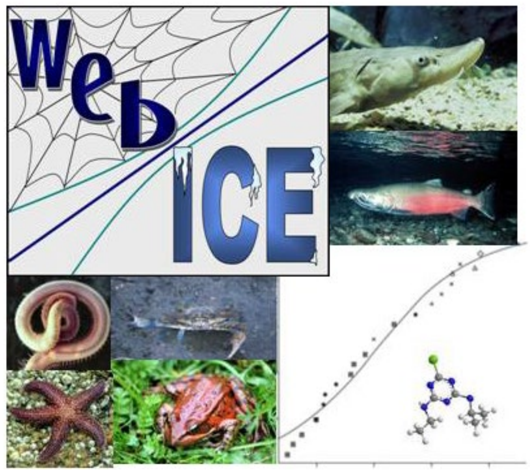
ICE Aquatic Toxicity Database Version 4.0 Documentation



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Operating Procedure for ICE Aquatic Toxicity Database Version 4.0 Quality Assurance Report

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1 Introduction

This document describes the compilation, review, standardization, and quality assurance/quality control (QA/QC) of the ICE Aquatic Toxicity Database (herein, database) developed and maintained by the US EPA Gulf Ecosystem Measurement and Modeling Division. Additional QA/QC of the development, validation, and application of ICE models is found in the QAPP associated with this project (*Interspecies extrapolation for chemical sensitivity,* QAPP ID: J-GEMMD-0033006-QP-1-0).

The database is composed of acute toxicity records for aquatic animal species and is used in the development of toxicological models that include, but may not be limited to, Interspecies Correlation Estimation (ICE) models (Raimondo et al., 2010), Species Sensitivity Distributions (SSDs) (Barron et al. 2012), and Quantitative Structure-Activity Relationship (QSAR) models (Lambert et al. 2022). ICE models are least squares linear regressions of the relative sensitivity between the taxa of interest (i.e., predicted taxa) and that of a surrogate species (e.g., standard test species). Validated ICE models are available on the US Environmental Protection Agency (US EPA) internet application, Web-based Interspecies Correlation Estimation (Web-ICE) (https://www3.epa.gov/webice/). SSDs are cumulative probability distributions of toxicity values for multiple species that may be used to derive a hazard level for ecological risk assessment based on a specified percentile of the distribution. QSARs are regression models describing the relationship between chemical structures and biological activity and can be used to predict activity of new chemicals.

The document is organized by section, including: 1) Introduction, 2) data sources used in developing the ICE database, 3) the quality acceptance criteria applied to the master database, 4) additional standardization applied to data used in ICE models, 5) quality assurance and control procedures, 6) data fields, 7) references, and technical appendices.

A separate database is maintained for algae toxicity data, the documentation for which is listed in Appendix B.

2 Data Sources

The database is composed entirely of secondary data (data previously collected for a different intended use). This section describes each data source in detail, including its acquisition and format. All data sources for the ICE version associated with documentation were obtained in electronic formats. Hard copy sources previously entered for prior versions of the ICE database (i.e., ICE v3.3) were retained within the project study file for that version and duplicate electronic copies are available for reference. Data received electronically for all database versions including the present one were saved as original, unaltered files and housed on a GEMMD network drive. All data sources went through an extensive review process to ensure that each record meets acceptance criteria.

2.1 ECOTOX

The ECOTOX Knowledgebase (http://cfpub.epa.gov/ecotox/), developed by the USEPA/ORD/CCTE Great Lakes Toxicology and Ecology Division, provides chemical toxicity information for aquatic organisms, terrestrial plants, and wildlife. It consists of toxicity data predominately from peer-reviewed literature, although there are some EPA records within the database as well. To obtain records for the database, ECOTOX was queried for acute, aquatic, animal records, which were downloaded in excel format. The ECOTOX columns used in the ICE database are provided in Appendix A-2.

2.2 Ambient Water Quality Criteria (AWQC)

EPA is required by the Clean Water Act (Section 304(a)(1)) to develop criteria for water quality that accurately reflects the latest scientific knowledge. These criteria are based on data and scientific judgment on pollutant concentrations and environmental or human health effects. EPA's compilation of national recommended Ambient Water Quality Criteria (AWCQ) are published and publically available sources of toxicity data for fresh and saltwater organisms that maybe exposed to surface water pollutants. The ICE database contains data from 67 AWQC documents published from 1987-2022 (Appendix A-3). Minimum data provided from the document's Table 1 are chemical name, species tested, water type, test and concentration type (e.g. static, measured), and toxicities (EC/LC50). Additional information provided by some documents include active ingredient, age, hardness, pH and corrected toxicity values for metals. Toxicity data were entered if records meet database acceptance criteria.

2.3 Office of Pesticide Program (OPP) Ecotoxicity Database

The Office of Pesticide Program's Ecological Fate and Effects Division (EFED) Pesticide Ecotoxicity Database contains published and registrant submitted toxicity data for pesticides. Their database was acquired for the current database in July 2020 and contained acute toxicity records for aquatic organisms. Data fields include chemical information, active ingredient, use category, taxa, test organism, age, test conditions, toxicity values, and acceptance category (i.e. core, supplemental). Water quality parameters are not provided, however each study is evaluated by EFED for conformance to Office of Chemical Safety and Pollution Prevention guidelines. Studies that contain major deviations from guidelines that affected the scientific integrity of the study are classified as unacceptable. Supplemental studies are those that are generally well conducted and employed Good Laboratory Practice (GLP), but the study did not meet all requirements listed for satisfaction of the OPP testing requirements (e.g. raw data not submitted). Core studies meet all OPP testing requirements, are well conducted, and all reported endpoints are validated by independent statistical analysis Only core and supplemental data were accepted into the ICE database.

2.4 OPPT Premanufacture Notification (PMN)

Premanufacture Notification (PMN) data that is submitted to EPA under the Toxic Substance Control Act (TSCA) is Confidential Business Information (CBI). GEMMD personnel with CBI certifications obtained PMN toxicity data summaries in pdf format for ICE database v3.1 (released 2010). Those data that met the database acceptance criteria were entered into excel spreadsheets and retained in all ICE database updates. Information includes chemical tested, species information and toxicities. In accordance with CBI procedures, the chemical identities were masked and data are not identifiable by chemical name and CAS number in files accessible by network connections. To censure data, a confidential identifier number (CIN) less than 100 (e.g., 1, 2, 3) is assigned to each CBI chemical in place of the chemical CAS, and a letter assigned in place of the chemical name. All chemicals with that same CAS number, regardless if they are CBI, were also assigned the same CIN in the database to allow development of ICE models while maintaining CBI requirements. There were no new CBI data incorporated into the current version of the ICE database.

2.5 High Production Volume (HPV)

Under the High Production Volume (HPV) Challenge Program, companies make health and environmental effects data publicly available on chemicals produced or imported in the United States in quantities of 1 million pounds or more per year. HPV chemicals and associated information are publically available through the EPA (www.epa.gov/HPV/) as downloadable pdf documents for each chemical. HPV toxicity studies are encouraged to follow GLP and report test quality information for each chemical/species tested. Information obtained included chemical information and active ingredients, species information, toxicities, test information and water quality parameters. In addition, notes on test guidance were included (i.e. ASTM, OECD 203). Questionable data (i.e. missing information, species name errors) were not included into the database. All HPV data in the current database were retained from previous database versions; no new HPV data were acquired for the current version.

2.6 Mayer and Ellersieck 1986

The *Manual of Acute Toxicity: Interpretation and Data Base for 410 Chemicals and 66 Species of Freshwater Animals* (Mayer and Ellersieck 1986) is a compilation of records for freshwater aquatic organisms assembled to assess the influence of test conditions and physical, biological, and chemical properties on species sensitivity. Tests were conducted at the Columbia National Fisheries Research Laboratory (U.S. Department of Interior) from 1965-1984. The manual includes 4901 tests and provides information on chemicals tested, active ingredients, species and age information, test conditions, toxicities (EC/LC50), temperature, pH and hardness.

2.7 ORD

Mayer 1987. The *Acute Toxicity Handbook of Chemicals to Estuarine Organisms* includes toxicity tests conducted at the U.S. EPA Office of Research and Development (ORD) Gulf Breeze Environmental Research Laboratory from 1961-1986. Data included chemical tested, active ingredient, species tested and age, test conditions, toxicities, temperature and salinity.

