Development of Acute-to-Chronic Ratios (ACRs) to Support Ecotoxicity Prediction for Surfactants

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Data availability statement

All retrieved and curated acute and chronic data are provided in Excel format and attached as supplemental files, in accordance with the Findable, Accessible, Interoperable, and Reusable (FAIR) principles (Wilkinson et al., 2016). These includes (i) the training dataset used for acute-to-chronic ratio (ACR) derivation (*SI ACR derivation dataset.xlsx*) and (ii) an independent external test dataset derived from the HERA database (*SI HERA dataset.xlsx*).

In addition, we developed a spreadsheet-based calculation tool that allows users to populate new acute and/or chronic data for ACR computation, and predict chronic values from given acute toxicity, using established ACR for a certain trophic level. This tool is deposited online and can be accessed at https://doi.org/10.5281/zenodo.20161634.

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Conflicts of interest

The authors declare no conflicts of interest.

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Abstract: Surfactants are used extensively in both industrial and domestic products. Whilst they degrade rapidly, their high production volumes necessitate recurrent safety assessments to ensure robust environmental management. Although substantial historical ecotoxicological data exist, gaps remain for some surfactants particularly with respect to chronic toxicity endpoints. One approach to fill these gaps, especially in light of a growing transition towards non-animal approaches, is to use acute-to-chronic extrapolation by leveraging existing surfactants' ecotoxicity data. Therefore, we established a dataset of 49 unique surfactants identified by Chemical Abstracts Service (CAS) number, including both acute and chronic data, to derive the surfactant-class-specific acute-to-chronic ratios (ACRs) for three regulatory used taxonomic groups, i.e., algae, daphnids, and fish, to represent different trophic levels. The relationship between acute and chronic toxicity was well fitted by linear regression across aquatic species. Regardless of the surfactant class, the median ACR values for algae, daphnids, and fish were 3.8, 7.1, and 4.5, respectively, and the corresponding 90th percentile ACRs were 9.4, 19.4, and 26.4. Furthermore, by employing an external test set (i.e., consisting of data independent from that used for the purposes of training) in order evaluate the predictivity of these derived ACRs, we demonstrated that the derived 90th percentile ACRs performed well with regards to predicting in vivo chronic toxicity from the respective acute value across these three taxonomic groups, with the predicted chronic toxicity of all surfactants no more than one order of magnitude higher than the measured chronic values. These transparent and robust surfactant-tailored ACR values are thus recommended for further application in regulatory safety assessment, supporting a weight-of-evidence justification for waiving additional chronic testing of surfactants.

Keywords: Acute-to-chronic extrapolation; Chronic aquatic toxicity; New approach methodology (NAM), Regulatory safety assessment; Weight-of-evidence;

1. Introduction

Over the last few years, chemical safety regulations have begun to shift away from a position primarily reliant upon generating *in vivo* data for hazard assessment, to one that increasingly encourages reduction or elimination of vertebrate animal testing. In the European Union, Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation states that testing on vertebrates can only be used as a last resort in order to fulfil data requirements for the registration of chemicals (European Chemicals Agency [ECHA], 2020). Similarly, in the United States, the revised Toxic Substances Control Act (TSCA) encourages the reduction and replacement of vertebrate animal tests and supports adoption of alternative methods (U. S. Environmental Protection Agency [USEPA], 2024). There are up to 100,000 chemicals registered in Europe and North America with up to 60,000 chemicals

estimated to be in routine use globally (ECHA, 2023; International Council of Chemical Associations, 2019; United Nations Environment Programme, 2019; USEPA, 2025). Since many of these have incomplete hazard data to meet regulatory requirements, there is a clear need for robust and reliable new approach methodologies (NAMs) to help fill these gaps especially without the need to generate further in vivo studies.

Surfactants are a diverse class of chemicals which are widely used globally in a range of industrial and consumer products, with various functions such as detergents, emulsifiers, wetting agents, and foaming (Cowan-Ellsberry et al., 2014). It is estimated that global surfactant production amounted to 18.8 million tons in 2025, expanding to 22.2 million tons by 2030 (Mordor Intelligence, 2025). Surfactants are distinguished by their amphiphilic character, which arises through their possession of both hydrophilic and hydrophobic structural components. They can be classified into four sub-classes according to the overall charge present on the hydrophilic headgroup, that is, anionic (negative charge), cationic (positive charge), non-ionic (no charge), and zwitterionic (both negative and positive charge). Anionic surfactants accounted for the highest global market share (ca. 48%) in 2024, followed by non-ionic, cationic, and then zwitterionic (Mordor Intelligence, 2025).

Despite being rapidly degradable within both wastewater treatment and within natural surface waters (Ying, 2006), surfactants may be present in the environment due to their widespread use and function. Understanding chronic toxicity of chemicals to aquatic organisms, including fish, invertebrates, and algae, is a regulatory cornerstone for chemical safety assessments under frameworks such as REACH, supporting both risk assessment and also more hazard based requirements such as the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) or the European Union Classification and Labelling and Packaging (EU CLP) regulation (EU, 2008; Kienzler et al., 2016; United Nations Economic Commission for Europe, 2023). As such, chronic aquatic toxicity data constitute part of the Standard Information Requirements (SIR) under REACH for all substances registered at or above 100 tonnes in the European Union (European Commission [EC], 2006). Historically, extensive ecotoxicological data for surfactants were generated to fulfill these data requirements using regulatory accepted environmental species across the three taxonomic groups, namely, algae, invertebrates, and fish. However, data gaps remain, particularly with respect to chronic toxicity. With the move away from the use of *in vivo* testing, the use of robust acute-to-chronic ratios (ACRs) provides an alternative and practical method to assess chronic toxicity, where only existing acute data are available (Kienzler et al., 2017b; Raimondo et al., 2007).

