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# Comparisons of PNEC derivation logic flows under example regulatory schemes and implications for ecoTTC

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## Abstract

Derivation of Predicted No Effect Concentrations (PNECs) for aquatic systems is the primary deterministic form of hazard extrapolation used in environmental risk assessment. Depending on the data availability, different regulatory jurisdictions apply application factors (AFs) to the most sensitive measured endpoint to derive the PNEC for a chemical. To assess differences in estimated PNEC values, two PNEC determination methodologies were applied to a curated public database using the EnviroTox Platform (www.EnviroToxdatabase.org). PNECs were derived for



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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yrtph.2021.104933.

3647 compounds using derivation procedures based on example US EPA and a modified European Union chemical registration procedure to allow for comparisons. Ranked probability distributions of PNEC values were developed and 5th percentile values were calculated for the entire dataset and scenarios where full acute or full chronic data sets were available. The lowest PNEC values indicated categorization based on chemical attributes and modes of action would lead to improved extrapolations. Full acute or chronic datasets gave measurably higher 5th percentile PNEC values. Algae were under-represented in available ecotoxicity data but drove PNECs disproportionately. Including algal inhibition studies will be important in understanding chemical hazards. The PNEC derivation logic flows are embedded in the EnviroTox Platform providing transparent and consistent PNEC derivations and PNEC distribution calculations.

**Keywords**

Threshold of toxicologic concern; Predicted No Effect concentration; Aquatic; Statistic; Hazard

## 1. Introduction

The Predicted-No-Effect-Concentration, or PNEC, is the most frequently used endpoint applied to summarize the overall hazard assessment in environmental risk assessment regardless of the environmental compartment being addressed. In regulatory ecotoxicology, environmental effect results from QSARs (quantitative structure activity relationships), acute and chronic ecotoxicity studies, and semi-field (microcosm, mesocosm) or field data are divided by a nominative safety, assessment or application factor (AF) to derive a PNEC for risk assessment. It is well recognized that AFs vary not only as a function of uncertainty in data extrapolation, but also due to the regional scope, regulatory program, and purpose of the assessment. Thus, PNECs can vary widely across regulatory boundaries even when considering the exact same dataset (Hahn et al., 2014). Ecotoxicology experts from the US, Canada, Japan, Australia and the EU were asked to generate PNEC derivations for five different chemicals as reported in Hahn et al. (2014). The experts used their own regional assessment processes and data qualification systems, and the derived PNECs (one per compound per assessor) spanned 3 orders of magnitude with coefficients of variation as high as 186%. These differences were not a function of the chemistry assessed, but the PNEC derivation logic employed and the choice of data used to derive the PNEC. This second aspect is reflected in the individual assessor's expert judgment applied to the various studies. Weber and Hemmelskamp (2005) further identify that differences in PNEC conclusions may also be a result of the heritage of individual chemical management programs, socio-economic considerations, and environmental policy goals. Flexibility within a chemical management framework to accommodate different amounts and quality of data are important as some compounds are intrinsically more hazardous, may have multiple uses, and differ in their usage volumes. Therefore, data requirements to meet risk assessment and management objectives should also be flexible. Further, regulations within a jurisdictional region may result in different data needs and AFs applied at the various stages of a hazard assessment. For example, the aquatic hazard characterization process under the US Federal Insecticide, Fungicide, and Rodenticide Act (US EPA 1996, 40 CFR Part 152 amending the Act of 1947 7 U.S.C. §136 et seq. 1996) differs from the processes used under the Lautenberg

Chemical Safety Act (LCSA) (US EPA 2016) that amended the Toxic Substances Control Act (TSCA) of 1976) in that the extrapolations for pesticides for environmental protection differ markedly from those of general chemicals. Pesticides generally require more data and rely heavily on chronic exposure information (although not exclusively). The contexts for exposures to these different chemical groups also differ greatly and can result in different data needs or requirements. QSARs for example are used extensively as primary data for many general chemicals.

Evaluations of individual ecotoxicity data used to derive PNECs is an important aspect of the effects assessment process. This is often broken down into evaluation of study validity, reliability and relevance. Study validity is defined in test guidelines for standard species and can be more subjective for non-standard test species. Uneven application of weight of evidence in hazard assessment by risk assessors is common, frequently as a result of individual familiarity with test methods, experience, and the regulatory structure in which the weight of evidence is applied (Hall et al., 2017). Reliability of ecotoxicological tests goes beyond test validity as discussed in detail by Moermond et al. (2017). In addition to reliability, study inclusion in an environmental risk assessment that is developed from a solid problem formulation step will require an understanding of relevance as well (Rudén et al., 2017). Reliable, valid, and relevant ecotoxicological data are candidates for assessing environmental risk of a particular chemical exposure in regulatory contexts.

PNEC derivation is particularly difficult and varied at the earliest tiers of hazard assessment, including screening and prioritization. Some frameworks incorporate quantitative structure activity relationships (QSARs) for ecotoxicity, as is the case in the US under TSCA (Zeeman et al., 1995) or LCSA whereas in Europe, QSARs are generally only used as a means to support read-across interpolations and are not used as primary ecotoxicity data inputs (ECHA 2017a). In most instances, acute toxicity studies are required for a representative alga, invertebrate (typically *Daphnia magna*), and fish. Internationally accepted taxa for these studies are identified in OECD (OECD 1992a; 1992b, 2004, 2006, 2012; 2013a; 2013b) or federal/national test guidelines (e.g., USEPA 850 series, https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines; ECHA 2008). AFs applied to acute toxicity data, can range from 100 to 1000 with some variants in between, depending on the number of trophic level taxa for which information is available (Zeeman et al., 2005; ECHA 2017a; Japan METI, 2016 [http://www.meti.go.jp/policy/chemical_management/english/cscl/about.html, Japan Chemical Substance Control Law description, accessed March 28, 2018]) (Table 1). AFs for extrapolating chronic toxicity data are also somewhat variable until a full data set (all three trophic levels and taxa tested) is achieved and in this circumstance, AFs are universally 10. Beyond “full” data sets, AFs for higher tiered data also vary considerably, mostly due to the level of expertise that is required and the greater variety of information that must be present to derive scientifically robust PNECs (Belanger et al., 2017; Belanger and Carr 2019). Examples of these larger types of higher tier data sets include those suitable for Species Sensitivity Distribution (SSD) analysis and semi-field (microcosm, mesocosm) studies where AFs range from 1 to 10 depending on the jurisdiction and data quality. The span of AF choices available to regulatory toxicologists is largely encompassed in Table 1 and variations on these themes exist for Australia, China, New Zealand, and South

Africa. It is important to note that very similar approaches are used in the derivation of water quality criteria and standards with some nuances across geographies; however, the principles are the same. The greater the breadth of data, the greater the emphasis on long-term ecotoxicological effects, and the lower the AF applied.

Another hazard assessment tool is the ecological Threshold of Toxicologic Concern (ecoTTC) (Belanger et al., 2015). EcoTTCs are derived based on the distribution of a large array of PNECs within a chemical category or mode of action (MOA) and provide a conservative hazard prediction for similar, but untested chemicals. The TTC concept is used to establish exposure levels for chemicals below which no appreciable risk to human health or the environment is expected, based on a *de minimis* value for toxicity identified for many chemicals. In the human health context, this *de minimis* value is set at the 5th percentile value DNEL (Derived No Effect Level) from a statistical distribution of similarly acting chemicals (Kroes et al., 2004). For ecoTTCs, the 5th percentile is derived from a statistical distribution of PNECs of similarly acting chemicals (HESI 2018; 2020). Of particular importance then is the categorization of “similar acting” compounds (Kienzler et al. 2017, 2019) as well as the derivation of the PNEC value. From above, it is clear then that ecoTTCs based on PNECs would be regionally based due to the differences in PNEC derivation because AFs applied to toxicity data are not globally aligned (Belanger and Carr 2019). Further, it is readily apparent that such an approach would have tremendous value for evaluations of innumerable compounds in commerce that lack data entirely (other than structure), are used at low volumes, and/or for which other assessment options are not possible such as groups lacking QSARs (e.g., certain UVCBs such as polymers, surfactants, dyes and pigments).

In the present paper we develop an explicit logic flow to derive PNECs based on two representative example chemical regulatory programs in the US and European Union (EU). Due to complexities associated with a wide variety of combinations of acute and chronic data, taxonomic coverage, and AFs, the logic flows are more complex than the simple summation presented in Table 1. We use example datasets to evaluate the logic flow of EU and US-based PNEC derivations and provide a preliminary assessment for the development of ecoTTCs based on the entirety of the EnviroTox data set (Connors et al., 2019; Kienzler et al. 2017, 2019). Using complete acute and chronic data sets we identify some trends as a function of PNEC drivers based on taxonomy and regulatory program being addressed.

