EnviroTox Platform

EnviroTox Database
PNEC Calculator
ecoTTC
Chemical Toxicity Distribution (CTD)

USER GUIDE

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EnviroTox Database Team

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HESI Animal Alternatives in ERA Committee

This user guide and associated EnviroTox Database website and tools are a work product of the Health and Environmental Sciences Institute (HESI) Animal Alternatives in Environmental Risk Assessment Technical Committee. The Committee's mission is:

> *To ensure the development of a sound technical basis for alternative test methods as a means to reduce, refine, or replace standard ecotoxicity test procedures around the globe.*
>
> *To provide a forum to coordinate the debates and best emerging practices of the alternatives and animal model development sciences to meet existing hazard assessment, effluent assessment, risk assessment, classification and labeling, and other regulatory needs.*

Information regarding the Technical Committee and HESI can be found at http://hesiglobal.org/. This committee is one of several active programs managed by a tripartite consortium of members representing academia, government, and industry in a public-private partnership with global representation.

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Table of Contents

User Guide Authors ii
Project Team ii
Acknowledgements iii
HESI Animal Alternatives in ERA Committee iii
Supporting Organizations iii
2017 Workshop Participants 1
Disclaimer 1
List of Figures 3
List of Tables 4
Terminology / Acronyms 4
Preface 6
Introduction 6
Need for alternative approaches 6
ecoTTC concept 6
EnviroTox Platform – Overview 7
Architecture 7
EnviroTox Database 9
Summary 9
Data collection 10
Physical-Chemical Information 12
Taxa Descriptions 13
Toxicity endpoints 14
Mode of Action Information 21
Metals 23
Salts 23
EnviroTox Platform: Database searching & Tools 23
Access 23
Database searching 23
Exporting a search to Excel 27
PNEC Calculator Tool 30
ecoTTC Tool 37
ecoTTC analysis 38
Chemical Toxicity Distributions (CTD) 42
References 47

List of Figures

Figure 1. Overall architecture of the EnviroTox web platform 8
Figure 2. Schematic of Envirotox platform tools and functionality 9
Figure 3. Determination of acute and chronic toxicity for photosynthetic microbial taxa 15
Figure 4. Determination of acute and chronic toxicity for microinvertebrate toxicity 16
Figure 5. Determination of acute and chronic toxicity for (macro)invertebrate taxa 17
Figure 6. Determination of acute and chronic toxicity for fish 18
Figure 7. Determination of acute and chronic toxicity for amphibians 19
Figure 8. Determination of acute and chronic toxicity for macrophytes 20
Figure 9. General search 25
Figure 10. Advanced search 25
Figure 11. Summary information provided after a search 26
Figure 12. Excel file export 27
Figure 13. Application Factors assigned to different data combinations under US chemical assessment conditions. 32
Figure 14. Application Factors assigned to different data combinations under European chemical assessment conditions. 33
Figure 15. PNEC tool table output 35
Figure 16. Heat-map of acute and chronic data within the PNEC tool 36
Figure 17. Table with geometric means by species 37
Figure 18. ecoTTC tool page 37
Figure 19. ecoTTC analysis 38
Figure 20. Excel download of ecoTTC analysis 40
Figure 21. Scale-location diagram 41
Figure 22. Cumulative probability distribution 41
Figure 23. Distribution density 42
Figure 24. CTD tool page 44
Figure 25. CTD analysis 45

List of Tables

Table 1. Sources of aquatic ecotoxicology data for the EnviroTox Database 10
Table 2. SIFT criteria used to ascertain inclusion of ecotoxicological data in the EnviroTox database 11
Table 3. Description of information included in the physical-chemical file for the EnviroTox database 12
Table 4. Information included in the standalone (non-interactive) species file for taxa found in the EnviroTox database 14
Table 5. MOA classification table and concordance “Bins” 22
Table 6. Output columns in an EnviroTox database search including the ecotoxicological information for each chemical as found in the “test” output tab. 28
Table 7. A summary of various aquatic PNEC assessment factors 31
Table 8. PNEC codes associated with various combinations of acute and chronic data and the application factors associated with each. 34
Table 9. Contents of the Full PNEC Table with explanations 36
Table 10. Contents of the CTD summary table with explanations 46

Terminology / Acronyms

AiiDA	Aquatic Impact Indicator Database
CAS	Chemical abstracts service
CTD	Chemical toxicity distribution
DNEL	Derived negligible effect level
EC_{50}	Half maximal effective concentration
ECCC	Environment and Climate Change Canada
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency
ecoTTC	Ecological threshold of toxicological concern
FET	Fish embryo test
HC_5	5th percentile hazardous concentration
HESI	Health and Environmental Sciences Institute
HPV	High Production Volume
ICCA	International Council of Chemical Associations
METI	Japanese Ministry of the Environment
MOA	Mode of action
NAM	New approach methodology
OECD	Organization for Economic Cooperation and Development
PNEC	Predicted no effect concentration
QSAR	Quantitative structure activity relationship
REACH	Registration, evaluation, and authorization of chemicals
SSD	Species sensitivity distribution
TTC	Threshold of toxicological concern
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
USGS	United States Geological Survey

Preface

The User's Guide contains general instructions on the information available at www.envirotoxdatabase.org.

- The EnviroTox Database: development, content, structure, and search functions
- The PNEC calculator tool
- The ecoTTC distribution tool
- The CTD tool

Introduction

Need for alternative approaches

The need for rapid and predictive methods to address aquatic ecological hazards of diverse substances remains essential, as the chemical universe remains largely untested. Flexible approaches that do not require the use of large numbers of vertebrate test animals (fish, amphibians, birds, etc.) are needed to address broad animal welfare concerns. To appropriately develop new approaches methodologies (NAMs) and non-testing approaches, existing information must be made available via integrated and curated datasets. Increasing regulatory requirements have laid the foundation for the development of more standardized and extensive data sets for a broader range of chemicals. Regulatory programs such as REACH; (EC 2007), ICCA (International Council of Chemical Association) High Production Volume (HPV), Chemicals Challenge (ICCA 2018) and Canada's Domestic Substance List (ECCC 2018) have helped to create unprecedented levels of available toxicity data along with continuing investigations of hazards of substances to aquatic life published in the scientific literature.

ecoTTC concept

Risk assessment of chemicals inherently involves an assessment of toxicity, exposure and the resulting likelihood or probability of observing an adverse response. Further, it requires ethical and resource consideration as to how much data is attainable and should be derived (e.g., via use of animal testing) versus what is considered an acceptable level of extrapolation (Belanger et al. 2015). One such methodology is the concept of the Threshold for Toxicological Concern, or TTC. The TTC establishes an exposure level for chemicals, below which no appreciable risk to human health or the environment is expected based upon a *de minimis* value for toxicity identified for many chemicals (US FDA 1993; Kroes et al. 2004). This level can then be compared to an estimate of the likely exposure to a chemical to complete a screening level safety assessment for a given route of exposure or environmental compartment/species of concern. The TTC concept is well-established to assess human safety of indirect food-contact substances and has been reapplied for a variety of endpoints including carcinogenicity, teratogenicity, and reproductive toxicity. TTCs have benefits for screening-level risk assessments, including the potential for rapid decision-making, fully utilizing existing knowledge, reasonable conservativeness for chemicals used in lower volumes, and reduction or elimination of unnecessary animal tests.

TTC approaches have only recently been explored in environmental assessments. It is not the intent to review the literature here for the purpose of this User's Guide, but assessors can refer to a series of papers such as those of De Wolf et al. (2005), Gross et al. (2010), Williams et al. (2011), Mons et al. (2013), Hendricks et al. (2013), Gutsell et al. (2015) and Belanger et al. (2015). Examples can be found therein that are varied in approach, but are close derivatives of the concept termed ecological Thresholds of Toxicologic Concern, or ecoTTCs, as used here. Aspects of information management, tracking of test species, use of categorization principles, and applications of statistical analyses to identify thresholds are common among the historical efforts (see Table 1 of Belanger et al., 2015).

EcoTTCs summarize the distribution of a large array of species level toxicity data as ecosystem PNECs (Predicted No Effect Concentrations). PNECs are derived for a chemical category or mode of action and project a conservative prediction for similar, but untested chemicals. In human safety, the TTCs are typically set as a 5th percentile value DNEL (Derived Negligible Effect Level) from a statistical distribution of similarly acting chemicals. EcoTTCs are defined here as the 5th percentile value derived from a statistical distribution of PNECs of similarly acting chemicals. The categorization of "similar acting" compounds can be based on Mode of Action (*sensu* Kienzler et al. 2017), a formed chemical category, or for a functional use of a chemical (e.g., pharmaceutical, detergent surfactant, etc.). Because PNECs are regionally based in their development, reflecting local attitudes around hazard conservatism and local Application Factors (AFs), PNEC distributions are expected to vary by region (Hahn et al. 2014). There may be situations where a researcher or assessor is interested in the toxicity distributions, without the added conservatism introduced by the assessment factors or the regional overtones of their application. A chemical toxicity distribution (CTD) can be used to perform this type of analysis, and a tool to evaluate this has been added as a component of this work.

EnviroTox Platform – Overview

Architecture

The overall structure of the database and tools housed in the EnviroTox platform, along with how this information is to be used is depicted in Figure 1. Three broad categories of data are housed within the EnviroTox database and include physical-chemical information (including mode of action (MoA) assignments), information on test species, and ecotoxicological information. The database is subject to query using the application interface and is described below. Queries are constructed based on the user's specific questions and interest. An output file containing data that matches the user query and can be subjected to further analyses ad hoc, outside the application, and within the application. The content of files, query, and outputs is described in greater detail below.

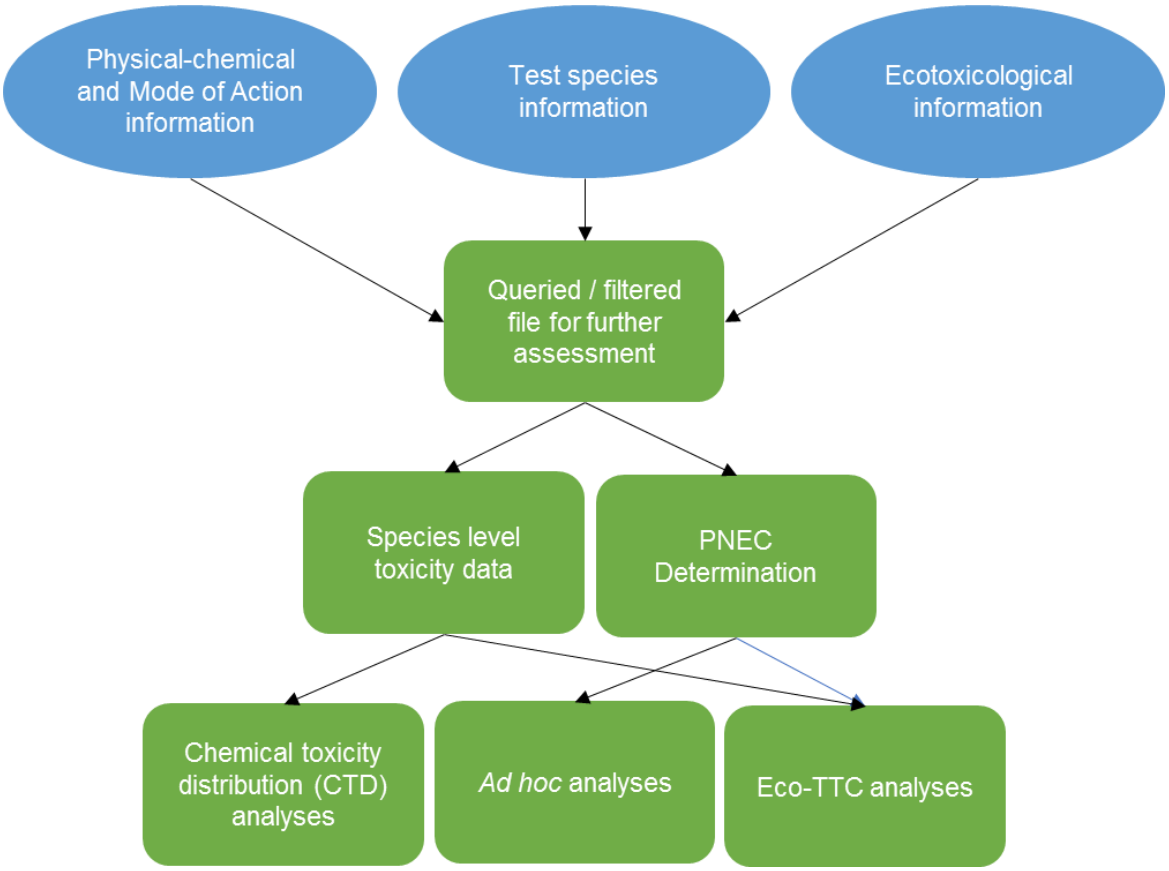


Figure 1. Overall architecture of the EnviroTox web platform

In the following sections, the tabs of the web tool and their various functionalities are described. A schematic overview is provided in Figure 2. The user can search the database to construct and customize the base ecotoxicological information from which an ecoTTC could be calculated. Analysis includes choosing a geographic region for deriving chemical-specific PNECs (different regions use different processes). Once PNECs are derived, the ecoTTC calculation can proceed with additional customization that is also user-defined, such as including or excluding PNECs that are supported with less or more ecotoxicological data. Chemicals with less data are extrapolated to the PNEC with larger uncertainty or application factors than those with more complete information. Finally, once the PNEC distributions are determined, the outputs are provided in Excel and graphical formats. At this point users can do further analyses or evaluate outputs directly for the purposes that were chosen. This is a highly genericized diagram of information flow and many others are possible. There may be situations where a researcher or assessor is interested in the toxicity distributions, without the added conservatism introduced by the assessment factors or the regional overtones of their application. A chemical toxicity distribution (CTD) can be used to perform this type of analysis.

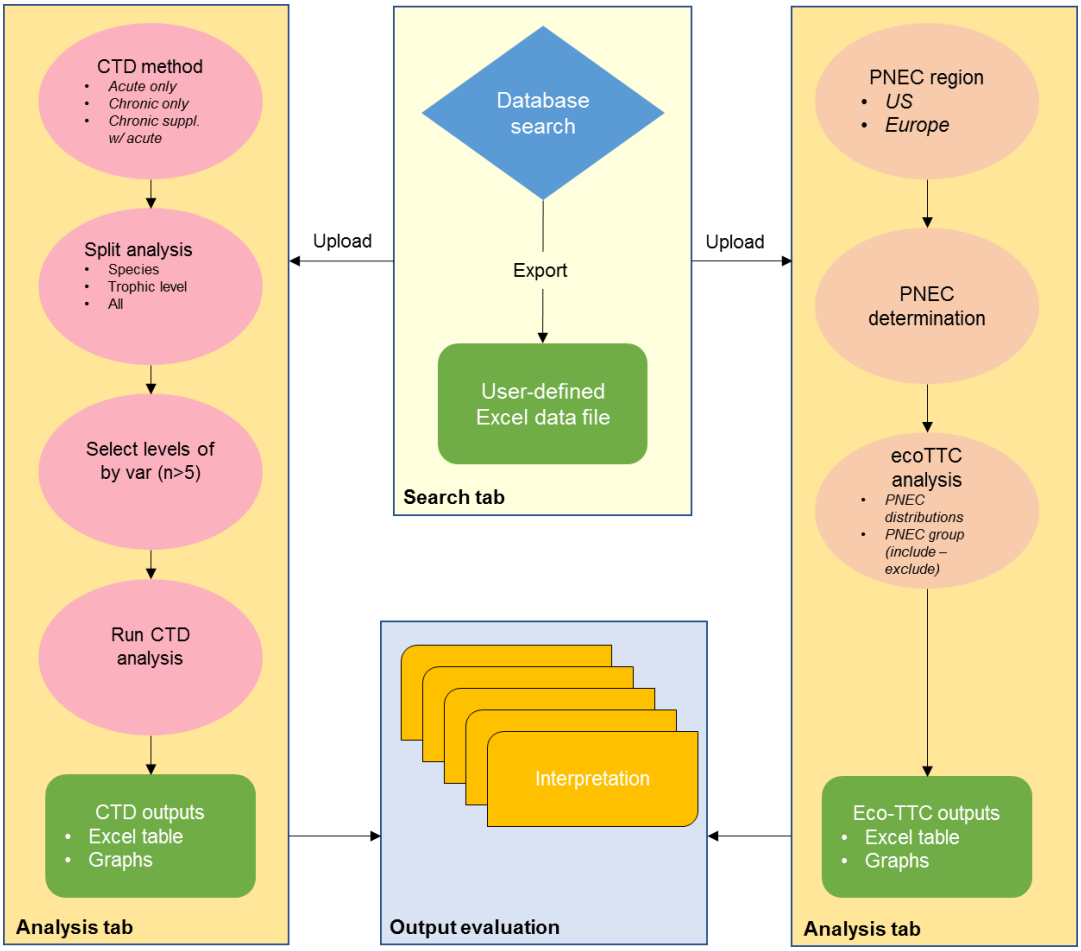


Figure 2. Schematic of Envirotox platform tools and functionality

EnviroTox Database

Summary

The EnviroTox database contains aquatic toxicity 91,217 records representing 1,563 species, and 4,016 unique chemical CAS. Chemical-specific information is also linked to each record and includes physical chemical information, chemical descriptors, and MoA classifications (Kienzler et al. 2017). Taxonomic descriptions of test species (phylogeny, trophic level, etc.) are also included and all records include the original source citations. Toxicity data is associated with the physical chemistry data, MoA classifications, and curated taxonomic information for the organisms tested. The database also includes a systematic process for including acute and chronic effects, as well as computing a predicted no effect concentration (PNEC) for exposed ecosystems based on depth and breadth of data included in the statistical computation. Additional discussion of the development of this database will be available in a forthcoming publication (Connors et al., in preparation). Information included in the database is provided in the sections below.

