-fitting distribution minimizes statistical uncertainty in the $EQS_{bioavailable}$ derivation. According to the EQS Technical Guidance (EU Water Directors 2018), statistical uncertainty can be further quantified by comparing the median HC5 estimate, HC50-50, with the lower bound of its 90% confidence interval, i.e., HC5-5. Within the Norwegian dataset, used to derive the proposed $EQS_{bioavailable}$, these values differ on average by 1.4-fold, indicating limited statistical uncertainty around the HC5-estimate.

Protectiveness of the EQS in relation to Zn toxicity in field and mesocosm studies - Environmental threshold derived from laboratory single-species toxicity tests may oversimplify effects of toxicants to field communities. This is mainly because ecological interactions, such as competition, predation, etc. are not accounted for in simple single-species experiments (Del Signore et al. 2016).

Several mesocosm and field studies have evaluated Zn toxicity at the community level. Mesocosm studies published prior to 2008 were reviewed in the EU risk Assessment report (RAR) on zinc (Munn et al. 2008). When re-assessed against the same criteria for reliability and relevancy as the single species data (see section "*Ecotoxicity database update"*) most studies had limitations, including the use of nominal concentrations, short exposure durations not relevant for long-term community-level effects, incomplete physico-chemistry descriptions, water conditions outside the ranges relevant for EU freshwaters and/or communities sampled from conditions with low Zn background concentrations not representative for European waters (Munn et al. 2008, Van Sprang et al. 2009). Re-evaluation of the EU RAR indicated that only one study, on macro-invertebrate communities, was relevant for the present assessment: Clements (2004) which reported a NOEC of 502 µg/L for abundance and structural endpoints.

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Two more recent multi-species studies evaluated the effect of Zn on temperate planktonic systems (Van de Perre et al. 2016), and a tropical freshwater planktonic and periphyton system (Hoang et al. 2021). Van de Perre et al (2016) investigated the effect of zinc on the structure and function of a temperate plankton community (sampled in The Netherlands) during a 5-week indoor microcosm exposure, with Zn concentrations maintained by repeated spiking and physicochemical conditions controlled within ranges relevant for EU waters. The community included zooplankton (e.g., cladocera, copepods, rotifers), phytoplankton (e.g., chlorophyta, bacillariophyta, cryptophyta, chrysophyta, dinophyta), and protozoa. Cladocerans were the most sensitive group, while phytoplankton was not or less affected. This highly relevant study derived a consistent community NOEC of 75 µg dissolved Zn/L, indicating that the proposed $EQS_{bioavailable}$ of 11.2 µg dissolved Zn/L is protective for community-level effects in temperate European freshwaters. The microcosm-specific HC5, derived with the updated EQS derivation approach, was 40.5 µg Zn/L (based on average physico-chemistry conditions: pH 8.1, DOC 10.7 mg/L and Ca 25.7 mg/L), further supporting the protectiveness of the updated EQS derivation approach. In contrast, the study from Hoang et al. (2021) was performed under tropical conditions, and had strongly varying zinc concentrations during the test. Moreover, the study suffered from test conditions outside the range relevant for EU waters, particularly pH>9, and was therefore considered not relevant.

Several field studies further support the protectiveness of the proposed $EQS_{bioavailable}$. In a large scale UK field study, Crane et al. (2007) assessed whether water quality standards protect benthic macro-invertebrate biodiversity, including the sensitive Epheromptera, Plecoptera and Trichoptera taxa, across 291 freshwater sites. They concluded that macro-invertebrate communities were generally not impacted below 20-27 µg dissolved Zn/L. Similarly, analysis of long-term macro-invertebrate monitoring data by Johnson et al. (2025) suggested that family richness was generally not affected below concentrations of 14.2 µg dissolved Zn/L. Recently, Peters et al (2023b) evaluated the protectiveness of a bioavailability-based EQS using field evidence and derived a EC10 of 14.8 $\mu g\, L^{-1}$ bioavailable zinc for the three most sensitive taxa based on 95th quantile regression analysis. This supported the conclusion, that the UK EQS of 10.9 $\mu g\, L^{-1}$ bioavailable zinc, is sufficiently protective of sensitive members of the invertebrate community under real environmental conditions, including a multitude of chemical and physical stressors. Overall,

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these field data indicate that the proposed $EQS_{bioavailable}$ is protective at the ecological scale, particularly for the sensitive benthic invertebrate communities, including inorganic and organic mixture conditions relevant for European waters.