## 2. Materials and methods

### 2.1. Approach overview

First, publications of regulatory programs were reviewed for their general principles regarding establishing PNECs for use in aquatic risk assessment. The approach used consisted of several distinct parts presented in sequence below and in Fig. 1:

1. PNEC logic flows based on general principles from historical US TSCA (Toxic Substances Control Act) and EU regulatory programs were devised and programed in R for integration into the EnviroTox platform.

2. The PNEC logic flows were assessed using model acute and chronic toxicity datasets which could be mathematically verified by hand and processed using the PNEC logic flow. This step was used to ensure that the logic flow provided predictions as expected.
3. The EnviroTox database was probed to establish input values using full acute and full chronic data sets to identify general trends associated with AFs described in the PNEC logic flow.
4. Various facets of the toxicity and PNEC data set were evaluated to ascertain possible drivers of PNECs and the corresponding 5th percentile PNEC values. These included assessment of the drivers of PNECs based on taxonomy of the included species and the influence of the most conservative (lowest) PNECs on the 5th percentile predictions for the entire PNEC distributions.
5. Future needs and relevant refinements to establish ecoTTCs based on ranked PNECs were identified.

### 2.2. PNEC logic flows

PNEC logic flows were derived from guidance documents and tables of AFs based on regulatory programs in the U.S. and EU. Nabholz et al. (1993) and Zeeman et al. (1995) provided summaries of the procedures historically used in the assessment of chemicals under the US Toxic Substances Control Act (TSCA) Pre-Manufacturing Notification (PMN) program, which applies to new chemicals. The US EPA has since modified the AF approach utilized by the Agency (US EPA, 2013). ECHA (2016) provides a similar view of chemical evaluation under the European REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) Regulation (EC, 2007). Diagrams of decision-making logic for each region are given in Figs. 2 and 3. Various combinations of acute and chronic data are explicitly handled. In instances where the source article used to guide the logic flow did not provide guidance, the placement of the AF decision is made explicit in the diagram in order to accommodate foreseeable data combinations. For example, most regulatory schemes assume the generation of a full set of acute data would precede the development of chronic data, but on occasion this is not the case; therefore, allowances for this type of data set were made to broaden the coverage.

The PNEC derivation processes were arranged into logic flows representing these general principles such that comparisons could be made across programs using the same input data. It is recognized that very specific toxicity summarization choices are often made within the regulatory programs. For example, US EPA often uses the Chronic Value, or the geometric mean of the NOEC and LOEC, to summarize toxicity data but such an approach is not viable when dealing with ECx determinations as specified in OECD Test Guidelines as the preferred toxicity metric. Thus, the choice was made to use NOEC and a range of small.

ECx values where x is small (<20) to represent chronic responses across all PNEC logic flows and treat these as equivalent. Note that in this sense, the PNEC logic flows are meant to characterize general trends for subsequent use in determinant the 5th percentile of ranked PNEC values and not necessarily a specific PNEC for a specific chemical assessment. PNEC

logic flows were then coded into R and programmed in the EnviroTox database program to link toxicity data to predictions.

### 2.3. Database and sources of information

Prior to PNEC derivation, additional aspects were addressed starting at the database construction level discussed in detail by Connors et al. (2019). A master dataset of ecotoxicological data and chemical property information was constructed using pre-determined inclusion criteria following the SIFT methodology of Beasley et al. (2015) and is publicly available at www.EnviroToxdatabase.org (HESI, 2018; Connors et al., 2019). Aquatic toxicity endpoints and associated metadata were selected from sources including peer-reviewed literature, the European Chemicals Agency REACH database (ECHA 2017b), the U.S. EPA ECOTOX database (USEPA, 2017), the Fish Embryo Test (FET) validation dataset (Belanger et al., 2013; Busquet et al., 2014), the USGS Columbia database (Mayer and Ellersieck 1986), the AiiDA database (AiiDA , 2017), the ECETOC Aquatic Hazard Assessment database (ECETOC, 2003), Japan's NITE-CHRIP database (Japan NITE, 2017), a proprietary dataset of pharmaceutical chemicals (Vestel et al., 2016), and selected, non-duplicative records from the ECOSAR Training data set (Mayo-Bean et al., 2011), USEPA Pesticide data from www.epa.gov/pesticides, and OECD QSAR Toolbox records from www.qsartoolbox.org. The master dataset included approximately 200,000 data records at Step 1 and a final dataset of 65,199 records (individual toxicity test data entries) on 3819 compounds by Step 4 following sifting. Updates to the database occur approximately annually and data is curated. EnviroTox database search engine version 1.3 and PNEC Derivation version 1.7.1 were employed for this paper (accessed on May 17, 2020). The EnviroTox Platform is fully described with an associated search engine, and calculation tools to determine ecological Thresholds of Toxicologic Concern (ecoTTC, as defined in Belanger et al., 2015) and Chemical Toxicity Distributions (CTD, as defined by Williams et al., 2011). Chemical name, 2-D structure using SMILES notation, key physical-chemical parameters (log Kow, solubility, molecular weight, Henry's Law constant) were aligned for each chemical entity (Connors et al., 2109). Kienzler et al. (2017, 2019) associated several MOA assignments using various assignment tools for each compound in the final data set to allow chemical grouping. Individual toxicity tests for such a large database span an incredibly diverse range of test designs, test species, endpoints, and durations. In order to enter information into a decision/logic diagram where "acute" and "chronic" toxicity is transparent, a determination was made for each test with the goal of assigning each test to a single acute or chronic designation. The process emphasized the use of endpoints which were of known regulatory application and significance (e.g., growth, survival, reproduction). Acute or chronic is also identified by the duration and the test statistic for each general group of organisms (microbes, invertebrates, fish, macrophytes, amphibians, etc.). The categorization diagrams are shown in Supplemental Information 1. It is recognized that these will vary across regulatory programs. Specific examples that represent how such a process was implemented are given here:

1. The No-Observed-Effect-Concentration (NOEC) for a 96-h acute fish study (OECD, 1992), which is a required endpoint for the test guideline, was not used as either the acute statistic (generally a 96-h LC50) or a chronic endpoint

(generally a 30-d NOEC or 30-d EC10 for small fish species). This endpoint was therefore not utilized.

2. The reproduction EC50 for a 21-d chronic *Daphnia magna* reproduction and survival study, also required for reporting by the test guideline (OECD, 2012), is not used by regulators as a chronic toxicity input value for hazard assessment. This type of endpoint was therefore not utilized.

3. Toxicity tests conducted on non-standard organisms that were sufficiently long to be considered in a chronic time frame *sensu* Sprague (1973) where the exposure may be "a long time; often signifying one-tenth of the life span" were considered. For example, a 30-d unionid mussel study (e.g., *Elliptio complanata*), where growth was measured as the effect and NOEC was the statistic was included and designated as "chronic" even though this is a non-standard species.

### 2.4. PNEC derivation, probability distributions for acute and chronic data sets

Each chemical was processed through a program written in R, embedded into the functionality of EnviroTox, that follows the visual logic shown in Fig. 2. As described by Connors et al. (2019), chemical information, MOA determination, taxonomic information, and ecotoxicological data were merged using an on-line customized SQL web platform to merge information and devise focused queries, followed by PNEC derivation in R, and outputs in EXCEL and pdf (graphical) formats. PNECs were identified using numerical codes associated with the various combinations of acute and or chronic data (Table 2, Fig. 3). Granularity in PNEC combinations is important for subsequent ecoTTC assessment within EnviroTox since they can be used to elucidate the data types that drive statistical distributions, especially at the tails.