Mayer et al. 2008. This U.S. EPA report contains acute toxicity for 29 endangered and surrogate species using five chemicals. The report provided chemical and species information, toxicities, test conditions.

2.8 Open Literature

Data from published studies that were not currently in ECOTOX were acquired for taxa (e.g. mussels, endangered species) or chemicals (e.g., PFAS) of interest based on EPA's priorities at the time the ICE data were updated. For the current database version, a review of literature for PFAS compounds identified potential sources for this priority group of contaminants. Careful review of each source determined if the information met acceptance criteria. The source must provide chemical tested, active ingredient, test species, age, test conditions, and toxicity. In addition, temperature and dissolved oxygen or indication that an appropriate test guidelines was used (i.e. ASTM) must be provided. Appendix A-4 provides a comprehensive list of peer reviewed studies included in the database.

2.9 Procter & Gamble

Algal and zebrafish embryo toxicity data were collected under a Cooperative Research and Development Agreement between the Office of Research and Development of the U.S. EPA and the Procter and Gamble Company (P&G). The development of the algal database is described in Appendix B. Zebrafish embryo toxicity data were compiled from public (ECOTOX and scientific literature), and P&G-owned sources, with the majority of data from information reported in OECD (2012), Belanger et al. (2013), Scholz et al. (2014), and Busquet et al. (2014).

3 Master Database QC and Acceptance Criteria

Data were only included in the database if they adhered to pre-determined acceptance criteria. These criteria evaluate test organisms (e.g., taxa, species confirmation), test chemicals (e.g. active ingredient), test duration and reported toxicity endpoint (e.g. mortality). The original source of data must clearly provide adequate information to assess these criteria for inclusion.

Data were subjected to two rounds of filtering; 1) first-round filter for general criteria which determined data suitability for primary database, described in Table 1 and 2) second-round filter for species-specific test conditions which determined data suitability for ICE model subset detailed in Section 4. It should be noted that some records included in hard copy data (e.g. Mayer and Ellersieck 1986) were not entered into electronic format if they did not meet some of the standardization criteria (e.g. active ingredient $\geq$ 90%) described below.

Standardization/quality criteria that were applied to all data sources in the first round of filtering are summarized in Table 1 and described in Sections 3.1- 3.4.

Table 1. Checklist of standardization criteria for inclusion into primary database.

Category	Data Information	Criteria
Chemical	Identity	Reported CAS, name or structure confirmed
		CAS corresponds to single compound or element
	Compound	Mixtures excluded except for chemical salts and specific congener mixtures[1]
	Purity	Active ingredient ≥ 90%
	Grade	If Purity is "NR", test grade must be one listed in Appendix A-5
	Name	Synonyms conformed to ICE chemical name
Organism	Species	Fish, invertebrates, amphibians
		Name & taxonomy verified
	Life stage	Eggs excluded except for zebrafish embryos[2]
Test Conditions	Test Media	Aquatic (no sediment, dietary, mixed dose or phototoxicity)
	Exposure type	Flow through (F), static (S), or static renewal (R)
	Exposure duration	Acute; 24 (fairy shrimp), 48 & 96 hrs
	Endpoint	EC50 or LC50
	Measurement	Mortality or immobility
	Test Location	Laboratory
Toxicity Value	Concentration	~, > or < excluded
	Units	µg/L, converted if needed
	Chemical Normalization[3]	Pentachlorophenol to pH 6.5; Ammonia to TAN[4], FW to pH 7, FW inverts to 20°C; Specific metals[5] hardness normalized
	Element Conversions[6]	Ag, Al, Cu, Cd, Co, Cr(III), Cr(VI), Hg, NH_4, Ni, Pb, Zn

[1] Included metal and other chemical salts, and specific congener mixtures

[2] Zebrafish embryo toxicity tests conducted using methods similar to OECD (2013) fish embryo toxicity test (FET).

[3]FW only, normalized according to AWQC

[4] Total Ammonia Nitrogen

[5] Ag, Cu, Cd, Cr(III), Pb, Ni, Zn

[6] Metals reported as salts were normalized to element

3.1 Chemicals

3.1.1 Active Ingredient and Mixtures

Inclusion of chemicals in the database required that the chemical tested have an active ingredient purity of $\geq$90%. This was determined from either the reported purity or the source/grade of the tested compound. Chemicals whose purity was not reported were accepted if the reported chemical grade is listed in Appendix A-5. If the chemical purity or grade was not reported or could not be determined through internet searches of commercial products, the record was not included. Mixtures were excluded, except for tests of single chemical salts and specific congener mixtures such as PCB, Arochlors, and toxaphene. Any degredates and metabolites were also excluded unless they were identified as the tested compound (e.g., met identity and purity requirements). Formulations of chemicals were excluded unless they contained 90% or greater of the test compound as the active ingredient.

3.1.2 Chemical names and CAS QA/QC

Each toxicity record in the database required a Chemical Abstracts Service (CAS) registry number or a chemical name for the compound tested. A toxicity record was only included if the source provides sufficient information to identify the test compound (e.g., chemical name, formula, smiles string, CAS). CAS and chemical name congruency were checked and/or assigned using public domain databases: the Allanwood Compendium of Pesticides (http://www.alanwood.net/pesticides/), Chemical Book (http://www.chemicalbook.com), or Sigma-Aldrich (http://www.sigmaaldrich.com). The CAS and name associated with each toxicity record were entered into the database as either the tested compound, as the element for Aluminum, Cadmium, Cobalt, Copper, Chromium (III), Chromium (VI), Lead, Mercury, Nickel, Silver, and Zinc, or as Pentachlorophenol or Ammonia for salts containing these chemicals . For records where CAS and chemical name were inconsistent or uncertain, additional internet sources, such as PubMed Compound (http://www.ncbi.nlm.nih.gov/pccompound), were consulted. The CAS or chemical name is either corrected or, in the case of uncertain chemical identity, the record removed. Chemical name as reported in the original source is maintained in the database, as well as the assignment of an ICE chemical name for synonym control. ICE chemical names were curated using DSSTox (www.epa.gov/ncct/dsstox/). A single name and the confirmed chemical abstract services registry number (CAS-RN) from the source material were checked against DSSTox to validate their consistency. Names that were not contained within DSSTox's list of synonyms for a particular chemical were manually checked to validate the agreement between the chemical identifiers and confirm the chemical-data linkage with ICE.

A separate database is maintained for mode of action (MOA) assignments. For complete description of MOA assignments see the Mode of Action and QSAR Databases and Modeling Quality Assurance Project Plan (QAPP-GED/BPRB/MB/2014-01-001). In brief, chemicals were assigned a broad MOA (e.g. AChE inhibition) and a specific MOA (e.g. AChE inhibition -

Organophosphate) in accordance with Barron et al. (2015). Data fields in the MOA chemical database included CAS, chemical name, broad and specific MOA assignments, chemical class assignment (for narcosis chemicals only), MOA source, and a notes column.

3.2 Organism

The aquatic database contains only animal records. Data sources must provide either common name and/or species names of the organisms tested. Verification of species, genus and family names was performed with the Integrated Taxonomic Information System (ITIS; www.itis.gov, last accessed Sept 2022). If verification could not be found in ITIS, other public domain internet websites (i.e. www.fishbase.com) or literature were used. Species names that could not be verified were excluded. After verification, species were grouped into broader taxonomic categories (e.g., fish, crustaceans). If only a common name was provided that was too general to determine species, genus or family (i.e. Ostracod, Amphipod) then the record was not included. Any organism that could only be verified at or was tested at taxonomic level of Order or higher was not included. Test organisms identified by only genus or family were accepted for use in genus and family-level models, respectively. Species synonyms were standardized to reflect the most current nomenclature and common name.

3.3 Test Conditions

No sediment, dietary, mixed dose exposures**,** or photo-enhanced toxicity results were included in the database. The database includes exposure types: static (S), flow through (F), and static renewal (R). Toxicity values reported as both measured (M) and nominal/unmeasured (U) were included. Acute toxicity results must be either immobilization (EC50) or mortality (EC/LC50). Test durations accepted were 24h (fairy shrimp), 48h and 96h tests.

Each species was designated as freshwater (FW) or saltwater (SW; estuarine or marine) based on the salinity of the test media and general knowledge of the species habitat requirements. If water type could not be determined, records were designated as not reported (NR). Toxicity records classified as FW are stenohaline FW species or where reported test salinity is $\leq$ 1 ppt. Records classified as SW are SW species or where the salinity recorded is > 1 ppt.