Data collection

Aquatic ecotoxicological information was gathered from a wide variety of sources listed in Table 1. Information was compiled by associating individual CAS numbers with ecotoxicological data. Each individual data point within a study was considered as a separate entity. The potential to include a data point or study was based on the SIFT methodology where predefined inclusion criteria is used to address relevance, validity and acceptability of data (Beasley et al. 2015) (Table 2).

Table 1. Sources of aquatic ecotoxicology data for the EnviroTox Database

Data source	***Description***
ECHA (REACH)	Obtained by query of the REACH data from eChemPortal database of publicly available substance data, submitted to ECHA (European Chemicals Agency) under the REACH (Registration, Evaluation and Authorisation of Chemicals) regulations. (OECD 2018). http://echemportal.org
USEPA ECOTOX	Obtained by query of the USEPA's ECOTOX Knowledgebase, including EPA-generated test data and data from the public literature. (USEPA 2018a). https://cfpub.epa.gov
Peer-reviewed literature	Original dataset foundational to Species Sensitivity Distribution work by De Zwart (2002) and colleagues, personal communication to the HESI Technical Committee, containing data and metadata stripped from peer-reviewed literature
ECETOC OASIS	Aquatic toxicity results from a variety of sources, available via the OECD QSAR Toolbox. http://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
AiiDA	Aquatic Impact Indicator DAtabase; contains data sourced from ECHA, ECOTOX and others. Queried to supplement for data not found in REACH. aiida.tools4env.com
METI	Summary of aquatic toxicity test results from OECD guideline tests conducted by the Japanese Ministry of the Environment (METI). Some data publicly available via the OECD Toolbox. Also known as the NITE-CHRIP database. http://www.nite.go.jp/en/chem/chrip/chrip_search/srhInput
FET	Dataset of acute aquatic toxicity test results from the OECD validation study to evaluate the reproducibility of the ZebraFish Embryo Test (ZFET). (Belanger et al. 2013; Busquet et al. 2014)
USGS Columbia	Summary dataset of acute aquatic toxicity tests conducted by the USGS Columbia Environmental Research Center, including Mayer and R. Ellersieck (1986). http://www.cerc.usgs.gov
Pharmaceuticals	Summary of acute and chronic aquatic toxicity data for active pharmaceutical ingredients. Provided by Sanofi S.A. and detailed in Vestel et al. (2016).

ECOSAR training set	Set of aquatic toxicity data used to train the computational QSAR tool ECOSAR (ECOlogical Structure Activity Relationship) developed by the U.S. Environmental Protection Agency for hazard estimation; sourced from the Help files for the ECOSAR program (USEPA 2012) https://www.epa.gov/tsca-screening-tools/ecological-structure-activity-relationships-ecosar-predictive-model
EPA Pesticide Data	Pesticide Ecotoxicity Database (formerly the Ecological Effects Database); aquatic toxicity data provided by the U.S. Environmental Protection Agency's Office of Pesticide Programs Environmental Fate and Effects Division. http://www.epa.gov/pesticides
OECD QSAR Toolbox	Queried to supplement aquatic toxicity data from ECOTOX and ECHA. Contents include data from Aquatic ECETOC and Aquatic Japan MoE. http://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm

Table 2. SIFT criteria used to ascertain inclusion of ecotoxicological data in the EnviroTox database

Step	Criteria	Specifics	~# Records
0: Purpose	Aquatic toxicity data and metadata	Initial pull of available information from databases listed in Table 1.	220,000
1: Relevance	Trophic designations	Fish, amphibian, invertebrate, algae	158,000
2: Validity	CAS	CAS present	132,000
	Required fields	Effect value/units, duration, test statistic, effect measured, source present	
	Qualifiers	Exclude effect values with qualifiers (e.g. <>)	
	Effect	Specific effect measurement (e.g. EC50)	
3: Acceptability	Duration	≥ 24h	123,500
	Test Statistic	≥5% and ≤70% effect measure (e.g. IC10, LC50), NOEC, LOEC, MATC	
	Effect	Abundance, biomass, cells, chlorophyll, emergence, filtration rate, gross primary productivity, growth, hatchability, intoxication, mortality, nitrogen fixation, population growth, population reduction, population change, primary production, regeneration, reproduction, shell deposition, teratogenesis Focus is on endpoints of regulatory significance and known use in decision-making	

Step	Criteria	Specifics	~# Records
4: Additional criteria	CAS, Chemical name SMILES	Harmonized. Database trimmed to only contain validated chemicals	91,000
	Metals	Inorganic compounds were collapsed to a 'dummy metal ion' CAS	
	Identification of duplicates	Removed records that were full duplicates (e.g., Citation, species, test duration, test statistics, measured effect, effect level)	
	Removal of extreme outliers	Solubility, effect concentrations	

Physical-Chemical Information

Several compound identifiers were included in the database as shown in Table 3. Information on specific chemicals is associated with Chemical Abstracts Service registry number (CAS). CAS numbers in the database are absent spaces or dashes. Chemical CAS, chemical name, and SMILES were systematically verified. This process involved first running all CAS through the USEPA Chemistry Dashboard (comptox.epa.gov/dashboard); if chemicals had a CAS and chemical name match through this tool, they were considered validated and the corresponding SMILES was extracted. For those chemicals where there was not a match through the USEPA Chemistry Dashboard, the chemical CAS was run through SciFinder and checked against several chemical identification tools to determine the name and CAS, then the SMILES were extracted.

Table 3. Description of information included in the physical-chemical file for the EnviroTox database

CAS	Chemical Abstracts Service (CAS) number, no dashes or spaces
Chemical descriptors	
Chemical name	Commonly employed chemical name.
SMILES	Unified SMILES (Simplified Molecular Input Line Entry Specification) code associated with the chemical and CAS.
Desalted canonical SMILES	Open Babel (Open Babel 2018) was used to generate desalted and canonicalized SMILES for subsequent modeling and chemical categorization.
Molecular weight	Molecular weight in g/mol; generated from desalted SMILES using EpiSuite DermWin (USEPA 2018b)
Log K_{ow}	Octanol-water partition coefficient; unitless; EpiSuite KOWWIN (USEPA 2018b) used to populate Log K_{ow} from desalted SMILES. Experimental used if available; modeled if no experimental available
Water Solubility	Solubility of the chemical in pure water (25˚C, 1 atmosphere) in mg/L; EpiSuite WSKOW (USEPA 2018b) used to populate water solubility from desalted canonical SMILES. Experimental used if available; modeled if no experimental available.

	Effect values that are greater than 5x of the water solubility level were flagged but not removed.
ECOSAR Classification	Assignment of chemical class based on desalted, canonical SMILES input to OECD QSAR Toolbox ((https://www.qsartoolbox.org/home). Note that compounds may be assigned to multiple ECOSAR groups depending on types of substitutions.
ECOSAR Classification – collapsed	For chemicals where multiple classifications were generated by ECOSAR, the first reported was used. These categories were further collapsed into 46 more general categories. The complete list of ECOSAR classification collapsed assignments is available as Supplementary Information
USEPA New Chemical Categories	Original categories cited in the document "TSCA New Chemicals Program (NCP)/ Chemical Categories" (USEPA 2010).
MOA Classifications	
Verhaar	Verhaar classes obtained via OECD QSAR Toolbox (https://www.qsartoolbox.org/home).
TEST	Toxicity Estimation Software Tool (TEST) based on the MOAtox broad assignments as described by Barron et al. (2015).
OASIS	OASIS acute aquatic toxicity MOA obtained via OECD QSAR Toolbox. (https://www.qsartoolbox.org/home)
ASTER	ASTER (ASsessment Tool for Evaluating Risk) is a rule-based expert system and is operated on a proprietary basis by US EPA based on the MOA categories in Russom et al. (1997).
Consensus MOA assignment	A 'consensus' MOA assignment of narcotic (N), specifically-acting (S), or unknown (U) was assigned to each chemical based on a consensus from the 4 classification schemes.
Chemical Categories	Determined from SMILES
Halogenated	Contains F, Cl, Br, I.
Heavy Metal	Contains a heavy metal (metallic element with a density greater than 5)

All of the listed parameters can be employed in various ways to query the available information.

Taxa Descriptions

Information shown in Table 4 has been collated for species present in the database. Current taxonomic status has been harmonized as of 2017. It is recognized that some designations can be somewhat arbitrary. For example, functionally photosynthetic/non-photosynthetic protists may be categorized as algae (photosynthetic microbes) when they may have been tested in a state absent of chloroplasts in some situation(s). Common authoritative taxonomic websites including https://www.ncbi.nlm.nih.gov, http://www.algaebase.org/, http://www.marinespecies.org/about.php, and http://fishbase.org/home.htm were consulted to derive final classifications. A few ecotoxicologically important species are among these and ecotoxicologists should recognize their transitions to new names: *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), *Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*), *Danio rerio* (formerly *Brachydanio rerio*), *Oncorhynchus mykiss* (formerly *Salmo gairdneri*) and *Americamysis bahia* (formerly *Mysidopsis bahia*) have all undergone taxonomic revision in recent years. All species identified in the database were also assigned to a freshwater or saltwater habitat. For estuarine or facultatively freshwater to saltwater species, the primary habitat in which they are known or tested in was used to assign habitat. A species file is maintained in the database and will be updated as needed.

Table 4. Information included in the standalone (non-interactive) species file for taxa found in the EnviroTox database

Latin Name	Linnaean Genus and species name
Trophic level	Algae, Invertebrate, Fish, Amphibian, Macrophyte, Fungi
Taxonomic Kingdom	Consensus based designation
Taxonomic Phylum or Division	Phylum (animal) or Division (plant)
Taxonomic Sub-phylum	Not always available
Taxonomic Superclass	Not always available
Taxonomic Class	Taxonomic Class
Taxonomic Order	Taxonomic Order
Taxonomic Family	Taxonomic Family

It should be noted that ecotoxicological tests performed on mixed communities of organisms (more than one taxon, well-described at the genus level) are not included in the database. Tests performed on organisms designated above the genus level (i.e., Family or higher) are not included. Note that the tests are identified in the database, but are excluded from consideration due to the structure of queries and the SIFT process (Beasley et al. 2015, also see below).

Toxicity endpoints

Central to the development of the EnviroTox database were decisions on endpoints to include and how to ascertain if the study was an acute or chronic test. Endpoints for ecotoxicity studies were evaluated for their utility in regulatory evaluations of ecotoxicity data (Moermond et al. 2017; Rudén et al. 2017; Hall et al. 2017). Further, the endpoint was then associated with appropriate statistical evaluations to arrive at a conclusion of “acute” or “chronic” toxicity or unassignable. As an illustrative example, a study on the ecotoxicity of a chemical to *Daphnia magna* (an accepted cladoceran) was performed over 17 days and response to a biomarker was measured and positioned as a No-observed effect-concentration (NOEC). While the species, duration and statistic may be appropriate for a “chronic” interpretation, the biomarker is not presently used in any regulatory framework for environmental risk assessment so it would not be further used. Decision logics were established for studies on all taxonomic groups so that transparency for assignment as acute or chronic determinations based on endpoint, species, and statistic that were operationally defined. The logic used to classify non-photosynthetic microinvertebrates, macroinvertebrates, amphibians, fish, and macrophytes as acute or chronic are included in Figures 3 – 8).

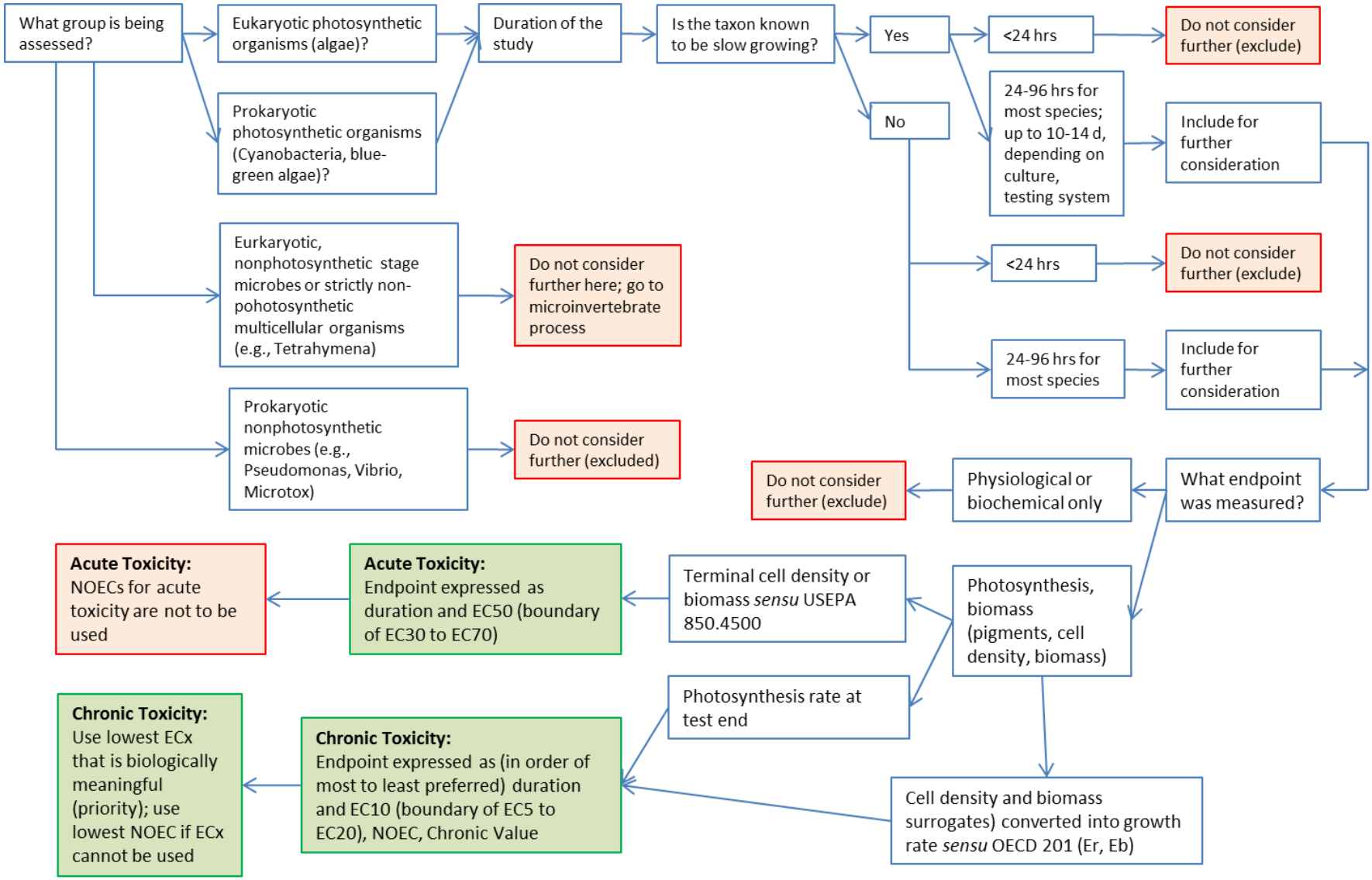


Figure 3. Determination of acute and chronic toxicity for photosynthetic microbial taxa