To test the PNEC logic, subsets of data were manipulated to transparently provide easy hand checks for geometric mean calculations per taxon and study duration. The entire data set was evaluated to provide a glimpse into the distribution of PNEC types (codes per Table 2) that are represented in the database regardless of MOA or chemical functional grouping. Example outputs and plots for three representative chemical groupings were calculated to provide insight into what will be possible with future ecoTTC determinations. These are not meant to provide proposed ecoTTCs *per se* but rather as examples of what is possible using the calculation tool and database of EnviroTox. Distributions of PNEC types (Table 2) for the US (PNEC 4 through 10) and Europe (PNEC13 through 19) were determined using the entire database to evaluate the most and least common toxicity test combinations and the resulting outcomes of the respective PNEC determinations by geographic region (Fig. 3). Evaluations of the most common taxon driving PNEC determinations were developed because of the utility for informing trends of most sensitive trophic levels and for future data gap-filling opportunities. Overall PNEC distributions in ranked order were developed using ecoTTC calculation tools to get a sense of the span of available PNECs regardless of data types for each geography. The distribution of PNECs is reflected in a series of centile values representing the 1st, 5th, 10th and 50th percentile and were determined for both US and European scenarios. PNEC distributions at the 5th percentile (PNEC0.05) are considered the ecoTTC values and indicative of a conservative hazard value for a chemical category or

MOA when data are lacking (Belanger et al., 2015; Connors et al., 2019; Kienzler et al., 2020). In addition to the "all data in" PNEC distributions, a second set was constructed only considering full acute and chronic data sets (all three taxa present – algae, invertebrates, and fish). As described in Connors et al. (2019), PNEC distributions are summarized following fitting to either a log-normal or log-logistic distribution programmed in R (Becker et al., 1988). The underlying distributions were verified via Anderson-Darling Goodness of Fit tests. In cases where either model provided adequate fit, the one with the more conservative 5th percentile was chosen for comparative purposes. Confidence intervals for the PNEC0.05 are also calculated. The ecoTTC calculation utilizes an estimation methodology similar to the Species Sensitivity Distribution Analysis (Belanger and Carr 2019; Carr and Belanger 2019, note that stand-alone R script for the distribution analyses is available as Supplemental Information for this pair of related papers).

As part of the output from the PNEC calculator in EnviroTox, the taxonomic group that drives an individual compound's PNEC is also identified (algae, invertebrates or fish). Summaries of the driving taxonomic group for full acute and full chronic data sets were enumerated to identify groups which drive PNECs in general. These were supplemented by the assessment of all data present in EnviroTox as an indication of the frequency of each test type in general which directly influenced the likelihood that a given group will be gauged as more or less sensitive than others.

Lastly, the ranked PNEC values (lowest to highest) are programmed to be part of the output from the PNEC calculator in the EnviroTox platform. These were inspected to evaluate the influence of the lowest PNECs on the eventual 5th percentile value of all PNECs analyzed. MOA and the functional use of the chemical (e.g., pesticide, industrial chemical, biocide, pharmaceutical, etc.) were identified and qualitative trend and pattern searches were conducted. For example, inspection as to whether a particular MOA was identified in the extremes of the distribution could be highly informative. Fifth percentile PNECs were recalculated with selective deletion of compounds to ascertain how large an influence these have on the overall result. Summaries of various scenarios were devised in tabular and graphical form. In some instances, statistical probing of comparisons was conducted beyond the calculation of 5th percentiles of PNEC distributions. Pairwise t-tests were utilized to identify most sensitive trophic level (algae, invertebrate, fish) for acute and chronic data using chemicals that were tested in common. Significance was inferred at $\alpha = 0.05$. All statistical tests were performed in R (Becker et al., 1988).

## 3. Results and discussion

### 3.1. Verification of PNEC logic

Using the search features of the EnviroTox Platform, a sub-set of the database was generated by identifying chemicals that had algal, invertebrate, or fish data available. Chemicals that have a metal component or were metal only cations were removed for consideration as CAS descriptions and MOA assignments are particularly problematic (including SMILES notations for their salts; these are termed "dummy metal ion" CAS as described in Connors et al., 2019). The final dataset used in this analysis contained 3818 chemicals comprising 65,199 toxicity records (accessed on May 17, 2020). Of these records, 55,863 were

classified as acute and 9336 as chronic tests, respectively. The dominant PNEC types were those with one or two available trophic levels (n = 2282 CASNO). A total of 1555 chemicals had complete (all three main trophic levels) acute or chronic data sets with 320 of these being full chronic data sets with all three trophic levels.

To test the underlying logic of the PNEC derivation program, approximately 50 combinations of acute and chronic data and trophic level compositions were used to derive PNECs in each scenario. The full span of PNEC types were probed (PNEC4 to PNEC10 for the US and PNEC13 to PNEC 19 for Europe). Representative example data sets are given in Table 3. In Scenario 1, acute toxicity data is available for all three trophic levels and algae, the most sensitive taxonomic group also has chronic data available. Therefore, under a representative example of a US regulatory approach, an AF of 10 was assigned to the most sensitive acute value that has chronic data as well and the PNEC is 80 μg/L (PNEC8). In a representative EU regulatory approach, the algal chronic information is not considered when it is the sole chronic data point and an AF of 1000 is assigned to the most sensitive acute value, which also happens to be algae. Thus, the PNEC is 1 μg/L (PNEC16). In Scenario 2, acute toxicity data were again available for all three taxa, but the most sensitive acute taxon *Daphnia* also had available chronic data. The US and EU-based PNECs were 44.7 and 4.47 μg/L respectively (PNEC types of PNEC8 and PNEC17 for US and EU, respectively). In Scenario 3, algae were the most sensitive species with acute toxicity data, but a full set of chronic data showed that fish were the most sensitive taxon. Complete datasets satisfying requirements for both US and EU-based approaches (PNEC10 and PNEC19) resulted in the same overall PNEC of 6.12 μg/L (AF of 10 applied to the geometric mean of the two available fish tests).

PNECs derived for the entirety of the EnviroTox database and the prevalence of different PNEC types based on trophic level combinations, sensitivity, and availability of acute and/or chronic data are summarized in Fig. 4. The most frequent PNEC grouping was for 1 trophic level being assessed (PNEC4-US and PNEC-13 EU). This group also had the largest AF and thus dominated the most sensitive end of the overall PNEC distribution.

Acute toxicity data were approximately six times more prevalent than chronic data in the Envirotox database, with fish the most prevalent taxa (34,299 records; Connors et al., 2019, Table 4). Invertebrates were also well represented (16,339 records), whereas algae were generally poorly represented (5225 records). This observation on the available richness of acute data by trophic group is consistent with other publicly available data sources, with limited differences across trophic groups when considering chronic toxicity data (Table 4). Interestingly, the numbers of individual tests per compound tested was low for algae confirming a trend within the EnviroTox database that algae were not only less frequently assessed, but that they are also less frequently re-assessed (many studies per taxon or substance). Fig. 5 provides an overview of the different available data combinations for unique chemicals categorized by test type (acute versus chronic) and trophic level (algae, invertebrates and fish). Information available for chronic toxicity was well-balanced across taxa, also indicating that all taxa are often assessed for compounds where knowledge of environmental hazard might be of an overall higher priority. Thus, PNECs derived from this smaller group of substances are expected to be likely be more robust overall and more

cohesive for interpretation across the substances that are assessed, however considerations on applicability domain (e.g., chemical category, MOA) is likely still needed.

### 3.2. Overall drivers of PNEC values for compounds in EnviroTox

While testing prevalence gives a picture of what data are available, it is also very relevant to understand what trophic level or groups of taxa tend to drive PNEC conclusions (i.e., the most sensitive taxa used in PNEC derivation). For this part of the exercise, only complete data sets were used, defined as at least one algal study, one invertebrate study, and one fish study available for the chemical. The acute data sets included chemicals that had complete acute and chronic datasets; however, the chronic data was ignored for this exercise. In the EnviroTox PNEC derivation, multiple values per trophic level are computed as a geometric mean to evaluate the central tendency for that trophic group to drive substance-specific PNECs. Acute and chronic full data sets were evaluated separately. A total of 736 and 320 data sets were available that had all 3 trophic groups present for acute and chronic toxicity, respectively (Table 5). Algae, invertebrates and fish were most sensitive 48.6, 28.5, and 22.8% of the time, respectively. This is consistent with trends noted from other investigations that assessed inter-group sensitivity but conducted for other purposes (Hutchinson et al., 2003; Jeram et al., 2005; Rawlings et al., 2019). In these other investigations, fish were more sensitive only 15–20% of the time. Chronic toxicity data sets were more balanced with respect to taxa sensitivity with algae, invertebrates, and fish being most sensitive 24.4, 43.7 and 31.9%, respectively. These patterns should also be considered in light of the sheer availability of each data type. Algae comprised just 9.3% of the all acute studies whereas invertebrates and fish are represented in 29.2 and 61.4% of acute tests within the Envirotox Platform, respectively. Thus, from the perspective of this database, much more is known of fish and invertebrate sensitivity to chemicals than for algae. Algal testing in particular measures a chronic like effect (population growth rate) even when the test is interpreted as acute growth inhibition (interpreted in regulatory frameworks as acute toxicity, see OECD, 2006). The difference between “acute” and “chronic” inhibition for algae is based entirely on the statistical metric applied (EC50 versus EC10 or NOEC) to the exact same data and thus acute-chronic relationships are inherently different for algae than for invertebrates or fish. Algal acute-chronic ratios are rarely 10 and for a large range of substances is approximately 4 (Brill et al. in press; Mayo-Bean et al., 2011). Even though algae were the least represented trophic group in the database, they were the most frequent group driving PNECs when chronic data was not available (Table 5). Fish were the most tested and the least frequent driver of PNECs acutely and were less frequently a driver when chronic testing was considered relative to invertebrates.