CONCLUSION

The above discussion indicates that the key considerations for selecting the assessment factor for the $EQS_{bioavailable}$ are met. The completeness of the dataset and the general robustness of the approach taken in the derivation of the $EQS_{bioavailable}$ derivation in the presented study, supports the application of an assessment factor of 1. This conclusion is further supported by the observation that the value of 11.2 µg dissolved Zn/L is protective for more than 98% of the FOREGS freshwaters sites within the applicability ranges of the bioavailability models (Figure S5.2), while only approximately 2% of the freshwaters sites have a site-specific HC5 that is lower than 11.2 µg dissolved Zn/L. Field evidence also indicates that the proposed $EQS_{bioavailable}$ is also protective under environmentally relevant mixed-exposure conditions.

The applicability ranges of the updated bioavailability models cover 81% of the European freshwaters in the FOREGS database (Figure S6.1). Based on the above considerations and the robustness of the approach, an $EQS_{bioavailable}$ of 11.2 µg dissolved Zn/L is proposed for implementation in the first tier of the compliance assessments for Zn as a River Basin Specific Pollutant under the WFD. In higher tiers, the updated bioavailability normalisation approach, can be used to calculate site-specific bioavailable Zn concentrations (EU Water Directors 2021) or site-specific environmental thresholds (i.e., local HC5, see Figure 1). This can either be done using the simplified bioavailability tool, bio-met v6.0, recently updated to reflect the updated bioavailability correction approach, or using the full bioavailability normalisation presented here. Together, the $EQS_{bioavailable}$ and bioavailability normalisation approach increase the environmental relevance of compliance assessments for Zn and will help member states to direct mitigation measures where they are most needed.

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590 FIGURE CAPTIONS

591 Figure 1 Overview of the updated Zn Environmental Quality (EQS) derivation approach, which combines
592 the chronic Zn toxicity dataset with the updated chronic Zn bioavailability model set and the species-
593 sensitivity distribution (SSD) approach. A description of the different steps included in the updated Zn EQS
594 derivation approach is given in the main text (*Development of the updated Zn EQS derivation approach*)

595 Figure 1 – ALT TEXT: Flowchart of the updated zinc EQS derivation approach. Chronic Zn
596 ecotoxicity data are speciated using WHAM VII and processed with updated bioavailability models to derive
597 species-specific intrinsic sensitivities (Q10), after species averaging and selection of the most sensitive
598 endpoint. Target-water chemistry is also speciated using WHAM VII. The species-specific sensitivities and
599 target-water chemistry are combined using the updated bioavailability models to predict species-specific
600 Zn toxicity in the target water. These predictions are converted to dissolved Zn concentrations, fitted to a
601 species sensitivity distribution, and used to derive the site-specific or local HC5.

602 Figure 2. Observed versus predicted EC10 and NOEC (10% effect concentrations and no-observable effect
603 concentrations; expressed in µg dissolved Zn/L) for invertebrates (upper panel), algae (middle panel) and
604 vertebrates (lower panel) considered in the independent and cross-species validation. Predictions were
605 made with the updated chronic Zn bioavailability models, where the invertebrate model was used for all
606 invertebrates, the algae model for all algae and the fish model for all vertebrates (see Table 1 for
607 bioavailability model parameters). Filled symbols represent tests conducted in natural waters, open symbols
608 in synthetic waters. The full line represents the perfect prediction line, while dashed and dotted lines indicate
609 a two-fold and three-fold, respectively, prediction error on the observed Zn toxicity.

610 Figure 2 – ALT TEXT: Scatter plots compare observed and model-predicted chronic zinc toxicity
611 for invertebrates, algae, and vertebrates. Points generally cluster around the 1:1 prediction line, with most
612 invertebrate and vertebrate observations falling within the two-fold prediction bounds. Algal predictions
613 show greater variability, with most observations falling within the two- or three-fold bounds. Data span
614 multiple species and both natural and synthetic waters.