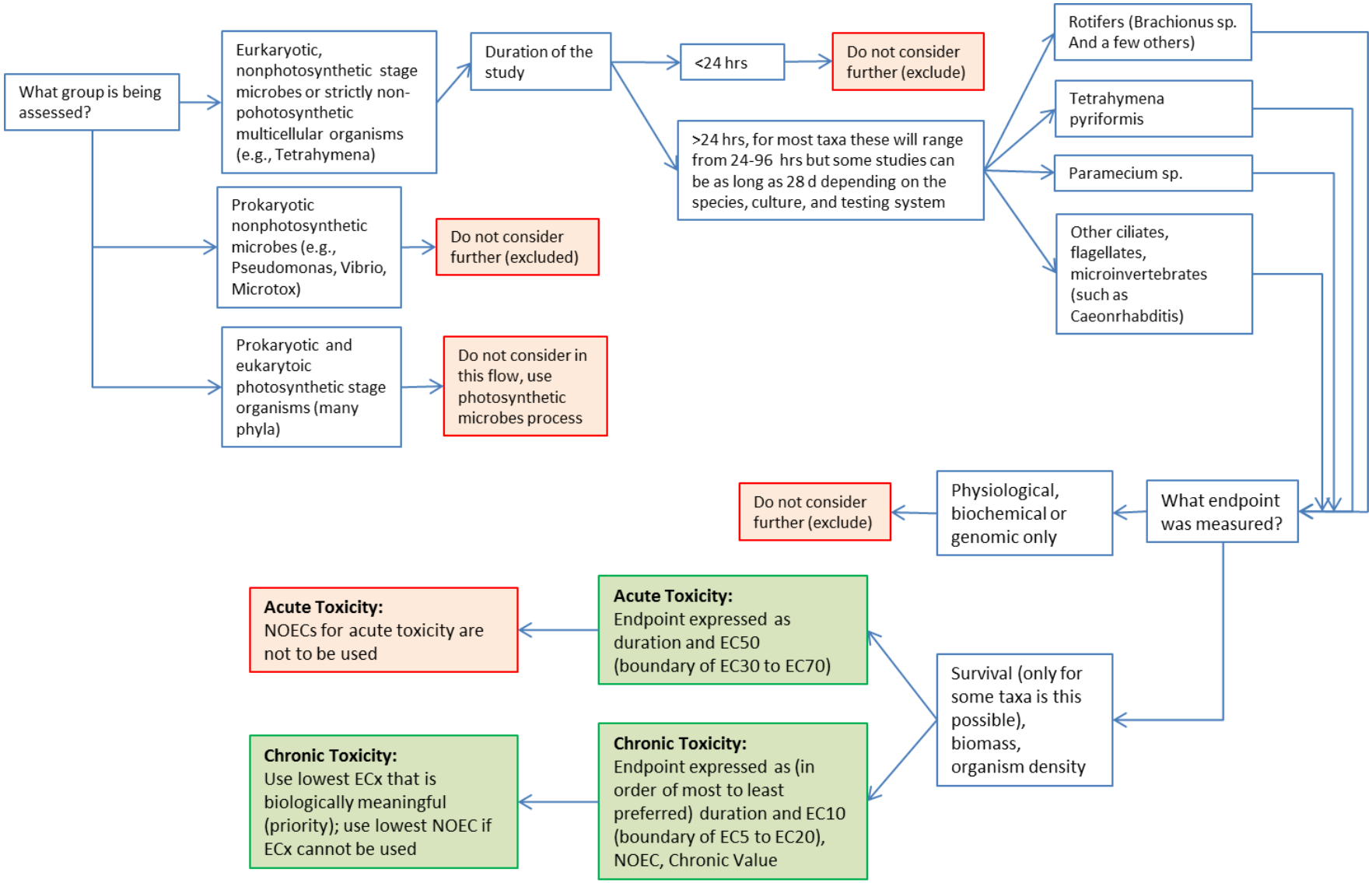


Figure 4. Determination of acute and chronic toxicity for microinvertebrate toxicity

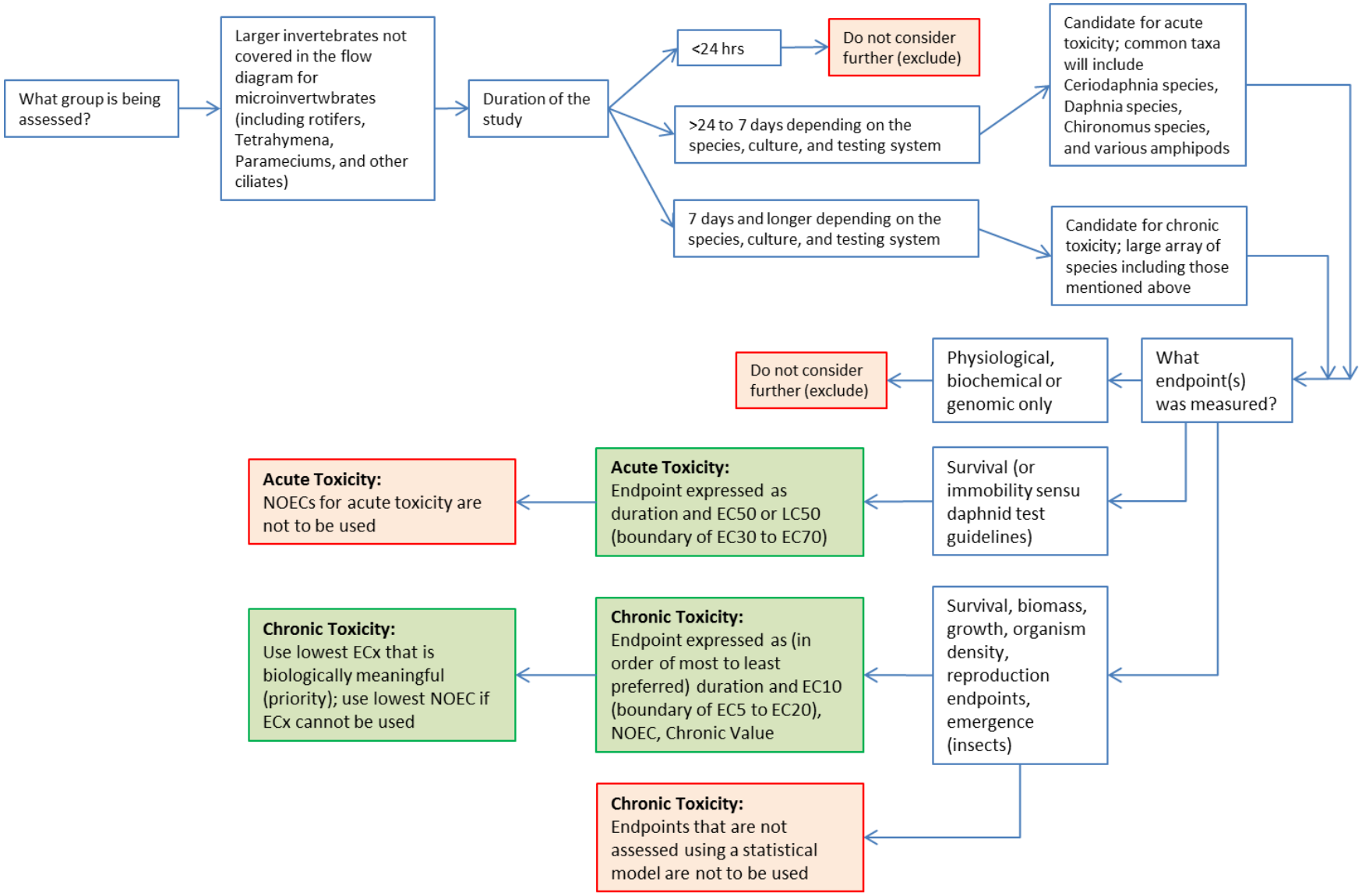


Figure 5. Determination of acute and chronic toxicity for (macro)invertebrate taxa

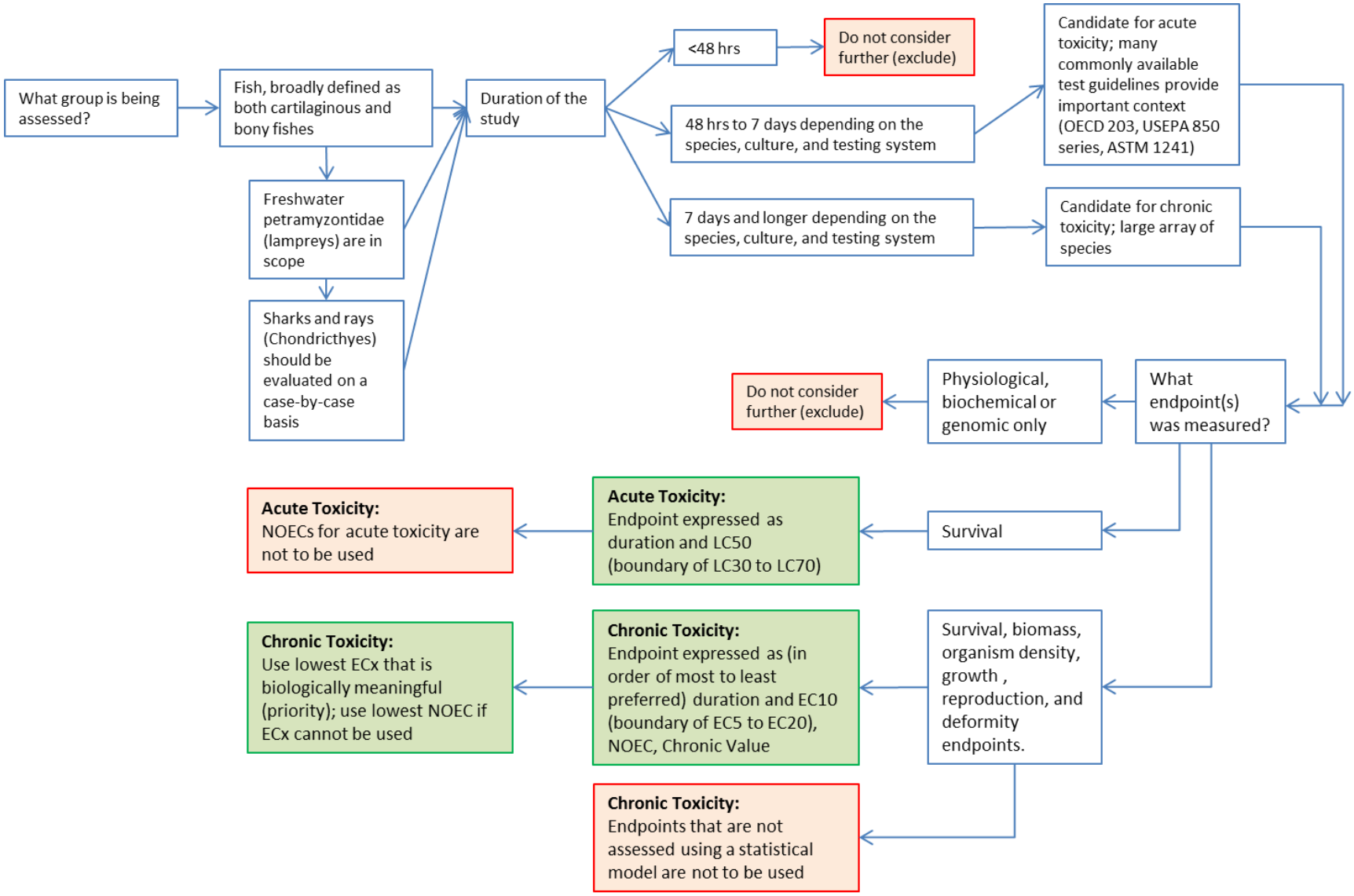


Figure 6. Determination of acute and chronic toxicity for fish

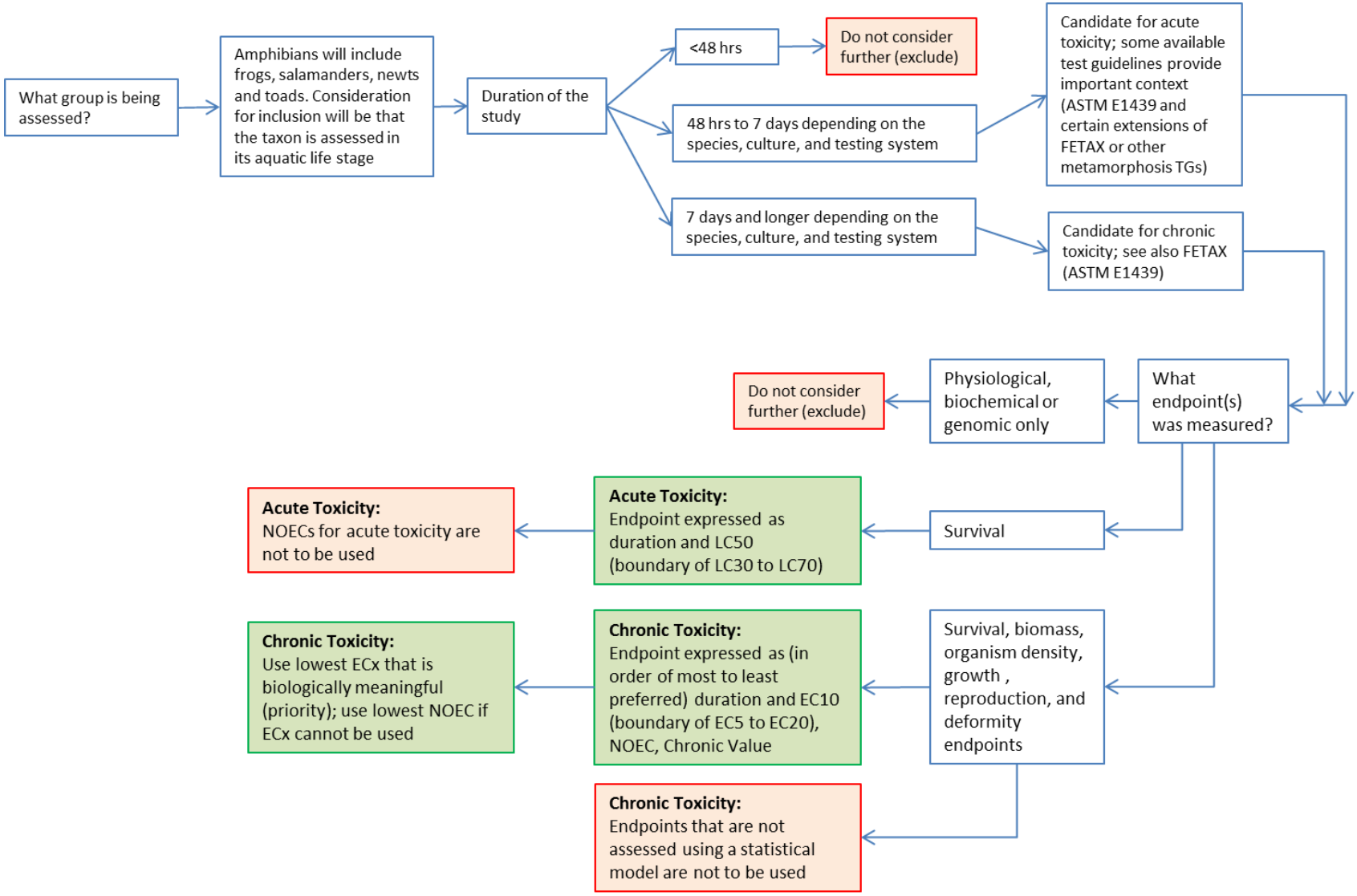


Figure 7. Determination of acute and chronic toxicity for amphibians

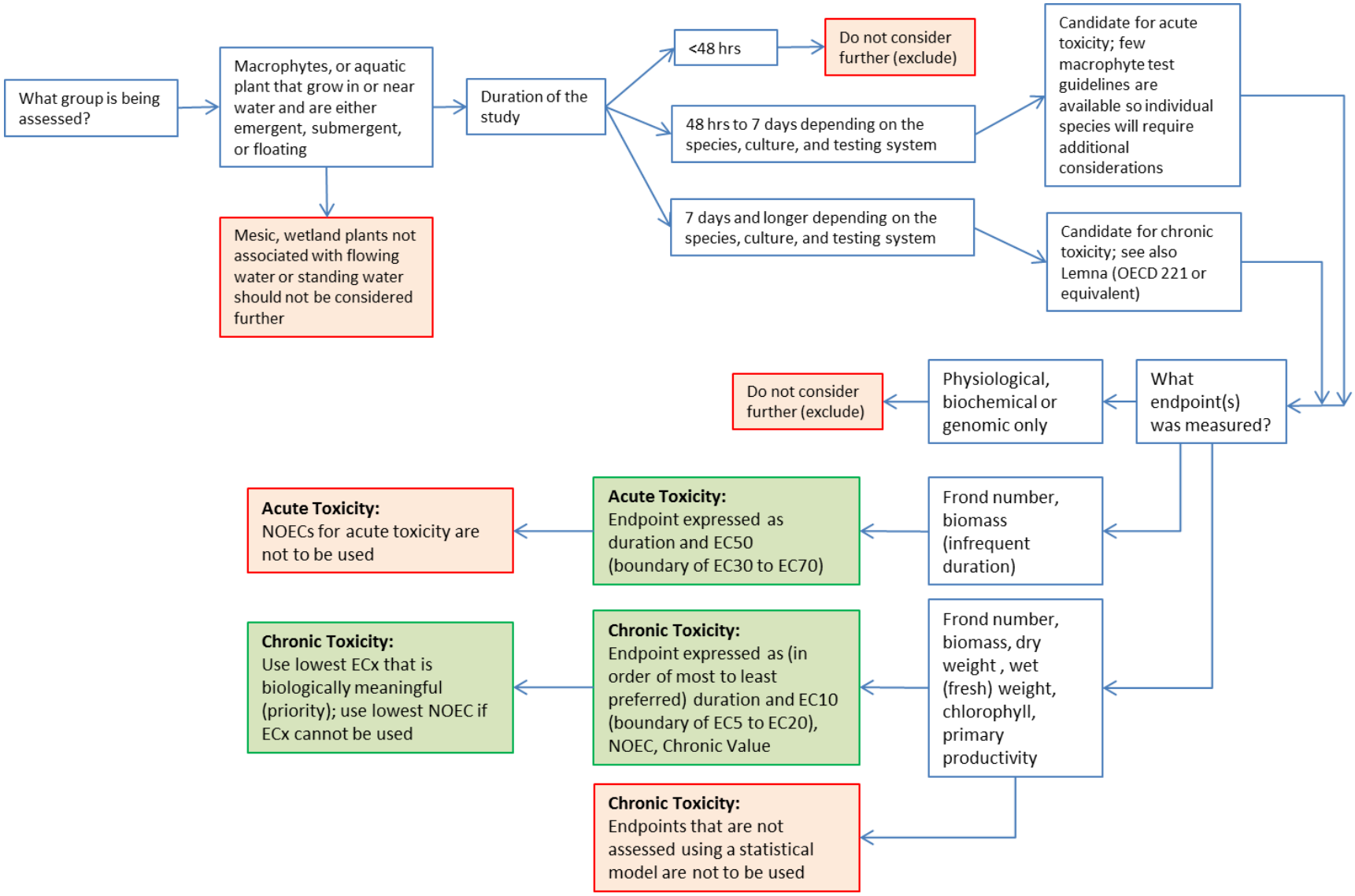


Figure 8. Determination of acute and chronic toxicity for macrophytes

Mode of Action Information

In order to allow grouping of chemicals for eventual ecoTTC or other analyses, four mode of action classification assignment schemes (Verhaar, TEST, OASIS, and ASTER) were applied to each chemical. This expands earlier work performed by Kienzler et al.,(2017) on a previous version of the database. The specific MOA assignments obtained from each of the schemes are included in the database. However, to facilitate simpler groupings for ecoTTC and other applications, each chemical was also assigned to one of three 'general' groupings based on the degree of consensus between the evaluated schemes and the concordance between schemes shown in Table 5: narcotic (N), specifically acting (S) or Unclassified (U).

