

# Unify Toxicity Module

## ECOTOXicology Knowledgebase System

### ECOTOX Coding Guidelines

Prepared for:

U.S. Environmental Protection Agency (EPA)  
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# REVIEW OF LITERATURE

## A. OVERVIEW OF ECOTOX

### 1. Introduction to Review of Literature

The data elements included in ECOTOX encompass standard test parameters typically reported within a publication. Each knowledgebase record contains information about the exposure and test conditions. Specific parameters include the test chemical, species, and endpoint or effect concentration.

The included literature is identified through standardized bibliographic retrievals. Each publication is reviewed and the applicable data is encoded by trained literature reviewers.

**Note:** Each publication included into the ECOTOX Knowledgebase must meet the five minimal criteria for acceptance (i.e. chemical, species, concentration, duration and effect) . If the paper is missing one or more of these criteria ECOTOX does not search other sources to obtain the missing data piece(s).

### 2. Literature Reviewer Training

#### Training

The training is designed to develop consistent, accurate, and versatile literature reviewers. This is accomplished through an intensive period of literature review, interactive quality assurance procedures, and mentoring with other ECOTOX Knowledgebase personnel.

The personnel available to support the reviewer include the data coordinator and trained ECOTOX reviewers. The following documentation and materials are used for training:

- ECOTOX Standard Operating Procedures (<http://cfpub.epa.gov/ecotox/blackbox/help/ecotoxsop.pdf> (this document) and <http://cfpub.epa.gov/ecotox/blackbox/help/codeappendix.pdf>);

The reviewer initially reviews the ECOTOX Standard Operating Procedures: Coding Guidelines. The primary emphasis is to understand the minimum criteria that characterize acceptable toxicity tests. These criteria must be reported in the toxicity publications selected for review in order to qualify for inclusion in the ECOTOX Knowledgebase.

The acceptance criteria are:

- Name of the test **chemical**;
- Name of the **test organism**;
- **Effect** of the test chemical on the organism;
- Test chemical **concentration** or application rate;

- Test **duration** (except for abstracts and non-English publications).

Once the general introductory materials are read, the standard training guidelines introduce the reviewer to each category of toxicity literature. The guidelines can be tailored to the specific areas of expertise and strengths that each person brings to the project. Three primary elements are emphasized in each component of the training sequence. The standard training sequence is:

1. Example review: Examination of previously encoded toxicity literature. The trainee reviews between 5 to 10 toxicity publications and compares each with its associated pre-completed coding printout.
2. Independent review: The trainee independently reviews a minimum of 10 to 20 toxicity publications. All 10 - 20 reviews are quality assured via a review of the publication and coding by the data coordinator. Inconsistent coding practices are resolved with the trainee. The trainee continues to review additional toxicity publications and the level of QA decreases from 100 percent to 10 percent as the reviewer's consistency and proficiency increase.
3. Measure of proficiency: Established ECOTOX quality assurance procedures require a close review of all reviewed publications by the data coordinator to ensure accurate reviewing is consistent with current test methodologies and SOPs. All discrepancies identified are noted by the data coordinator and discussed with the trainee.
4. A full time reviewer begins the training reviewing publications with mentoring and the interactive quality assurance procedures.

### **Measures of Competency for Trained Reviewers**

The quality assurance process is an ongoing component of literature reviewing. Emphasis is placed on quality assurance during the initial collaborative training period., As part of this process, consistency and concurrence between the Reviewers is attained.

### **Steps in the Quality Assurance Process**

1.
  1. All data fields with the exception of comment data fields have validation files to limit entries. For example, Response Site has a list of specific entries that limit entries to ensure that non-response sites are not allowed.
  2. Electronic quality assurance checks occur on 95 percent of ECOTOX data fields. This will check for integrity of codes between data fields (e.g. Chemical Grade and Concentration Type) consistency of data with in data fields.
  3. All entries are quality assured by review staff to ensure that all data have been addressed and are accurate.

### 3. General Coding Information

#### Overview

ECOTOX was developed using two databases – AQUIRE and TERRETOX, housing aquatic toxicity data and terrestrial toxicity data, respectively. These databases were developed independently but were merged together under the ECOTOX framework. Data elements for each test contained in ECOTOX are grouped by chemical, organism, exposure conditions, and effects. Test chemical parameters describe the toxicant and any associated carrier; the CAS registry number; and the grade, purity and/or composition. The test organism parameters include the scientific name, a species number and lifestage, source, and/or characteristics of the organism. The test conditions identify the test location; exposure type, time, and conditions; and any control parameters. Effect and endpoint parameters consist of codes to define lethal, sublethal, or residue effects and/or endpoints. The corresponding chemical concentration or dose is reported for both exposure and observation concentrations, if reported. Available data are extracted from the text, tables, and graphs of each publication. Any data fields that are specific to a habitat (aquatic, soil, non-soil) or test location (FieldN or Lab/FieldA) become available for coding when specific codes are selected.

The following sections are designed as an overview of the guidelines for reviewers. The information presented in this section identifies the common attributes of the knowledgebase. Each section heading corresponds to a data element. Any exceptions from these guidelines must be authorized by the EPA Database Manager and subsequently documented in these guidelines.

#### Coding Practices

This section provides an overview of the general coding practices used for the ECOTOX knowledgebase. These practices have been devised to ensure accuracy and consistency in transcribing data from the original publication to the final data file.