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Figure 3. Distribution of site-specific 5% hazardous concentrations (HC5; expressed in µg dissolved Zn/L) across European freshwaters based on the FOREGS database (Salminen et al. 2005). Colours indicate percentile classes of the overall HC5 distribution, with the corresponding ranges of normalised HC5 shown in the legend. All values were normalized using the updated bioavailability normalisation approach for Zn. Only sites with physicochemical conditions within the model's applicability domain are shown: pH 5.7-8.4, Ca 2.0-160 mg/L, dissolved organic carbon 0.1-30 mg/L.

Figure 3- ALT TEXT: Map of Europe showing the spatial distribution of site-specific 5% hazardous concentrations (HC5) for dissolved zinc at FOREGS freshwater monitoring sites. Sites are classified into six percentile groups, from ≤14.5 µg dissolved Zn/L for the lowest 5% of HC5 values (highest bioavailability conditions) to 61.9–260 µg dissolved/L for the highest 25% (lowest bioavailability conditions). The map shows substantial spatial variation in HC5 values across European freshwater sites, with clusters of both low and high values occurring across different regions.

Figure 4 Overview of distribution of species in the updated Zn ecotoxicity database over main taxonomic groups recognized in the Technical Guidance on the derivation of Environmental Quality Standards (European Union Water Directors 2018).

Figure 4 – ALT TEXT: Sunburst chart showing species in the updated zinc ecotoxicity database by major taxonomic group and subgroup. Invertebrates make up the largest proportion, followed by vertebrates and algae/plants. The outer ring further divides these groups into molluscs, crustaceans, insects, rotifers, fish and amphibian families, Chlorophyta, and Araceae.

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Table 1. Overview of characteristics, bioavailability model parameters and applicability ranges of different available Zn bioavailability models.

	Original chronic Zn bioavailability model set[a]			Zn invertebrate model refinement	Updated Zn bioavailability model set (present study)		
	Invertebrates[c]	Algae[d]	Vertebrates[e]	Invertebrates[f]	Invertebrates	Algae	Vertebrates
Bioavailability model type[b]	BLM	gBAM	BLM	gBAM	gBAM	gBAM	gBAM
Speciation model	WHAM V	WHAM V	WHAM V	WHAM V	WHAM VII	WHAM VII	WHAM VII
Bioavailability model parameters							
S_{pH}[g]	-	-0.754 (-0.652)	-	- (-0.130)	-0.262 (-0.150)	-0.625 (-0.596)	-0.406 (-0.252)
Log K_{MgBL}	2.69	-	3.1	2.69	2.69	-	3.1
Log K_{CaBL}	3.22	-	3.6	3.22	3.22	-	3.6
Log K_{NaBL}	1.90	-	2.4	1.90	1.90	-	2.4
Log K_{ZnBL}	5.31	-	5.5	-	-	-	-
Log K_{HBL}	5.77	-	6.2	-	-	-	-
Applicability ranges[h]							
pH	5.5-8.4	5.6-8.5	5.7-8.4	NA	5.5-8.6	5.7-8.5	5.7-8.4
Ca (mg/L)	4.8-160	0.8-159	1.4-156	NA	1.8-160	0.8-159	2.0-156
DOC (mg/L	0.3-17.3	0.3-22.3	0.3-22.8	NA	0.3-40.0	0.2-25.0	0.3-22.3

[a] The original chronic Zn bioavailability model set represents the bioavailability models that have been used in by Van Sprang et al. (2009).

[b] Two types of bioavailability models are represented in the table: I) Biotic ligand model (BLM): a classic linear competition model, II) generalized bioavailability model (gBAM): a model that integrates a log-linear pH effect that is super-imposed on the linear competition effects of other competing ions besides H^+ (see Eq. 1 for model structure)

[c] Model developed by Heijerick et al. (2005)

[d] Model developed by De Schamphelaere et al. (2005)

[e] Model developed by De Schamphelaere & Janssen (2004)

[f] Model developed by Van Regenmortel et al. (2017b)

[g] Values reported represent S_{pH} derived based on 10% effect concentration, (EC10) and between parentheses S_{pH} derived based on 50% effect concentration. Values derived based on EC10 have been taken forward in the updated Zn EQS derivation approach.