An additional means to compare sensitivities was employed using all available data in EnviroTox without the constraint of focusing on “complete” data bases. Paired comparisons for acute and chronic toxicity were made for algae-invertebrates, algae-fish, and invertebrates-fish. Fig. 6 depicts these paired comparisons. Values less than 0 indicate that taxon is more sensitive. In ~60% of cases, algae are more sensitive than either invertebrates or fish on an acute basis. Invertebrates are somewhat more sensitive than fish acutely. In all instances (comparison of algae, invertebrates, and fish both acutely and chronically) indicate differences are significant ($p < 0.0001$ for all paired comparisons).

Algae are somewhat less sensitive than invertebrates and fish on a chronic basis. This interchanging of order of sensitivity is not unexpected as algae inhibition tests determine short-(acute) and long-term (chronic) effects using the same data. This results in an ACR (acute:chronic ratio) of approximately 4 on average mostly independent of MOA (Brill et al., 2021). On the other hand, invertebrate and fish acute and chronic tests often display ACRs in the range of 10 or more (Raimondo et al., 2007).

PNEC0.05 (5th percentile) distributions were computed for all data available using historic TSCA US and modified current European PNEC derivation scenarios. The US-based "all-in" PNEC0.05 was $2.73 \times 10^{-5}$ mg/L (95% lower-upper confidence limit of 2.25–3.31 $\times 10^{-5}$ mg/L) whereas the EU-based "all-in" PNEC0.05 was $3.45 \times 10^{-6}$ mg/L (95% lower-upper confidence limits of 2.83–4.17 $\times 10^{-6}$ mg/L, a difference of 7.9-fold (Fig 7). When the PNEC derivations were constrained to PNEC6 (acute, all trophic levels tested) sets the PNEC0.05 for US acute data was $7.14 \times 10^{-5}$ mg/L and for Europe was $7.14 \times 10^{-6}$ mg/L exactly as expected given AF differences. PNECs derived from full chronic data sets gave a PNEC0.05 values of $4.96 \times 10^{-5}$ mg/L (Fig. 7) and were the same for both US and European scenarios owing to the utilization of the same AF of 10. These outputs of PNEC0.05 likely have limited utility as they encompass only a subset of PNEC derivation scenarios but are useful to develop broad comparisons for the application of different PNEC assessment schemes and AFs. A more refined approach would be to utilize the input data sets based on MOAs or chemical classes. The ten smallest PNECs (all data considered) in these calculations are from organotin, pyrethroid compounds and di-benzofuran/dioxin compounds (e.g., tributyltin, alpha-cypermethrin, and 2,3,7,8 tetrachlorodibenzodioxin) most acting by specifically known MOA. In addition to being mostly specifically acting compounds (Kienzler et al. 2017, 2019), eight of the ten are derived from data sets with only one or two of the three trophic levels at the level of acute toxicity. Only one had any chronic data (ethynylestradiol) and is known already to be amongst the most potent of endocrine disruptors. An "all-in" PNEC distribution based on selective removal of the most sensitive lowest ten PNECs altered the PNEC0.05 to 3.00 $\times 10^{-5}$ mg/L for the US, an increase of 8.9%. The lowest PNEC for a non-specifically acting compound was for benzo-*a*--pyrene, a known carcinogen which had the 26th lowest PNEC in EnviroTox. If assessing a polar narcotic compound, for example, it would make sense to constrain to that MOA as can be done in the ecoTTC application of EnviroTox (Kienzler et al. 2017, 2019; Connors et al., 2019). Future evaluations of representative and vetted ecoTTC values by MOA and various categories of functional use (grouped as cationic polymers, neurotoxicants, etc.) will be developed and probed more deeply. Consideration will be given to intervals of hydrophobicity as well, for example, developing ecoTTC values for non-polar narcotics between log Kow of 2 and 4 that might provide additional coherence in the putative PNEC0.05 values. As has previously been discussed in Hahn et al. (2014), PNECs for the same compound can vary by orders of magnitude based on risk assessor/risk management decisions regarding quality of input data, interpretation of the jurisdiction's regulatory requirements, and scenario-based flexibility in assigning AFs. Clearly, the level of conservatism in PNEC derivation exists across region programs based on both science and policy – all PNECs may at times be equally viable because the true safe concentration in the environment cannot be known with certainty. PNEC0.05 trends based on all data for

the US-based approach were approximately 8 times higher than the EU-based approach, reflecting the order of magnitude differences in AFs for datasets without a complete set chronic values. It is also interesting to note that for US PNEC0.05 where full acute data sets were analyzed ($7.14 \times 10^{-5}$ mg/L) is not too different to the PNEC0.05 for full chronic data sets ($4.96 \times 10^{-5}$ mg/L) which may provide some support to the confidence applied for acute extrapolations in the absence of chronic information. The low PNEC0.05 values should not be overly concerning as refined assessments based on MOAs or category groupings within a narrower slice of physical-chemical properties will be more useful for regulatory and assessment prioritization purposes. Because the EnviroTox database makes use of all available algal, invertebrate and fish studies that were curated through the SIFT process (Beasley et al., 2015), additional refinements can also be evaluated. For example, PNEC inputs may be constrained to only taxa with an available standard test guideline. Novel test species may increase uncertainty and variability in trophic level computations, but are recognized as being important for addressing the broad range of sensitivity as well.

### 3.3. Future challenges and research

Historically, AF or uncertainty factors applied to toxicity data to estimate safe concentrations of chemicals for ecosystems was relatively crude. Simple factors of 10 were applied to the appropriate tiers of collected data. However, increased sophistication in understanding acute and chronic toxicity relationships and improved knowledge of MOA is impacting how regulatory authorities and ecotoxicologists apply AFs. Japan METI (2017) identifies the use of an AF = 20 for application to daphnid chronic toxicity data when the compound assessed is a quaternary ammonium cationic, known to impact this group more than others. Environment Canada's PNEC derivation approach is undergoing a significant refinement where PNEC derivation is by a novel assessment factor method as an alternative to traditional factors of 10 (personal communication, Alexander Okonski, ECCC). The proposed method breaks the factor down into three components - endpoint standardization, extrapolation for species variation, and MOA consideration (each of which has sub-components). US EPA (2013) has used refined ACRs based on trophic group and certain chemical classes and the ACRs are embedded in the PNEC derivation process. This diversity of approaches complicates chemical registration and evaluation practices globally as PNECs are regionally based, emphasize different aspects of MoA, and different forms of chemical classification. These will likely be addressed in future versions of EnviroTox, but will have limitations. EnviroTox utilizes a unified determination of MoA classification (Kienzler et al. 2017, 2019) and broad categorization of chemical classes is not without challenges, especially for simple mixtures of homologous compounds (such as alcohols).

The US and EU-based PNEC derivation processes presently programmed in EnviroTox are examples only and future additions (e.g., Japan, Canada) are being explored. Formalization of specific ecoTTC values and approaches are in development for various case studies involving input into mixture hazard assessment, water quality criteria, private sector product development and others. Additions to the EnviroTox database are planned, and on-going on an annual basis. The web application at www.EnviroToxdatabase.org is also allows privately held data to be appended to searches of the public database and as input into the PNEC calculation and extrapolation tools (HESI 2018; Connors et al., 2019). The PNEC

calculators themselves can be directly useful to consistently and repeatedly develop PNEC extrapolations for specific CASNO and data sets. The calculators should thus prove useful for transparent presentation of PNECs in hazard assessments.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The subject of the paper (non-chemical-specific, multi-sector and international) and co-authors came from a cross section of academic/industry/government (including regulatory) interests. This HESI scientific initiative is primarily supported by in-kind contributions (from public and private sector participants) of time, expertise, and experimental effort. These contributions are supplemented by direct funding (that largely supports program infrastructure and management) that was provided by HESI's corporate sponsors. A list of supporting organizations (public and private) is available at http://hesiglobal.org.