- Each reported test exposure requires a separate test entry into UNIFY. The UNIFY system guides the user through the required data fields using dropdown menus and text boxes.
- When endpoints, effect or exposure concentrations/doses, control data and exposure times reported in graphic format are coded (additional data was reported in text and/or table) the following are used to represent the data: Data extracted from graphs are presented as range or <, > values, unless an exact value is clearly presented. The values taken from the graph must be ranged using the author's X- and Y- axis values. Do not interpolate values that lie between axis values. A result remark is added to the observed response field denoting that the data were extracted from a graph. †Data extracted from a graph must be accompanied by a comment in the COMMENTS field "FROM GRAPH" in the corresponding remark data field. However, it is acceptable to estimate values from the graph for Exposure Durations (see Exposure Duration (ETIME) for further details).
- If there are discrepancies between data presented in the text and tables, data in the text is coded. If there are multiple discrepancies in the text, the most reported value is coded.

- To ensure completeness and accuracy, if information is unavailable for a coding field, the field must still be completed using either NR (not reported) or occasionally, NA (not applicable), with the exception of comment and operator fields. Note that for all fields that have a Unit data field, entering “NR” in the unit field will populate “NR” in the mean, min and max data fields.
- To ensure accuracy in entering data values, all numbers between zero and one should be reported with a zero preceding the decimal point (e.g., 0.5 not .5). Periods are only used to represent a decimal point, never an abbreviation.
- To ensure consistency as well as accuracy, report the significant figures as the author reports them. Do not add or round off numbers. Report only the actual values, do not code variance information (e.g. +/-).
- When entering numbers do not use commas. They can be mistaken for decimal points or numbers.
- Use “per” or a colon (:) instead of a slash (/) to designate ratios. Reserve the slash for designating units.
- Use commas to separate notes in the Comments fields. Do not use any slashes (/) in the text fields, which was used historically.
- A COMMENT field is available for data fields in UNIFY, e.g. Formulation, Measurement, etc. and are located next to the data field.
  - ✓ Comments are used to better define or capture the researcher's intent. THESE ARE USED SPARINGLY. Comments are made in the associated data field’s comment field. For example, an ENDPOINT comment of median LC50 is located in ENDPOINT remarks data field as “MEDIAN LC50.
  - ✓ For all Comment data fields, reviewers should not use the percent or apostrophe symbols as these will cause errors to be thrown. The alternative is to omit apostrophes, and use the word for "percent" in the comment data fields. In addition, the < sign will cause information proceeding it to be stripped from excel files. The words “LESS THAN” should be used instead
- When a reviewer has completed the review of a paper, they must write their last name preceded with “R=” on the bottom center of the paper. If the paper is coded in entirety, the total number of result records coded is also recorded as “TT=xx”.
- 
- If the author provides data from another publication, the reviewer should search the UNIFY reference module to determine if this reference has been previously identified as applicable to ECOTOX. If the paper has not been identified, the reviewer should have the paper ordered by the literature acquisition staff. If the cited data is from a publication which is within the ECOREF file, the ECOREF number should be noted on the original paper. Data should only be coded from the original publication to avoid duplicate data within the knowledgebase. The bibliographic citations for references containing the common data must be cross referenced. If a publication is missing pages, pages are cut off or illegible, the first step is to reorder the publication. If all versions of the publication are incomplete, then the data is coded as possible from the available publication. The missing information is documented as part of the bibliographic information.
- For supplementary data published by journal and not printed: If the supplementary journal materials are needed for coding the publication and the pages are not

attached to the paper, an email is sent to appropriate staff to request supplementary journal materials for the article. This ensures that the pdf copy of the article is complete.

- All information from a paper is abstracted using the author's terminology and numeric values. Exceptions to this include the expansion of exponential numbers and when the author's "words" match the standard definition effects and endpoints. If an endpoint is "interpreted" by an abstractor, it is noted by an 'R' in the ASSIGNED P/R data field.
- To improve the QA process, the reviewer should write the a number (1, 2, 3, etc.) in the paper next to the result encoded. The number written next to the result encoded in the paper will correspond with the Reviewer Number entered on the Result screen.
- Reviewers should note any comments about abstraction in the margins or on the tables/graphs of the original paper. This would include the Test ID Number for each unique test design, the Reviewer number for each record coded, reason for data not being coded, LD50s outside of confidence intervals, errors between text and tables, or other anomalies.
- For mean values outside the min/max values, the abstractor encodes only the endpoint value and notes that the range was not coded in the original publication. The min/max values are placed into the comments field.

## B. ECOTOX CODING GUIDELINES

### Quality Assurance Parameters

Note that all of the quality assurance parameters discussed below are automatically updated by the Unify Toxicity module and are found in the data fields at the top right section of the entry screen.

Record History	(Modified)
Created by: csuomi	on 03/22/2013
Modified by: csuomi	on 04/29/2013
Citation QAed by: csuomi	on 03/29/2013

Data Maintenance  
 Attachment

<input checked="" type="checkbox"/> Citation QAed	Unreviewed	Edit Paper Status
PAPER STATUS: Returned (En Route)	Checkout: 03/28/2013	
DATA STATUS: REVIEWED - UNIFY - COMPLETE	Checkin:	
DUPLICATE STATUS: No duplicates	Last Returned: 04/29/2013	
<input type="button" value="Recall"/>	<input type="button" value="To Recall"/>	<input type="button" value="File"/>
<input type="button" value="Delete"/>		

TOTAL TESTS: 30      Last Reviewed: Brian Kinziger on 04/24/2013  
 TOTAL RESULTS: 54      Last RQAed: Amy Puglisi on 04/25/2013  
 TOTAL DOSE RESPONSES: 0      Last Maintenance QAed:

ECOREF#: 159926

*Efficacy of Some Insecticides on Field Populations of Culex pipiens (Linnaeus) from Egypt by Emtithal, A.E.S., and A.E.B. Thanaa, 2012*

Figure 1. Toxicity Module Quality Assurance Parameters

**QA Date/Initials**

Once the person conducting the first Quality Assurance Check selects QA Complete, the Toxicity module marks the date of the QA check and their name.