[h] Applicability ranges represent the pH and Ca range over which each of the models have been calibrated and validation. For the original chronic Zn bioavailability model, ranges have been taken over from European Union Water Directors (2021).

BLM=biotic ligand model, gBAM=generalised BioAvailability Model, WHAM=Windermere Humic Aqueous Model, NA: not assessed, DOC= dissolved organic carbon, EQS= environmental quality standard

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Table 2 Overview of prediction performance of the updated chronic Zn bioavailability models for independent data[a] based on the Factor of Agreement[b]

Bioavailability model	Species	Reference [c]	Test duration – effect size	Number of datapoints[d]	Factor of Agreement[b]
Invertebrate model	*Daphnia magna*	[1]	21d-EC10	5 (4/1)	1.00
		[2]	21d-EC10	5 (4/1)	0.80
	Lymnaea stagnalis	[3]	28d-EC10	6 (6/0)	1.00
	Brachionus calyciflorus	[3]	48h-EC10	5 (5/0)	0.80
	Ceriodaphnia dubia	[4]	7d-EC10	4 (0/4)	1.00
		[5]	7d-EC10	6 (6/0)	0.50
		[6]	7d-LC10	2 (2/0)	1.00
	Daphnia thomsoni	[6]	21d-EC10	3 (3/0)	1.00
	Neocleon triangulifer	[7]	14d-EC10	3 (3/0)	1.00
Algae model	*Raphidocelis subcapitata*	[8]	72h-NOEC	19 (0/19)	0.84
		[2]	48h/72h-EC10	20 (20/0)	0.90
		[6]	72h-EC10	5 (5/0)	0.60
	Chlorella sp.	[9]	48h-NOEC	5 (0/5)	0.60/1.00[e]
		[10]	72h-EC10	6 (0/6)	0.33/1.00[e]
		[11]	72h-EC10	17 (0/17)	0.88/0.94[e]
		[12]	72h-EC10	12 (0/12)	0.58/0.75[e]
		[13]	72h-EC10	8 (8/0)	0.63/0.88[e]
Vertebrate model	*Oncorhynchus mykiss*	[1]	28d-EC10	5 (5/0)	1.00
	Salmo trutta	[14]	120d-NOEC	4 (4/0)	0.75

[a] Only the prediction performance of low effect values, EC10 (10% effect concentrations) and NOEC (no-observable effect concentration) are given, a more in-depth evaluation of prediction performance across different effect levels is available in Supplemental information.

[b] Factor of Agreement (FoA) represents the fraction of toxicity data predicted within 2-fold error.

[c] [1] De Schamphelaere et al. (2005) ; [2] Van Regenmortel et al. (2017b); [3] De Schamphelaere & Janssen (2010) ; [4] Naddy et al. (2015); [5] Nys et al. (2017); [6] Stauber et al. (2023); [7] Besser et al. (2021); [8] Heijerick et al. (2002); [9] Wilde et al. (2006) ; [10] Price et al. (2021); [11] Price et al. (2023a); [12] Price et al. (2022a); [13] Price et al. (2023b) ; [14] Kallqvist et al. (2003)

[d] Total number of datapoints considered in a dataset (only datapoints with reliable effect concentrations were included). Between parentheses #natural waters/#synthetic waters are indicated

[e] For *Chlorella sp.*, the Factor of Agreement represents the proportion of data points predicted within 2-fold error/3-fold error. For this species, toxicity results have been reported to vary more compared to other species (Price et al. 2022b).

EC10=10% effect concentration; LC10=10% lethal concentration ; NOEC=no-observable effect concentration.