Critical in underpinning the adoption of in silico predictive methods such as quantitative structure-activity relationships (QSARs), read-across and ACRs, is a strong understanding of both the mode and/or mechanism of toxic action (MoA/ MechoA; Brockmeier et al., 2022; Edwards et al., 2016; Firman et al., 2022; Kienzler et al., 2017b, 2017a). Whilst traditional profilers for MoA, such as those of Verhaar et al (1992) and Barron et al (2015), historically produced discrepancies in classification

between profilers, more recent advances in the development of mechanistic based profilers, such as the tool 'MechoA' (Bauer et al., 2018) and the Sapounidou-Firman scheme (Firman et al., 2022; Sapounidou et al., 2021), combined with more consensus-focused approaches (Kienzler et al., 2019) provide increasingly robust options for MoA/ MechoA classification. A number of such MoA or chemical class based ACR have been reported in the scientific literature (Ahlers et al., 2006; Brill et al., 2021; European Centre for Ecotoxicology and Toxicology of Chemicals [ECETOC], 2003; Kienzler et al., 2017b; May et al., 2016). For example, ACR median values for pesticides with specific MoAs and for general organics, relating to *Daphnia magna*, were 44 and 5.5, respectively (ECETOC, 2003). Previous studies also reported an ACR value of 100 to be protective for 90% of the examined chemicals, although it does not seem to be equally protective for each MoA (Kienzler et al., 2017b; May et al., 2016).

The aquatic toxicity of surfactants has been widely demonstrated to correlate positively with lipophilicity (Hodges et al., 2006a; Müller et al., 1999; Roberts, 1991). Combined with other evidence in the literature, including toxic unit approaches, membrane uptake correlation, and phenotypic and genotypic observations, this has led to a general consensus that surfactants behave by a narcosis MoA; evidence indicates that nonionic and ionisable/ ionised surfactants act through non-polar/ baseline narcosis and polar narcosis respectively (Brockmeier et al., 2022; Davies et al., 2004; Droge et al., 2023; Gredelj et al., 2025; Hodges et al., 2006b; Joshi et al., 2007; Roberts and Costello, 2003; Soap and Detergent Association/Alkylsulfate Consortium, 2007). Whilst cationic surfactants also act by polar narcosis (Roberts and Costello, 2003), the membrane binding is significantly stronger due to strong electrostatic interaction between the positively charged cationic nitrogen and the negative moiety of the phosphatidyl choline membrane lipids. This results in more effective partitioning into the membrane and hence enhanced toxicity (Roberts et al., 2013). Similar membrane binding interactions also have been observed with negatively charged microbial surfaces (Vereshchagin et al., 2021). Once a narcotic has passed through the cell membrane, it is able to disrupt vital functions, such as energy production, metabolism, and nutrient/oxygen transport (Brockmeier et al., 2022). This narcosis MoA makes it more readily predictable than other MoAs / MechoAs (Aurisano et al., 2019; Vaal et al., 1997). Usually, a lower value of ACR (e.g., 10) is justified to extrapolate acute to chronic toxicity for non-polar narcotic compounds (Ahlers et al., 2006; May et al., 2016; Raimondo et al., 2007). Furthermore, the USEPA Ecological Structure Activity Relationship (ECOSAR) model gives specific ACRs corresponding to several chemical classes, with values of 5 and 6.5 assigned to non-ionic and anionic surfactants, respectively. Unfortunately, detailed analyses describing ACR derivation within these published approaches is not well documented (Mayo-Bean et al., 2012; Zeeman, 1995).

This study aimed to develop robust and transparent surfactant-specific ACRs, sourcing existing acute and chronic ecotoxicological data from regulatory-relevant aquatic species across the three taxonomic groups of algae, invertebrates

(represented by daphnids), and fish. In addition to the training dataset used for ACR derivation, an external test set was employed to assess the robustness of the derived ACR values by comparing the predicted and measured values.

2. Methodology

2.1 Data source and curation

2.1.1 Identification and categorisation of surfactants

Details relating to substances recognised as possessing surfactant application were extracted from a series of reference sources (Johansson et al., 2012; Michael and Irene, 1993; Mohammed Taha et al., 2022; Williams et al., 2017), as outlined within online supplementary material, Table S1.

Associated with each entry was a Chemical Abstracts Service (CAS) identifier, alongside name and (where appropriate) a Simplified Molecular Input Line Entry System (SMILES) representation of chemical structure. Categorisation proceeded in line with fundamental surfactant structural class – be this anionic, cationic, non-ionic or zwitterionic.

2.1.2 Gathering of experimental data characterising surfactant aquatic toxicity

For the purposes of acquiring relevant aquatic toxicity data, searches were performed using two platforms – the Organisation for Economic Co-operation and Development (OECD) QSAR Toolbox v. 4.6 (Dimitrov et al. 2016), which provides access to the ECHA Chemicals Database (ECHA CHEM), and the U.S. Health and Environmental Sciences Institute (HESI) Envirotox Database v. 2.0.0, which additionally includes extracted data from the USEPA ECOTOX platform (Connors et al., 2019; Figure 1).

OECD QSAR Toolbox

In this instance, all appropriate CAS present within the OECD QSAR Toolbox (v. 4.6, https://qsartoolbox.org/) were screened. Under the *Ecotoxicological Information* heading, the data source options Aquatic ECETOC, Aquatic Japan MOE, Aquatic OASIS, ECHA REACH (as the ECHA CHEM database), Open Food Tox Hazard (EFSA) were selected. A search, limited to Aquatic Toxicity content, was subsequently conducted – with output collated ahead of manual curation.

Envirotox

The Envirotox Database (https://envirotoxdatabase.org/) was searched, with application of the following filters:

- USEPA New Chemical Categories: All containing the term "*surfactant*"
- Trophic level: *FISH*, *INVERT*, *ALGAE*

It should be noted that inspection of substances appearing within the USEPA *surfactant* lists was found necessary, in order to identify those deemed to fall beyond the range of this exercise. Amongst such compounds appeared assortments of

established pesticides (exemplified by spirotetramat) and surfactant synthetic precursors (e.g., triethylene glycol). Following exclusion of these, acceptable entries were categorised (i.e., anionic, cationic, non-ionic or zwitterionic) as previously described.

2.1.3 Attribution and subdivision of recovered data

Content retrieved from the OECD QSAR Toolbox and Envirotox resources were pooled, with duplicate entries excluded. From the resulting series, outcomes were classified in accordance with criteria outlined within Figure 1 – whereby distinction was drawn with respect to taxonomic group (i.e., fish, invertebrates, or algae) and to the acute or chronic nature of the study protocol. Data not matching these specifications were removed, as were those which aligned with the following conditions:

- Study conducted within artificial stream or mesocosm environment
- Describes effects relating to non-intact organisms (e.g., ex vivo)
- Effect concentrations expressed as a range or approximation

For acute toxicity endpoints, 50% effect concentration (EC50) and 50% lethal concentration (LC50) values were extracted and compiled from the data sources. For chronic toxicity, the EC10, EC20 and No Observed Effect Concentration (NOEC) results were collated. The preferential order of selection of EC10> EC20 > NOEC was considered when parallel values were identified for a unique substance (as defined by CAS) within a common species; both the EC10 and EC20 are statistical results from dose-response relationship, while NOEC is heavily dependent on the study design (i.e., defined by the concentration range selected) and EC10 is more conservative than EC20.