3.4 Toxicity Values

3.4.1 Concentrations and Units

Open-ended toxicity values (i.e. > 100 µg/L or <100 µg/L) and approximate values (~100 µg/L) were excluded. All toxicity records were converted to µg/L (Table 2). If units could not be determined, the toxicity records were not included.

Table 2. Toxicity units and conversion factors

Unit	Alternate name	Conversion to ug/L
µg/L	PPB	= µg/L
mg/L	PPM	=mg/L * 1000
ng/L	PPT	= ng/L/ 1000
µmol/L	micromolar	= (µmol/L)*MW

3.4.2 Data normalization

The AWQC documents outline normalization procedures for pentachlorophenol (normalized to pH 6.5), ammonium compounds (converted to total ammonia nitrogen, and normalized to a pH of 7 for all freshwater records and to 20°C for all freshwater invertebrates) and specific metal salts (hardness normalized; reporting as metal element). These normalizations were applied to records for these compounds prior to inclusion into the database according to the Operating Procedure for ICE Database Chemical Conversions and Normalizations (J-GEMMD-BEPRB-SOP-3740-1). Large metal salts and organometals were not normalized because of uncertainty in the relationship between their toxicity, hardness, and dissociation, and were treated as separate compounds in the database. These exceptions are further explained in the the Operating Procedure for ICE Database Chemical Conversions and Normalizations (J-GEMMD-BEPRB-SOP-3740-1).

4 Standardization for ICE Models

Data were further standardized for the development of ICE models to ensure models reflect species sensitivity and contained minimal extraneous variation. Toxicity records that met these requirements were designated as a “True” in the “Meets model requirements” column. This section explains the additional standardization for data used to develop ICE models (herein, model data subset), summarized in Table 3.

Table 3. Standardization criteria for data included in ICE model development

Component	Information required	Acceptance requirements
Test organism	Life stage[1]	Amphibians: embryo and larvae (tadpole) only Crabs, crayfish, and lobsters: juvenile and larvae only Fish: juvenile only Zebrafish: embryos[3] or juveniles (separated for models) Insects: immature aquatic lifestages Mollusc[2]: juvenile and spat All other species: all life stages
Test conditions	Test duration	24-48 hr: fairy shrimp 48 hr: water fleas, midges, mosquitoes 96 hr: all other species
	Temperature[4]	species specific ($\pm$ 3°C)
	Dissolved oxygen	Static (S): $\leq$48 hr 60-100%; >48 hr 40-100%. Static renewal (R) or flow-through (F): 60-100%.
	Salinity	<1 ppt: FW species[5] $\geq$15 ppt: SW species[6]

[1] If life stage not reported, determined through reported age/size; Appendix A-6
[2] Glochidia excluded
[3] Zebrafish embryo toxicity tests conducted using methods similar to OECD (2013) fish embryo toxicity test (FET).
[4] Based on ASTM and equivalent test guidelines for test species; Appendix A-7
[5] Anadromous fish (salmonid and sturgeon) tests included are freshwater
[6] Striped bass (*Morone saxatilis*) tests are saltwater

4.1 Life stage

The life stage of each species was broadly defined as embryo, larvae, juvenile, or adult. In the model data subset, only the juvenile stages of fish (with the exception of zebrafish embryos); embryo and larval (tadpole) stages of amphibians; juvenile and larval stages of crabs, crayfish and lobsters; juvenile and spat of molluscs; and immature aquatic lifestages of aquatic insects were used. For all other species, all life stages (except embryo) were included. If a specific stage was not identified in the original source, life history and organism size were used to determine life stage (Appendix A-6). Fish larvae include hatchlings through full fin development. Juvenile fish are those with full fin development lacking sexual maturity, and adult fish are those that are sexually mature. In cases where only a weight is provided for a fish species, life stage was determined using length-weight regressions in the Fish Base Life History Tool (Froese and Pauly 2008). When length-weight regressions were not available or adequate information was not provided, age class was designated as unknown. Records with an “unknown” life stage designation were only included in the model data subset for those species where all life stages were included, and for records where the egg and embryo stage could be ruled out. Zebrafish embryo data were included where the tests were conducted using methods similar to OECD (2013) fish embryo toxicity test (FET). The exception for zebrafish embryos is due to the number of embryotoxicity tests conducted on this species and its application in global efforts to replace the traditional acute fish toxicity test. In model development, zebrafish embryo were kept separate form zebrafish juveniles such that separate models were developed for each life stage.

4.2 Freshwater (FW) or Saltwater (SW) Water Type

Only records designated as freshwater (FW) or saltwater (SW) with ≥15 ppt salinity were included in the ICE model subset, with the following specific exceptions. Only FW records for anadromous fish (salmonids, sturgeons) species were accepted to limit potential variability due to wide differences in test salinity for these euryhaline species. Only SW records for striped bass (*Morone saxatilis*) were accepted because of their juvenile life history characteristics.

4.3 Temperatures

To limit variability associated with test temperature, a 6°C range (+/- 3°C) of temperatures optimal for each species was chosen based on standard test guidelines where provided, or life history where guidelines did not specify species-appropriate conditions. This range was chosen because (1) acceptable within-test temperature is typically +/-2°C and (2) it maximizes data retention while maintaining a relatively narrow temperature range. Temperature ranges were assigned for species where the reported temperatures exceed a 6°C range. Temperature ranges were generally consistent with ASTM and OPPTS recommend test ranges (Appendix A-7). If temperature was reported in a record as a range (i.e., 19-22°C), the median temperature was used to determine if temperature fell within the acceptable range. If the reported range of a toxicity test was greater than 6°C, then the record was excluded.

4.4 Dissolved Oxygen

Dissolved oxygen (DO) must be reported for inclusion into the model data subset or the record reported following standardized testing procedures which would meet the DO guidelines. If DO was reported as a range (i.e., 30-70%), then the average was used. Where necessary, DO values are converted to % saturation to verify compliance with ASTM standards. Conversions to % saturation are calculated as:

$$\text{DO (\% saturation)} = \frac{\text{measured DO (mg/L)}}{\text{DO (mg/L at 100 \% saturation and 760 mm Hg)}} \times 100$$

Only records that met ASTM (2007) dissolved oxygen requirements were included in the model data subset:

- S tests ≤ 48 h, 60-100%;
- S tests > 48 h, 40-100%;

- F or R tests, 60-100%.

4.5 Check for outliers

When more than one toxicity value was available for a chemical and species using the standardization criteria for model development outlined in this section, the ratio of the maximum and minimum values was calculated along with the mean and median. For data obtained for a species/chemical, a median that was approximately equal to the mean indicates a normal distribution of data around the mean and suggest that neither the minimum nor maximum value are an outlier. Toxicity records with max/min ratios greater than 10 and the mean and median not approximately equal were examined for outliers. This included sorting the data by chemical and toxicity value to evaluate the range of all species records for the chemical. Evaluating the spread of data for all species within a chemical allowed outliers to be identified as toxicity values that are an order of magnitude or greater from the range of data acquired for that chemical. Since chemical MOA may be different for vertebrates and invertebrates, the range of data for taxa related to the species in the record in question may be used in place of the full complex of species for which data are available. Such outliers may occur from error in the reported toxicity units which can be confirmed by reviewing the original source. Outliers identified through this process were 1) corrected if an error to reported unit was determined, 2) retained with MMR = False if a record was a suspected outlier but the test appeared to follow good laboratory practices, or 3) removed if the test appeared critically flawed.

5 Quality Assurance and Control

All records in the database were subjected to strict quality assurance and control in accordance to the Quality Assurance Project Plan (*Interspecies extrapolation for chemical sensitivity,* QAPP ID: J-GEMMD-0033006-QP-1-0). Once all standardization was complete, duplicate records were identified and removed. Duplicate records were defined by having the same source citation or authors, CAS, species, age and toxicity value. As new records were entered in the database, duplicates were identified between new and old records. Generally, new records were retained and the older records were removed, based on the assumption that data may have been updated to correct an error or missing information in the previous data. For example, records from the OPP 2020 download for Mayer and Ellersieck data were retained while the original Mayer and Ellersieck records were removed. This was based on personal communication from OPP that corrections to their database from this source were made.

6 Data fields

The data fields and associated code definitions included in the database are outlined in Appendix A-8.