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Table 3. Overview of ranges in physico-chemistry and normalized 5% hazardous concentrations in European freshwaters based on the FOREGS database (Salminen et al. 2005)

Country[a]	Number of sites[b]	pH[c]	DOC (mg/L)[c]	Ca (mg/L)[c]	Distribution of HC5 (µg/L)[d]		
					5th percentile	Median	95th percentile
All	643/795	7.7 (6.3-8.4)	5.0 (0.62-19.6)	45 (2.9-122.7)	14.5	44.0	91.0
Norway	22/53	6.5 (6.1-8.1)	3.3 (0.25-12.1)	5.6 (2.2-21.8)	7.7	29.3	67.2
Italy	34/44	8 (7.2-8.4)	1.3 (0.25-13.0)	55.4 (5.6-117.7)	9.4	33.3	103.2
Switzerland*	8/10	7.8 (6.5-8.3)	1.9 (0.38-4.6)	51.5 (5.0-79.0)	10.0	30.2	75.3
Austria	18/20	8.1 (7.3-8.4)	1.3 (0.50-5.4)	37.8 (13.0-102.3)	11.1	22.6	53.1
United Kingdom*	55/60	7.9 (7.1-8.3)	6.6 (1.4-15.3)	26.7 (2.9-138.7)	12.3	39.4	72.3
France	109/119	7.8 (6.7-8.4)	2.9 (0.76-9.1)	49.1 (4.5-125.0)	15.4	33.9	56.2
Czech Republic	9/10	7.5 (6.8-8.2)	3.5 (1.6-7.5)	28.7 (12.0-71.4)	15.9	30.8	48.3
Germany	63/74	7.5 (6.5-8.2)	3.0 (0.70-21.2)	62.8 (9.2-117.0)	15.9	45.1	90.9
Spain	58/87	8.1 (7.2-8.4)	4.4 (0.93-9.8)	43.5 (2.9-128.5)	16.0	36.1	66.7
Greece	23/27	8.1 (7.6-8.4)	1.4 (0.29-2.8)	58.8 (31.9-101.8)	16.9	30.9	56.6
Slovakia	15/15	7.8 (7.5-8.1)	4.4 (1.7-9.3)	51.5 (13.6-114.5)	20.2	43.5	70.5
Portugal	16/19	6.8 (6.2-8.3)	3.4 (1.7-12.8)	15.7 (3.9-108.2)	21.6	29.1	104.0
Slovenia	4/4	7.7 (7.5-7.8)	0.88 (0.51-1.3)	62.8 (43.0-66.8)	24.9	35.1	44.9
Poland	50/56	7.7 (7.1-8.1)	8.1 (1.8-16.1)	82.7 (41.3-127.2)	31.5	57.4	84.2
Hungary	10/10	8.0 (7.2-8.3)	4.4 (1.9-13.9)	97.8 (36.1-132.3)	32.0	60.7	80.7
Denmark	5/5	6.4 (6.1-7.6)	9.0 (6.7-25.5)	13.7 (9.0-65.3)	34.4	48.1	83.8
Finland	42/65	6.8 (6.1-7.4)	14.2 (5.7-26.8)	4.0 (2.2-11.6)	38.0	73.2	144.2
Sweden	44/50	6.7 (5.9-7.5)	13.3 (7.1-23.2)	4.9 (2.6-19.9)	39.3	72.8	100.5
Ireland	7/11	7.5 (5.8-7.9)	13.5 (6.6-19.2)	28.6 (5.4-149.8)	40.8	56.7	87.4
The Netherlands	8/9	7.4 (7.2-7.7)	11.5 (6.1-21.4)	67.0 (47.9-103.7)	42.8	76.4	105.6
Lithuania	12/14	7.7 (7.1-8.0)	10.2 (5.5-14.4)	96.6 (59.5-119.9)	45.9	71.7	108.4
Croatia	10/10	8.1 (7.2-8.3)	4.4 (2.5-11.5)	94.6 (28.3-117.1)	46.0	58.4	73.4
Belgium	4/5	6.3 (5.8-6.6)	4.5 (3.0-5.4)	37.9 (15.4-110.6)	47.8	61.0	233.1
Estonia	11/11	7.6 (7.3-8.0)	11.1 (3.5-16.6)	90.9 (55.3-132.8)	52.3	70.5	89.0
Latvia	7/7	7.7 (7.5-7.9)	16.5 (7.8-24.0)	76.1 (57.8-97.9)	67.5	80.0	97.6

[a] Stars indicate countries which have not implemented the Water Framework Directive (WFD). Although Norway is not a European Union (EU) member state, as member of the European Economic Area it has implemented the EU WFD since 2008. The United Kingdom implemented the WFD while it was an EU Member State. Following its withdrawal from the EU, the requirements of the WFD have continued through domestic legislation, although the United Kingdom is no longer bound by the Directive as EU law.