Each MOA scheme assignment was collapsed into one of the three bins as assigned in Table 5 below. A four-letter code, corresponding to the TEST, ASTER, OASIS, and Verhaar bins, respectively, was assigned to each chemical, a consensus MOA was determined, and a confidence score was assigned.

- All four in agreement (e.g., NNNN, SSSS, UUUU): Confidence score of 3
- Three schemes in agreement (e.g., NNNS, SSNS): Confidence score of 2
- Two schemes in agreement with these other two as "U" (e.g., NNUU, SUSU): Confidence score of 1 and assignment made on the non-"U" assignment
- All other combinations: assigned a consensus MOA of "U" and a confidence score of 0

Table 5. MOA classification table and concordance "Bins"

VERHAAR		ASTER		TEST		OASIS	
Classification	**Bin**	**Classification**	**Bin**	**Classification**	**Bin**	**Classification**	**Bin**
• Class 1 (narcosis or baseline toxicity) • Class 2 (less inert compounds)	N	• Non-polar narcosis • Polar narcosis • Ester narcosis	N	Narcosis	N	• Basesurface narcotics • Narcotic amines • Phenols and anilines • Alpha, beta-unsaturated alcohols • Esters	N
• Class 3 (unspecific reactivity) • Class 4 (compounds and groups of compounds acting by a specific mechanism)	S	• Diester toxicity • Reactive • Chloro-diester-based reactivity • Carbonyl (C=0)-based reactivity • Carbonyl reactivity • Alkylation/arylation-based reactivity • Acylation-based reactivity • Sulfhydryl (-S-H)-based reactivity • Reactive dinitroaromatic group • Nitroso-based reactivity • Quinoline reactivity • Acetamidophenol reactivity • Reactive diketones • Acrylate toxicity • N-halogenated acetophenone inhibition • Hydrazine-based reactivity • Isocyanate (-N=C=O)-based reactivity • Pyridnium compounds • Neurotoxicant: DDT-type • Neurotoxicant: pyrethroid • Neurotoxicant: cyclodiene-type • Neurotoxicant: strychnine • Neurotoxicant: nicotine • Organophosphate-mediated AChE inhibition • Carbamate-mediated AChE inhibition • Uncoupler of oxidative phosphorylation • Respiratory blocker: azides and cyanides	S	• Reactivity • Neurotoxicity • AChE inhibition • Electron transport inhibition • Iono/osmoregulatory / circulatory impairment	S	• Reactive unspecified • Aldehyde	S
Class 5	U	Unknown mode of action	U	Unknown	U	Unknown	U

Metals

The toxicity of some metal-containing compounds can be driven by the presence of the freely dissolved metal ion. Consistent with the U.S. derivation of water quality criteria for aquatic life and international screening values (Barron and Wharton, 2005), specific divalent metal compounds were grouped by metal ion. Inorganic compounds were assigned to a 'dummy metal ion CAS' (e.g., Metalgrp.Ag') 'if 1) the metal ion could dissociate from the compound (e.g., acetate, lactate), 2) the toxicity of the compound would be driven by the metal ion. A compound was not assigned to a dummy metal CAS if it was caustic or highly reactive, if the metal was associated with ammonia or hydroxides, or if more than one metal was present in the compound. A total of 140 compounds in the database were assigned one of 24 different 'dummy metal ion CAS'. The original CAS for the compound and dummy metal ion CAS are both provided in the database.

Salts

Chemical compounds were excluded from the database if the desalted canonicalized SMILES resulted in the individual hydroxide (OH-), chloride (Cl-), ammonium (NH4+) or amino cation (NH2+). The corresponding effects data for excluded compounds were removed from the database because of uncertainty regarding the moiety that would produce the toxicological effect.

EnviroTox Platform: Database searching & Tools

Access

The EnviroTox platform, which includes the database, search interface, and tools, can be accessed at www.envirotoxdatabase.org. Upon agreement with the HESI, users agree to appropriately utilize the tools and data. Primary and derivative works where the EnviroTox database and/or its calculation functions have been used for a scientific purpose should be cited when used. Information on how to cite use of the database and tools is available under the "About" tab on the website.

Database searching

On the top of the homepage the five clickable tabs of the EnviroTox web applications are found: Search, Analysis, Setup, Documentation and About. Two options are available to search the EnviroTox database: a "General Search" (Figure 9) and an "Advanced Search" (Figure 10). Both options result in the user being able to export their desired data as an Excel file, which will then be uploaded into the analysis tools. Additional search terms are available using the Advanced Tab.

General Search Fields:

Substance Properties
- CAS
- Chemical Name
- Desalted Canonical SMILES
- Log Kow
- Water Solubility (mg/L)
- MW (g/mol)
- ECOSAR classification
- US-EPA New Chemical Categories
- Consensus MOA

Taxonomy Properties
- Latin name
- Trophic Level

Test Properties
- Test type
- Test statistic
- Duration (A = Acute; C= Chronic)

Advanced Search Fields include those of the General Search, PLUS:

Substance Properties
- Canonical SMILES
- Heavy Metals
- Halogenated
- Desalted Canonical SMILES
- Log Kow
- TEST
- ASTER
- OASIS
- Actual Verhaar Category

Test Properties
- Test type
- Test statistic
- Duration (days)
- Duration (hours)
- Effect is 5x above water solubility

Begin either type of search by clicking on the “Select a Field (optional)” dropdown menu and selecting a field to filter the dataset. Next click the “contains” dropdown menu and select the qualifier you want to use for your chosen field (e.g., contains, =, <). Last, begin typing in the “Search for…” how you would like to filter the data based on the field chosen (this could be letters or numbers, depending the field chosen).

If you would like to filter the data by more than one field, then click the “+” box immediately to the right of the “Search for…”box to add another filter Field. All additional filter fields require you to specify the desired Boolean operator (AND or OR) for how you wish to link the search filters together.

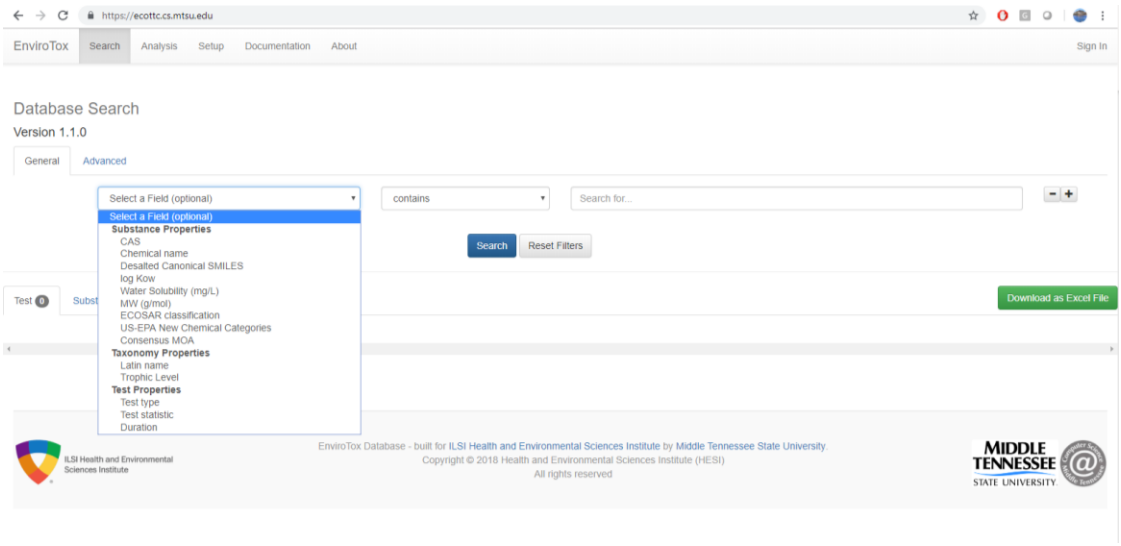


Figure 9. General search

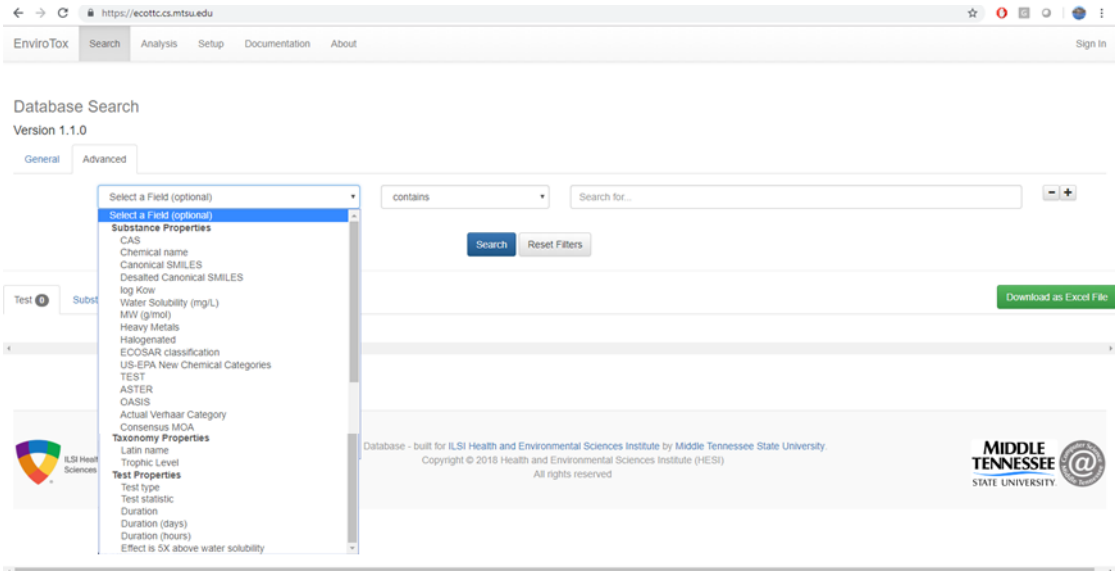


Figure 10. Advanced search

Once all filter Fields have been completed, click the blue “Search” button and a snapshot of the resulting data will appear with three tabs running across the top of the data that indicate the number of studies (Test), chemicals (Substances), and Species (Taxonomy) in the filtered dataset (Figure 11). If the search performed has returned the desired then click the green button on the “Download as Excel File” right side of the “Search for…” box to export the data file. You will want to save this to your local drive in a place easy to locate, as this file will serve as the input for the analysis tools. If the search performed has not returned the desired dataset then you can either modify your previous search or click the “Reset Filters” button and start the advanced search over.

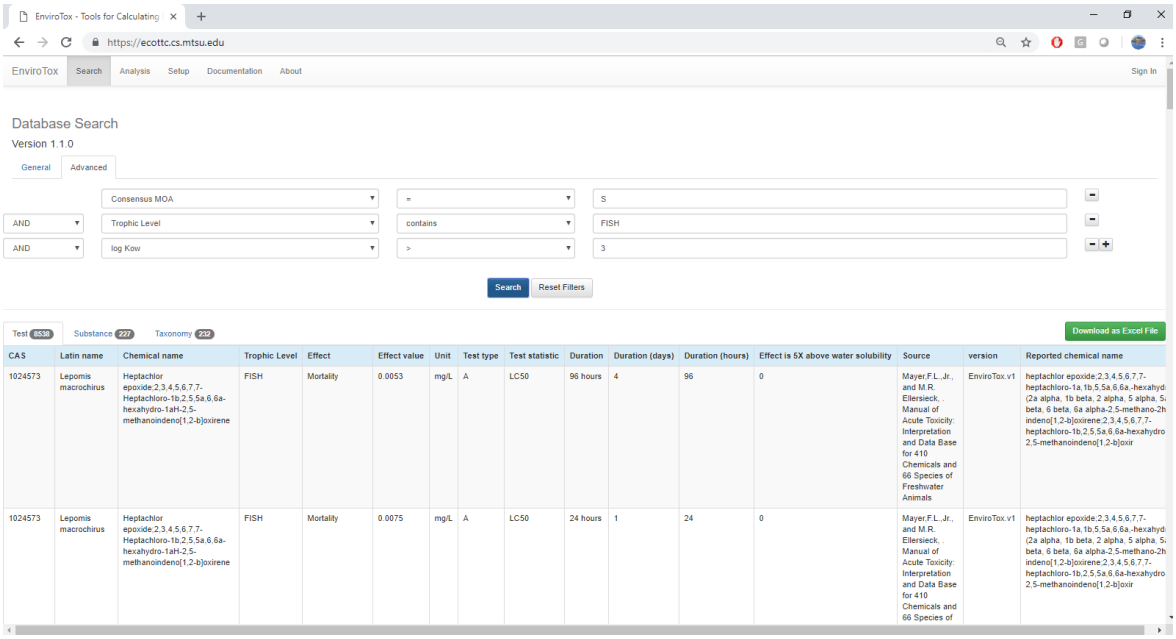


Figure 11. Summary information provided after a search

IMPORTANT NOTE: The web tool saves no searches, once filters have been reset; there is no way to retrieve the search parameters used.

The database query system is sensitive to characters entered in the “Search for” field (i.e., it employs smart search technology). The more characters added, the narrower the search becomes. The user can scroll into the dropdown box and highlight the group desired at any point. Refinement of the search can continue by adding additional search terms with the + sign or remove search terms with the – sign at the right of the “Search for” field. For example, if the user desired to narrow the data to be analyzed to those non-polar compounds that have log K_{ow} values between 3 and 5, these could appear as additional search terms.

At times, it may be desirable to perform a search that includes multiple identifiers for a selected search category. For example, a search for multiple CASNOs or multiple ECOSAR categories. A refined search functionality is provided to perform this action using the “=” sign within the dropdown field containing Boolean descriptors. After selecting the “equals sign” the user can enter multiple terms for the category into the “Search for” field. Terms should be separated by a space backwards slash. This function is particularly useful in the search for multiple CASNOs, as an example.

Please note that direct entry by the user into the “Search” box is fully enabled for CASNOs. Other Search categories, such as taxonomic names, mode of action assignments, and so forth need to be entered by first developing the search string in a text editor (Word, Wordpad, Notepad, etc.) and pasting the string into the “Search for” field. The “=” term requires an exact match to the entry in the field chosen. As an example, if “the ECOSAR category chosen by the user is “Phenols”, the term “Phenol” will not return a hit.

CAUTION: If direct entry into the box is utilized for multiple terms the user cannot using the highlighting function to select the terms. Each term must be typed in or copied into the box a string from a text editor. Otherwise, only the most recent entry will appear in the search, not the entire string. Capitalization and correct spelling are essential.

Exporting a search to Excel

After clicking on the green "Download as Excel file" button, your database filter search will save to your "downloads" folder. A screenshot of a representative data file is provided below in Figure 12. The file structure (column placement, content) is essential to being compatible with the analysis tools. A summary of the information included in the downloadable file is provided in **Error! Reference source not found.**

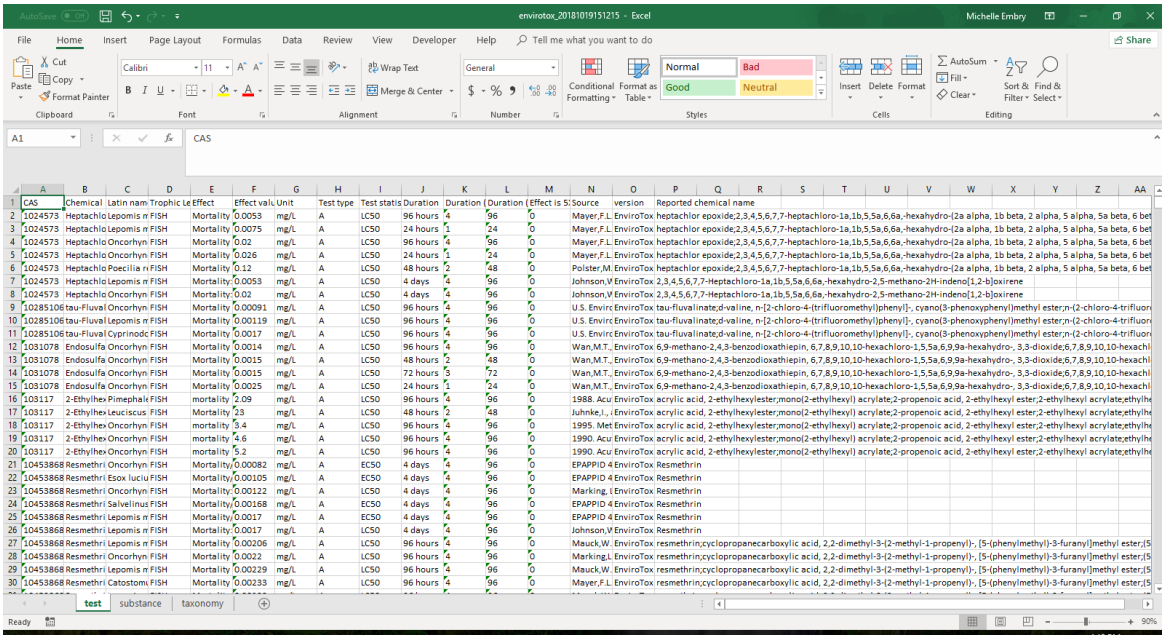


Figure 12. Excel file export

If a user desired to include additional data not in the EnviroTox Database, the additional data can be appended to the exported data file as described above. The file format for the output must be identical to those required by R analytical tools. If the file structure is maintained, additional analysis should be possible to conduct with no issue. The user is cautioned however that the analytics are on a publicly accessible server, with limited control. Analyses are not saved nor tracked by MTSU or HESI.