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8 Appendix A

Appendix A-1. Total number of records in the database and those used in models by source for database version 4.0.

Data Source	# records in database	# records used in models
AWQC	3601	2016
Mayer et al. 2008	87	73
ECOTOX	12403	4760
HPV	398	234
Literature	721	437
Mayer 1987	119	65
Mayer and Ellersieck 1986	1888	1667
OPP	4271	3170
OPPTS_PMN	59	58
P&G	351	309
Total	23898	12789

Appendix A-2. ECOTOX columns used in the ICE database.

ICE column	ECOTOX Columns	Use notes
Source Specific	Acquire Location	Added ECO before #
Source Citation	Author Title Source Publication Year Reference Number	
Chemical Tested	Chem Primary Name	
CAS Reported	Test Cas	
AI	Test Grade Desc Test Purity	Deleted records with <90% Purity or Grades not in Appendix 5.
Water Type	Water Type Desc	
Common Name	Common Name	
Species	Latin Name	
Age	Lifestage Desc Age Desc Organism Char	Deleted records for eggs or embryos except for amphibians or zebrafish.
Duration	Test Duration	Also referred to as Observed Duration. Only kept 48hr (2d) or 96hr (4d) with the exception of fairy shrimp 24hr (1d) test. Deleted records with operators (>,<,~).
Endpoint	Endpoint	
Test Type	Exposure Type Desc	Only kept flow-through, static or static renewal.
Concentration Type	Test Method Desc	
Temp	Temperature	
Salinity	Salinity	
DO	Dissolved Oxygen	
pH	PH	
Hardness	Hardness	
ICE toxicity (ug/L)	Conc 1 (all available columns)	Deleted records with operators (>,<,~) or ranges. Also reviewed Conc 2 and Conc 3 if reported but used Conc 1. Conversions and normalizations made to reported toxicity as needed.
Guidelines	Test Method New Desc	

Appendix A-3. List of AWQC documents and publication years entered into the database

Document Name	Year
AWQC updates	1995
2,4-dichlorophenol	1980
2,4-dimethylphenol	1980
2-chlorophenol	1980
Acenaphthene	1980
Acrolein	1980
Acrylonitrile	1980
Aldrin/Dieldrin	1980
Aluminum	1988
Ammonia	2013
Antimony	1980
Arsenic	1984
Atrazine (draft)	2003
Benzene	1980
Benzidine	1980
Beryllium	1980
Cadmium	2016
Carbon tetrachloride	1980
Chlordane	1980
Chloride	1988
Chlorinated benzenes	1980
Chlorinated ethanes	1980
Chlorinated napthalenes	1980
Chlorinated phenols	1980
Chlorine	1984
Chloroalkyl ethers	1980
Chloroform	1980
Chlorpyrifos	1986
Chromium	1984
Copper	1984
DDT	1980
Diazinon	2005
Dichlorobenzenes	1980
Dichloroethylenes	1980
Dichloropropane/propenes	1980

Document Name	Year
Dinitrotoluenes	1980
Diphenylhydrazine	1980
Endosulfan	1980
Endrin	1980
Ethylbenzene	1980
Fluoranthene	1980
Haloethers	1980
Halomethanes	1980
Heptachlor	1980
Hexachlorobutadiene	1980
Hexachlorocyclohexane	1980
Hexachlorocyclopentadiene	1980
Isophorone	1980
Lead (draft)	2008
Mercury	1984
Naphthalene	1980
Nickel	1986
Nitrobenzene	1980
Nitrophenols	1980
Nitrosamines	1980
Nonylphenol	2005
Parathion	1986
Pentachlorophenol	1986
Perfluorooctanoic acid (PFOA, draft)	2022
Perfluorooctane sulfonic acid (PFOS, draft)	2022
Phenol	1980
Phthalate esters	1980
Selenium (draft)	2004
Silver (update)	2007
Thallium	1980
Toluene	1980
Toxaphene	1986
Trichloroethylene	1980
Zinc	1987

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Appendix A-5. Acceptable Chemical Grades with purities ≥ 90%

Code	Definition	Code	Definition
A	Analytical Grade	NP	Normapur Grade
A or R	Analytical or Reagent Grade	PAN	Pestanal Grade
A or S	Analytical or Spectrophotometric Grade	PA	Proanalysis grade
A or GU	Analytical or Guaranteed Grade	PFG	Purified Grade
AASG	Atomic Absorbtion Spectometry Grade	PG	Pure Grade
ACS	American Chemical Society Grade	PH	Pharmaceutical Grade
AL	Analysis Grade	PRG	Pesticide Residue Grade
AN	Analar Grade	PST	Pesticide Grade
AN or R	Analar or Reagant Grade	R	Reagant Grade
AR	A.R. Grade (Analytical Reagant Grade)	RE	Reasearch Grade
CH	Chromatographic Grade	RE or A	Research or Analytical Grade
CL	Clinical Grade	RFG	Reference Grade
CT	Certified Grade	RS	Residue Grade
DG	Distilled in Glass Grade	S	Spectrophotometric Grade
EL	Electrophoresis Grade	SC	Scintillation Grade
FD	Food Grade	SO	Solvent Grade
GC	Gas Chromatography Grade	SPC	Spectrochemical Grade
GR	General Reagent Grade	T	Technical Grade
GU	Guaranteed Grade	T or P	Technical or Purified Grade
GUR	Guaranteed Reagant Grade	T or PU	Technical Grade or Pure
HG	Histological Grade	TA	Technical Acid Grade
HPLC	High Performance Liquid Chromatography Grade	TAR	Technical, Analytical or Reagant Grade
L	Laboratory Grade	TIS	Tissue Culture Grade
MBG	Molecular Biology Grade	ULV	ULV Grade
ME	Monsanto Electrical Grade	UP	Ultrapure Grade
MK	Merck Grade	USP	United States Pharmacopeia Grade
NAF	National Formulary Grade	UV	UV Grade

Appendix A-6. Age classifications used to designate life stage in the database.

Larvae also included nauplii, zoea (Crustaceans); Yolk-sac fry, fry alevin, glass eel stage (Fishes); glochidia (Mollusca); tadpole (Amphibians).
Juvenile also included immature, subadult, Young of year, black eel stage, fingerling, parr, yearling (Fishes); spat (Mollusca)

Family	Species	Larvae[a]			Juvenile[b]			Adult			Source
		Lengths (mm)	Age	Weights (g)	Lengths (mm)	Age	Weights (g)	Lengths (mm)	Age	Weights (g)	
Acipenseridae		< 30		< 0.2	30-700		0.2-900	> 700		> 900	Jones et al. 1978, Bath and O'Connor 1981, Hastings et al. 1987, Froese and Pauly 2008
Adrianichthyidae	*Oryzias latipes*		<2 w			>2-6 w			>5 w		Personal communitcation Rodney Johson, EPA, MED, 2015
Anabantidae	*Anabas testudineus*	< 10		< 0.1	10-110		0.1-25	> 110		> 25	Mookerjee and Mazumdar 1946, Froese and Pauly 2008
Anguillidae	*Anguilla anguilla*	<80		<.65	80-500		.65-236.47	>500		236.47	Froese and Pauly 2008
Anguillidae	*Anguilla sp.*	< 70		< 0.5	70-400		0.5-100	> 400		> 100	Hardy 1978a, Froese and Pauly 2008
Anostomidae	*Leporinus obtusidens*				<21.6		<189.54	>21.6		>189.54	Froese and Pauly 2008
Aplocheilidae	*Rivulus marmoratus*	< 12			12-40			> 40			Grageda et al. 2004; Froese and Pauly 2008
Ariidae	*Ariopsis felis*	<45			68-88			>126			Froese and Pauly 2008, Merriman 1940
Atherinopsidae	*Menidia beryllina*	<10			10t-50			>50			Wurtsbaugh and Li 1985, Froese and Pauly 2008
Atherinopsidae	*Menidia menidia*	5			<9.3			>9.3			Froese and Pauly 2008, Conover et al. 2005
Atherinopsidae	*Menidia peninsulae*	3.89			32.5			>42.5			Middaugh and Hemmer 1987
Atherinopsidae	*Menidia sp.*	< 10	<7-10 d	< 0.1	10-75	7- 60 d	0.1-2.5	> 75	>50-60 d	> 2.5	Martin and Drewry 1978, Froese and Pauly 2008, personal communication Scott Kellman, Aquatic Biosystems, Ft Collins, CO 2015
Bagridae		< 10			10-90			> 90			Rahman et al. 2004, Froese and Pauly 2008
Carangidae	*Trachinotus carolinus*	<10.9			10.9-400			>400			Rolf 2011
Catostomidae	*Catostomus sp.*	< 17		< 0.1	17-200		0.1-100	> 200		> 100	Jones et al. 1978, Froese and Pauly 2008
Centrarchidae	*Lepomis macrochirus*	<26		<.32	26-72	<1 y	.32-7.93	>72	>1 y	>7.93	Belk 1998, Froese and Pauly 2008
Centrarchidae	*Lepomis sp.*	< 13		< 0.1	13-125		0.1-25	> 125		> 25	Hardy 1978b, Ross 2001, Froese and Pauly 2008
Centrarchidae	*Micropterus salmoides*	<9		<.01	9-285	<9 m-1 y	.01-358.06	>285	>9 m-1 y	>358.06	Froese and Pauly 2008
Centrarchidae	*Micropterus sp.*	< 17		< 0.2	17-250		0.2-175	> 250		> 175	Hardy 1978b, Ross 2001, Froese and Pauly 2008
Centrarchidae	*Pomoxis sp.*	< 15		< 0.1	15-200		0.1-70	> 200		> 70	Hardy 1978b, Froese and Pauly 2008
Chanidae	*Chanos chanos*	<13		<.03	13-918		.03-5890	>918		>5890	Froese and Pauly 2008
Channidae	*Channa orientalis*							>201			Froese and Pauly 2008
Channidae	*Channa punctata*	<40		<.99	10-120		.99-23.27	>120		23.27	Froese and Pauly 2008, Dehadrai and Tripathi 1976