[b]The values represent the number of datapoints within the applicability ranges of the Zn bioavailability normalization approach (pH 5.7-8.4, Ca 2.0-160 mg/L)/ total number of datapoints.

[c] For the physico-chemistry parameters, median (5^{th}-95^{th} percentile) of the waters within the applicability range are reported.

[d] For each country and Europe, percentiles have been calculated based on the site-specific HC5 (in µg dissolved Zn/L) for the waters with physico-chemistry within bioavailability model parameters.

DOC=dissolved organic carbon; HC5= 5% hazardous concentration

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Table 4. Overview of ranges in normalized 5% hazardous concentrations in European freshwaters based on the country-specific monitoring databases for the countries identified to reflect the highest bioavailability conditions for Zn (see Table 3)

Country	Reference[a]	Monitoring year	Number of samples[b]	Number of sites [c]	Distribution in site-specific 5% hazardous concentrations (HC5; µg dissolved Zn/L)[c]		
					5th percentile	50th percentile	95th percentile
Norway	MERA 2025	2022	997/1460	189/235	11.2	25.3	64.3
Italy	MERA 2024	2020	2660/2854	432/438	15.4	34.7	65.9
Austria	MERA 2024	2017	1219/1316	110/110	15.1	27.8	46.9
France	MERA 2024	2017	6959/7458	2295/2345	15.6	41.9	67.6
Czech Republic	MERA 2024	2016	31/34	31/34	24.2	41.3	71.0
Germany	MERA 2025	2021	9506/11998	1261/1361	21.3	45.4	94.1

[a] MERA 2024: refers to the online database of the Metals Environmental Research Association (MERA 2024). MERA 2025: refers to the most recent data collected by the database of the Metals Environmental Research Association (MERA 2025).

[b]Number of samples within the applicability ranges of the Zn bioavailability normalization approach (pH 5.7-8.4, Ca 1.8-160 mg/L)/ total number of samples. Only samples with at least pH, DOC and Ca were retained.

[c]Number of sites with at least one sample within the applicability ranges of the Zn bioavailability normalization approach (pH 5.7-8.4, Ca 1.8-160 mg/L)/ total number of samples

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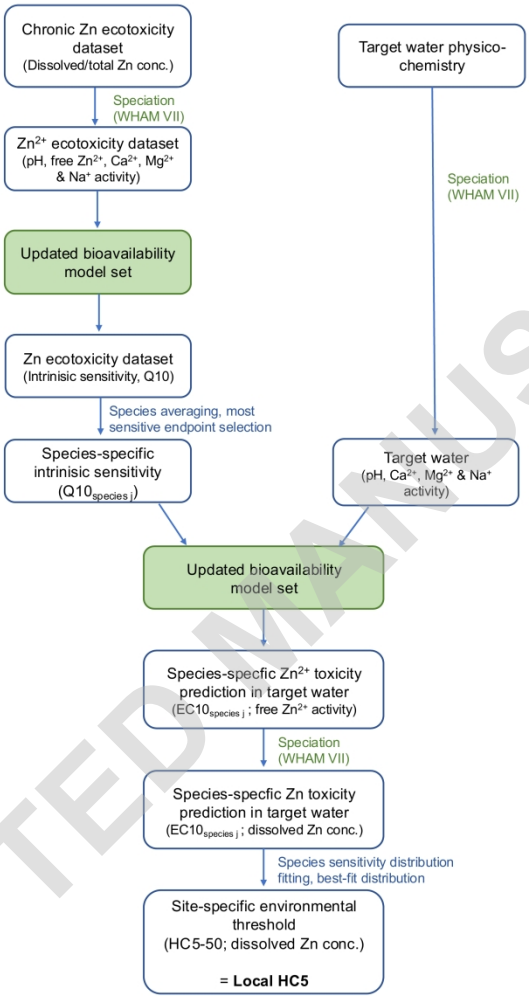