We acknowledge that across regulatory jurisdictions, NOEC may be the preferred chronic endpoint. Therefore, in parallel, an alternative endpoint preference order (NOEC > EC10 > EC20) was also evaluated for ACR derivation. Where multiple values from a given endpoint are reported, all were considered through varying methods of aggregation (see the next section for details). Besides the OECD-recommended toxicity effect (e.g., growth, and reproduction), other health- and development-related effects (e.g., intoxication, histology), if generated under qualified test conditions, were included to maximise the number of acute-chronic pairs and to support a more robust ACR derivation across taxa (Figure 1). The final data selection for ACR derivations is attached as a supplemental Excel file (see online supplementary material). Within entries explicitly labelled as having originated from studies conducted in accordance with OECD test guidelines, the identities of the adopted protocols were stated. It should be noted that the absence of such labels does not necessarily imply non-adherence.

Where possible, all effect potencies were expressed as representations of active surfactant concentration. Numerous outcomes were retrieved relating to the activities of formulations within which the surfactant of interest accounted for only a fraction of

the tested material. In such cases, effect concentrations were often reported by study authors in a manner which incorporated adjustments necessary to describe only the active component. However, this was not always so. Where test composition had been specified but such amendments not introduced prior, they were instead made in situ. By way of example, the median lethal dose of the commercial product “Savol Algaecide” was recorded at 9.60 mg/ml. In consideration of its 10% cocoalkonium chloride content, this value was revised to 0.96 mg/ml.

To enable the derivation of appropriate ACRs, comparable acute and chronic outcomes were first identified. The meeting of this definition was dependent upon the fulfilment by entries of two fundamental criteria:

- Relation to a common trophic level
- Correspondence to a shared CAS identifier

2.2 Acute-to-chronic ratio (ACR) derivation

To ensure a sufficiently large and representative dataset, three different approaches were considered for pairing acute and chronic toxicity data, in particular when there are multiple candidate values for a same CAS in the same trophic level. In each case, averages were calculated using the geometric mean since it reduces the influence of unusually high or low values, providing a more balanced and representative summary. This is especially important in ecotoxicology, where toxicity values can sometimes span 1–2 orders of magnitude for the same compound (Hickey et al., 2012; Hrovat et al., 2009). The pairing approaches differed in the level of precision used to group and match species-level toxicity data for each surfactant. They are presented in order of decreasing species specificity, beginning with the most detailed species-level matching and progressing toward broader aggregation methods:

All Species: Geometric means for both acute and chronic values were calculated across all available data for each CAS number, at a given trophic level, regardless of species. This approach maximised data inclusion but did not account for species-specific variability or species-specific effects, a major drawback which is addressed in the following two methods.

Group Species: For each CAS number within a trophic level, acute and chronic values were first summarised within each test species by calculating species-specific geometric means across all eligible studies. These species-level geometric means were then combined using a second geometric mean (equal weight per species) to obtain a single acute value and a single chronic value per CAS number. This two-stage aggregation reduces bias arising from unequal data availability among species, preventing species with disproportionately many datapoints from dominating the overall estimate.

Same Species: Acute and chronic values were paired only when both endpoints were available for the same species. This is the most sensitive of the approaches, as it ensures that the acute and chronic data reflect responses from the same species.

However, it significantly reduced the dataset size due to limited quantity of intra-species pairings present within the data.

Once acute–chronic pairs were established, ACRs were calculated using a logarithmic approach at the level of individual CAS, often referred to as the geometric mean of the ratio using Equation 1.

$$ACR = e^{\sqrt[n]{\prod_{i=1}^{n}(\ln(Acute_i)-\ln(Chronic_i))}} = \frac{GeoMean(Acute)}{GeoMean(Chronic)} \quad \text{(Equation 1)}$$

Where $Acute_i$, $Chronic_i$ are the acute and chronic values for pair i and n is the number of acute-chronic pairs, following the aggregation rules defined above. Natural logarithms were used throughout. Records with non-numeric effect concentrations were excluded during curation. No zero-valued concentrations were retained. Based on the distribution of ACR values for individual CAS within a certain trophic level, the corresponding median, 90th and 95th percentile ACRs were determined.

Within this framework, the geometric mean provides an appropriate measure of central tendency on a multiplicative scale, whereas the arithmetic mean can be disproportionately influenced by high-end values in right-skewed data; the median, while robust, depends primarily on rank order and the central observation(s) and does not incorporate the magnitudes of all datapoints, making it less aligned with the log-based regression and percentile approach used here. Working in log space also ensures symmetry between high and low ratios and supports straightforward derivation of percentiles. All statistical analyses and visualisations were conducted in Microsoft Excel (Office 365); see the Data Availability for the fully documented Excel-based tool.

2.3 Curation of external test set for ACR evaluation

Each of the categorisation, curation, and ACR determination stages described above were repeated, using data drawn from hitherto excluded sources. These consisted of reports issued through the Human and Environmental Risk Assessment (HERA) initiative relating to ingredients of household cleaning products (HERA, 2025), alongside further documentation compiled on behalf of the Dutch Association of Soap Manufacturers (NVZ; BKH Consulting Engineers, 1994). Appropriate acute and chronic entries were combined in order to form a dataset referred to as the external test set. Where matched, experimental chronic toxicity values were compared relative to those estimated values following application of ACR to the corresponding acute data.

The chronic toxicity of the surfactants was evaluated in this external test set using Equation 2.

$$Predicted\ chronic\ toxicity\ (mg/L) = \frac{Measured\ acute\ toxicity\ (mg/L)}{ACR} \quad \text{(Equation 2)}$$

When parallel values were identified for the same substance and species, the preferential selection order EC10 > EC20 > NOEC was applied. For cases where

multiple values for a given endpoint were reported, the geometric mean was taken, consistent with the approach used for the training set.