NOTE: The file output nomenclature does not contain the search terms used to develop the EXCEL output file. It is advisable to rename the output file to indicate the type of search and date for future reference by the user.

The output file is named as envirotox_YYYYMMDDHHMMSS where Y, D, H is the calendar date and H, M, S indicates military time at the moment the file was created. The file contains three tabs:

- Test – the exported data that was the subject of the database search;

- Substance – a listing of the substances and associated physical-chemical properties along with Mode of Action assignments;
- Taxonomy – a list of the taxa that were captured in the search.

Table 6. Output columns in an EnviroTox database search including the ecotoxicological information for each chemical as found in the "test" output tab.

CAS*	Harmonized CAS number as found in the Physical-chemical descriptor file
Chemical name*	Common name of the chemical
Latin name*	Genus and species name of the test organism
Trophic Level*	Designation as algae, invertebrate, fish, amphibian, plant, or fungi
Effect	Type of response, such as mortality, immobility, population growth rate
Effect value	Concentration at which the response was observed
Unit	Units associated with the Effect (universally mg/L)
Test type*	Acute or Chronic
Test statistic*	LC50, EC50, NOEC, etc.
Duration*	Duration of the test (varies, may be hours, days, months, etc.) given in text form
Duration (days)*	Duration of the test given numerically in days
Duration (hours)*	Duration of the test given numerically in hours
Effect is 5X above water solubility	Indicate as "0" or no or "1" as yes
Source	Information source
Version	Database release version
Reported chemical name	Common name as reported in the information source

**Terms that can be searched within the tool*

Table 7. Output columns in an EnviroTox database search including the physical-chemical property information for each chemical as found in the "substance" output tab.

CAS	Harmonized CAS number as found in the Physical-chemical descriptor file
original CAS	CASNO as cited in the original source may be incorrect based on all available information available
Chemical name	Common name of the chemical
Canonical SMILES	Smiles notation including counter ions (may not be usable in QSAR programs)
Desalted Canonical SMILES	Smiles notation without counter ions and used in MoA and chemical grouping assessments
log Kow	Octanol-water partition coefficient; measured values used if available, estimated values used if measured data is not available
Water Solubility (mg/L)	Aqueous solubility; measured values used if available, estimated values used if measured data is not available
MW (g/mol)	Molecular weight of desalted compound
Heavy Metals	Indicated as "0" if no metal is present, "1" if metals is present
Halogenated	Indicated as "0" if no halogen is present, "1" if halogen is present
ECOSAR classification	Primary ECOSAR classification based on chemical structure
US-EPA New Chemical Categories	Chemical class determined from ECOSAR plus expert judgement
TEST coded	Chemical found in TEST and its associated code
TEST	Expert judgement of TEST mode of action
ASTER coded	Chemical found in ASTER and its associated code
ASTER	Expert judgement of ASTER mode of action
OASIS coded	Chemical found in OASIS and its associated code
OASIS	Expert judgement of OASIS mode of action
Verhaar coded	Chemical found in Verhaar and its associated code
Actual Verhaar Category	Expert judgement of Verhaar mode of action
4 letter code	Combined codes of available mode of action assignments
Consensus MOA	Conclusion of the consensus mode of action
MOA Confidence score	Confidence in mode of action conclusion by expert panel

**Terms that can be searched within the tool*

Table 8. Output columns in an EnviroTox database search including the test species information for each chemical as found in the "species" output tab.

Latin name	Linnean hierarchical same in "Genus species" form
Trophic Level	Algae, Invertebrate, Fish, Plant, Amphibian, Fungi, bacteria. Conclusions on micro- and metazoan that are facultatively photosynthetic or heterotrophic made on the basis of physiological condition at the time of testing
Medium	Freshwater or saltwater; designation of medium based on the preferred ecological requirements of the test species and test conditions
Taxonomic kingdom	Taxonomic assignments based on authoritative entries taxonomic websites including https://www.ncbi.nlm.nih.gov, http://www.algaebase.org/ http://www.marinespecies.org/about.php, and http://fishbase.org/home.htm
Taxonomic phylum or division	As above
Taxonomic subphylum	As above
Taxonomic superclass	As above
Taxonomic class	As above
Taxonomic order	As above
Taxonomic family	As above

PNEC Calculator Tool

The user selects the geographic region for which the PNECs will be determined as seen below. Each region or federal regulatory authority has its own suite of considerations for PNEC determination. The combination of breadth of test species data with whether the data is of acute or chronic duration determines the Application Factors assigned to derive regional PNECs (see also Belanger et al., in preparation).

A data file is loaded by placing the cursor into the "Browse" box and navigating to the location of the Excel file containing the data be analyzed. As stated above, the file needs to conform to that structure initially distributed by the query to the user as this is the only file structure that is read by the system.

Once the file is uploaded, a quick summarization of the information is displayed that includes an enumeration of the number of toxicity data available (= Rows), the number of unique chemicals (= Chemicals), the breadth of taxa tested (= Species), the amount of acute data (= Acute) and the amount of chronic data (= Chronic) that is in the uploaded file.

The user can move to the Full PNEC Table tab which will display the chemical-by-chemical output. The information can be viewed by adjusting the vertical and horizontal scroll bars. The user can also indicate how many entries they would like to see at a time from 10, 25, 50 or 100 entries. This tab also provides the ability to perform two different exports of information associated with the chemical-specific PNECs in Excel format:
The full PNEC Table (see

- Table 9 below for a description of the PNEC Table contents)
- Geometric mean toxicity data by species for each chemical in the output

PNECs are regional in nature and reflect the level of conservatism applied to a local regulatory authority. Table 7 provides a summary of representative Application Factors applied to the most sensitive available aquatic toxicity data at various levels of data availability.

Table 7. A summary of various aquatic PNEC assessment factors

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

a) 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.
b) OECD (1992)
c) Zeeman and Gilford (1993), Nabholz (1991)
d) EU TGD refers to short and long term toxicity instead of acute and chronic toxicity.
e) see: http://www.meti.go.jp/policy/chemical_management/english/cscl/about.html, Japan Chemical Substance Control Law (accessed 28 March 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
f) Application factors generally assigned to the most sensitive data point available

The actual PNEC determination process is somewhat more complex than the above simplified table as a variety of data combinations may be available. For the purposes of the ecoTTC utilization of PNECs, the following logic diagrams were developed to allow the interpretation of EXCEL files and toxicity data by chemical into consistently applied Application Factors. Future regional PNEC determination logics will likely be added over time.

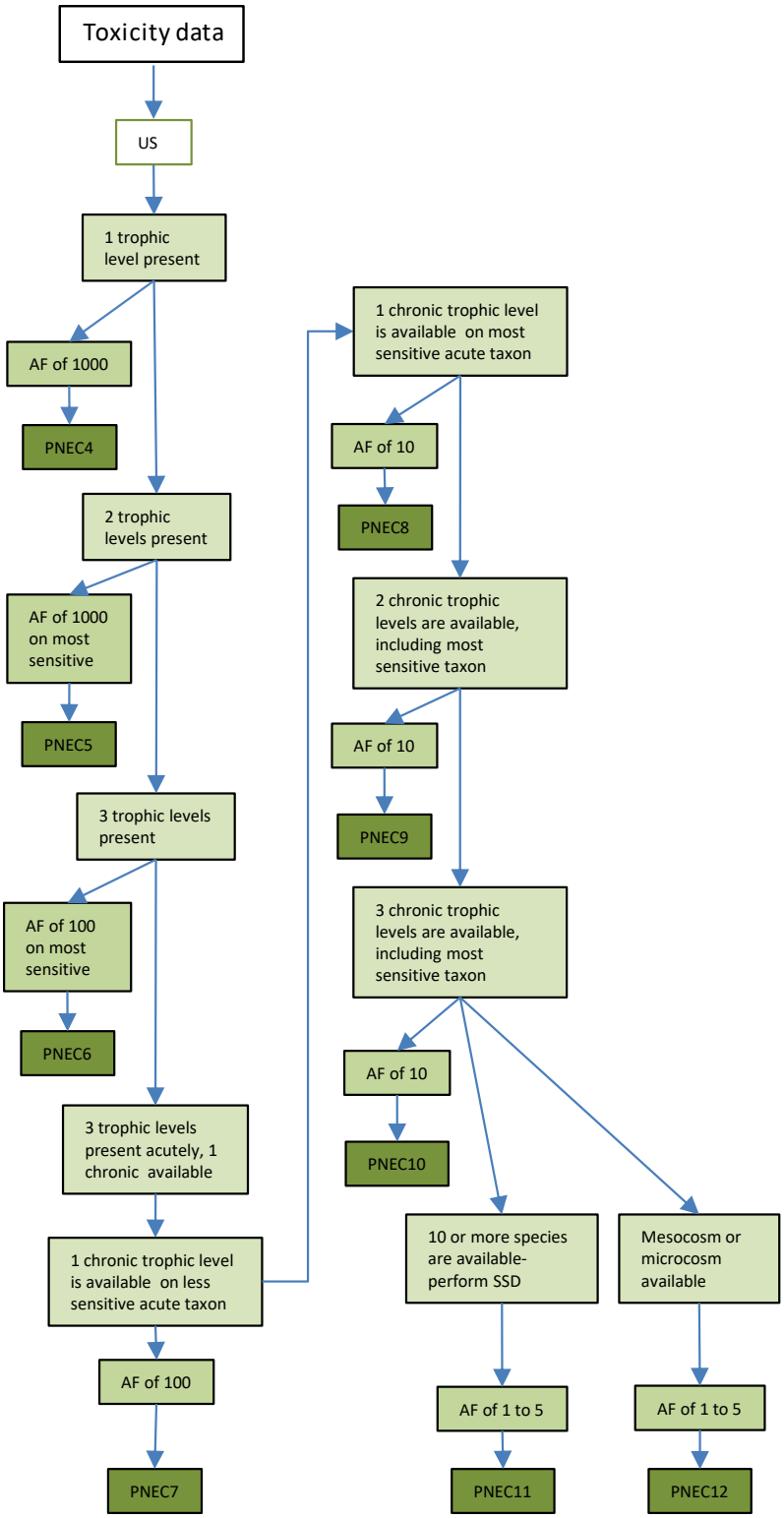


Figure 13. Application Factors assigned to different data combinations under US chemical assessment conditions.

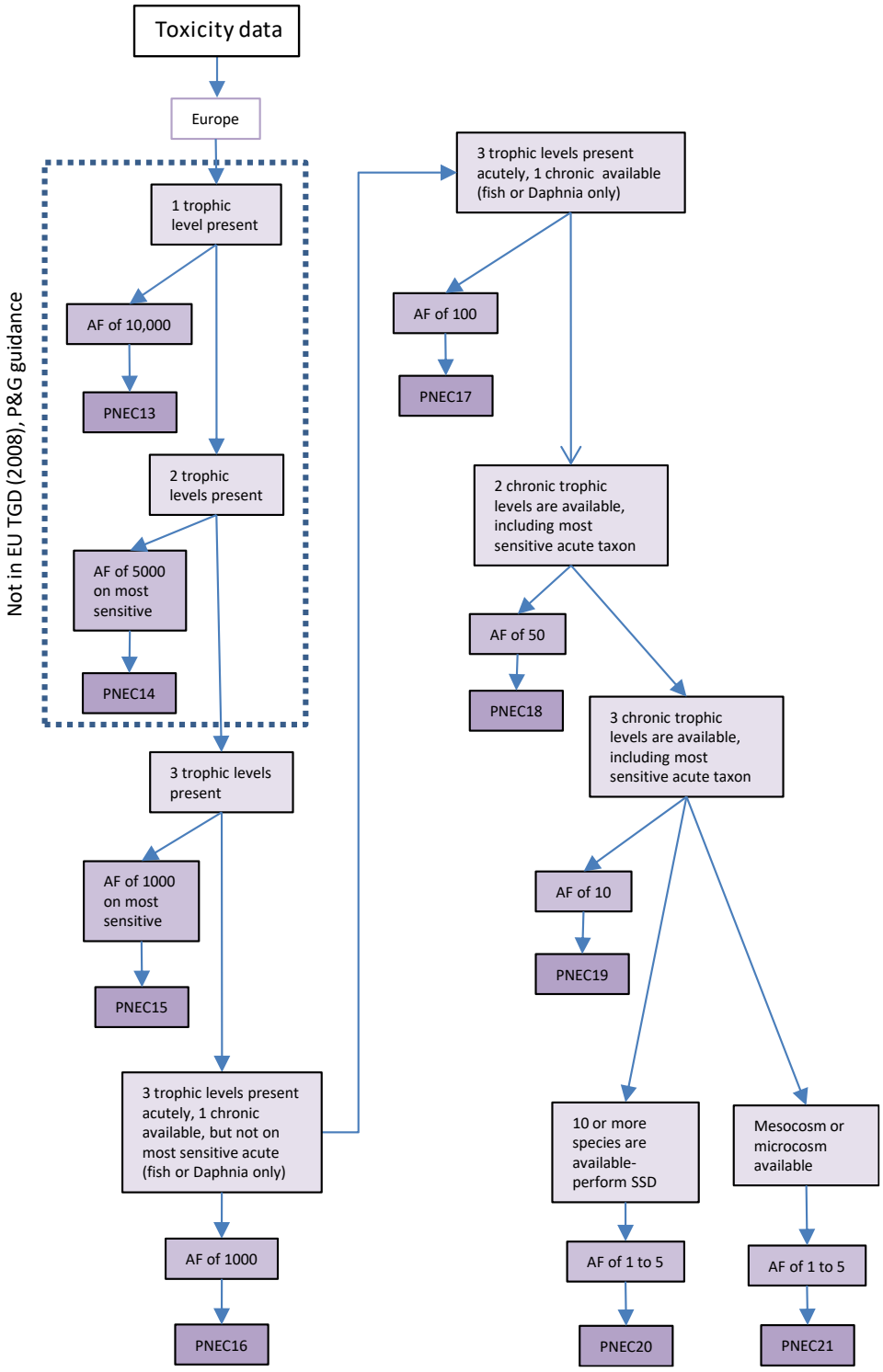


Figure 14. Application Factors assigned to different data combinations under European chemical assessment conditions.

Table 8 provides an overview of the different data combinations that are employed in the R-logic. Users should not interpret any of these as endorsements of the logic for regulatory application as actual decisions for chemical approval have many other factors associated with the decision-making process. Further, the PNEC derivations utilize a single AF assignment logic for a region when it is known that multiple assessment types may be employed. For example, in the US, industrial chemicals evaluated under TSCA (now The Lautenberg Chemical Safety Act of 2016) are evaluated differently from pesticides (evaluated and regulated under the Federal Insecticide, Fungicide, and Rodenticide Act). Below is a table describing the assignment of Applications Factors (AF) in the PNEC derivation logic.

Table 8. PNEC codes associated with various combinations of acute and chronic data and the application factors associated with each.