Family	Species	Larvae[a]			Juvenile[b]			Adult			Source
		Lengths (mm)	Age	Weights (g)	Lengths (mm)	Age	Weights (g)	Lengths (mm)	Age	Weights (g)	
Cichlidae		< 20		< 0.3	20-80		0.3-30	> 80		> 30	Global invasive species database 2005, Froese and Pauly 2008, Hassan-Williams and Bonner 2008, personal communication M.Peterson
Clariidae	*Heterobranchus longifilis*	>3.1		0.2	3.1-597		0.2-1588	>597		1588	Froese and Pauly 2008, Legendre 1986
Clupeidae		< 30		< 0.2	30-180		0.2-100	> 180		> 100	Jones et al. 1978, Froese and Pauly 2008
Cottidae	*Cottus bairdii*	<9			9-41			>41			Grossman et al. 2002, Froese and Pauly 2008
Cyprinidae	*Abramis brama*							>387		>607	Froese and Pauly 2008
Cyprinidae	*Barilius bendelisis*	<70			70-83			>83			Gairola et al. 1990, Froese and Pauly 2008
Cyprinidae	*Campostoma anomalum*	< 20		< 0.1	20-100		0.1-2	> 100		> 2	Buynak and Mohr 1980b, Froese and Pauly 2008
Cyprinidae	*Carassius sp.*	< 12		< 0.1	12-300		0.1-500	> 300		> 500	Jones et al. 1978, Froese and Pauly 2008
Cyprinidae	*Chrosomus eos*	5.6-15			15-28			>28			Froese and Pauly 2008
Cyprinidae	*Cirrhinus mrigala*	< 20		< 0.1	20-525		0.1-500	> 525		> 500	Alikunhi 1956, Chakrabarty and Murty 1972, Froese and Pauly 2008
Cyprinidae	*Cyprinella spiloptera*							>38			Gotelli and Pyron 1991
Cyprinidae	*Cyprinella whipplei*							>106			Gotelli and Pyron 1991
Cyprinidae	*Cyprinus carpio*	< 19		< 0.1	19-250		0.1-200	> 250		> 200	Jones et al. 1978, Scott and Crossman 1979, Froese and Pauly 2008
Cyprinidae	*Danio rerio*		3 - 21 d			21 d - 6 m		> 23	> 6 m		Harper and Lawrence 2011
Cyprinidae	*Gibelion catla*	< 20		< 0.1	20-440		0.1-500	> 440		> 500	Alikunhi 1956, Chakrabarty and Murty 1972, Froese and Pauly 2008
Cyprinidae	*Gila elegans*	<28			28-260			> 260			Kaeding and Zimmerman 1983, Marsh 2004, Froese and Pauly 2008
Cyprinidae	*Hybognathus amarus*	<9.2			9.2-18.8			>60	18 m		Magana 2007
Cyprinidae	*Hypophthalmichthys molitrix*	< 30	2 d – 15 d		> 30	15 d - 4 y			> 4 y	> 2500	Towers 2010, Ancevski 2011
Cyprinidae	*Labeo sp.*	< 20		< 0.2	20-100		0.2-20	> 100		> 20	Alikunhi 1956, Chakrabarty and Murty 1972, Cambray 1985, Weyl and Booth 1999, Tedesco and Hugueny 2006, Froese and Pauly 2008
Cyprinidae	*Leuciscus idus*							> 430	> 5 y		Siriwardena, 2008
Cyprinidae	*Notemigonus crysoleucas*	<14.7		0.09	14.7-64		.09-5.31	>64		>5.31	Buynak and Mohr 1980a, Froese and Pauly 2008
Cyprinidae	*Notropis sp.*	< 15		< 0.1	15-40		0.1-0.5	> 40		> 0.5	Saksena 1962, Ross 2001, Froese and Pauly 2008
Cyprinidae	*Pimephales promelas*	4-5.2		<.01	5.2-57	<4 m	.01-2	>57	>3-4 m	>2	Froese and Pauly 2008, personal communication Tim Dawson, EPA, MED, 2015
Cyprinidae	*Pimephales sp.*	< 10		< 0.1	10-50		0.1-1.4	> 50		> 1.4	Ross 2001, Froese and Pauly 2008

Family	Species	Larvae[a] Lengths (mm)	Larvae[a] Age	Larvae[a] Weights (g)	Juvenile[b] Lengths (mm)	Juvenile[b] Age	Juvenile[b] Weights (g)	Adult Lengths (mm)	Adult Age	Adult Weights (g)	Source
Cyprinidae	*Pseudorasbora parva*							>20			Froese and Pauly 2015
Cyprinidae	*Ptychocheilus lucius*	< 25			25-420	>25 d	>0.05 g	>420			Vanicek and Kramer 1969, Tyus and Haines 1991, Froese and Pauly 2008
Cyprinidae	*Puntius conchonius*	<8			8-60			>60			Amenla and Dey 2013
Cyprinidae	*Puntius sophore*							>50		>1.8	Hossain et al. 2012, Froese and Pauly 2008
Cyprinidae	*Puntius ticto*	<14	<14 d	<.1	14-80	14-48 d	.1-15.1	>80	>48 d	15.1	Banik and Saha 2012, Froese and Pauly 2008
Cyprinidae	*Rasbora daniconius*							>72		>3.3	Froese and Pauly 2008
Cyprinidae	*Rhinichthys osculus*	<9		<.1	9-40		.1-4.6	>40		4.6	COSEWIC 2006, Froese and Pauly 2008
Cyprinidae	*Scardinius erythropthalmus*	<12		<0.01	12-81		.01-6.37	>81		>6.37	Wolnicki et al. 2009, Froese and Pauly 2008
Cyprinidae	*Trigonostigma heteromorpha*	4+						>38			Froese and Pauly 2008
Cyprinodontidae	*Cyprinodon sp.*	< 12		< 0.1	12 - 30		0.1-0.5	> 30		> 0.5	Hardy 1978a, Cripe et al. 2009
Cyprinodontidae	*Jordanella floridae*	>4	<8 d		<25	>8 d	<0.3 g	>25		>0.3g	Nasuti 2006; Holdway and Dixon 1986
Embiotocidae	*Micrometrus minimus*							>106		>20	Schultz et al. 1991, Froese and Pauly 2008
Esocidae	*Esox sp.*	< 20		< 0.1	20-200		0.1-55	> 200		> 55	Jones et al. 1978, Froese and Pauly 2008
Fundulidae	*Fundulus sp.*	< 25		< 0.1	25-40		0.1-1	> 40		> 1	Hardy 1978a, Able and Fahay 1998, Froese and Pauly 2008
Gasterosteidae	*Culaea inconstans*	< 26			26-38			>38	>1 y		Acere 1986
Gasterosteidae	*Gasterosteus aculeatus*	< 16	<9 d	<.03	16-45	>9 d-1 yr	.03-.94	>45	>1 yr	>.94	Norenburg and Ritgers 2015, Froese and Pauly 2008
Gasterosteidae		< 15			15-45			> 45			Hardy 1978a, Able and Fahay 1998
Gobiidae	*Gobiosoma bosc*	< 7			7-30			> 30			Ruple 1984, Froese and Pauly 2008
Heteropneustidae		< 12			12-120			> 120			Thakur et al. 1974, Froese and Pauly 2008
Ictaluridae	*Ameiurus nebulosus*	22-Apr		<.13	22-178		0.13-71.06	>178		>71.06	Froese and Pauly 2008
Ictaluridae		< 20		< 0.1	20-250		0.1-100	> 250		> 100	Jones et al. 1978, Scott and Crossman 1979; Froese and Pauly 2008
Mastacembilidae	*Macrognathus aculeatus*	<10.8	<30 d	< 0.1	10.8-160		0.1-14.6	>160		14.6	Das and Kalita 2003, Froese and Pauly 2008
Melanotaeniidae	*Melanotaenia nigrans*	<21			21-70			>70			Crowley and Ivanstoff 1982
Melanotaeniidae	*Melanotaenia splendida*							>129			Crowley and Ivanstoff 1982
Melanotaeniidae	*Pseudomugil signifer*							>28			Froese and Pauly 2008