Figure 1 Overview of the updated Zn EQS derivation approach, which combines the chronic Zn toxicity dataset with the updated chronic Zn bioavailability model set and the species-sensitivity distribution (SSD) approach. A description of the different steps included in the updated Zn EQS derivation approach is given in the main text (Development of the updated Zn EQS derivation approach)

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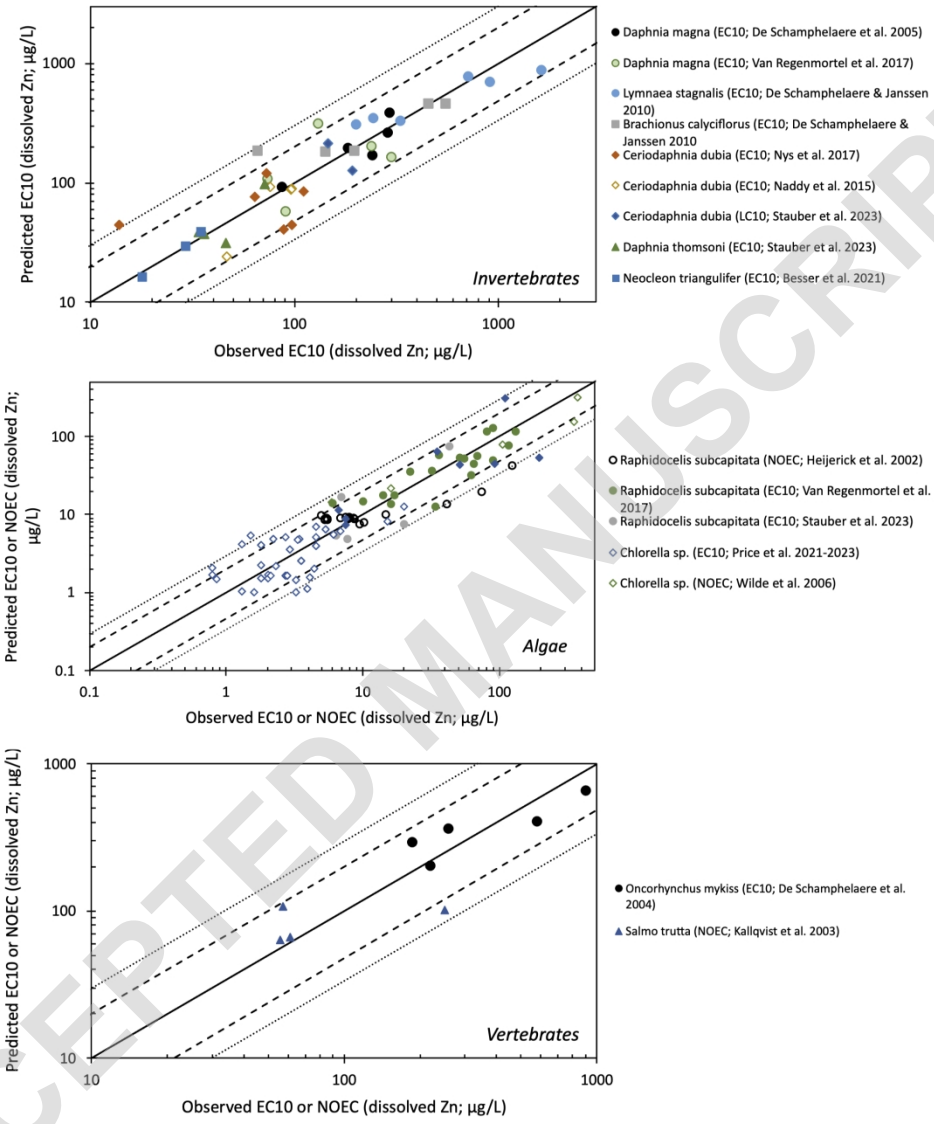


Figure 2. Observed versus predicted EC10 and NOEC (10% effect concentrations and no-observable effect concentrations; expressed in µg dissolved Zn/L) for invertebrates (upper panel), algae (middle panel) and vertebrates (lower panel) considered in the independent and cross-species validation. Predictions were made with the updated chronic Zn bioavailability models, where the invertebrate model was used for all invertebrates, the algae model for all algae and the fish model for all vertebrates (see Table 1 for bioavailability model parameters). Filled symbols represent tests conducted in natural waters, open symbols in synthetic waters. The full line represents the perfect prediction line, while dashed and dotted lines indicate a two-fold and three-fold, respectively, prediction error on the observed Zn toxicity.