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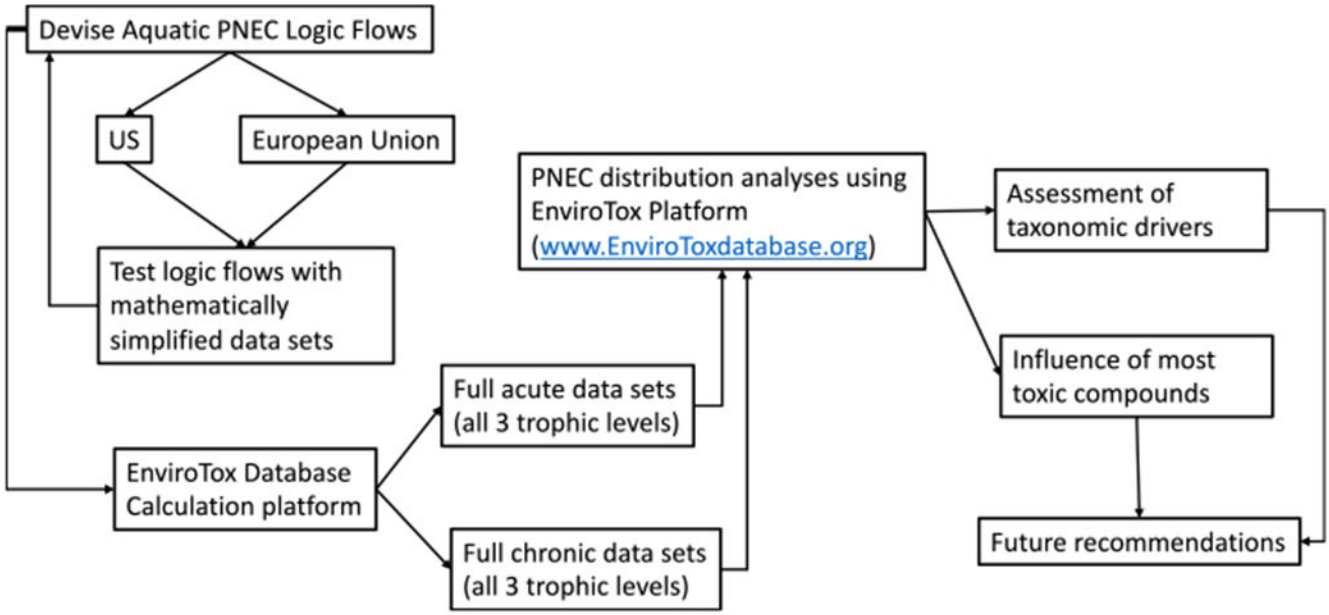


**Fig. 1.**

Process used to evaluate PNEC logic and outcomes for US and European Union-based assessments using the EnviroTox Platform (https://envirotoxdatabase.org/).

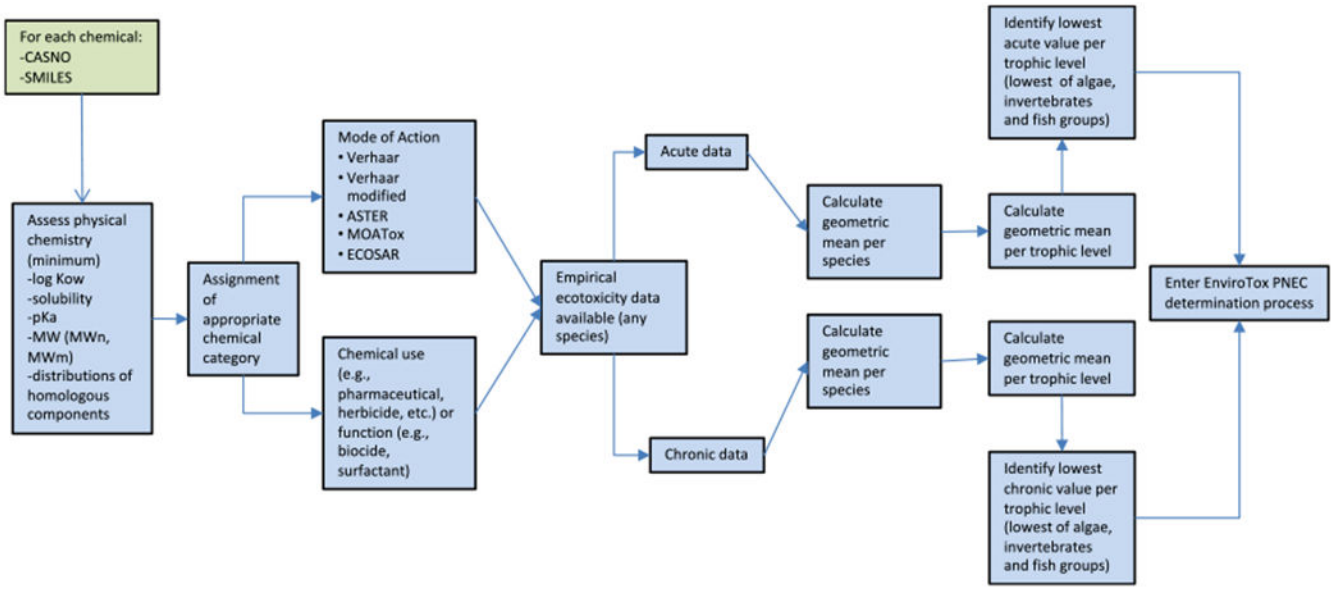


**Fig. 2.**

Pre-processing of raw ecotoxicity data prior to entry into PNEC derivation processes.