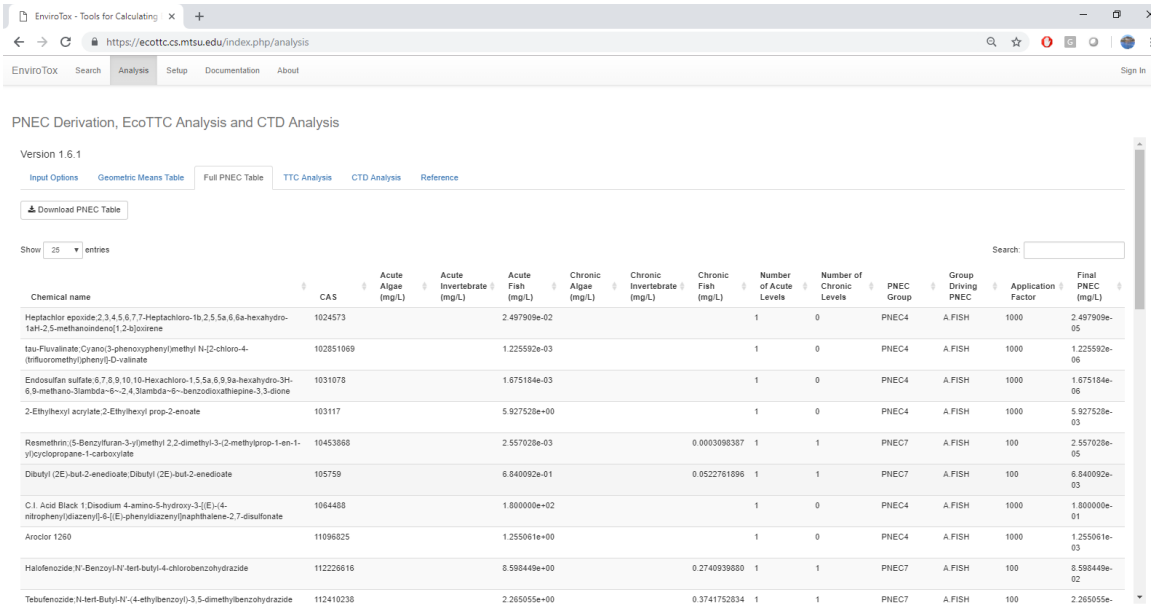
Region	PNEC Code	Data combination	AF Assigned
Unspecified, not part of a regulatory implementation; not currently implemented into ecoTTC			
	PNEC1	ecoTTC already available expressed as 5th percentile of PNECs in a group	1
	PNEC2	QSAR output for a local type QSAR (e.g., one for a specific group of homologous 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	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 including most sensitive acute taxon	10
	PNEC11	≥10 species chronic toxicity data; perform Species Sensitivity Distribution (SSD)	1-5[b]
	PNEC12	≥ 10 species chronic toxicity data; Mesocosm or microcosm	1-5[b]
Europe	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	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 including most sensitive taxon	10
	PNEC20	≥10 species chronic toxicity data; perform SSD	1-5[b]
	PNEC21	≥ 10 species chronic toxicity data; Mesocosm or microcosm	1-5[b]

[a] *Not formally a part of the European hazard assessment methodology (no data no market, with <3 acute species data)*

[b] *Decided on a case-by-case basis*

Using the PNEC derivation processes in Figure 13 and Figure 14 followed by description of the PNEC groups in Table 7, the user can interpret the PNEC output as given by the ecoTTC application.

Figure 15 provides a screen shot of the Full PNEC Table tab. The table was constructed for a query employing the US PNEC algorithm. PNEC groupings may be especially useful to evaluate in greater detail the consequences of having more and less data for a given chemical data set for example.



Chemical name	CAS	Acute Algae (mg/L)	Acute Invertebrate (mg/L)	Acute Fish (mg/L)	Chronic Algae (mg/L)	Chronic Invertebrate (mg/L)	Chronic Fish (mg/L)	Number of Acute Levels	Number of Chronic Levels	PNEC Group	Group Driving PNEC	Application Factor	Final PNEC (mg/L)
Heptachlor epoxide;2,3,4,5,6,7,7-Heptachloro-1b,2,5,5a,6,6a-hexahydro-1aH-2,5-methanoindeno[1,2-b]oxirene	1024573			2.497909e-02				1	0	PNEC4	A.FISH	1000	2.497909e-05
tau-Fluvalinate;Cyano(3-phenoxyphenyl)methyl N-[2-chloro-4-(trifluoromethyl)phenyl]-D-valinate	102851069			1.225592e-03				1	0	PNEC4	A.FISH	1000	1.225592e-06
Endosulfan sulfate;6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-3H-6,9-methano-3lambda~6~,2,4,3lambda~6~-benzodioxathiepine-3,3-dione	1031078			1.675184e-03				1	0	PNEC4	A.FISH	1000	1.675184e-06
2-Ethylhexyl acrylate;2-Ethylhexyl prop-2-enoate	103117			5.927528e+00				1	0	PNEC4	A.FISH	1000	5.927528e-03
Resmethrin;(5-Benzylfuran-3-yl)methyl 2,2-dimethyl-3-(2-methylprop-1-en-1-yl)cyclopropane-1-carboxylate	10453868			2.557028e-03			0.0003098387	1	1	PNEC7	A.FISH	100	2.557028e-05
Dibutyl (2E)-but-2-enedioate;Dibutyl (2E)-but-2-enedioate	105759			6.840092e-01			0.0522761896	1	1	PNEC7	A.FISH	100	6.840092e-03
C.I. Acid Black 1;Disodium 4-amino-5-hydroxy-3-[(E)-(4-nitrophenyl)diazenyl]-6-[(E)-phenyldiazenyl]naphthalene-2,7-disulfonate	1064488			1.800000e+02				1	0	PNEC4	A.FISH	1000	1.800000e-01
Aroclor 1260	11096825			1.255061e+00				1	0	PNEC4	A.FISH	1000	1.255061e-03
Halofenozide;N'-Benzoyl-N'-tert-butyl-4-chlorobenzohydrazide	112226616			8.598449e+00			0.2740939680	1	1	PNEC7	A.FISH	100	8.598449e-02
Tebufenozide;N-tert-Butyl-N'-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide	112410238			2.265055e+00			0.3741752834	1	1	PNEC7	A.FISH	100	2.265055e-

Figure 15. PNEC tool table output

Table 9. Contents of the Full PNEC Table with explanations

Chemical name	Common chemical name
CAS	CAS number
Acute Algae (ug/L)	Geometric mean acute algae result in µg/L
Acute Invertebrate (ug/L)	Geometric mean acute invertebrate result in µg/L
Acute Fish (ug/L)	Geometric mean acute fish result in µg/L
Chronic Algae (ug/L)	Geometric mean chronic algae result in µg/L
Chronic Invertebrate (ug/L)	Geometric mean chronic invertebrate result in µg/L
Chronic Fish (ug/L)	Geometric mean chronic fish result in µg/L
Number of Acute Levels	Number of trophic levels with acute data
Number of Chronic Levels	Number of trophic levels with chronic data
PNEC Group	PNEC group defined by the particular combination of acute and chronic data for a chemical
Group Driving PNEC	Most sensitive taxonomic group
Application Factor	Application Factor assigned for the particular combination of acute and chronic data for a chemical
Final PNEC (ug/L)	Final PNEC value (lowest relevant toxicity data/AF)

The PNEC Group is defined by the particular combination of acute and chronic toxicity data available and follows the regional PNEC determination logic.

Initial data visualization tools are provided to the user which may be useful and are visible by scrolling to the bottom of the PNEC table. This summary information depicts a heat map (Figure 16) of the available acute and chronic data and the associated acute or chronic categorizations for the associated PNEC Table file is displayed along with an enumeration of the different PNEC groupings.

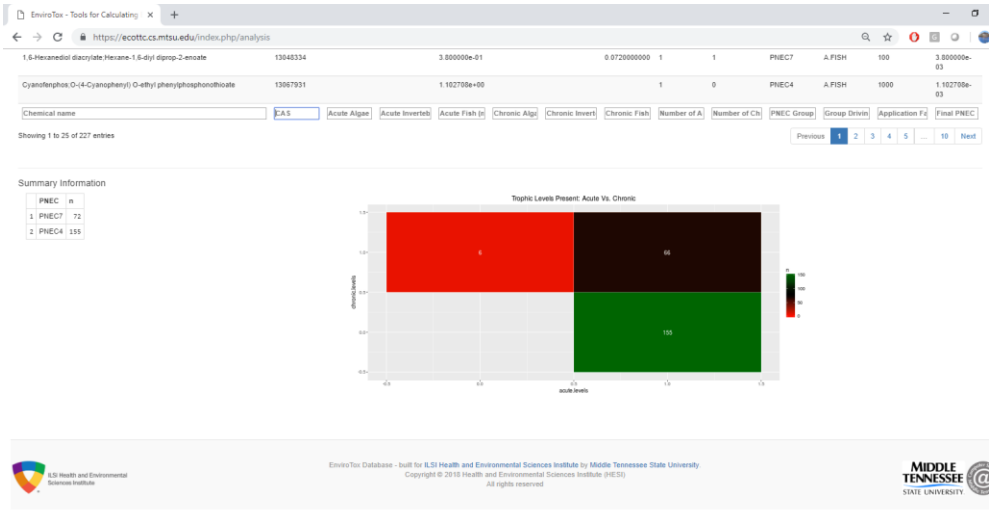


Figure 16. Heat-map of acute and chronic data within the PNEC tool

A table with geometric means by species (Figure 17) can also be viewed and downloaded by selecting the appropriate tab on the analysis screen. This table can also be downloaded and may be useful for a variety of other analytical purposes such as Species Sensitivity Distribution analysis.

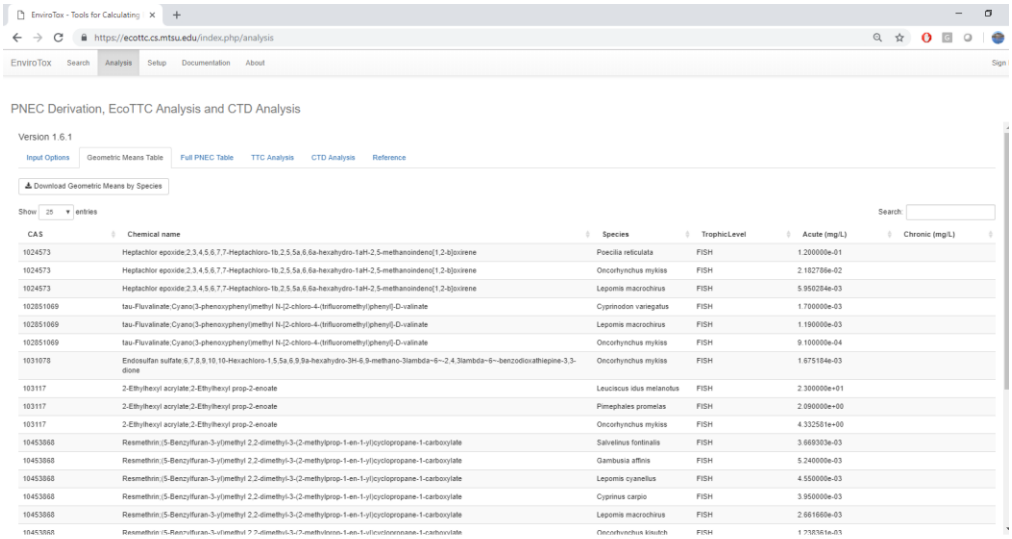


Figure 17. Table with geometric means by species

ecoTTC Tool

The tab labeled “TTC Analysis” is dedicated to the calculation of an ecoTTC for the chosen/queried data set which was uploaded for PNEC determination and the selected region. The user will see several items in this display which deserve attention (Figure 18).

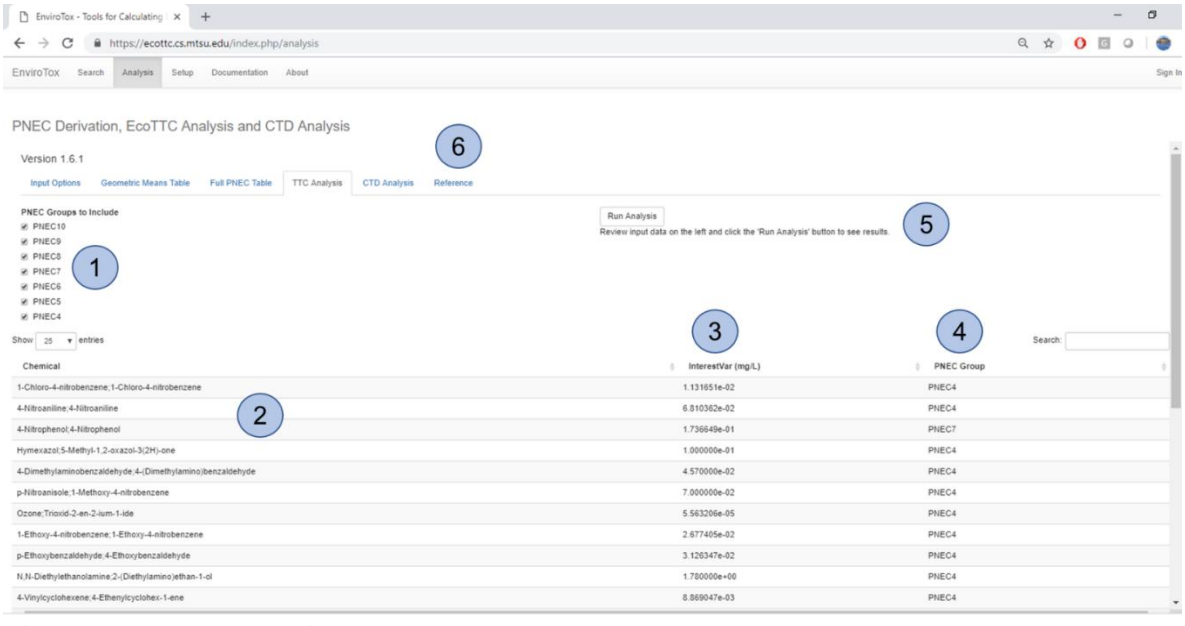


Figure 18. ecoTTC tool page

1. PNEC Groups to Include: PNECs can vary in their “quality” in that the underlying ecotoxicological data may be composed of only acute data on one species to numerous acute and chronic data on a range of species. The actual combination of available data dictates the Application Factor applied to usually the most sensitive acute or chronic information available. The default ecoTTC is calculated using all available PNECs but under some conditions, the user can choose which PNECs to include. Very large data sets may have sufficient information to utilize only chemical data sets where the underlying ecotoxicological data is fully complete as an example. The definition of PNEC Groups is provided in the Reference tab in this screen.
2. Chemical: This region displays the compounds included in the initial upload and for which chosen PNEC groups are to be used. The user chooses to view 10, 25, 50, or 100 entries using the drop down to the left and above the Chemical name column.
3. InterestVar: This is the final PNEC value for the chemical.
4. PNEC Group: The PNEC grouping to which the chemical belongs based on the completeness of the underlying ecotoxicological data.
5. Run Analysis: This button initiates the computation of the ecoTTC.
6. Reference: This tab provides a convenient table of the generic Application Factors assigned to the available ecotoxicological data for the chemicals. Depending on whether US or Europe was chosen for the initial PNEC determination, a different table of Application Factors will be displayed.

ecoTTC analysis

Once the analysis is initiated (Run Analysis button), progress towards completion is tracked in the upper right of the screen (progress tracker). Upon completion, a screen similar to the one shown in Figure 19 is displayed. Depending on the user’s display settings, horizontal and vertical scroll bars are often displayed which can provide a view of additional information.

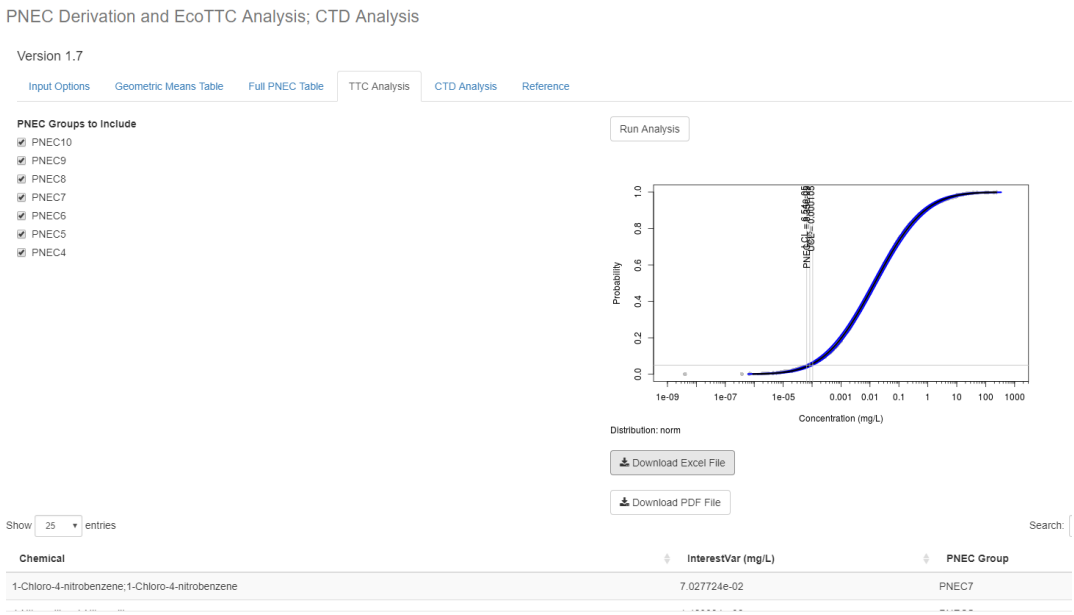


Figure 19. ecoTTC analysis

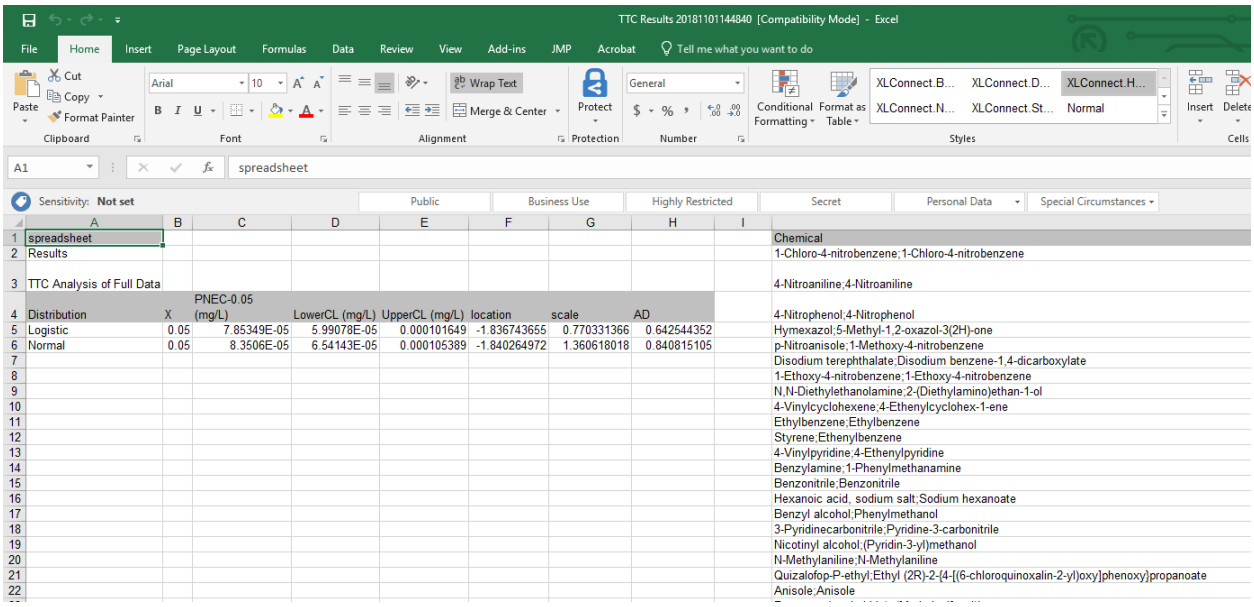
A thumbnail representation of the log normal cumulative frequency distribution of PNECs is displayed for convenience (note that the log-logistic distribution will also be found in the pdf output). The fifth percentile PNEC (=ecoTTC or PNEC0.05) and associated upper and lower confidence intervals are also given. Very importantly, a high- resolution pdf of all graphical outputs can be downloaded as well as a file of all statistical characterizations available for the calculated distributions. Note that the thumbnail will only be for the assumed log-normal distribution but both a log-normal and log-logistic analysis are available in the downloaded files.