**Publication Reference Number, Author, Year**

The Reference Number (REFERENCE #) is the unique number which identifies a particular publication. This number, assigned by the data entry program, provides the link between the data entered and the original publication. It also displays the Author and Year.

**Total Tests**

The total tests encoded for a publication are recorded by the Toxicity module. The total test number equals the total number of individual effect records that are coded for each publication.

**Reviewer/Date**

The reviewer's name and date of review is tracked by the Toxicity module.

**Test Number (TESTID)**

A new Test is added by searching on the Reference Number and selecting the review button. On the entry screen select Add Test. Select the test parameters that define the from the dropdown boxes that are populated from the Skim module.

Each unique test is assigned a unique test number (TEST ID) by the Toxicity module. A unique test design may be characterized by a new test chemical, test species, test location, or exposure type. Additionally, there are experimental design parameters that will influence a test scenario sufficiently to warrant an independent Test ID. Such parameters include tests conducted at different test temperatures or conducted during different seasons. Some examples are found in Tables 1 & 2.

Table 1: A study is conducted with 2 different chemicals and the exposure for 2 species is started at 3 different lifestages. The Test IDs would be:

TEST ID	Unique Test Design
1	Benzene, Fish, Egg
2	Benzene, Fish, Juvenile
3	Benzene, Fish, Adult

TEST ID	Unique Test Design
10	Toluene, Invert, Egg
11	Toluene, Invert, Larvae
12	Toluene, Invert, Adult

Table 2: A study is conducted with 1 chemical and the exposure for 1 species is conducted at 3 different temperatures. The Test IDs would be:

TEST ID	Unique Test Design	Temperature
1	Benzene, Fish, 15C	15 C
2	Benzene, Fish, 20C	20 C
3	Benzene, Fish, 25C	25 C

If appropriate, include information about the varying Experimental Design parameters in the OTHER EFFECT field for each independent record coded. For example, if the temperatures varied as in Table 2 directly above, TEMP EFCTS is coded in the OTHER EFFECT field.

## 1. Test Chemical Parameters (Chemical Information Tab)

### Test Chemical

Seven data fields comprise the Test Chemicals Parameters in ECOTOX; Chemical, In a Mixture, Grade, Purity, Formulation, Radiolabel and Characteristics.

Please see ‘Instructions for encoding combinations of Grade, Purity, Formulation and Characteristics information’ examples below and Concentration Type section for concentration type assignment by Formulation.

### Chemical

Select the chemical name tested from the drop-down menu populated from the chemical information skimmed to the reference.

Reviewers should confirm that the chemical selected was entered correctly during skimming. If the name differs, the reviewer must return to the Skim screen and update skimming prior to proceeding with the review. If several chemical identifiers (e.g., trade names, synonyms, molecular formulas) are reported refer to the ECOTOX Chemical Verification and Entry Procedure SOP for additional guidelines.

### In a Mixture?

Select the check box next to this question if the chemical tested is also part of a mixture study in the reference being coded. Report 'MIXTURE' in the Other Effects comment filed. If it is not part of a mixture, no selection is made.

### Grade (GRADE)

Select the chemical grade information from the GRADE drop-down menu (refer to ECOTOX Code Appendix - Appendix B). If the author does not report the chemical grade information, select Not Reported 'NR'.

### Purity (PURITY)

Enter the numeric percentage information about the purity or active ingredient of the chemical in the PURITY data field (e.g., if the author reports 97% purity, 97 would be entered into this data field).

NOTE: The number preceding the formulation suffix for dry or granular formulations, e.g. 50WP, 50 is the percent active ingredient. For liquid formulations, e.g. 3 EC, the number represents the quantity of active ingredient in the liquid, in this case 3 lbs active ingredient per gallon (from Farm Chemicals Handbook, 1994).

### Formulation (FORM)

Select the chemical formulation code for the chemical reported. The formulation is only entered when explicitly identified by the authors as the form (e.g., commercial) or when presented in a standard format (e.g., 25EC) (refer to ECOTOX Code Appendix - Appendix C). If the author does not report chemical formulation information, enter 'NR'.

### Radiolabel (RADIOLABEL)

If a radiolabeled chemical is tested, select the isotope, according to the ECOTOX Code Appendix - Appendix D, in the RADIOLABEL field. If a specific isotope is not reported, the RADIOLABEL comment field should be coded as 'NO ISOTOPE REPORTED'. When both radiolabeled and unlabeled test chemicals are used in a test, report the radiolabel isotope and code "labeled and unlabeled" in the CHARACTERISTICS field.

### Characteristics

Additional information regarding the chemical is entered, the list below are examples.

- Additional chemical names or isomers (not product numbers)
- Particle size, surface area or particle shape (e.g. nanorods) for Nanoparticle chemicals if additional information e.g. Hydrodynamic size and Zeta potential is reported, 'OTHER PARTICLE PARAMETERS REPORTED' is entered

- 'SALT TESTED' is entered if a specific metal salt is not reported
- 'LABELED AND UNLABELED' if both a radiolabel and unlabeled form are used
- 'SOLVENT EVAPORATED' is entered if the solvent is evaporated prior to testing
- Carriers without CAS numbers, 'CARRIER NATIONAL STICKER, 0.05 UL/L, USED'
- Hydrated forms of a reported chemical - Copper Sulfate pentahydrate (CuSO<sub>4</sub> \* 5H<sub>2</sub>O)
- If the exposure is based a single ion, e.g. Copper but the exposure is due to 2 metal compounds, copper sulfate and copper chloride 'COPPER SULFATE and COPPER CHLORIDE' is entered.