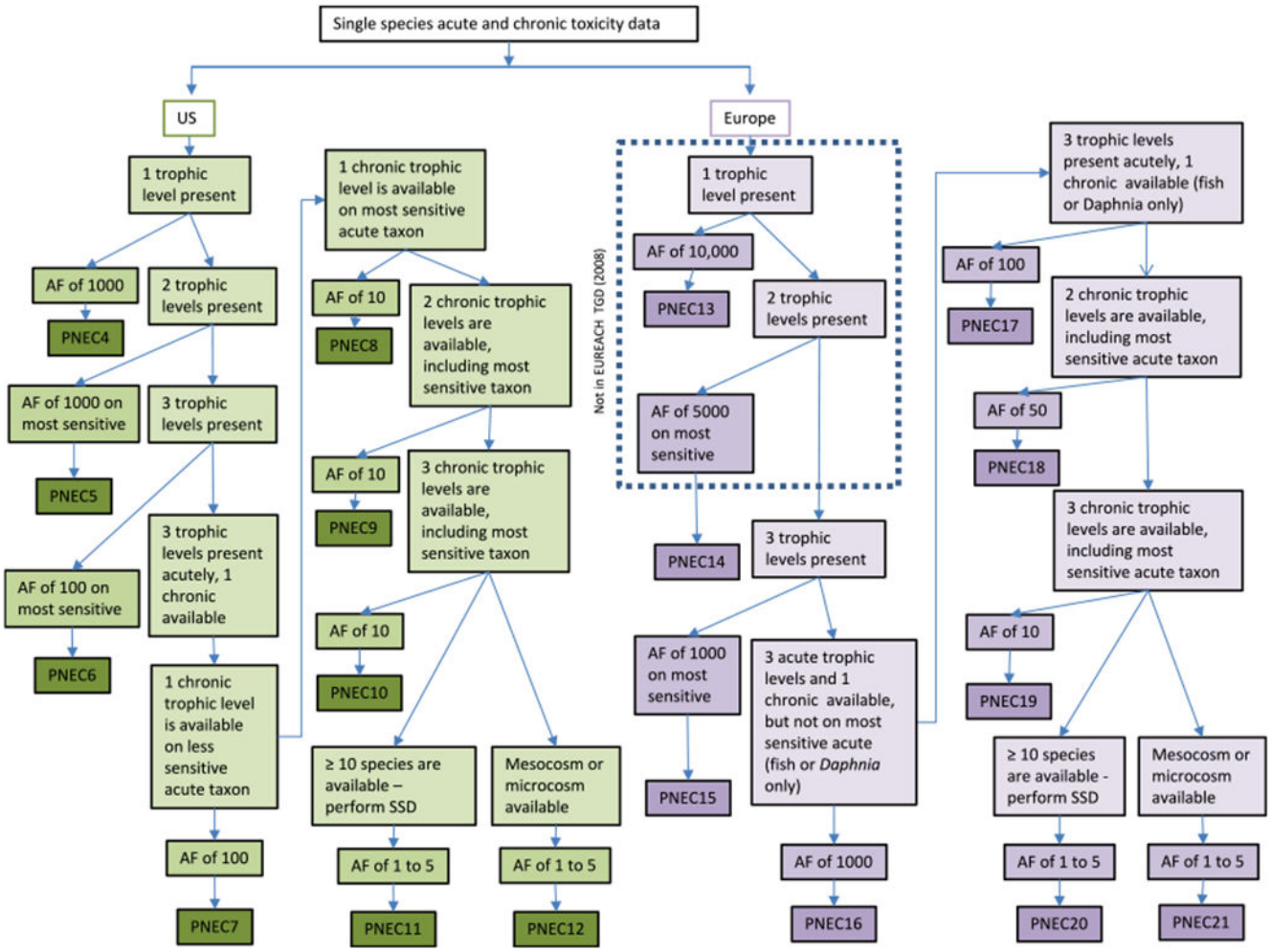


**Fig. 3.**
PNEC derivation processes for US and EU regions.

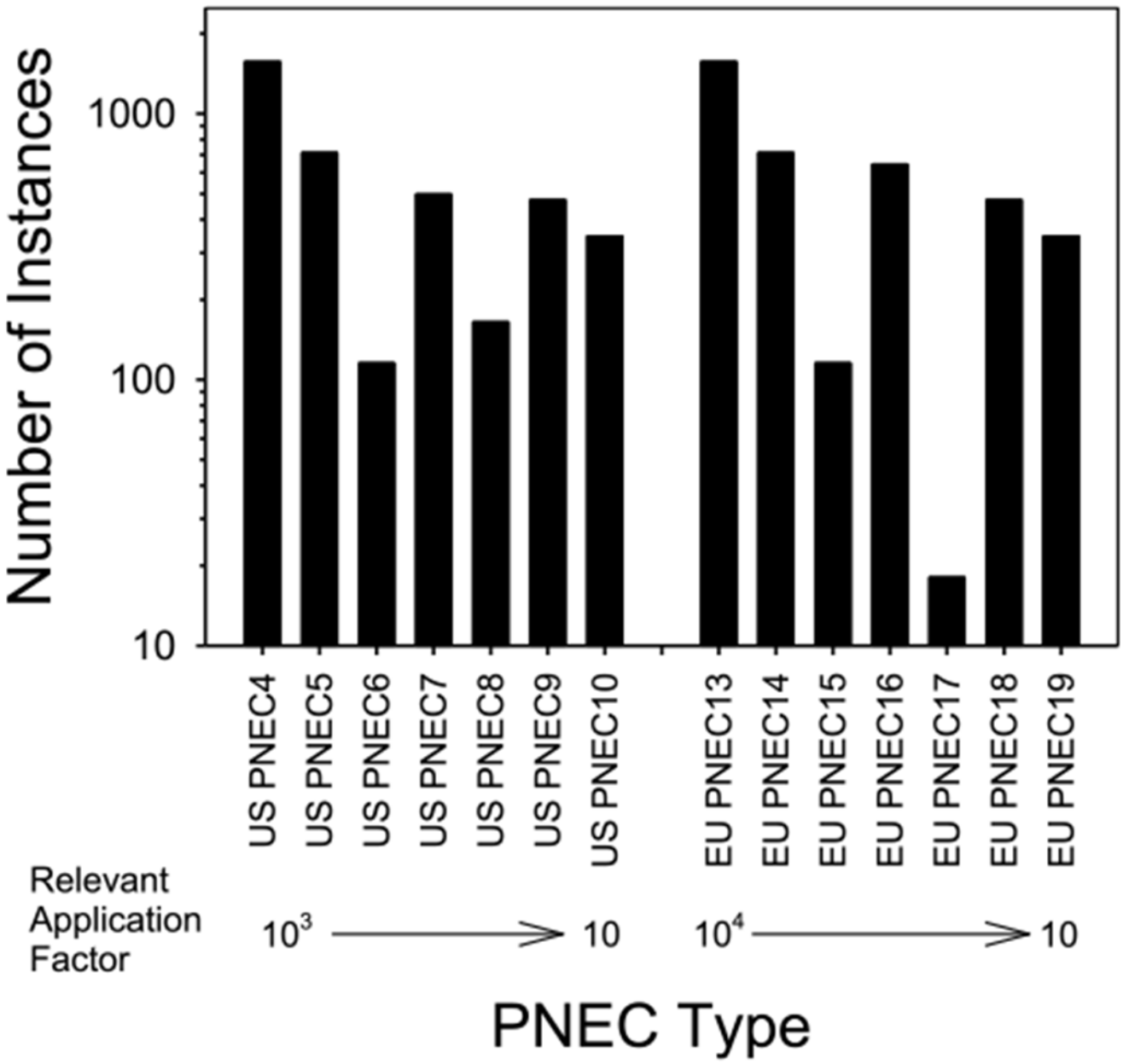


**Fig. 4.**

Distribution of PNEC types by region and data availability. See Table 2 for definitions of data combinations and a complete listing of Application Factors per PNEC type.

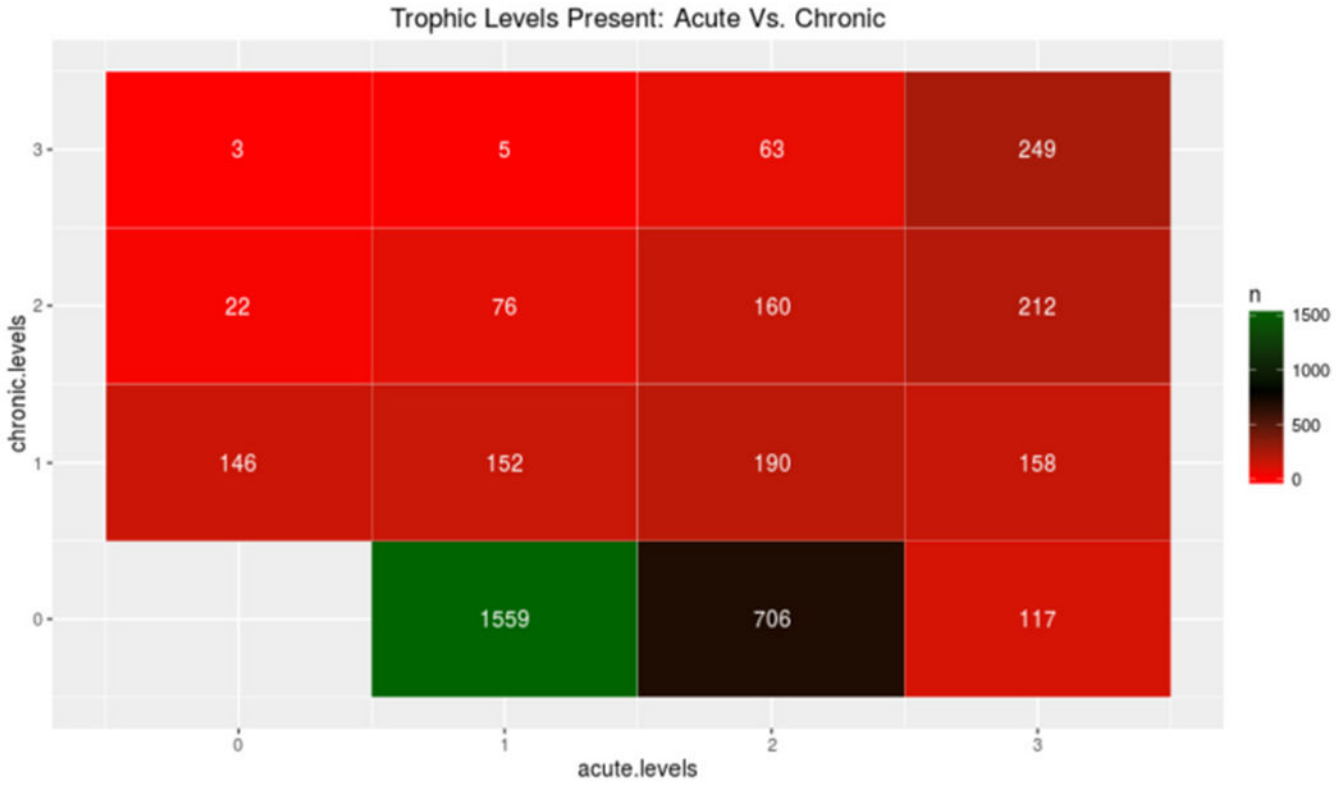


**Fig. 5.**

Distributions of all available acute and chronic data for US and European-based hazard assessment (PNEC determination for “all data in”) scenarios based on datasets comprised of algal, invertebrate and/or fish test species. Values in each cell indicate the number of CASNOs fulfilling a certain combination. Acute and chronic “levels” indicate numbers of trophic groups represented.