The ecoTTC tool fits two distributions to each set of data provided: normal and logistic. Technically, all calculations are performed by fitting normal and logistic distributions to the log10-transformed concentration values, and fifth percentile estimates from these distributions are reported by back-transforming to the measured concentration scale. Note that this is equivalent to fitting log-normal/log-logistic distributions to the measured concentration scale of the data.

Both the normal and logistic distributions are symmetric about their centers and are generally difficult to differentiate in data analyses unless there is a large amount of data provided. Generally speaking, the logistic distribution has heavier tails, or a slightly wider spread than does the normal distribution. Both the logistic and normal distributions are defined by two parameters: a location parameter, relating to the mean of the data, and a scale parameter, relating to the variability or spread of the data.

For each distribution, calculations are performed to estimate these parameters, and subsequently derive a joint confidence set for the two parameters of the distribution, from which confidence intervals on percentiles of the distribution can be calculated. Concrete guidance to the user cannot be advised solely on the statistical outcomes as with all PNEC implementations, these can also reflect matters of environmental policy (e.g., the level of conservativeness employed). In general, the developers advise to use the distribution which provides the best empirical fit to the data, regardless of the 5^{th} percentile calculated outcome. Individual data points at the tails, which may profoundly influence the distribution fits can, be further inspected as to their inherent study qualities. Censoring of data can be done but should be fully documented and justified.

The analysis output file in Excel is shown in Figure 20, and for each distribution, the PNEC0.05 estimate (the 5th percentile of the PNEC distribution), a confidence interval on the PNEC0.05, the location and scale estimates from the log-scale fit, and an Anderson-Darling (AD) goodness-of-fit test p-value are provided. A significant AD p-value generally indicates that distribution is not a good fit to the data, however, it could also reflect the presence of outliers, which should be considered. For large sets of data, it may prove more difficult to identify a distribution that fits the data well. Note that because the logistic distribution has heavier tails than the log-normal distribution, the log-logistic is less sensitive to the presence of outliers.



	A	B	C	D	E	F	G	H	I	
1	spreadsheet									Chemical
2	Results									1-Chloro-4-nitrobenzene;1-Chloro-4-nitrobenzene
3	TTC Analysis of Full Data									4-Nitroaniline;4-Nitroaniline
4	Distribution	X	PNEC-0.05 (mg/L)	LowerCL (mg/L)	UpperCL (mg/L)	location	scale	AD		4-Nitrophenol;4-Nitrophenol
5	Logistic	0.05	7.85349E-05	5.99078E-05	0.000101649	-1.836743655	0.770331366	0.642544352		Hymexazol;5-Methyl-1,2-oxazol-3(2H)-one
6	Normal	0.05	8.3506E-05	6.54143E-05	0.000105389	-1.840264972	1.360618018	0.840815105		p-Nitroanisole;1-Methoxy-4-nitrobenzene
7										Disodium terephthalate;Disodium benzene-1,4-dicarboxylate
8										1-Ethoxy-4-nitrobenzene;1-Ethoxy-4-nitrobenzene
9										N,N-Diethylethanolamine;2-(Diethylamino)ethan-1-ol
10										4-Vinylcyclohexene;4-Ethenylcyclohex-1-ene
11										Ethylbenzene;Ethylbenzene
12										Styrene;Ethenylbenzene
13										4-Vinylpyridine;4-Ethenylpyridine
14										Benzylamine;1-Phenylmethanamine
15										Benzonitrile;Benzonitrile
16										Hexanoic acid, sodium salt;Sodium hexanoate
17										Benzyl alcohol;Phenylmethanol
18										3-Pyridinecarbonitrile;Pyridine-3-carbonitrile
19										Nicotinyl alcohol;(Pyridin-3-yl)methanol
20										N-Methylaniline;N-Methylaniline
21										Quizalofop-P-ethyl;Ethyl (2R)-2-[4-[(6-chloroquinoxalin-2-yl)oxy]phenoxy]propanoate
22										Anisole;Anisole

Figure 20. Excel download of ecoTTC analysis

The pdf plot output consists of scale location diagrams, cumulative probability plots and distribution density plots for both normal and logistic distributions. It is up to the user to ascertain the most appropriate model and plot for their application.

- *Scale-location diagram*: This plot (Figure 21) is primarily included as a quality check. The points represent combinations of the two parameters (after log10 transformation, location on the x-axis and scale on the y-axis) that determine the shape of the distribution. Points inside of the light-blue line represent parameter combinations that would be considered reasonable for the data. The colored region should be approximately elliptical. If the shape of this region is not elliptical, it may be an indication that the distribution is not a good fit, or other problems with the data. Each point inside of the ellipse corresponds to a unique distribution, with its own HC5 estimate. The range of these HC5 estimates from within the confidence region defines the HC5 confidence interval. Other percentile confidence intervals (blue lines in the following figure) are calculated similarly. The plot below is for a representative data set conforming to the desire for an elliptical shape and visual confidence boundaries.

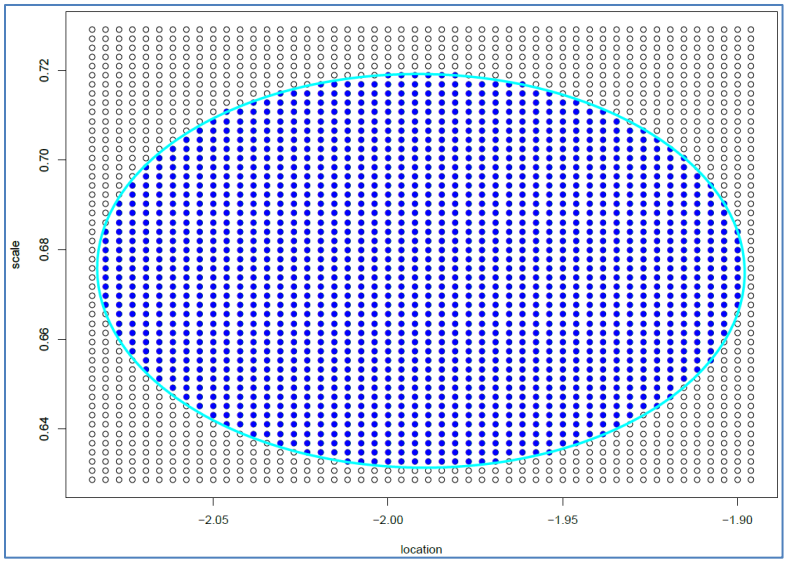


Figure 21. Scale-location diagram

- *Cumulative Probability*: The black line in Figure 22 represents the best reasonable fit for each distribution and the blue lines show the 95% confidence interval at each concentration. Systematic departures of the raw data points from these lines, especially those falling outside of the confidence interval may indicate that the distribution type (logistic or normal) is not a good fit for the data. It could also indicate that there are outliers in the data that are influencing the estimation of distribution parameters. Data at the tails of the distribution (very high and/or very low concentrations) can heavily influence estimation of distribution parameters and cause a poor fit. The ecoTTC point estimate (HC5 label in the graph as a vertical line) is equivalent to the 5th percentile PNEC of the distribution. Individual points (gray) represent a distinct chemical. LCL and UCL are the Lower and Upper 95% confidence limit estimates around each concentration.

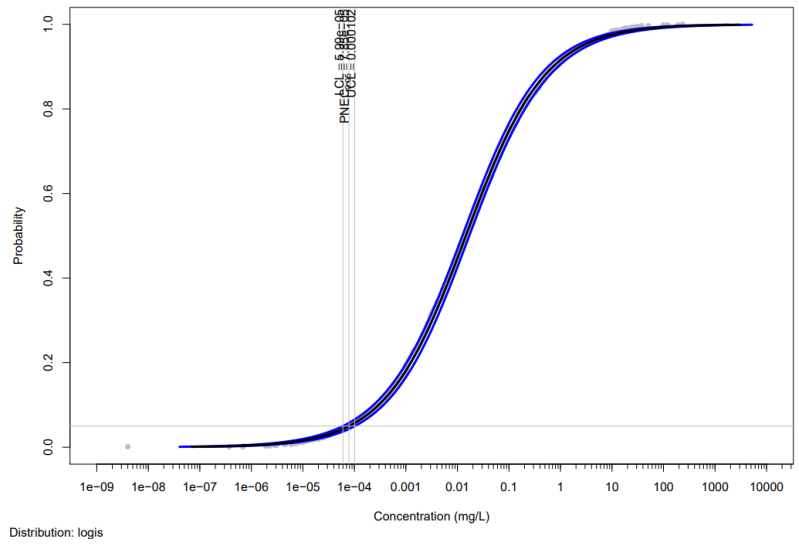


Figure 22. Cumulative probability distribution

- *Distribution Density*: This is an alternative visualization of the fit and confidence interval (Figure 23). The best-fitting distribution to the data is shown in black, while the best-fitting distributions that are restricted to have 5th percentiles equal to the confidence interval limits are shown in color. The plot is intended to show the range of reasonable distributions that could fit the data. The raw data density is represented just above the x-axis with the gray ticks. The higher the sample size, the closer these distributions will look to each other. HC_5, LCL and UCL have the same meanings as in the cumulative distribution.

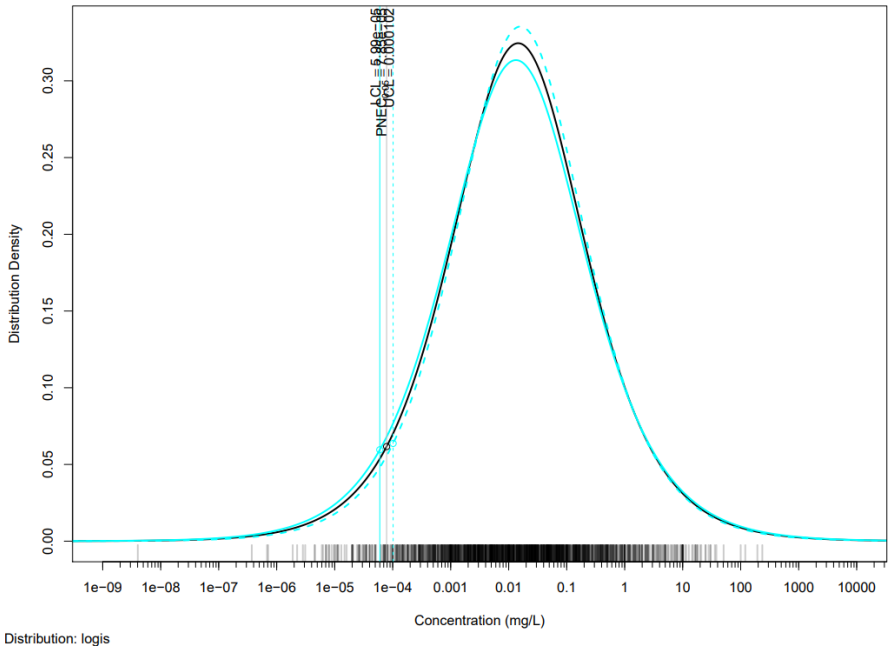


Figure 23. Distribution density

In cases where both the normal and logistic clearly do not fit the data, the expertise of a trained statistician may be required. While alternative distributional choices can be explored, the ecoTTC analysis may contain sufficient data (N>=80) that nonparametric estimation of the PNEC0.05 is possible (see for example, Hahn and Meeker 1991), avoiding the need to choose a distribution, and being robust to outliers, particularly on the high end of the distribution. Future versions of this tool may expand the distributions available for analysis.

Most outputs from the ecoTTC database search, PNEC algorithms, and ecoTTC calculations can easily be re-formatted and applied to other statistical and/or graphics display programs as they are essentially Excel flat files.

Chemical Toxicity Distributions (CTD)

The ecoTTC approach relies on the development of chemical and region specific PNEC values using regulatory assigned application factors. These PNEC values are then statistically modeled to derive a PNEC0.05. Note here that there is a clear distinction between the PNEC0.05 and an HC5 typically developed as an output from Species Sensitivity Distribution analysis. There may be situations where a researcher or assessor is interested in the toxicity distributions, without

the added conservatism introduced by the assessment factors or the regional overtones of their application. A chemical toxicity distribution (CTD) can be used to perform this type of analysis.

ecoTTCs and CTDs rely on the same underlying theory and statistical methods. These approaches differ solely based on the type of input data: ecoTTCs contain distributions of PNECs and CTDs contain distributions of hazard values. The output from CTD analysis is termed the CTD0.XX, where XX indicates a chosen percentile of the distribution.

Traditionally, CTDs have been used to probabilistically model hazard values from a single species and test type (e.g., acute *Daphnia* toxicity tests; Williams et al 2011). Slight modifications of this approach have been included in the CTD Analysis tool to allow for CTDs to be performed at single species level, trophic level, or incorporating all trophic levels. Additionally, the tool allows for CTDs to be constructed with just acute or just chronic data, or chronic data supplemented with acute values. Users need to carefully examine the data being loaded into the CTD tool and critically think about how the CTD results may be influenced by the relative contribution of data from different species, trophic levels, and experimental durations.

CTD analysis can be conducted with the same exported Excel data file used in an ecoTTC analysis. To initiate data analysis, the user should click on the “Analysis Tab”.

A data file is loaded in the usual manner by placing the cursor into the “Browse” box and navigating to the location of the Excel file containing the data to be analyzed. A “PNEC region” does not need to be selected, as no assessment factors will be used and no PNECs will need to be derived.

Once the file is uploaded a quick summarization of the information is displayed that includes an enumeration of the number of toxicity data available (=Rows), the number of unique chemicals (= Chemicals), the breadth of taxa tested (=Species), the amount of acute data (=Acute) and the amount of chronic data (=Chronic) that is in the uploaded file.

After the data file has been loaded, the user should click on the “CTD Analysis” tab. Here, the user will decide what type of data will be included within the CTD analysis (Figure 24).

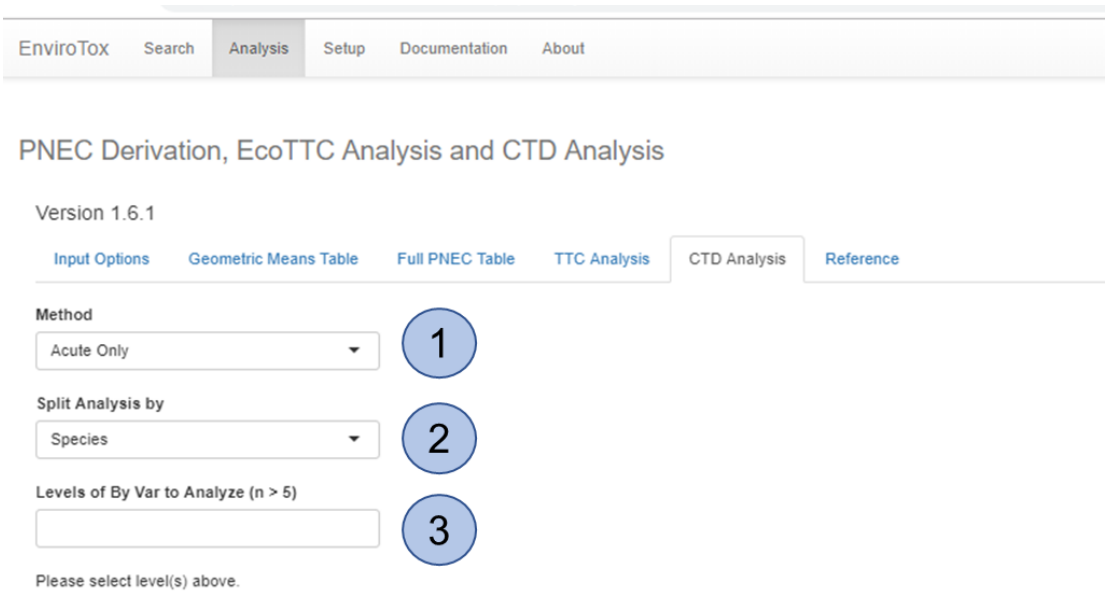


Figure 24. CTD tool page

1. Method: Here the user choses which type of data will be included within the CTD:
    a. "Acute Only".
    b. "Chronic Only".
    c. "Chronic Supplemented with Acute"

    The "Acute Only" and "Chronic Only" data are self-explanatory. This selects only data that is of a specific test type.