Instructions for encoding combinations of Grade, Purity, Formulation and Characteristics information

When coding the Grade, Purity, Formulation and Characteristics information for a chemical, code all information into the discrete data fields. If all pieces of information from the chemical name have been coded into a discrete data field, no comment is made in the Characteristics data field. If part of the name was not coded in a discrete data field, a comment should be added to the Chemical Characteristics Comment field. If the piece of information is a number only, also code the formulation suffix associated with the number in the Chemical Characteristics data field. See examples below.

- Technical grade chemical, 99.5% active ingredient was tested, code Grade = T, Purity = 99.5, Formulation = NR and Chem Char = NR
- Technical grade chemical, 99.5+ active ingredient was tested, code Grade = T, Purity >= 99.5, Formulation = NR and Chem Char = NR
- Atrazine 70WP, code Grade = NR, Purity = 70, Formulation = WP and Chem Char = NR
- Diuron 4EC, code Grade = NR, Purity = NR, Formulation = EC, Formulation Comment = '4EC' and Chem Char = NR
- Provado 1.6F containing 17.4% active ingredient, code Grade = NR, Purity = 17.4, Formulation = FF, Formulation Comment = '1.6F' and Chem Char = NR
- Chlorpyrifos, 25.3% Active Ingredient, 30% Cis and 68% Trans, code Grade = NR, Purity = 25.3, Formulation = NR and Chem Char = '30 PERCENT CIS AND 68 PERCENT TRANS'
- Ivermectin containing 80% 22,23-dihydroavermectin B1a and 20% 22,23-dihydroavermectin B1b, code Grade = NR, Purity = NR, Formulation = NR, and Chem Char = 80 PERCENT 22,23-DIHYDROAVERMECTIN B1A, 20 PERCENT 22,23-DIHYDROAVERMECTIN B1B

## Carrier

Seven data fields comprise the Test Carrier Parameters in ECOTOX; Chemical, Purpose, Grade, Purity, Formulation, Radiolabel and Characteristics. If more than one carrier is used, enter each carrier separately.

The following guidelines describe how to enter Carriers, Solvents, Adjuvants and Stickers for skimming and reviewing:

- If there is a clean and solvent control, enter the solvent as a test chemical in skimming. It should be coded providing it meets all criteria (concentration, etc.). If there is not a concentration, it should be skimmed as NO CONC.
  - If a CAS number cannot be located and is ARCHIVED, it should be noted in the Chemical Characteristics data field for the primary test chemical (e.g. CARRIER NATIONAL STICKER, 0.05 UL/L). Additionally, the Solvent would be skimmed as ARCHIVED CHEM.
- If the only Control is a solvent control, it should not be entered in skimming as a test chemical and will not be coded.
  - If a Carrier CAS number cannot be located and is ARCHIVED, it should be noted in the Chemical Characteristics data field for the primary test chemical (e.g. CARRIER NATIONAL STICKER, 0.05 UL/L). It would not be entered in skimming.
- Carriers, Solvents, Adjuvants and Stickers are to be treated as Mixtures if it is clear that the focus of the paper is to examine how they may play a role in affecting the toxicity of the primary test chemical(s). In which case, they would be skimmed as MIXTURE.
- If Carriers, Solvents, Adjuvants and Stickers are used as the primary test chemical, they will be skimmed and reviewed.

### Chemical (Carrier/Positive Control)

Enter and select the carrier or positive control name used in the paper. A carrier must be verified as a solvent in the chemical module. If you receive a QA error, "Carrier XXXXX must be a solvent.", forward the chemical and paper to the verification staff for solvent verification.

### Purpose

Select either Carrier or Positive Control as specified in the paper.

### Grade (GRADE)

Select the carrier/positive control grade information from the GRADE drop-down menu (refer to ECOTOX Code Appendix - Appendix B). If the author does not report the chemical grade information, select Not Reported 'NR'.

### Purity (PURITY)

Enter the numeric percentage information about the purity or active ingredient of the carrier/positive control in the PURITY data field (e.g., if the author reports 97% purity, 97 would be entered into this data field.

### Formulation (FORM)

Select the carrier/positive control formulation code for the chemical reported and is only entered when explicitly identified by the authors as the form (e.g., commercial) or when presented in a standard format (e.g., 25EC). If there is more than one formulation code enter the code most closely related to the chemical purity, and enter the rest in the Formulation comment field (refer to ECOTOX Code Appendix - Appendix C). If the author does not report chemical formulation information, enter 'NR'.

### Radiolabel (RADIOLABEL)

If a radiolabeled carrier/positive control is tested, select the isotope, according to the ECOTOX Code Appendix - Appendix D, in the RADIOLABEL field. If a specific isotope is not reported, the RADIOLABEL comment field should be coded as 'NO ISOTOPE REPORTED'. When both radiolabeled and unlabeled test chemicals are used in a test, report the radiolabel isotope and code "labeled and unlabeled" in the CHARACTERISTICS field.

### Characteristics

Additional information regarding the carrier/positive control is entered, the list below are examples.