3. Results and Discussion

3.1. Composition of ACR-derivation dataset

Following each of the curation steps described within section “Data source and curation”, a dataset consisting of 899 valid experimental outcomes remained, related to 115 distinct substances (as distinguished through CAS identifier). Of these, 27 represented forms of cationic surfactant (almost exclusively quaternary ammonium salts), 39 of anionic (sulphates and sulphonates, alongside a carboxylate minority), 39 of non-ionic (ethoxylated alcohols and amines) and ten of zwitterionic (a split between betaines and N-oxides). A total of 65 test species were noted – the overwhelming majority of which (56) constituted varieties of teleost fish. Alongside these were found seven algal and two daphnid species.

With regards to fish toxicity, 81 surfactants were found to have acute data (Table 1), whilst only 22 possessed chronic. From these, 19 were identified as having both acute and chronic values for a shared CAS number, and thus could be considered suitable candidates for derivation of ACR. Within the daphnid taxon, species were limited to *Daphnia magna* and *Ceriodaphnia dubia*, and 18 pairings emerged from out of 62 substances with acute data and 28 substances with chronic data. A total of 31 matches were apparent amongst the 38 and 34 substances holding, respectively, acute and chronic algal data. Across each of the three trophic levels, 49 unique surfactants were represented, on at least a single occasion, by such pairings – including seven cationic, 17 anionic, 18 non-ionic and seven zwitterionic.

It should be noted that substances falling under the bounds of a single CAS may not necessarily possess absolute uniformity with respect to their chemical structure. Whilst a defining polar head unit is almost certain to remain conserved, it was not uncommon to observe variation either in accompanying alkyl chain length or in extent of ethoxylation. Given that each of these characteristics is acknowledged as holding influence upon toxicity, it is plausible that inappropriate comparison between compounds very different in terms of intrinsic potency could bias subsequent ACR estimation.

In practice, however, such variability is often minor: not only is the range of alkyl groups permissible under a given CAS typically restricted (e.g., from C12 to C14), but the compositions of commercially-available products corresponding to an identifier are liable to converge (as exemplified by similarity in chain-length averages appearing under CAS 42615-29-2, linear alkylbenzene sulphonate). Ethoxylate number is less constrained – with many CAS relating instead to free “polyethoxylated” forms either of alkyl alcohols or sulphates. In extreme instances, such that of polyethylene glycol nonylphenyl ether (CAS 9016-45-9), this figure may range from one to as many as thirty units. Often, however, it is left unspecified. On account of such ambiguity, referral to CAS alone was considered the most practical means of differentiation.

3.2 Considerations in data analysis of acute-to-chronic extrapolation

Within the curated dataset, each surfactant (represented by a CAS number) may have multiple data entries for acute or chronic toxicity, extracted from across different studies and tested species. The latter is particularly true within fish, for which outcomes relating to 45 species were adopted. To derive robust ACR values, therefore, we first evaluated three approaches of species grouping and acute-to-chronic pairing: All Species, Group Species, and Same Species.

The “All species” grouping was prone to bias, in instances where certain species held multiple data entries corresponding to a given surfactant. For each CAS number, the ‘Group Species’ approach calculated the mean toxicity for each species first and then computed a geomean across these species-level averages. This helps to reduce potential bias resulting from species with disproportionately high data representation. The ‘Same Species’ method, while considered more robust and accurate, excluded a substantial portion of the dataset due to limited pairings in species-specific data (e.g., from 19 to 13 surfactants for fish). Although different normalisation approaches can lead to differences in individual ACR values, these differences did not translate into statistically significant differences in ACR distributions at the trophic-level scale ($p > 0.05$, see online supplementary material, Figure S1). This finding is consistent with a previous study (May et al., 2016), which demonstrated that fish ACRs (both median and 90th percentile), derived using the same test species, were comparable to those derived regardless of species across a broad class of industrial chemicals. The “Group Species” method was therefore selected for deriving the final ACRs, since this approach offered a practical balance between robustness and data availability.

It has been reported that fish life stage and species can substantially affect the derived ACRs, particularly 90th percentile ones (Ahlers et al., 2006; May et al., 2016; Raimondo et al., 2007). Use of only early life-stage tests and identical fish species can yield a more conservative ACR (Ahlers et al., 2006; May et al., 2016). In practice, within the data compilation for surfactants, given that reduced sample size would affect the credibility of relationships, all test types and species were employed in deriving ACRs. This is in line with existing studies (Ahlers et al., 2006; May et al., 2016).

Beyond the pooling of outcomes taken from non-identical species, further compromises were necessary in order to ensure that the availability of data was adequate. Each of these may, alone, hold the potential to contribute towards uncertainty within the emerging ACRs. Variations in experimental protocol and conditions (e.g., considering organism life-stage, constitution of water or testing medium, dosing range and interval, etc.) are likely to impact upon measured toxicity, rendering such comparisons intrinsically imperfect. Effect sizes may, furthermore, be reported in terms either of product formulation or of active surfactant concentration and expressed as a nominal (i.e., assuming no compound degradation) or, alternatively, as an analytically-determined quantity.

Steps were taken in order to guarantee that confounding influences in the data were minimised – with the exclusion of studies known either to have been performed outside of the laboratory setting, to have adopted irregular time-scales or endpoints, or to have focused upon toxicity recorded in non-intact organisms. On occasion, however, such details were undisclosed. As a consequence, some ambiguity with respect to extent of inter-study correspondence was liable to remain. By way of example, whilst effort was made to ensure that effect concentrations were represented consistently as surfactant component alone (rather than as the tested substance), this aim was not always achievable. In all, outputs from 400 (out of 899 entries) related explicitly to active surfactant content – including those 79 which were adjusted in accordance with steps described within section "*Attribution and subdivision of recovered data*". Across the remaining 499, details relating to material composition were absent. Of course, should one value relate solely to active ingredient and another (unknowingly) to a solution in which it comprises only a fraction, then a clear potential for misstatement of potency exists. Similar challenges were noted with respect to discerning the measured or nominal origins of such figures. In the majority of instances (470 from 899), this was left ambiguous. Amongst the remainder, 288 entries were described as nominal and 141 as explicitly measured.