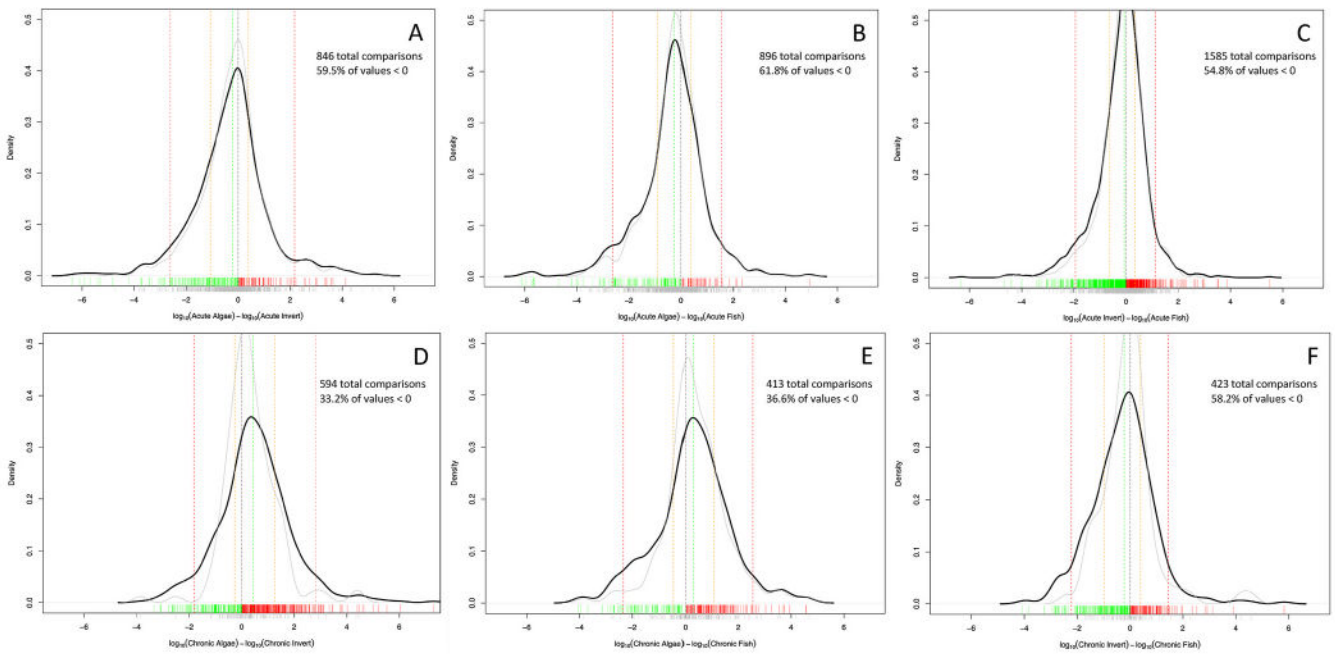


**Fig. 6.**

Comparisons of the relative sensitivities of algae-invertebrate, algae-fish, and invertebrate-fish toxicities for acute (A, B, and C, respectively) and chronic (D, E, and F, respectively) exposures. Note that the number of comparisons available is different than that in Table 4, which include only comparisons where all three trophic level data for a compound are available. The centered, vertical black line in each graph is drawn at zero and represents equality in sensitivity. The inner orange vertical lines denote 50% of all of the data, which are represented by individual tick marks on the x-axis. The outer red vertical reference line indicates 90% of all data shown. The green vertical reference line is at the median. Short vertical green tick marks on the x-axis indicate when the first trophic level identified in the graph is less sensitive and red when the first trophic level is more sensitive. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

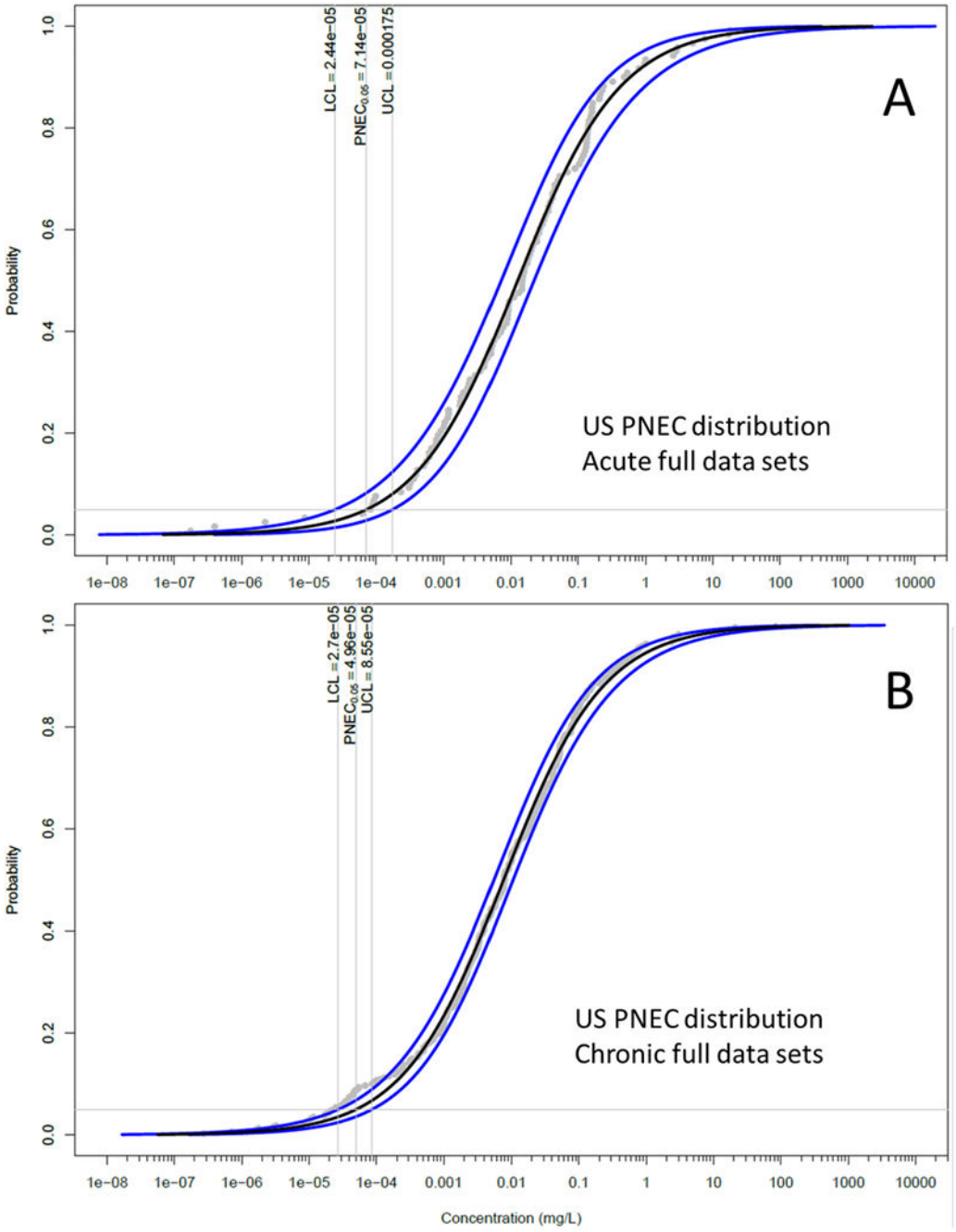


**Fig. 7.**

Representative US PNEC distributions for complete (all three trophic levels present) acute (A) and chronic toxicity (B) data sets. The 5th percentile PNEC (PNEC0.05) and its 95% confidence (Lower, L; Upper U) limits are indicated.

## Table 1

Brief summary of various aquatic PNEC assessment factors[a].

| Data | Canada[b] | Japan[c] | OECD[c] | US EPA[e] | EU TGD[f] |
|---|---|---|---|---|---|
| QSAR | | | 1000 | 1000 | |
| Acute Data (one or two species) | 1000 | 100 × ACR[g] | 1000 | 1000 | |
| Acute Data (3 taxa) | 100 | 10 × ACR[g] | 100 | 100 | 1000 |
| Chronic Data (1 taxa) | | 100 | | 10[h] | 100[i] |
| Chronic Data (2 taxa) | | 50 | | 10 | 50 |
| Chronic Data (3 taxa) | 10 | 10 | 10 | 10 | 10 |
| Chronic Probabilistic | | | | | 1–5 |
| Microcosm/Mesocosm Data | Case-by-case | – | Case-by-case | 1 | Case by case; 1-10 |

[d] OECD (1992).

[a] Not all possibilities are explicitly identified here; consult source documentation for more information.

[b] Environment Canada (1997). Maximum factors; however, new PNEC derivation approach is under development where AF are calculated based on a variety of criteria and not predefined.