- Additional chemical names or isomers (not product numbers)
- Percent or volume of carrier used, e.g. 2% v/v or 0.01 ML/L
- 'LABELED AND UNLABELED' if both a radiolabel and unlabeled form are used
- Trade name of carrier/positive control if the trade name is archived and coded under the active ingredient
- '1 OF 2 SOLVENT USED' solvents used if different solvents are noted, but not specified by test chemical

### Additional Chemical and Carrier Information

A differentiation of a mixture is drawn when nutrients are added to an experimental set up. If the author states that a nutrient is added at a level that is needed for growth, ECOTOX does not consider this as a mixture. If, however, the author adds a series of nutrients and toxicants to test interactive effects, ECOTOX considers this a mixture. The following example illustrates how nutrients and toxicants are coded.

**A. Effect of copper on plant growth**

**B. Effect of copper and nutrient on plant growth**

Nutrient	20 ug/l	40 ug/l
Copper		
0 ug/l	100%	120%
10 ug/l	100%	90%
20 ug/l	80%	80%
30 ug/l	60%	50%
40 ug/l	30%	20%

Nutrient	0 ug/l	10 ug/l	20 ug/l	30 ug/l	40 ug/l
Copper					
0 ug/l	100%	100%	100%	110%	120%
10 ug/l	100%	100%	100%	100%	90%
20 ug/l	95%	90%	80%	85%	80%
30 ug/l	60%	60%	60%	65%	50%
40 ug/l	20%	15%	30%	35%	30%

A. Author states that nutrients are added for growth. All results coded. Each nutrient level is coded as a separate test and the nutrient level is noted in an experimental design set-up comment in the EXPERIMENTAL DESIGN field.

B. Author does not state that nutrient is added for growth. Two tests are coded, one for the nutrient tested alone and a second result for the copper tested alone. The shaded area is not coded. Mixture is noted in general remarks.

If the author does not document the basal level value, a determination may be possible for deficient, basal and toxic dose levels. The suggested guideline for making this determination would be interpreting dose response results for mortality, growth and reproduction to determine the deficient, basal (or basal range) and toxic levels. This could be accomplished by interpreting the trends for these effects. The deficient results would be excluded and basal level is coded as the control. If multiple basal values are reported, the most optimal dose for mortality, growth, reproduction would be considered the control value.

If the toxicant added does not produce a toxicity test result (i.e., only deficient and/or basal levels), then the paper would be rejected as nutrient study.

The only situation in which a mixture exposure can be coded is when an *in situ* field study is conducted with the test organisms transplanted from a clean source and caged in the polluted source. The duration and concentration must be provided. For these *in situ* studies where the exposure is by default an exposure to a chemical mixture; code residue effects or endpoints (BCF) only. No other effects or endpoints are strictly attributable to a single chemical in the same way as a residue concentration. If the author provides only the species age for the duration, it is unacceptable to code. Data for all chemicals in the mixture with reported water concentrations and residue effects are coded.

If the author reports that a precipitate was formed during the addition of the chemical or during the exposure period, code “precipitate formed” the EXP DESIGN field.

Effect responses from exposures to hormones (e.g. estradiol, testosterone) are included in the knowledgebase. There are studies where the hormone is administered as a toxicant to observe the andro/estrogenic effects.

Studies involving carbon dioxide (CO<sub>2</sub>) or ozone (O<sub>3</sub>) as the toxicant are not coded into the ECOTOX knowledgebase.

Lead shot papers are not encoded into ECOTOX. Authors often report the number of pellets fed to the animals, but not the actual dose of lead or concentration of lead/pellet. There are also instances in which the lead pellet is actually a mixture of two metals (i.e. Lead and tin).

Nutrients such as phosphorus, nitrogen, potassium are coded for ECOTOX if the exposure system is dosed rather than an ambient exposure.

For example, code phosphorus as an exposure chemical if, in the given paper, all of the following are true:

- The phosphorus was added to the ecosystem in a direct discrete manner, i.e., code "nylon mesh bags of Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> placed in streams at beginning of test", do not code "system may have received added phosphorus in overland runoff due to fertilizers used in nearby agricultural operations". Aerial applications are acceptable if the other conditions are met.
- The concentration in the water should be measured, or at a minimum, the application rate should be available. Application rate may be calculated using the flow volume and the phosphorus-containing compound's dissolution rate.
- The effects of the phosphorus are tested on a biological test organism; water quality or chemical-fate only papers are not coded.

Effect responses from exposures to humic acid only are not coded for ECOTOX. Humic acids are any various complex organic acids obtained from humus which are insoluble in acids and organic solvents. However, tests that include an exposure with a toxicant and humic acid should not be interpreted as a mixture. The humic acid information should be coded into the HUMIC ACID field (see water chemistries section for specifics on coding) if the concentration is given and “Humic Acid Efcts” should be coded in the OTHER EFFECTS field.

Example:           The toxicant is Copper and Humic acid is added at 10 mg/l.  
                           Code the concentration of Humic acid in the Humic acid field as 10 mg/L .