3.3 Derivation of acute-to-chronic ratios across trophic levels

3.3.1 Algal ACRs

The derived median, 90th and 95th percentile ACRs for algae are 3.8, 9.4, and 20.0 (with a range of 1.7–32.4), respectively (Table 2). It is worth noting that unlike daphnids and fish, acute and chronic toxicity data in algae represent different statistical interpretations often derived from the same experiment (OECD Test Guideline 201), both so-called acute and chronic outcomes often arise as different statistical endpoints (e.g., growth rate vs. yield) from the same test, not from biologically distinct exposure durations (Brill et al., 2021). This results in a greater number directly corresponding acute and chronic pairs and an improved goodness of fit ($R^2 = 0.86$).

3.3.2 ACRs for daphnids and fish

Median ACR values for daphnids and fish are 7.1 (1.7–78.7), and 4.5 (1.6–50.7), respectively, and the 90th and 95th percentile ACRs for daphnids are 19.4 and 28.5, and 26.4 and 30.6 for fish, respectively. Interestingly, daphnids had a higher median ACR, but a lower 90th percentile ACR than fish (Table 2), reflecting the narrower distribution of values for this taxon (see online supplementary material, Figure S2).

As mentioned above, besides those well-accepted effects within the regulatory context, other effects with health relevance, if tested in qualified conditions, were included in the testing dataset. This allowed for increased sample size, particularly in the cases of fish and daphnids, where data availability is otherwise limited. Specifically, applying a strict filtration to include only entries that can be confidently attributed to OECD Test Guideline-compliant studies would result in only three and five acute–chronic pairs for

fish and daphnids, respectively, which are insufficient to derive any robust ACRs for these taxa. While this broader inclusion inevitably introduces additional uncertainty, the relationship between their acute and chronic toxicity data demonstrated a significant and strong fit when tested using linear regression (Figure 2), ensuring the reliability and accuracy of derived ACRs.

3.3.3 Metrics and robustness considerations

In a more conservative manner, 90th or 95th percentile values may be used to predict chronic toxicity. Of these, the 90th percentile is most commonly reported (Ahlers et al., 2006; Kienzler et al., 2017b; May et al., 2016; Raimondo et al., 2007). Given the variability and limited size of the dataset, especially at the extreme ends of the toxicity distribution, the 90th percentile was selected as the preferred summary statistic over median and 95th percentile values in the present study. While the 95th percentile is often used in conservative risk assessments, it was deemed less reliable in this instance due to the reduced number of data points in the upper tail. The 90th percentile, by contrast, lies within a denser region of the distribution, making it statistically more robust while still erring on the side of conservatism (see online supplementary material, Figure S2). This choice ensures that the derived ACRs are protective for the majority of surfactants, without being overly influenced by outliers or limited data. In addition, the preference for NOEC or EC10 might deliver different ACRs (Brill et al., 2021). In the present study, using an alternative chronic endpoint preference hierarchy (NOEC > EC10 > EC20) affected ACR derivation for 1 of 19 fish CAS, 3 of 18 daphnid CAS, and 9 of 31 algal CAS, resulting in a noticeable change in the 90th percentile ACR for algae, as summarised in online supplementary material, Table S2. Nevertheless, EC10 is retained as the preferred chronic endpoint in the present study, as explained in section "*Attribution and subdivision of recovered data*". We acknowledge that NOECs may be preferred by some readers; therefore, all raw data are provided in the spreadsheet-based tool, allowing users to derive ACRs using NOECs if desired.

ACRs have been derived for individual surfactant classes at each trophic level (see online supplementary material, Table S3). However, in a few cases given the limited pairings of corresponding acute and chronic toxicity outcomes at each trophic level, some of these values may be considered of lower reliability where $n < 5$. It is usually accepted that, following the Topliss ratio, at least five individual chemicals per descriptor are needed for an effective QSAR model, and a similar philosophy can be applied to the development of ACRs (Dearden et al., 2009; Gissi et al., 2021). Some exceptions to this can be seen for the algae ACRs of anionic and non-ionic surfactants which provide reliable ACR values (R^2 =0.73, and 0.88, respectively) and sample size (both n = 12; see online supplementary material, Table S3). The ACRs derived for all surfactants can predict conservative and robust chronic toxicity for each class, since all qualified ($n \geq 5$) ACR values are higher than class-specific ones (see online supplementary material, Table S3). Interestingly, the dataset showed that cationic surfactants had an apparent tendency of displaying higher toxic potencies (both acute and chronic) than other surfactant classes (Figure 2). This is concordant with previous

observations where it has been proposed that cationic surfactants can partition into the membrane in a more effective way than other classes, thus inducing a more toxic, whilst still non-specific, effect (Roberts et al., 2013).

3.4 Comparisons of ACRs across chemical groups and MoAs

To compare the derived ACR with existing ACRs, values across chemical groups and MoAs were directly extracted from published studies and regulatory sources, as reported therein, without any further interpretation (see online supplementary material, Table S4). The results show that the ACR median values in this study are consistent with the majority of existing literature ACRs for chemicals which share the same relevant narcosis MoAs (Figure 3; Ahlers et al., 2006; May et al., 2016; Raimondo et al., 2007; Roex et al., 2000). In particular, our median ACRs were similar to the values for surfactants reported by the USEPA and listed within the ECOSAR software (Mayo-Bean et al., 2012). One clear exception in the literature is reported ACR values of 51.9 and 541 for median and 90th percentile respectively for polar narcosis (May et al., 2016). However, these values are clearly anomalous to all other reported values in the literature. Unfortunately, without access to the original dataset used in the derivation of these values it is impossible to understand why they appear erroneous compared to all other literature reported values. However, this does exemplify the need for careful data curation and transparency in order to justify and support robust ACR derivation. As such, importantly, this study presents a transparent and robust dataset for surfactant ACR derivation, alongside conservative ACRs for 90% and even 95% of chemicals.

In general, the existing literature ACRs (both median and 90th percentile values) derived from all chemicals, including pesticides and/or metals, are larger than the surfactant-specific ACRs acquired in this study. This likely arises due to chemicals such as biocides usually have specific MoAs, which are likely to result in higher chronic toxicity potential (Scholz et al., 2018). Thus, the derivation of surfactant-specific ACRs enables more realistic and reliable predictions of chronic toxicity relevant to each regulatory relevant trophic level, whilst supporting increased robustness of safety assessments.