Family	Species	Larvae[a]			Juvenile[b]			Adult			Source
		Lengths (mm)	Age	Weights (g)	Lengths (mm)	Age	Weights (g)	Lengths (mm)	Age	Weights (g)	
Moronidae	*Morone americana*	< 20			20-150			> 150			Hardy 1978b, Froese and Pauly 2008
Moronidae	*Morone chrysops*	<17.2			17.2-280	>4 w	>5.9	>280	>1 y	>250g	Denson and Smith 1996, Froese and Pauly 2008, Smith 1995
Moronidae	*Morone saxatilis*	< 25	5-30 d		25-400	30 d-2 y		> 400	>2 y		Hardy 1978b, Froese and Pauly 2008, Fay et al. 1983
Mugilidae		< 35		< 0.2	35-350		0.2-300	> 350		> 300	Martin and Drewry 1978, Froese and Pauly 2008
Percidae	*Etheostoma sp.*	< 18		< 0.1	18-35		0.1-0.4	> 35		> 0.4	Johnson 1984, Fisher 1990, Froese and Pauly 2008
Percidae	*Perca flavescens*	< 20		< 0.1	20-125		0.1-20	> 125		> 20	Hardy 1978b, Froese and Pauly 2008
Percidae	*Sander vitreus*	< 20		< 0.1	20-250		0.1-177	> 250		> 177	Hardy 1978b, Froese and Pauly 2008
Pleuronectidae	*Platichthys sp.*	< 7		< 0.1	7-200		0.1-80	> 200		> 80	Ahlstrom et al. 1984, Froese and Pauly 2008
Poeciliidae	*Poecilia reticulata*	6			<20		<.58	>20	>1 m	>.58	Reznick 1983, Reznick et al. 1990, Froese and Pauly 2008
Poeciliidae	*Xiphophorus maculatus*							>31		0.7	Froese and Pauly 2008
Poeciliidae		< 10		< 0.1	10-25		0.1-0.25	> 25		> 0.25	Hardy 1978b, Froese and Pauly 2008
Polyodontidae	*Polyodon spathula*							>565		>3644	Mims and Knaub 1993, Froese and Pauly 2008
Salmonidae	*Oncorhynchus mykiss*	<40		<0.3	40-192	>19 d	0.3-70	>192		>70	Froese and Pauly 2008; USEPA 1996
Salmonidae	*Oncorhynchus sp.*	< 25		< 0.2	25-200		0.2-100	> 200		> 100	Kendall and Behnke 1984, Ross 2001, Froese and Pauly 2008, Ueberschar and Froese 2008
Salmonidae	*Prosopium williamsoni*	<60			60-200			>200	>2 y	>100g	McPhail and Troffe 1998, Stalnaker and Gresswell 1974
Salmonidae	*Salmo sp.*	< 25		< 0.2	25-200		0.2-5.3	> 200		> 75	Kendall and Behnke 1984, Jonsson 1985, Gorodilov 1996, Marschall et al. 1998, Froese and Pauly 2008, Ueberschar and Froese 2008
Salmonidae	*Salvelinus fontinalis*	<18		<.1	18-150		0.1-42.1	>150		>42.1	Froese and Pauly 2008
Salmonidae	*Salvelinus sp.*	<20		< 0.2	20-200		0.2-100	> 200		> 100	Kendall and Behnke 1984, Froese and Pauly 2008, Ueberschar and Froese 2008
Sciaenidae	*Leiostomus xanthurus*	< 15		< 0.1	15-200		0.1-90	> 200		> 90	Johnson 1978, Froese and Pauly 2008
Sparidae	*Lagodon rhomboides*	< 15		< 0.1	15-120		0.1-60	> 120		> 60	Johnson 1978, Froese and Pauly 2008
Syngnathidae	*Syngnathus fuscus*	9+						>99		>1.1	Froese and Pauly 2015, Campbell and Able 1998
Terapontidae	*Bidyanus bidyanus*	3.6						>238		>412.7	Rowland 2004
Terapontidae	*Terapon jarbua*	< 23		< 0.3	23-130		0.3-46.92	>130		>46.92	Froese and Pauly 2008
Umbridae	*Umbra pygmaea*	< 8.5		<.01	8.5-37		.01-.44	>37		>.44	Froese and Pauly 2008

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Appendix A-7. Temperature ranges used to standardize species in the model data subset.

Species	ICE Temp Acceptance Range	Species from Guidelines	ASTM[1]	OPP[2]	OPPTS 1996[3]	OECD 203[4]	EPA 1975[5]	EPA 1993[6]
Acartia tonsa	20-26	x	x	x	x	x	x	x
Aedes aegypti	22-28	x	x	x	x	x	x	x
Aldrichetta forsteri	19-25	x	x	x	x	x	x	x
Ameiurus melas	18-24	x	x	x	x	x	x	x
Ameiurus nebulosus	18-24	x	x	x	x	x	x	x
Americamysis bahia	21-27	*Americamysis bahia*	25-29	21-23	23-27	x	x	19-21, 24-26
Ampelisca abdita	20-26	x	x	x	x	x	x	x
Asellus sp.	18-24	x	x	x	x	x	x	x
Asellus aquaticus	13-19	x	x	x	x	x	x	x
Astropecten sp.	19-25	x	x	x	x	x	x	x
Baetis sp.	15-21	x	x	x	x	x	x	x
Bidyanus bidyanus	20-26	x	x	x	x	x	x	x
Bufo bufo	16-22	x	x	x	x	x	x	x
Caecidotea brevicauda	15-21	x	x	x	x	x	x	x
Carassius auratus	17-23	*Carassius auratus*	15-24	x	x	x	20-24	x
Catostomus commersonii	10-16	x	x	x	x	x	x	x
Ceriodaphnia dubia	21-27	*Ceriodaphnia dubia*	23-27	x	x	x	x	19-21, 24-26
Chironomus sp.	17-23	*Chironomus* sp.	20-24	x	x	x	20-24	x
Chironomus plumosus	17-23	*Chironomus* sp.	20-24	x	x	x	20-24	x
Chironomus riparius	17-23	*Chironomus* sp.	20-24	x	x	x	20-24	x
Chironomus tentans	19-25	*Chironomus* sp.	20-24	x	x	x	20-24	x
Chironomus zealandicus	17-23	*Chironomus* sp.	20-24	x	x	x	20-24	x
Clarias batrachus	20-26	x	x	x	x	x	x	x
Coregonus fera	8-14	x	x	x	x	x	x	x
Corophium volutator	9-15	x	x	x	x	x	x	x
Crangonyx pseudogracilis	8-14	x	x	x	x	x	x	x
Crassostrea virginica	19-25	*Crassostrea virginica*	20-24	x	x	x	x	x
Ctenopharyngodon idella	19-25	x	x	x	x	x	x	x
Culicoides furens	19-25	x	x	x	x	x	x	x