The screenshot shows the 'Chemical Information' tab with the following fields and values:

- Chemical:** 101463698 - Cascade - 488.7734
- Grade:** NR
- Purity:** Mean: 5, Min: NR, Max: NR
- Formulation:** EC
- Radiolabel:** NR
- Characteristics:** NR
- Chemical Carriers:** A table with one entry:
 

Purpose	CAS Number	Name	Molecular Weight	Grade	Purity	Formulation	Radiolabel	Characteristics
Carrier	64175	Ethanol	46.069	NR	NR	NR	NR	NR

Figure 2. Toxicity Module Chemical Information Tab

## 2. Test Organism Parameters (Species Information Tab)

Level	Name
Kingdom:	Animalia
Phylum/Division:	Arthropoda
Subphylum:	Hexapoda
Class:	Insecta
Order:	Diptera
Family:	Culicidae
Genus:	Culex sp.
Species:	Culex pipiens

Level	Name
Subspecies:	Culex pipiens ssp. f

Figure 3. Toxicity Module Species Information Tab

### Species (LATIN NAME/SPECIES #)

Select the species Scientific name as reported in the publication from the dropdown populated during the skim process. Refer to Species Procedures for additional information about the species data file and verification procedures. Field studies may report results for a target community (e.g. benthic macroinvertebrates) or for an entire enclosed ecosystem (e.g. system-level primary productivity or respiration). If a community of organisms was tested, be as specific as the author is about the species grouping.

For efficacy papers, where the paper reports percent plants diseased (usually by fungus) or percent plants damaged (usually by insects or fungus) and the chemical applied is to reduce the amount of fungus or the number of insects, the effect is coded on the fungus or insects. These types of studies generally occur in the field.

#### Examples:

The author is testing peanut plants that are infected with a fungus. A fungicide is sprayed on the infected plants and the paper reports the “percent plants infected”. A population abundance (POP ABND) is coded for the Fungus and “MEASURED AS PERCENT PLANTS INFECTED” is noted in MEASUREMENTS COMMENTS field.

The author is conducting a field study where rice is sprayed with an insecticide to control insect pests. The paper reports the percent damaged plants and grain yield. A population abundance (POP ABND) is coded for the insects and “MEASURED AS PERCENT DAMAGED PLANTS” is noted in the MEASUREMENT COMMENTS field. The grain yield is coded as population biomass (POP BMAS) for the rice.

In some cases, authors will report an effect on a group of organisms or will specify a species composition. If a group of organisms is reported, reviewers should code at the lowest taxonomic level the species composition has in common. An Organism Comment noting the species composition is made.

If the Authors do not refer to a species composition or the species composition crosses taxonomic kingdoms, a generic species term, e.g. Aquatic Community, Soil Community or Microbial Community is encoded. An Organism Comment noting the species composition is made. If this is not possible note "Aquatic Composition", "Soil Composition" or "Microbial Composition" Undefined.

The approved definition of aquatic dependent wildlife is:

"Aquatic-dependent wildlife is comprised of waterfowl (e.g. ducks), shorebirds (e.g. sandpipers), terrestrial mammals that feed extensively on aquatic organisms (e.g. otter, beaver), terrestrial insects, invertebrates and nematodes that have an aquatic lifestage or can live in wetlands (e.g. mosquito, dragonfly) and wetland plants including but not limited to rice, cattails and mangrove species."

#### Habitat (HAB)

Select the habitat in which the organism is exposed; water for aquatic studies, soil for plant and organisms that live in soil or non-soil for organisms that do not live in soil.

The Habitat selection will enable different parameters for field data (Habitat Information Tab in the Test section) and Media Characteristics (Media Characteristics tab in the Result section).

#### Organism Source (ORGANISM SOURCE)

Select the source of the test organism (see ECOTOX Code Appendix - Appendix E for codes). The source describes where the organisms tested were obtained, e.g. collected from the wild, grown in the author's laboratory, purchased from a vendor.

**Laboratory:** An organism cultured or bred for use in any experimental or scientific procedure. This includes organisms from breeding establishments, supplying establishments and user establishments such as universities, aquacultures, fish farms, laboratories, hatcheries and commercial stores. This also includes domesticated organisms such as cows or chickens or when a laboratory strain name is given such as Nostoc sp. Strain PCC 7120.

**Wild:** An organism collected from the natural environment; not cultivated or specifically bred for the use in any experimental or scientific procedure.

**Not reported.** Used when no specific source information is given or it cannot be determined using other information from the paper. This includes organisms procured from "local fish markets" as it is unclear if the organisms have been caught in the wild by local fisherpersons and brought to the markets live. If the author(s) compare results based on the source of the organisms. "ORGANISM SOURCE EFCTS" should also be noted in the OTHER EFCT field.

Example:

1st test: Organisms from a Wild source

ORGANISM SOURCE = WLD

OTHER EFFECT Comment: ORGANISM SOURCE EFCTS

2nd Test: Organisms from a Laboratory source  
ORGANISM SOURCE = LAB  
OTHER EFFECT Comment: ORGANISM SOURCE EFCTS

Record as 'NR' if the information is not reported in the publication.

If the author describes the location where the organism was obtained and/or reared in the laboratory, enter the location as described by the author in the ORGANISM SOURCE COMMENT data field. Do not include any latitude or longitude information.

Example:

ORGANISM SOURCE = WLD  
ORGANISM SOURCE COMMENT: FROM GRASSLAND NEAR EERSTE RIVER,  
STELLENBOSCH, WESTERN CAPE, SOUTH AFRICA

ORGANISM SOURCE = LAB  
ORGANISM SOURCE COMMENT: KARNATAKA FISHERIES DEPARTMENT, DHARWAD,  
INDIA

ORGANISM SOURCE = LAB  
ORGANISM SOURCE COMMENT: ORIGINALLY OBTAINED FROM ERL-DULUTH,  
MAINTAINED IN LABORATORY FOR 50 GENERATIONS

ORGANISM SOURCE = NR  
ORGANISM SOURCE COMMENT: LOCAL FISH MARKET OF JODHPUR

If specific strains are noted, code in the ORGANISM CHARACTERISTICS data field. This would include organisms whose original source was the field, but have been cultured in the lab for generations.