3.5 Evaluation of the predictivity of derived ACRs in chronic toxicity

Following the derivation of ACRs it was considered important to examine their performance in estimating chronic toxicity of surfactants not included within the training set. Since the ecotoxicological data from the HERA project were difficult to assign to specific CAS numbers, due to factors such as complexity of chain length, these results were kept independent from those used for the purposes of training. Thus, they provided an opportunity to objectively assess ACR performance, as an external test set. Importantly, acute and chronic data from the HERA project were paired only for substances with matching chain length, ethoxylation degree, and structural configuration, minimising uncertainty arising from compositional mismatch. In this evaluation, we considered it acceptable if the difference between predicted and

measured chronic values was less than one order of magnitude, consistent with recent literature approach making similar data comparisons (Nyman et al., 2025). If the predicted figure was more than one order of magnitude lower than the measured chronic toxicity, it was also considered acceptable in a conservative manner.

Results demonstrated that the ACRs derived here performed well with respect to predicting chronic toxicity for surfactants (Figure 4). Specifically, 24 from 25 (96%), 17 from 18 (94%), and 5 from 5 (algae, daphnid and fish, respectively) had comparable predictions, i.e. no more than one order of magnitude higher than the experimentally derived chronic toxicity value, when applying the median ACRs values (see online supplementary material, Figure S3). Application of the 90th and 95th percentile ACRs resulted in all predicted chronic toxicity values being comparable to experimental values (see online supplementary material, Figure S3). This suggests that the 90th percentile ACR can provide an acceptable and sometimes conservative estimate of surfactant chronic toxicity, regardless of classes, based upon measured acute toxicity. As discussed above, from the statistical perspective, the 90th percentile ACR is also more accurate and reliable than the 95th percentile. Therefore, the 90th percentile ACR values are recommended for potential further application in environmental risk assessment.

3.6 Potential applications of the derived ACRs

This study represents a significant development in terms of ACRs applicable to surfactants. Through extending existing knowledge and compiling surfactant-specific data, a credible alternative to default values is offered. Such tailored ACRs enhance transparency and relevance by ensuring alignment within a given chemical class or taxonomic group. Demonstrated with scientific robustness and reliability, the derived ACRs (90th percentile values being recommended) for each of the three regulatory taxonomic groups have potential application in environmental risk assessment and regulatory decision-making (Aurisano et al., 2019).

A key application of the derived ACR values lies in the filling of data gaps relating to long-term aquatic toxicity, where only acute endpoint data are available, thereby fulfilling regulatory endpoint requirements and supporting the derivation of predicted-no-effect-concentrations (PNECs). Within the regulatory context, for example, chronic aquatic toxicity data for invertebrates and fish are required under the EU REACH regulation when substance tonnage is or exceeds 100 tons per year (EC, 2006). The surfactant-tailored ACRs offer a cost-effective and ethical alternative route by which to derive missing chronic endpoint values for such substances. By refining extrapolation factors specific to surfactants, these ACRs have the potential to provide improved accuracy and confidence in PNEC derivation. In regulatory risk assessment, ACR-derived chronic values are typically followed by the application of assessment factors (AFs) to derive PNECs (ECHA, 2008), reflecting remaining uncertainty and data completeness and commonly range from 10 to 100 when chronic datasets are limited, or may be reduced when robust species sensitivity distributions and HC5

values are available. Applied on top of the conservative 90th percentile ACR values recommended here, such AFs would add a further margin of precaution, supporting protective risk management decisions. In addition, the derived ACR could contribute to the enrichment of chronic toxicity datasets used in species sensitivity distributions (Cao et al., 2023).

The ACR approach can offer a further line of evidence, in combination with data from other NAMs (e.g., QSAR and read-across), supporting a weight-of-evidence justification for waiving additional chronic testing of surfactants.

3.7 Considerations and limitations for the application of derived ACRs

Surfactants are often recognised as difficult-to-test substances, meaning that actual exposure concentrations during ecotoxicity tests can deviate substantially from nominal values due to rapid biodegradation, adsorption to test vessels, solubility constraints, and micelle formation near or above the critical micelle concentration (CMC). These processes can lead to pronounced reductions in freely dissolved concentrations over time, sometimes falling below 80% of nominal levels, making time-weighted measurements necessary for accurate exposure characterisation.

Although every effort was made to ensure data quality, including prioritising measured concentrations whenever both nominal and measured values were available within a given study and cross-checking reported effect levels against water solubility when necessary, most of the legacy studies included in the dataset did not explicitly report exposure verification for difficult-to-test chemicals in compliance with OECD Guidance Document 23 (OECD, 2019). Consequently, key information on exposure maintenance, analytical confirmation, or surfactant-specific behaviour was often unavailable, limiting ability to fully assess the accuracy of reported concentrations.

This incomplete exposure reporting represents an unavoidable limitation of the underlying dataset. Nonetheless, despite such uncertainties at the level of individual acute or chronic values, the data still exhibited strong linear relationships between acute and chronic endpoints, indicating that these sources of variability may not necessarily translate into substantial deviations in the derived ACRs. More importantly, evaluation using an external test set showed that the derived ACRs performed well in estimating chronic toxicity, suggesting that they are robust to underlying data uncertainties. However, such uncertainties should still be taken into account when applying the ACRs in regulatory or predictive contexts.

4. Conclusions

Acute-to-chronic extrapolation is a promising and practical approach towards the estimation of long-term toxicity from acute endpoints, without the requirement for additional animal testing. In this study, we established surfactant-specific ACRs for regulatory relevant taxonomic groups (i.e., algae, invertebrates, and fish), through a transparent and scientific process, incorporating data collection, curation, and analysis, aligned with regulatory guidance documents (as possible). The derived 90th percentile

ACR values (i.e., 9.4 for algae, 19.4 for daphnids, and 26.4 for fish) were shown to produce comparable estimates of chronic toxicity (i.e., not exceeding one order of magnitude higher than experimental values) from acute data across three trophic levels within an external test set. Therefore, they are recommended for further application as a line of evidence within regulatory and environmental risk assessment contexts.

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Figure 1. Workflow outlining the collection and curation of surfactant ecotoxicity data. Following the identification of relevant CAS numbers, corresponding outcomes were retrieved from Organisation for Economic Co-operation and Development QSAR Toolbox and Envirotox databases. Upon exclusion of unsuitable entries, remaining data were organised, in accordance with the criteria listed, by associated taxonomic group (i.e., fish, invertebrates, algae) and by experimental protocol (i.e., acute or chronic). Abbreviations: CAS: Chemical Abstracts Service; OECD: Organisation for Economic Co-operation and Development; QSAR: quantitative structure-activity relationship; EC10/EC20/EC50, 10%/20%/50% effect concentration; LC50, 50% lethal concentration; NOEC, no observed effect concentration.