Species	ICE Temp Acceptance Range	Species from Guidelines	ASTM[1]	OPP[2]	OPPTS 1996[3]	OECD 203[4]	EPA 1975[5]	EPA 1993[6]
Cyclops sp.	18-24	x	x	x	x	x	x	x
Cymatogaster aggregata	12-18	x	x	x	x	x	x	x
Cyprinodon variegatus	20-26	*Cyprinodon variegatus*	20-24	21-23	20-24	x	20-24	19-21, 24-26
Cyprinus carpio	18-24	*Cyprinus carpio*	x	x	20-24	20-24	x	x
Daphnia carinata	18-24	x	x	x	x	x	x	x
Daphnia magna	18-24	*Daphnia magna*	18-22	x	18-22	x	15-19	19-21, 24-26
Daphnia pulex	15-21	*Daphnia pulex*	18-22	x	18-22	x	15-19	19-21, 24-26
Diaptomus clavipes	16-22	x	x	x	x	x	x	x
Esox lucius	12-18	x	x	x	x	x	x	x
Eurytemora affinis	19-25	x	x	x	x	x	x	x
Farfantepenaeus duorarum	19-25	*Farfantepenaeus duorarum*	20-24	x	x	x	20-24	x
Faxonius nais	15-21	*Orconectes* sp.	15-24	x	x	x	20-24	x
Fenneropenaeus indicus	23-29	x	x	x	x	x	x	x
Fenneropenaeus merguiensis	29-35	x	x	x	x	x	x	x
Gambusia affinis	14-20	x	x	x	x	x	x	x
Gammarus fasciatus	15-21	*Gammarus fasciatus*	15-19	x	17-19	x	15-19	x
Gammarus lacustris	14-20	*Gammarus lacustris*	15-19	x	17-19	x	15-19	x
Gammarus pseudolimnaeus	15-21	*Gammarus pseudolimnaeus*	15-19	x	17-19	x	15-19	x
Gammarus pulex	13-19	x	x	x	x	x	x	x
Gasterosteus aculeatus	18-24	*Gasterosteus aculeatus*	15-19	x	10-14	x	20-24	x
Gibelion catla	24-30	x	x	x	x	x	x	x
Heteropneustes fossilis	20-26	x	x	x	x	x	x	x
Hexagenia bilineata	18-24	x	x	x	x	x	x	x
Hyalella azteca	18-25	x	x	x	x	x	x	x
Ictalurus punctatus	17-23	*Ictalurus punctatus*	15-24	x	20-24	x	20-24	x
Ischnura sp.	13-19	x	x	x	x	x	x	x
Ischnura verticalis	15-21	x	x	x	x	x	x	x
Labeo rohita	24-30	x	x	x	x	x	x	x
Lagodon rhomboides	20-26	*Lagodon rhomboides*	20-24	x	x	x	20-24	x
Lates calcarifer	24-30	x	x	x	x	x	x	x
Leiostomus xanthurus	21-27	x	x	x	x	x	x	x
Lepomis cyanellus	17-23	*Lepomis cyanellus*	15-24	x	x	x	x	x
Lepomis macrochirus	18-24	*Lepomis macrochirus*	15-24	x	20-24	21-25	20-24	x
Lepomis microlophus	18-24	x	x	x	x	x	x	x

Species	ICE Temp Acceptance Range	Species from Guidelines	ASTM[1]	OPP[2]	OPPTS 1996[3]	OECD 203[4]	EPA 1975[5]	EPA 1993[6]
Lestes congener	19-25	x	x	x	x	x	x	x
Limnodrilus hoffmeisteri	20-26	x	x	x	x	x	x	x
Lithobates catesbeianus	17-23	x	x	x	x	x	x	x
Lithobates clamitans	17-23	x	x	x	x	x	x	x
Lithobates pipiens	17-24	x	x	x	x	x	x	x
Lumbriculus variegatus	19-25	x	x	x	x	x	x	x
Menidia beryllina	19-25	*Menidia* sp.	20-24	21-23	20-24	x	20-24	19-21, 24-26
Menidia menidia	22-28	*Menidia* sp.	20-25	21-24	20-25	x	20-25	19-21, 24-27
Micropterus dolomieu	17-23	x	x	x	x	x	x	x
Micropterus salmoides	17-23	x	x	x	x	x	x	x
Morone saxatilis	20	x	x	x	x	x	x	x
Mystus vittatus	22-28	x	x	x	x	x	x	x
Neanthes arenaceodentata	17-23	x	x	x	x	x	x	x
Neomysis americana	19-25	x	x	x	x	x	x	x
Nereis diversicolor	10-16	x	x	x	x	x	x	x
Notemigonus crysoleucas	16-22	x	x	x	x	x	x	x
Notropis topeka	19-25	x	x	x	x	x	x	x
Oncorhynchus clarkii	9-15	x	x	x	x	x	x	x
Oncorhynchus gorbuscha	9-15	x	x	x	x	x	x	x
Oncorhynchus keta	9-15	x	x	x	x	x	x	x
Oncorhynchus kisutch	9-15	*Oncorhynchus kisutch*	10-14	x	10-14	x	10-14	x
Oncorhynchus mykiss	9-15	*Oncorhynchus mykiss*	10-14	x	10-14	13-17	10-14	11-13
Oncorhynchus nerka	7-13	x	x	x	x	x	x	x
Oncorhynchus tshawytscha	9-15	x	x	x	x	x	x	x
Ophiogomphus sp.	15-21	x	x	x	x	x	x	x
Oreochromis mossambicus	23-29	x	x	x	x	x	x	x
Oreochromis niloticus	21-27	x	x	x	x	x	x	x
Ortmanniana pectorosa	20-26	x	x	x	x	x	x	x
Oryzias latipes	19-25	*Oryzias latipes*	x	x	x	21-25	x	x
Palaemonetes sp.	19-25	x	x	x	x	x	x	x
Palaemonetes kadiakensis	15-21	x	x	x	x	x	x	x
Paratanytarsus dissimilis	18-24	x	x	x	x	x	x	x
Pelophylax nigromaculatus	15-21	x	x	x	x	x	x	x
Penaeus monodon	23-29	x	x	x	x	x	x	x

Species	ICE Temp Acceptance Range	Species from Guidelines	ASTM[1]	OPP[2]	OPPTS 1996[3]	OECD 203[4]	EPA 1975[5]	EPA 1993[6]
Penaeus semisulcatus	18-24	x	x	x	x	x	x	x
Perca flavescens	12-18	x	x	x	x	x	x	x
Pimephales promelas	20-26	*Pimephales promelas*	23-27	x	21-25	21-25	20-24	19-21, 24-26
Poecilia reticulata	23-29	*Poecilia reticulata*	x	x	21-25	21-25	x	x
Polypedilum sp.	18-24	x	x	x	x	x	x	x
Praunus flexuosus	9-15	x	x	x	x	x	x	x
Pseudacris regilla	17-23	x	x	x	x	x	x	x
Pteronarcella badia	10-16	x	x	x	x	x	x	x
Pteronarcys californica	10-16	*Pteronarcys* sp.	10-14	x	x	x	10-14	x
Puntius conchonius	13-19	x	x	x	x	x	x	x
Salmo salar	11-17	*Salmo salar*	x	x	10-14	x	x	x
Salmo trutta	11-17	x	x	x	x	x	x	x
Salvelinus confluentus	7-13	x	x	x	x	x	x	x
Salvelinus fontinalis	11-17	*Salvelinus fontinalis*	10-14	x	10-14	x	10-14	11-13
Salvelinus namaycush	9-15	x	x	x	x	x	x	x
Sander vitreus	12-18	x	x	x	x	x	x	x
Scylla serrata	23-29	x	x	x	x	x	x	x
Simocephalus serrulatus	15-21	x	x	x	x	x	x	x
Simocephalus vetulus	19-25	x	x	x	x	x	x	x
Streptocephalus proboscideus	19.5-25.5	x	x	x	x	x	x	x
Tilapia zillii	24-30	x	x	x	x	x	x	x
Tubifex tubifex	19-25	x	x	x	x	x	x	x
Utterbackia imbecillis	19-25	x	x	x	x	x	x	x
Villosa iris	19-25	x	x	x	x	x	x	x
Villosa lienosa	25-31	x	x	x	x	x	x	x
Villosa villosa	25-31	x	x	x	x	x	x	x
Xenopus laevis	22-28	x	x	x	x	x	x	x