    The "Chronic Supplemented with Acute" data is generated in several steps. First, all chronic data is collected. If a chemical has acute toxicity data, but not chronic toxicity data, an Acute-to-Chronic ratio (ACR) is applied to convert the acute data point into a "chronic" value. This value is supplemented to the "Chronic Only" dataset.

    The applied ACR is chosen based on the trophic level of the test organism. Acute fish and invertebrate data are divided by an ACR of 10 to generate a chronic toxicity value. Acute algae data is divided by an ACR of 4 to generate a chronic toxicity value. The algal ACR is a special case of acute-chronic toxicity extrapolation. Algal chronic toxicity endpoints and statistics are developed on the exact same information as required for acute inhibition. The statistical algorithm is altered to provide a lower level of inhibition for chronic versus acute toxicity (Brill, et al., in preparation).

2. Split Analysis by: This drop-down menu lets the user decide if the CTD should be performed with:
    a. "Species". An individual species, like a traditional CTD.
    b. "Trophic level". A specific trophic level (e.g. Fish, Invert, Algae).
    c. "All". This selects all data.

3. Levels of by Var to Analyze (n>5): The user manually types in the name of the species or trophic level they wish to analyze. Only options that have a minimum of 5 unique CAS will be accepted. This prevents the CTD tool from generating non-sensical distributions. This window will not be present if you have selected to run “Split Analysis by: All”, as no further data parsing is required to run this option.

It is worth noting that a 5-data point CTD (or ecoTTC) is not considered to be robust, and should be interpreted with extreme caution. A minimum of 8 data points is suggested, though the predictive power of the model will increase with 10+ data points.

After you have made your selection, a completed summary table of the data will appear (Figure 25) In the example below, the following selections have been made: “Chronic Supplemented with Acute”. “Trophic Level”, “ALGAE”.

Table 10 provides an overview of the data used in the CTD analysis.

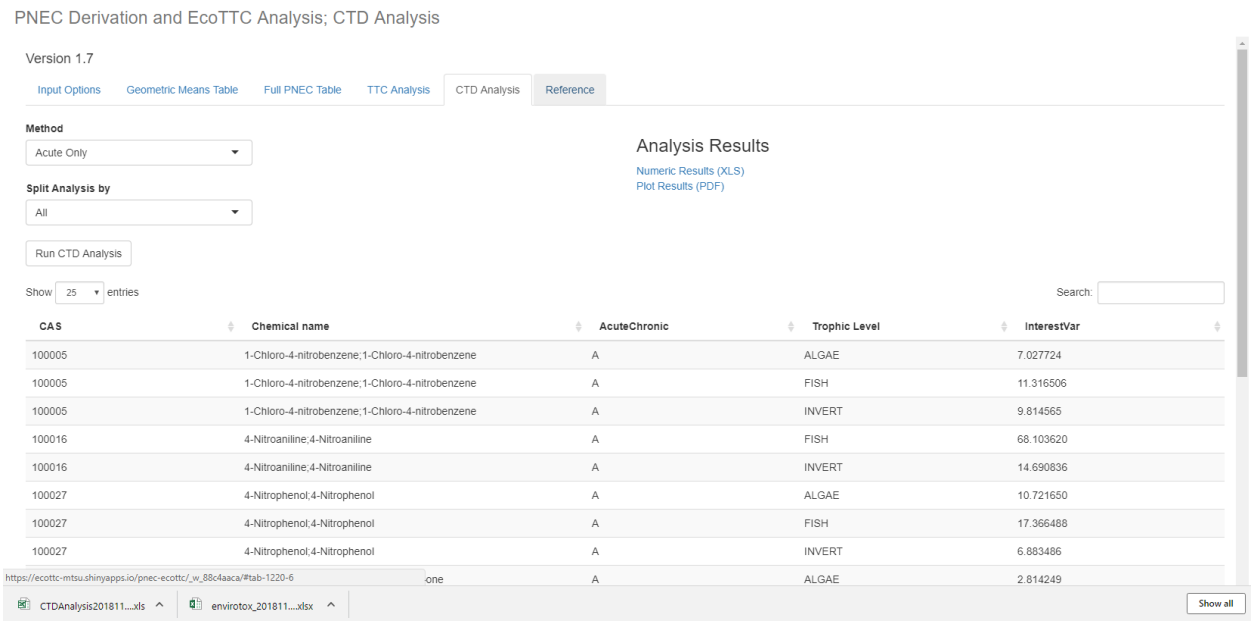


Figure 25. CTD analysis

Table 10. Contents of the CTD summary table with explanations

CAS	CAS number
Chemical name	Common chemical name
Trophic level	Trophic level of the species contained within the CTD. This column reflects the choices made in the "Split Analysis By" drop-down menu.
Acute (mg/L)	A geometric mean of all acute toxicity values contained within this database subset for a specific CAS.
Chronic (mg/L)	A geometric mean of all chronic toxicity values contained within this database subset for a specific CAS.
ACR Adjustment	The Acute to Chronic Ratio (ACR) that was applied to derive the final value. If no chronic toxicity value is present, and ACR of 3 (algae) or 10 (fish, inverts) would be applied to acute toxicity data. If chronic toxicity data is present, no ACR is needed. An ACR of 1 is written to show that the chronic toxicity value was used.
InterestVar	The final toxicity value (mg/L) that will be loaded into the CTD calculation.

After the analysis methods have been assigned, a geometric mean is calculated for each CAS. If data is summarized on a "Trophic Level", a CAS-level geometric mean would be calculated for all species within that trophic level. If the data is summarized using "All" data, a geometric mean would be calculated using all data. This geometric mean is *not* weighed based on species representation or by trophic level representation.

We recommend carefully examining the data file you want to analyze before making decisions on how to best summarize the data in a CTD. Trophic level CTDs or CTDs using all data may be heavily influenced by the relative make-up of the data. For example, if a chemical had 4 studies with *Daphnia magna* and 30 studies by various Algae species, all studies would be treated equally. The geometric mean for this CAS would be heavily influenced by the Algal data.

After the data analysis decisions have been made the button "Run CTD Analysis" will appear. Clicking this button will initiate the CTD analysis to occur. Two outputs from this analysis will be generated: "Numeric Results (XLS)" and "Plot Results (PDF)". These results are analogous to the ecoTTC outputs.

The Numeric Results in the CTD run file page is named CTDAnalysisYYYMMDDHHMMSS using the same time-date stamp sequence as in the ecoTTC output file. The CTD analysis includes both logistic and normal distribution statistics and also provides estimates of the 1st, 5th, 10th, 25th, and 50th percentile of the CTD distribution. The 1st percentile is absent 95% confidence limits as experience has shown that these are so wide as to require advanced estimation methodologies not presently in the R scripts running behind the EnviroTox database. Graphical output is similarly named and downloadable as a pdf file.

References

Barron MG, Lilavois CR, Martin TM. 2015. MoaTox: A comprehensive mode of action and acute aquatic toxicity database for predictive model development. Aquatic toxicology (Amsterdam, Netherlands). 161:102-107.

Barron MG, Wharton SR. 2005. Survey of methodologies for developing media screening values for ecological risk assessment. Int Env Ass Man 1:320-332.

Beasley A, Belanger SE, Otter RR. 2015. Stepwise information-filtering tool (sift): A method for using risk assessment metadata in a nontraditional way. Environmental toxicology and chemistry. 34(6):1436-1442.

Bejarano AC, Farr JK, Jenne P, Chu V, Hielscher A. 2016. The chemical aquatic fate and effects database (cafe), a tool that supports assessments of chemical spills in aquatic environments. Environmental toxicology and chemistry. 35(6):1576-1586.

Belanger SE, Rawlings JM, Carr GJ. 2013. Use of fish embryo toxicity tests for the prediction of acute fish toxicity to chemicals. Environmental toxicology and chemistry. 32(8):1768-1783.

Belanger SE, Sanderson H, Embry MR, Coady K, DeZwart D, Farr BA, Gutsell S, Halder M, Sternberg R, Wilson P. 2015. It is time to develop ecological thresholds of toxicological concern to assist environmental hazard assessment. Environmental toxicology and chemistry. 34(12):2864-2869.

Belanger SE, Beasley A, Brill JL, Krailler J, Connors KA, Embry MR, Barron M, Kienzler A. Comparisons of PNEC Derivation Logic Flows Under Various Regulatory Schemes and Implications for eco-TTC. In Preparation for Environmental Toxicology and Chemistry.

Brill, JL, Belanger SE, Carr GJ, Connors KA, Barron MG, Embry MR. Acute-chronic toxicity relationships for algal inhibition and toxicity assessment. In preparation for Environmental Toxicology and Chemistry.

Busquet F, Strecker R, Rawlings JM, Belanger SE, Braunbeck T, Carr GJ, Cenijn P, Fochtman P, Gourmelon A, Hubler N et al. 2014. Oecd validation study to assess intra- and inter-laboratory reproducibility of the zebrafish embryo toxicity test for acute aquatic toxicity testing. Regulatory toxicology and pharmacology. 69(3):496-511.

Connors KA, BeasleyA, Barron M, Belanger SE, Bonnell M, Brill JL, de Zwart D, Kienzler A, Krailler J, Otter R, Phillips J, Embry MR. Creation of a Curated Aquatic Toxicology Database: EnviroTox. In Review.

de Wolf W, Siebel-Sauer A, Lecloux A, Koch V, Holt M, Feijtel T, Comber M, Boeije G. 2005. Mode of action and aquatic exposure thresholds of no concern. Environmental toxicology and chemistry. 24(2):479-485.

De Zwart D. 2002. Observed regularities in species sensitivity distributions for aquatic species.

ECCC (Environment and Climate Change Canada). 2018. Canadian Domestic Substances List. Accessed 02 October 2018. Available at: https://www.canada.ca/en/environment-climate-change/services/canadian-environmental-protection-act-registry/substances-list/domestic.html

ECHA (European Chemicals Agency). 2018. Registered Substances Database. Accessed 09 September 2016. Available at: https://echa.europa.eu/information-on-chemicals/registered-substances

EC (European Commission). 2007. Corrigendum to Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Offic J Eur Union L136:3–282. See also: http://eur-lex.europa.eu/

Gross M, Daginnus K, Deviller G, de Wolf W, Dungey S, Galli C, Gourmelon A, Jacobs M, Matthiessen P, Micheletti C, Nestmann E, Pavan M, Paya-Perez A, Ratte HT, Safford B, Sokull-Kl€uttgen B, Stock F, Stolzenberg HC, Wheeler J, Willuhn M, Worth A, Comenges JMZ, Crane M. 2010. Thresholds of toxicological concern for endocrine active substances in the aquatic environment. Integr Environ Assess Manag 6:2–11.

Gutsell S, Hodges G, Marshall S, Roberts J. 2015. Ecotoxicological thresholds—Practical aplications in an industrial inventory. Environ Toxicol Chem 34:935–942.

Hahn T, Diamond J, Dobson S, Howe P, Kielhorn J, Koennecker G, Lee-Steere C, Mangelsdorf I, Schneider U, Sugaya Y, Taylor K, Van Dam R, Stauber JL. 2014. Predicted No Effect Concentration derivation as a significant source of variability in environmental hazard assessments of chemicals in aquatic systems: An international analysis. Integr Environ Assess Manag 10:30–36.

Hall AT, Belanger SE, Guiney PD, Galay-Burgos M, Maack G, Stubblefield W, Martin O. 2017. New approach to weight-of-evidence assessment of ecotoxicological effects in regulatory decision-making. Integrated environmental assessment and management. 13(4):573-579.

Hendriks AJ, Awkerman JA, de Zwart D, Huijbregts MAJ. 2013. Sensitivity of species to chemicals: Dose-response characteristics for various test types (LC50, LR50 and LD50) and modes of action. Ecotoxicol Environ Saf 97:10–16.

Hutchinson TH, Barrett S, Buzby M, Constable D, Hartmann A, Hayes E, Huggett D, Laenge R, Lillicrap AD, Straub JO et al. 2003. A strategy to reduce the numbers of fish used in acute ecotoxicity testing of pharmaceuticals. Environmental toxicology and chemistry. 22(12):3031-3036.

ICCA (International Council of Chemical Associations). 2018. High Production Volume (HPV) Chemicals Challenge Program. Accessed 02 October 2018. Available at: http://www.icca-chem.org/

Jeram S, Sintes JM, Halder M, Fentanes JB, Sokull-Kluttgen B, Hutchinson TH. 2005. A strategy to reduce the use of fish in acute ecotoxicity testing of new chemical substances notified in the european union. Regulatory toxicology and pharmacology : RTP. 42(2):218-224.

Kienzler A, Barron MG, Belanger SE, Beasley A, Embry MR. 2017. Mode of action (moa) assignment classifications for ecotoxicology: An evaluation of approaches. Environmental science & technology. 51(17):10203-10211.

Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG et al. 2004. Structure-based thresholds of toxicological concern (ttc): Guidance for application to substances present at low levels in the diet.

Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 42(1):65-83.
Mayer F, R. Ellersieck M. 1986. Manual of acute toxicity:Interpretation and data base for 410 chemicals and 66 species of freshwater animals.
Moermond C, Beasley A, Breton R, Junghans M, Laskowski R, Solomon K, Zahner H. 2017. Assessing the reliability of ecotoxicological studies: An overview of current needs and approaches. Integrated environmental assessment and management. 13(4):640-651.
Mons MN, Heringa MB, van Genderen J, Puijker LM, Brand W, Van Leeuwen CJ, Stoks P, van der Hoek JP, van der Kooij D. 2013. Use of the threshold of toxicological concern (TTC) approach for deriving target values for drinking water contaminants. Water Res 47:1666– 1678.
Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B. 2011. How many species are there on earth and in the ocean? PLoS Biology. 9(8):e1001127.
Open Babel Package. 2018. Version 2.3.1 http://openbabel.org (accessed July 2017)
Organization for Economic Cooperation and Development. 2018. The Global Portal to Information on Chemical Substances (eChem Portal). Accessed 02 October 2018. Available at: https://echemportal.org/
Raimondo, S., C.R. Lilavois, and M.G. Barron. 2016. Web-based Interspecies Correlation Estimation (Web-ICE) for Acute Toxicity: User Manual. Version 3.3. EPA/600/R-15/192. Office of Research and Development, U. S. Environmental Protection Agency. Gulf Breeze, FL, USA.
Ruden C, Adams J, Agerstrand M, Brock TC, Poulsen V, Schlekat CE, Wheeler JR, Henry TR. 2017. Assessing the relevance of ecotoxicological studies for regulatory decision making. Integrated environmental assessment and management. 13(4):652-663.
Russom C, Bradbury S, Broderius S, Hammermeister D, Drummond R. 1997. Predicting modes of toxic action from chemical structure: Acute toxicity in the fathead minnow (pimephales promelas).
Sanderson H, Thomsen M. 2009. Comparative analysis of pharmaceuticals versus industrial chemicals acute aquatic toxicity classification according to the united nations classification system for chemicals. Assessment of the (q)sar predictability of pharmaceuticals acute aquatic toxicity and their predominant acute toxic mode-of-action. Toxicology letters. 187(2):84-93.
Staveley J, Wentsel R. 2016. The challenge: How can we improve the quality of ecotoxicology research to increase relevance and use in regulatory decision making? Environmental toxicology and chemistry. 35(1):14.
USEPA (United States Environmental Protection Agency). 2010. TSCA new chemicals program (NCP) Chemical Categories. Accessed 02 October 2018. Available at: https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf
USEPA (United States Environmental Protection Agency). 2018a. ECOTOXicology knowledgebase (ECOTOX). Accessed 02 October 2018. Available at: https://cfpub.epa.gov/ecotox/
USEPA (United States Environmental Protection Agency). 2018b. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. United States Environmental Protection Agency, Washington, DC, USA.

US FDA (United States Food and Drug Administration). 1993. Center for Food Safety and Applied Nutrition toxicological principles for the safety assessment of direct food additives and color additives used in food, Redbook II. Fed Reg 58:16536.USFDA 1993

Vestel J, Caldwell DJ, Constantine L, D'Aco VJ, Davidson T, Dolan DG, Millard SP, Murray-Smith R, Parke NJ, Ryan JJ et al. 2016. Use of acute and chronic ecotoxicity data in environmental risk assessment of pharmaceuticals. Environmental toxicology and chemistry. 35(5):1201-1212.

Williams ES, Berninger JP, Brooks BW. 2011. Application of chemical toxicity distributions to ecotoxicology data requirements under REACH. Environmental toxicology and chemistry. 30:1943–1954.