Figure 2. Linear regression between acute and chronic toxicity of various kinds of surfactants to algae (a), daphnids (b), and fish (c). Dash lines represent 95% confidence interval of the linear regression. For algae, acute and chronic toxicity often arise as different statistical interpretations in the same test, rather than biological extrapolations across different exposure durations. The presented linear regressions are shown for descriptive purposes only and are not used for acute-to-chronic ratio (ACR) estimation. ACRs are calculated independently using Equation 1 from the distribution of natural-log-transformed ACRs.

Figure 3. Comparison of median acute-to-chronic ratios (ACRs) derived through this study, alongside existing median ACRs. "All chemicals" may include pesticides and/or metals, depending on the study (Ahlers et al., 2006; Brill et al., 2021; Kienzler et al., 2017b; May et al., 2016; Raimondo et al., 2007). "Organics" represent chemicals without active pesticides, organometallics, and inorganics (ECETOC, 2003), whereas "non-polar narcotics" represent chemicals holding such a mode of action (Ahlers et al., 2006). "An- and non-ionic surfactants" are taken from a U.S. Environmental Protection Agency document (Mayo-Bean et al., 2012). Detailed comparisons of both median and 90th percentile ACRs are listed in online supplementary material, Table S4. Abbreviations: USEPA, U.S. Environmental Protection Agency.

Figure 4. Validation of derived acute-to-chronic ratios (ACRs), through comparison of measured and predicted chronic toxicity of various surfactant classes across three trophic levels. The predicted chronic toxicity = measured acute toxicity / 90th percentile ACR, considering all classes (9.4, 19.4, and 26.4 for algae, daphnids, and fish, respectively). Both measured acute and chronic toxicity data were taken from the Human and Environmental Risk Assessment Project (Human and Environmental Risk Assessment, 2025). The solid line is the 1:1 line, and the dashed represents a deviation of 1 log unit.

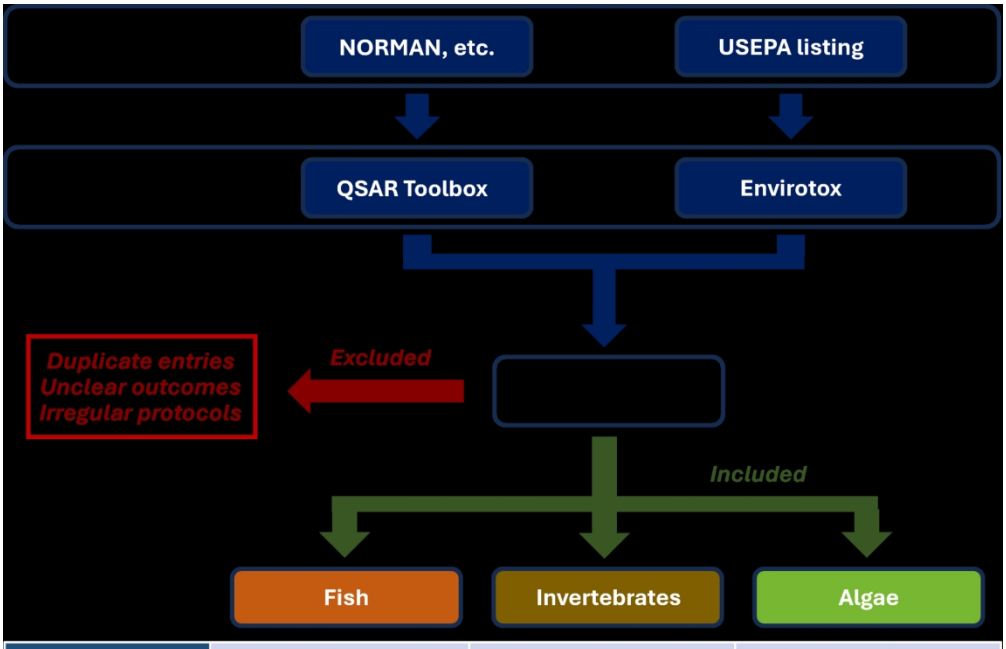
Table 1. Distribution of surfactant ecotoxicological data employed for the purposes of ACR generation, in accordance with trophic level and structural class.

			Fish	Invertebrate	Algae	Total
n. Data entries	Total		505	263	131	899
	Acute		446	223	63	732
	Chronic		59	40	68	167
n. Surfactants [a]	By sub-class	Total				115
		Cationic				27
		Anionic				39
		Non-ionic				39
		Zwitterionic				10
	With acute toxicity data	Total	81	62	38	
		Cationic	25	10	7	
		Anionic	25	21	15	
		Non-ionic	24	22	12	
		Zwitterionic	7	9	4	
	With chronic toxicity data	Total	22	28	34	
		Cationic	6	3	3	
		Anionic	8	11	15	
		Non-ionic	6	9	12	
		Zwitterionic	2	5	4	
	Acute-chronic data pairings [b]	Total	19	18	31	
		Cationic	6	2	3	
		Anionic	6	7	13	
		Non-ionic	5	5	11	
		Zwitterionic	2	4	4	

[a] As defined by shared CAS identifier
[b] CAS identifiers for which both acute and chronic data are present

Table 2. Derived acute-to-chronic ratios (ACRs) covering all surfactant classes across trophic levels

Trophic level	ACR values				
	Minimum	Median	90th percentile	95th percentile	Maximum
Algae	1.7	3.8	9.4	20.0	32.4
Daphnids	1.7	7.1	19.4	28.5	78.7
Fish	1.6	4.5	26.4	30.6	50.7



Test species		All teleost (e.g., *P. promelas*)	*Daphnia magna*, *Ceriodaphnia dubia*	Common test species (e.g., *D. subspicatus*)
Acute	Duration	96 hr	48 hr	72 or 96 hr
	Effect	Lethality	Lethality, immobility, intoxication	Growth rate (preferred), biomass, cell count
	Endpoint	LC50, EC50	LC50, EC50	EC50
Chronic	Duration	≥ 21 day	*D. magna*: ≥ 21 day *C. dubia*: 7 day	72 or 96 hr
	Effect	Growth, lethality, other (e.g., histology)	Reproduction (preferred), lethality	Growth rate (preferred), biomass, cell count
	Endpoint	EC10 > EC20 > NOEC (order of preference)	EC10 > EC20 > NOEC (order of preference)	EC10 > EC20 > NOEC (order of preference)