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3. Ecological Effects Test Guidelines. OPPTS 850.1075 Fish Acute Toxicity Test, Freshwater and Marine. EPA 712-C-96-118. April 1996
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Appendix A-8. List of data fields in master database

Data Field	Description
ID	Unique Web-ICE record identification number
Source Specific	Specific data source (e.g. ECO12207 = Ecotox Acquire # 12207)
Source Category	General category of data (i.e. literature, ECOTOX, AWQC)
Source Citation	Citation of original source of data (i.e. the source listed in ECOTOX or AWQC for where they obtained the data)
Chemical Tested	Chemical name as reported in original source
CAS Reported	CAS reported by original source
ICE Chemical	Standardized chemical name
ICE CAS	CAS registry number for standardized chemical name
AI	Active ingredient or chemical grade of chemical tested
Water Type	Freshwater (FW); Saltwater (SW); NR (not recorded)
Taxa	Broad taxa of test species
Common Name	Common name of test species
Species	Standardized species tested ("none" = genus only e.g. Daphnia sp.)
Genus	Genus name of test species
Family	Family name of test species
Age	Age as reported (size, weight, etc.)
Age Class	Assigned ICE age class basef on reported age. (L = larvae, J = juvenile, A = adult, U = unknown, E = embryo)
Duration	Duration of test in hours. 48h; 96h; NR (not recorded)
Endpoint	LC50; EC50; NR (not recorded)
Test Type	F (flow through); S (static); R (static renewal); NR (not recorded)
Concentration Type	M (measured); U (nominal/unmeasured); NR (not recorded)
Temp	Test temperature as reported
Salinity	Test salinity as reported
DO	Test dissolved oxygen as reported
pH	Test pH as reported
Hardness	Test hardness as reported
ICE toxicity (µg/L)	Toxicity used for ICE models after normalizations
Guidelines	Guidelines reported for test (i.e. ASTM). If field says confirmed then record was verified to meet ICE standardizations
Meets Model Requirements	True/False field - does record meet ICE model standardization requirements
Notes	Any additional information from source that could be useful
False for MMR	If record does not meet model requirements (MMR), this column contains the reason(s) why record is false (e.g. age)

9 Appendix B. Algae ICE Module; Technical Basis of the Development of Algae ICE Models for Web-ICE

This technical basis was last updated April 10, 2013

Introduction

This document summarizes the data used in the Web-ICE v3.2.1 Algae Modules. The Algae Modules were developed under a Cooperative Research and Development Agreement between the Office of Research and Development of the U.S. EPA and the Procter and Gamble Company (P&G).

The Algae Modules allow estimation of toxicity in selected species or genera of freshwater or marine algae by inputting the known toxicity in another algal species. Both the Algae Modules and this technical basis document will be updated periodically as the database, interspecies algal models, or functionality is revised. Users are encouraged to report any issues to EPA via the Web-ICE contact page.

Overview of Algae Database and Model Development

The process of obtaining data and ICE model creation is provided below:

1. A compilation of public (ECOTOX and scientific literature), EPA (Office of Pesticide Programs Toxicity Database) and P&G-owned algal toxicity data were compiled into an ACCESS database. The database of acute toxicity data for freshwater or marine algae: EC50 or equivalent values for short-term algal growth in biomass or cell number.
2. Duplicate records were removed, as well as records containing open ended (greater than or less than) toxicity values. After initial processing, over 17,000 studies comprising over 500 species and nearly 1500 chemicals were included in the initial database.
3. A general quality review of each algal acute study was performed by assessing the source of the record for conformance to standard methods and guidelines, such as OECD, USEPA and ASTM.
4. The database was then restructured to include: (1) the 11 algal genera with sufficient toxicity records (EC50 or equivalent) to allow ICE model development, (2) only 72 or 96-hr acute toxicity data, (3) newly calculated toxicity values (i.e., over 80 EC50s were recalculated), (4) additional P&G studies, (5) harmonized algal taxonomic names, (6) test material names that were confirmed and coordinated, and (7) calculated geometric means and variance per taxon per chemical. This restructured database contained approximately 3500 EC50 records with 791 unique chemicals and 74 species of algae.
5. A preliminary assessment of the influence of type of EC50 (e.g., E_rC_{50} and E_bC_{50}) separately and combined was completed. An ErC50 was based on growth rate while an EbC50 was based on biomass. The same data is used to determine each endpoint but different statistical approaches are used. The biomass parameter generally provides a

lower value compared with growth rate, but both types of EC50s were included based on correlation analysis.

6. An extensive quality assurance review of the records in the restructured database was completed following general USEPA Science Advisory Board recommendations (Table 1) The final database used in Web-ICE models consisted of 1647 unique studies with approximately 457 chemicals, and 69 Species of Green Algae, Blue-Green Algae and Diatoms.
7. The final database was used to generate 44 Genus-level models and 58 species level models that were cross-validated (Raimondo et al. 2007).
8. Only significant models ($p<0.05$) that had three or more chemicals were included in the Algae Module.

References

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Table 1. Checklist of standardization criteria for inclusion into algal database used to create ICE models.

Category	Data Information	Criteria
Chemical	Identity	Reported CAS, name or structure confirmed [a]
		CAS corresponds to single compound or element
	Compound	Mixtures excluded except for metal and specific chemical salts
	Purity	Active ingredient $\geq$ 90% [b, c]
	Grade	If Purity is "NR", test grade conformed to Web-ICE requirements
	Name	Harmonized within the algal database
Organism	Species	Algae and diatoms
		Name & taxonomy verified
Test Conditions	Test Media	Aquatic (FW/SW identified)
	Exposure type	F, S, SR (no sediment, dietary, mixed dose or phototoxicity)
	Exposure duration	Acute; 72 & 96 hrs
	Endpoint	EC50
	Measurement	growth rate, biomass or cell density
	Test Location	Laboratory only
Toxicity Value	Concentration	> or < excluded
	Units	ug/L, converted if needed
	Chemical Normalization	Metals: no hardness correction [c]
	Element Normalization [d]	Ag, Al, Cu, Cd, Co, Cr(III), Cr(VI), Hg, Ni, Pb, Zn

[a] Some proprietary data encoded with false CAS number to avoid chemical identification

[b] Included chemicals with AI <90% if equivalent for all species tested with that chemical.

[c] Tests performed in standard test media [e.g., OECD 201: OECD Guideline for Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006); ASTM E1218-20: Standard Guide for Conducting Static Toxicity Tests with Microalgae (2009); EU Method C_3: Algal Inhibition Test]

[d] Metals reported as salts were normalized to element

10 Appendix C

Appendix C-1. Chemical mode of action assignments

Chemicals were assigned a mode of action (MOA) through a weight of evidence approach, utilizing online pesticide databases, literature sources, and analysis of chemical structure. A list of chemicals with their respective Chemical Abstract Service (CAS) numbers needing a MOA assignment was recorded in an Excel spreadsheet. Chemicals were first checked to see if they had an existing MOA assignment in Web-ICE. These chemicals were then run through the EPA's TEST (Toxicity Estimation Software Tool, https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test) which used QSAR methodologies, chemical structure, and existing data to generate experimental and predicted MOAs for most chemicals in the list. Chemicals that were obvious pesticides (based on chemical name) were then examined using the online MOA databases of three major pesticide action committees; namely IRAC (Insecticide Resistance Action Committee, https://irac-online.org/), FRAC (Fungicide Resistance Action Committee, https://www.frac.info/home), and HRAC (Herbicide Resistance Action Committee, https://hracglobal.com/). Subsequently, for chemicals that were not pesticides, we checked a range of online sources (Google Scholar, PubChem, and ChemicalBook) for relevant literature and structural information on MOA. All relevant information from all sources was recorded in the Excel spreadsheet. If no relevant information could be found, the chemical was skipped.

Once all relevant information was recorded, MOAs were assigned using the following process. If there was a general concordance among the sources of information gathered, a broad MOA (e.g. AChE inhibition) was assigned. Specific MOA (e.g. Organophosphate) was assigned only if there was sufficient evidence and concordance among sources. Where there were contradictions among sources, the chemical was re-evaluated focusing on chemical structure and the strongest source of evidence, and a different broad or specific MOA was assigned. For chemicals with insufficient information or too many contradicting points of evidence, we marked the MOA as Uncertain. For a flowchart outlining the MOA assignment methodology, please see Appendix C-2.

Appendix C-2. Chemical mode of action assignment Flow-Chart