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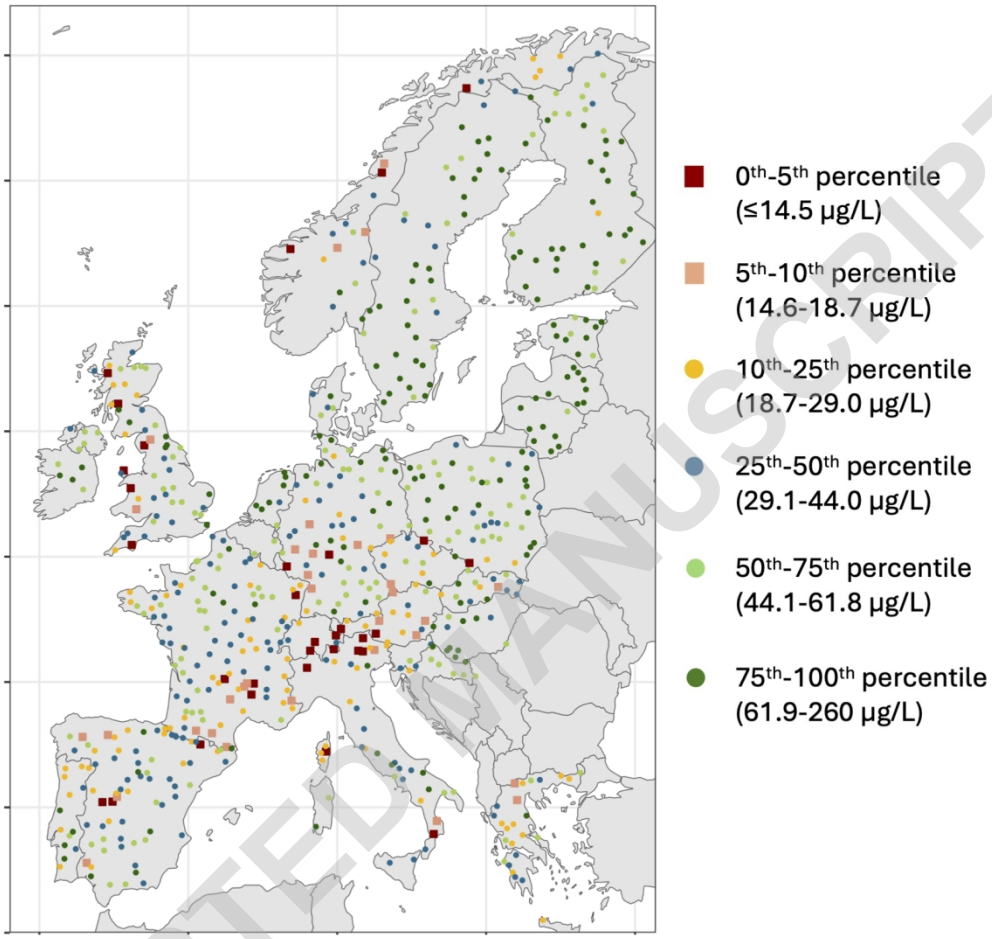


Figure 3. Overview of the distribution of site-specific 5% hazardous concentrations (HC5; expressed in µg dissolved Zn/L) in European freshwaters based on the FOREGS database (Salminen et al. 2005). The colours relate to the different percentiles of the entire HC5 distribution. All normalizations were performed with the updated bioavailability normalisation approach for Zn. Only sites for which physico-chemistry falls within the applicability ranges are plotted (i.e., pH 5.7-8.4, Ca 2.0-160 mg/L, DOC 0.1-30 mg/L).

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Figure 4 Overview of distribution of species in the updated Zn ecotoxicity database over main taxonomic groups recognized in the Technical Guidance on the derivation of Environmental Quality Standards (EU Water Directors 2018).

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