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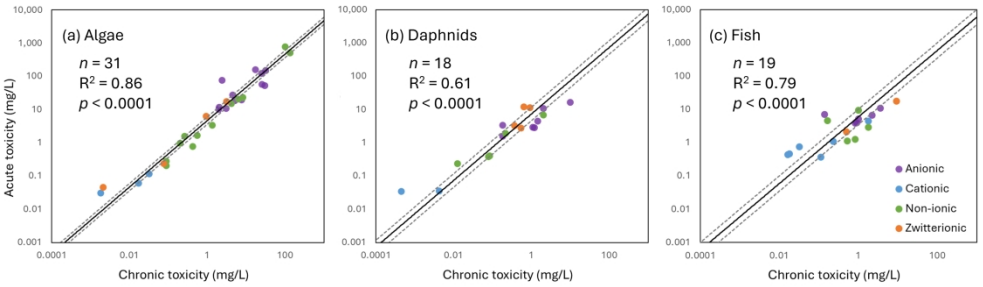

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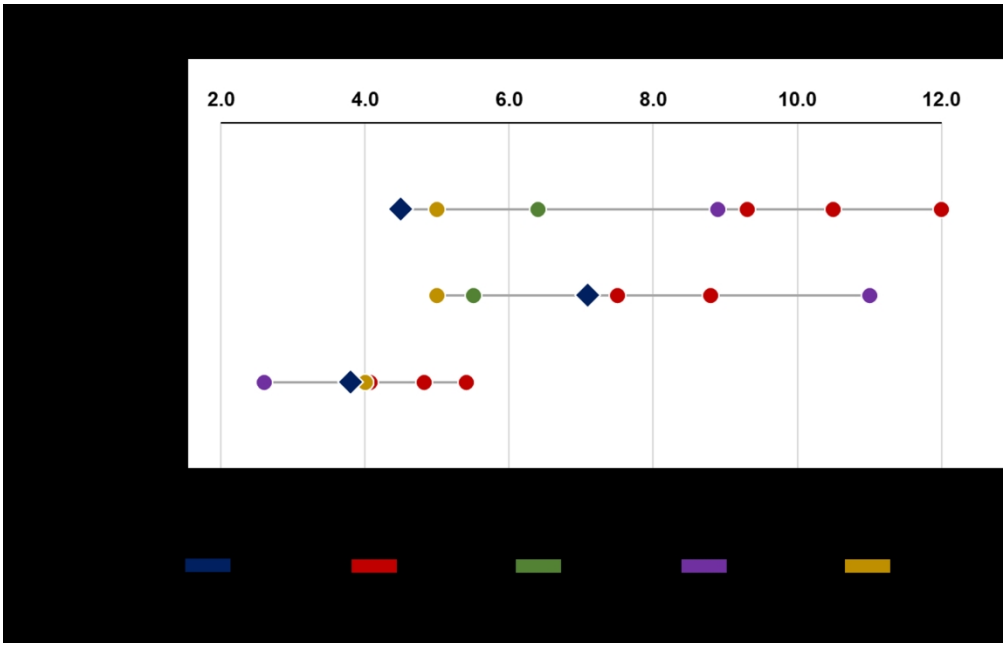

Figure 3. Comparison of median acute-to-chronic ratios (ACRs) derived through this study, alongside existing median ACRs. "All chemicals" may include pesticides and/or metals, depending on the study (Ahlers et al., 2006; Brill et al., 2021; Kienzler et al., 2017b; May et al., 2016; Raimondo et al., 2007). "Organics" represent chemicals without active pesticides, organometallics, and inorganics (ECETOC, 2003), whereas "non-polar narcotics" represent chemicals holding such a mode of action (Ahlers et al., 2006). "An- and non-ionic surfactants" are taken from a USEPA document (Mayo-Bean et al., 2012). Detailed comparisons of both median and 90th percentile ACRs are listed in Table S4 (see online supplementary material). Abbreviations: USEPA, U.S. Environmental Protection Agency.

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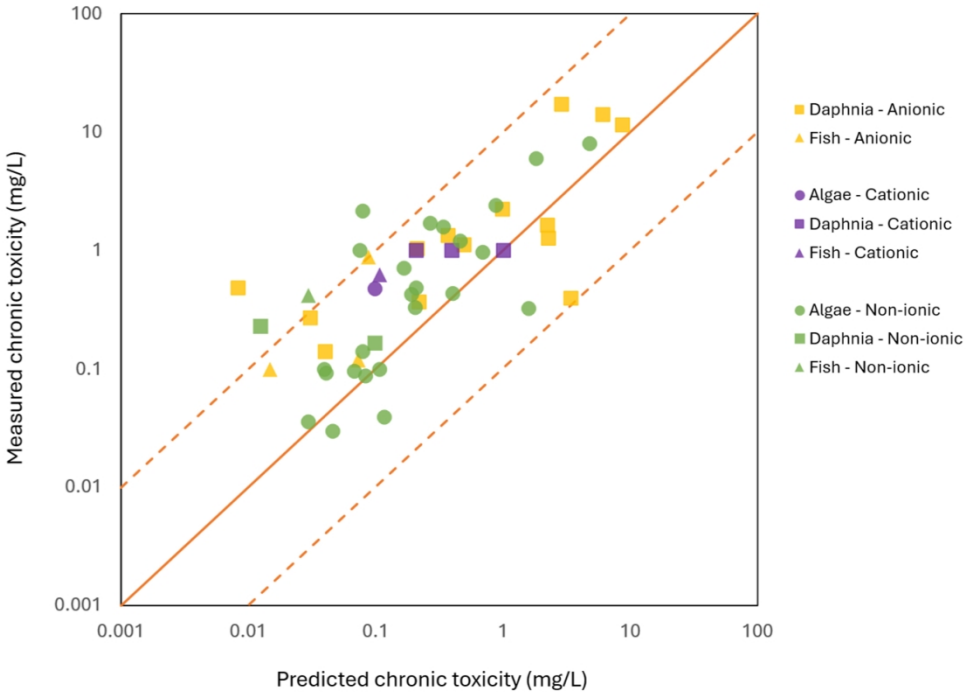

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230x170mm (150 x 150 DPI)