#### Lifestage (LIFESTAGE)

Select the specific lifestage for the test organism at the beginning of exposure, as reported in the paper (see ECOTOX Code Appendix - Appendix F). Record as 'NR' if the information is not reported in the publication. The following are examples of data coded in this field:

a) The author reports adults are tested

Lifestage: AD

b) The author reports exponential growth phase algae are tested

Lifestage: EX

Tests in which eggs are initially exposed, and the exposure continues through adulthood to the first generation etc., are represented as "EG" in LIFESTAGE and the stage of the organism at the time of measurement is recorded in the Result Sample Unit field for the results reported.

Example: Exposed eggs resulting in mortality of fry

LIFESTAGE: EG EFCT: MOR MEASUREMENT: MORT RESULT SAMPLE UNIT: FY

If data for multiple lifestages within the same test are presented along with statistical results, report results for each lifestage separately. If statistics are not presented, combine the results into one data record.

### Age (AGE) and Age Unit (AGE UNIT)

Enter the age and age unit for each test organism at beginning of exposure, as reported in the paper (see ECOTOX Code Appendix - Appendix I for associated time units). The age may be a development stage if no specific time is reported.

For example: The author reports that 4<sup>th</sup> instar larvae were used in the study. The following is coded:

LIFESTAGE: LV      AGE: 4      AGE UNIT: ins

### Organism Initial Weight (WTINT)

If the information is not reported in the publication, record the organism's age as 'NR'.  
Initial Weight

Report the weight and weight unit for each test organism at beginning of exposure, as reported in the paper.

If the author does not report an initial body weight, enter 'NR' in the body weight field.

### Gender (SEX)

This field identifies the sex of the organism (male (M), female (F) or both (B)) at each exposure level. The importance of this field becomes apparent where organisms of both sexes are exposed at a given treatment level, but the observations are conducted on either the male or female. In this instance, the SEX entry would be coded as B, with individual results reported for M and F in the SAMPLE NUMBER UNIT field.

Record as 'NR' if the information is not reported in the publication.

### Organism Characteristics (ORGANISM CHARACTERISTICS)

Report any general information provided about the initial condition of the test organism that is not coded in the LIFESTAGE, INITIAL WEIGHT and/or AGE and AGE UNIT fields. This includes information for both the control and test organisms. Organism comments include information such as length, developmental stage, type of culture (e.g., axenic) and/or initial cell concentration (e.g. 10000 cells/ml) to describe the organism being tested. Each piece of information is separated by a comma. The value and range, if reported, are recorded for each available parameter (e.g. 3(2-4) in). However, deviations are not coded (e.g. 33 +- 4 mm is coded as 33 mm). Record strains, hybrids or taxonomic groupings, if reported. When reporting a cultivar, include 'cv.' before the name of the cultivar. Include 'var.' for variety in a similar manner. Common names and Taxonomy Author names are not coded in this field. List individual species Scientific names when 3 or fewer species are included within a grouping; when more than 3 individual species are included within a grouping, code as "# species."

For example:

The author reports the abundance Branchiopoda and reports the specific species names

SPECIES = Branchiopoda

ORGANISM CHARACTERISTICS: DAPHNIA MAGNA, DAPHNIA PULEX AND BOSMINA SP

The author reports the abundance of weeds in an agricultural crop and gives a list of 25 weed species found in the samples but analyzes all as a group:

\_\_\_\_\_ SPECIES: PLANTAE

\_\_\_\_\_ ORGANISM CHARACTERISTICS: 25 WEED TAXA

Standard terms used for recording organism length include standard length (SL), (e.g. 3.1 cm SL), total length (TL), fork length (FL), carapace length (CL), carapace width (CW).

If a paper reports results using organisms from a polluted source AND organisms from a non-polluted source, only the non-polluted source test results are coded. No mention of 'non-polluted' needs to be presented in the ORGANISM CHARACTERISTICS field, but 'polluted organisms' is entered into OTHER EFCT data field. If data are presented for organisms from a polluted source and no other concurrent data with organisms from a clean source are presented, the reviewer should code the test results, but enter "POLLUTED ORGANISM" in the ORGANISM CHARACTERISTICS data field.

If a paper reports that the organisms used for testing were diseased, "Diseased Organisms" is entered into the EXPERIMENTAL DESIGN field. If the study was conducted on diseased organisms compared to non-diseased organisms, the data from the non-diseased organism test is coded, but the data from the diseased organisms is not. In this case, "Diseased Organism Test" is entered into the OTHER EFCT field.

If a paper reports that the organisms used for testing were altered organisms (e.g. surgically, genetically, etc.) and also reports data for unaltered organisms, only the data for the unaltered organisms is coded. A comment of "ALTERED ORGANISM STUDY" is entered into the OTHER EFCT field. If only altered organisms are used, the data is coded and the specific alteration is reported in the EXPERIMENTAL DESIGN field.

If a paper reports that the organisms used for the measurements were dead and it is unknown how long the organisms have been dead, do not code these data, but enter "DEAD ORGANISMS" in OTHER EFCT. An author may measure the growth or weight of a dead organism. The weights of dead animals can be biased, especially for juveniles, depending on the time between death and measurement. Also, temperature may affect the measurement after death (e.g. higher brooder temperatures may cause rapid desiccation which would result in weight changes).

If the study was conducted on dead organisms compared to living organisms, the data from the living organism is coded but the data from the dead organism test is not coded. In this case, "DEAD ORGANISM" is entered into the OTHER EFCT field.