[c] see: http://www.meti.go.jp/policy/chemical_management/english/cscl/about.html, Japan Chemical Substance Control Law (accessed March 28, 2017), ACR applied to algae is 20, for *Daphnia* ACR for amine and non-amine compounds are 100 and 10, respectively; ACR for fish = 100.

[e] Example historic values from Zeeman and Gilford (1993) and Nabholz (1991).

[f] EU REACH TGD (2008) refers to short and long term toxicity instead of acute and chronic toxicity; an additional AF of 10 is also identified for freshwater to marine extrapolation.

[g] ACR – acute to chronic ratio.

[h] Applicable when most sensitive acute taxon is also tested with respect to chronic toxicity.

[i] Must be either a fish or an invertebrate.

**Table 2**

PNEC codes associated with various combinations of acute and chronic data and the application factors associated with each. When indicated, “acute” or “chronic” indicate empirical test data is present.

| Region | PNEC Code | Data combination | Application Factor Assigned |
|---|---|---|---|
| Unspecified, not part of a specific regulatory program | PNEC1 | Eco-TTC already available expressed as 5th percentile of PNECs in a group | 1 |
| | PNEC2 | QSAR output for a local type QSAR (e.g., one for a specific group of homologous compounds), applied to most sensitive taxon | 10,000 |
| | PNEC3 | QSAR output for a generalized QSAR (e.g., ECOSAR class) applied to most sensitive taxon | 10,000 |
| United States | PNEC4 | 1 trophic level acute | 1000 |
| | PNEC5 | 2 trophic levels acute; use most sensitive taxon | 1000 |
| | PNEC6 | 3 trophic levels acute; use most sensitive taxon | 100 |
| | PNEC7 | Up to 3 trophic level acutes; 1 chronic on less sensitive acute taxon | 100 |
| | PNEC8 | 3 trophic level acutes; 1 chronic on most sensitive acute taxon | 10 |
| | PNEC9 | 3 trophic level acutes; 2 chronics including most sensitive acute taxon | 10 |
| | PNEC10 | 3 trophic level acutes; 3 trophic level chronics | 10 |
| | PNEC11 | ≥10 species chronic toxicity data; perform Species Sensitivity Distribution | 1–5[b] |
| | PNEC12 | ≥10 species chronic toxicity data; Mesocosm or microcosm | 1–5[b] |
| European Union | PNEC13 | 1 trophic level acute | 10,000[a] |
| | PNEC14 | 2 trophic levels acute; use most sensitive taxon | 5000[a] |
| | PNEC15 | 3 trophic levels acute; use most sensitive taxon | 1000 |
| | PNEC16 | Up to 3 trophic levels acute; 1 chronic available (fish or invertebrate) but not on most sensitive acute | 1000 |
| | PNEC17 | 3 trophic levels acute; 1 chronic available (fish or invertebrate) which is also most sensitive acute | 100 |
| | PNEC18 | 3 trophic levels acute; 2 chronics available including most sensitive acute taxon | 50 |
| | PNEC19 | 3 trophic levels acute; 3 trophic levels chronic | 10 |
| | PNEC20 | ≥10 species chronic toxicity data; perform Species Sensitivity Distribution | 1–5[b] |
| | PNEC21 | ≥10 species chronic toxicity data; Mesocosm or microcosm | 1–5[b] |

[a] Not formally a part of the freshwater European hazard assessment methodology (no data no market, with <3 acute species data); note that within EnviroTox, separate freshwater and marine assessments can be made. An additional factor of 10 for marine assessments can be added unless marine echinoderm and mollusc ecotoxicity data is absent.

[b] Decided on a case-by-case basis based on expert judgements of the assessor based on data quality, number of taxa tested, types of studies, and knowledge of the chemical.

**Table 3**

Example scenarios used to test PNEC derivation logic. Test Type A and C indicate acute or chronic toxicity (see Supplemental information). The structure of the data sets allows computation of geometric means per taxon and geometric means per trophic level for acute and chronic scenarios for entry into the PNEC logic.

| Test ID | Latin name | Trophic Level | Effect value (μg/L) | Duration | Test type | Test statistic |
|---|---|---|---|---|---|---|
| *Scenario 1 – PNEC8 and PNEC16 type* | | | | | | |
| 1 | Algal taxon 1 | ALGAE | 1000 | 3 d | A | EC50 |
| 2 | Algal taxon 2 | ALGAE | 1000 | 3 d | A | EC50 |
| 3 | Algal taxon 3 | ALGAE | 800 | 3d | C | NOEC |
| 4 | *Daphnia magna* | INVERT | 1000 | 2 d | A | EC50 |
| 5 | *Brachionus plicatilus* | INVERT | 2000 | 2 d | A | EC50 |
| 6 | *Pimephales promelas* | FISH | 5000 | 4 d | A | LC50 |
| 7 | *Pimephales promelas* | FISH | 6000 | 4 d | A | LC50 |
| *Scenario 2 – PNEC8 and PNEC17 type* | | | | | | |
| 8 | Algal taxon 1 | ALGAE | 1000 | 3 d | A | EC50 |
| 9 | Algal taxon 2 | ALGAE | 2000 | 3 d | A | EC50 |
| 10 | *Daphnia magna* | INVERT | 900 | 2 d | A | EC50 |
| 11 | *Ceriodaphnia dubia* | INVERT | 950 | 2 d | A | EC50 |
| 12 | *Daphnia magna* | INVERT | 500 | 21 d | C | NOEC |
| 13 | *Daphnia magna* | INVERT | 400 | 21 d | C | EC10 |
| 14 | *Pimephales promelas* | FISH | 5000 | 4 d | A | LC50 |
| 15 | *Pimephales promelas* | FISH | 6000 | 4 d | A | LC50 |
| *Scenario 3 – PNEC10 and PNEC19 type* | | | | | | |
| 16 | Algal taxon 1 | ALGAE | 10000 | 3d | A | EC50 |
| 17 | Algal taxon 2 | ALGAE | 20000 | 3d | A | EC50 |
| 18 | Algal taxon 3 | ALGAE | 800 | 3d | C | EC10 |
| 19 | *Daphnia magna* | INVERT | 1000 | 2 d | A | EC50 |
| 20 | *Hyallela azteca* | INVERT | 2000 | 2 d | A | EC50 |
| 21 | *Chironomus riparius* | INVERT | 900 | 21d | C | NOEC |
| 22 | *Daphnia magna* | INVERT | 950 | 21d | C | NOEC |
| 23 | *Pimephales promelas* | FISH | 5000 | 4 d | A | LC50 |
| 24 | *Pimephales promelas* | FISH | 6000 | 4 d | A | LC50 |
| 25 | *Danio rerio* | FISH | 50 | 30d | C | EC10 |
| 26 | *Pimephales promelas* | FISH | 75 | 30d | C | EC10 |

**Table 4**

Distribution of all tests, substances (or compounds) tested, and numbers of species across algal, invertebrates and fish taxonomic groupings. These data were used in subsequent “all data in” analyses. Relative percentages per grouping are given in parentheses below the raw count. For Tests and Species, sum totals indicate unique tests and taxa but Substances are not unique in these counts (most substances are assessed across many taxa). Therefore, the percentage for Substances represent the percent of substances tested from the total of unique substances in the database overall (i.e., the sum of percentages across all taxa exceeds 100%).

| Group | Acute data sets | | | Chronic data sets | | |
|---|---|---|---|---|---|---|
| | Tests | Substances | Species | Tests | Substances | Species |
| Algae | 5225 (9.3) | 1196 (32.8) | 169 (13.4) | 3373 (36.1) | 1023 (71.2) | 83 (34.3) |
| Invertebrates | 16,339 (29.2) | 2282 (62.6) | 709 (56.3) | 2883 (30.9) | 876 (61.0) | 91 (37.6) |
| Fish | 34,299 (61.4) | 2760 (75.7) | 381 (30.3) | 3080 (33.0) | 647 (45.1) | 68 (28.1) |
| Totals | 55,863 | 3647 | 1286 | 9336 | 1436 | 242 |

**Table 5**

The number of instances that a given trophic group drove the PNEC derivation for acute and chronic toxicity input data sets when all data were present.

| | Total Acute | Total Chronic | Percent of Total – Acute | Percent of Total - Chronic |
|---|---|---|---|---|
| Algae | 358 | 78 | 48.6 | 24.4 |
| Invertebrates | 210 | 140 | 28.5 | 43.7 |
| Fish | 168 | 102 | 22.8 | 31.9 |
| Total number of “full” data sets | 736 | 320 | | |