If some of the organisms tested are fed differing amounts and/or some of the organisms are not fed during the study and the authors are comparing the data (e.g. 10 organisms fed, 10 organisms not fed and/or some organisms fed 10 pellets and some organisms fed 5 pellets), all data are coded, "FED", "NOT FED" or the food amount is coded in the EXP DESIGN field, and "FEEDING EFCTS" is coded in the OTHER EFCT field.

If an unverified species has been sent to the species verification staff and it was unable to be verified, the species verification staff will assign the next highest taxonomic level and the unverifiable name reported by the author is recorded in the ORGANISM CHARACTERISTICS field.

For in Vitro studies, report the cell types or organs used in the study. If the isolation method is reported, encode the isolation method. For example, HEPATOCYTES AT A FINAL CONCENTRATION OF 2000000 CELLS/ML. HEPATOCYTES WERE ISOLATED BY THE IN SITU PERFUSION METHOD OF MOMMSEN ET AL., 1994.

### 3. Test Information Parameters (Test Information Tab)

The screenshot displays the 'Test Information' tab within a software interface, organized into three main sections: Chemical Information, Species Information, and Test Information. The 'Test Information' section contains the following fields and values:

- Study Duration:** Mean 24, Min NR, Max NR, Unit h.
- Exposure Duration:** Mean 24, Min NR, Max NR, Unit h.
- Concentration:** Empty field.
- Study Type:** NR.
- Test Type:** NR.
- Test Location:** LAB.
- Test Method:** WHO.
- Exposure Type:** AQUA - S.
- Media Type:** FW.
- Application Frequency:** Mean 1, Min NR, Max NR, Unit X.
- Control/Dose Types:** Empty field.
- # Doses:** Mean 8, Min NR, Max NR.
- Doses Table:**

Dose Number	Control	Conc	Type	Dose	Unit	Organism #	Comments
4	REPLICATES						
- Experimental Design:** 4 REPLICATES.
- Additional Comments:** Empty text area.
- Other Effects:** STRAIN EFFECTS, RESISTANCE RATIO.

Figure 4. Toxicity Module Test Information Tab

Exposure and Study Duration

A toxicity test may range in duration from a pre-treatment period through the actual toxicant exposure and conclude with observations of the organisms post-exposure. Duration information is coded using the units reported in the publication (see ECOTOX Code Appendix – Appendix I for valid units). Refer to Table 4 for a coding example. Exposure and study durations are reported with the Test Information. Observation Duration is reported with the Results Information.

Table 4. Example 17-day experimental period with 2-day pre-treatment, 5-day exposure, and 10-day observation. Note: Pre-treatment days are not included in the study duration and are considered Baseline Control data.

	Duration of Experiment																
	Pre-Trt		Exposure					Observation									
Calendar Days	1	2	3	4	5	6	7	8	9	10	11*	12	13	14	15	16	17
Test Periods	1	2	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10
Reported Days (Study Duration)	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

**Note:** In test scenarios where incubation times are reported, i.e. enzyme fixation assays, be careful to report the toxicant exposure time *not* the assay incubation time.

For studies using laboratory species and where exposures are noted as a text description, e.g. from mating through lactation, an approximate duration may be calculated using default duration values as noted in the table below. A comment should be added to the specific duration comment data field noting the calculation of the duration.

For example, an exposure to adult mice starts 3 weeks prior to mating and continues through gestation and lactation. The exposure duration would be 3 weeks + 21 days gestation + 21 days weaning = ~63 days. Code ~63 days in the exposure duration field and an Exposure Duration comment "3 weeks + 21 days gestation + 21 days weaning = ~63 days".

Default Weaning and Gestation duration for Laboratory Species

Group	Species	Weaning (days)	Gestation (Days)	Puberty (days)	Reference
Laboratory Rodents	Mouse	21	21	50	USEPA, 1988
	Rat	21	21	56	USEPA, 1988
	Guinea Pig	14	60-72	70	USEPA, 1988
	Hamster	21	16	60	USEPA, 1988
	Gerbil	21	24-26	70	USEPA, 1988
Other Laboratory Mammals	Cat	49	52-65	240	USEPA, 1988
	Dog, Beagle	42	53-71	240	USEPA, 1988
	Rabbit, New Zealand	56	30-32	190	USEPA, 1988
Avian Species	57 Species	See Reference	See Reference	See Reference	Data for life-history parameters including time between eggs laid, incubation start, time to hatch, time to fledging, etc. In Bennett, R.S. and M.A. Etterson (2013). Avian Life-History Profiles for Use in the Markov Chain Nest Productivity Model (MCnest). Version: 12 December 2013 (EPA/600/R-14/009).

Study Duration (STUDY DURATION)

The study duration is the total time of the study *excluding* pre-treatment times. In the example in Table 4, the study duration is equal to 15D (5D exposure plus a 10D observation). In cases where the observation time is the only duration reported, it is assumed that the study duration is equivalent to the observation time. The study duration will be reported as 'NR' if no observation or study time is reported.

**Note:** for most field studies the exposure and study duration are identical because it is difficult to determine when the exposure ends. It is difficult to know when the application has completely dissipated in the environment. For lab studies the exposure and study duration may be different. This difference will be seen when there is a recovery period from exposure duration. For lab studies when the treatment is some type of injection or diet (e.g. intraperitoneally or by gavage), study duration and exposure duration are the same.

**Note:** for regressed/extrapolated duration endpoints, e.g. LTxx, ETxx, etc., if the duration of the regressed/extrapolated endpoint is outside of the study duration reported by the author, include the maximum regressed/extrapolated duration as the Study Duration Maximum value. The Minimum Study Duration is either the Minimum duration of a range reported by the author OR the Mean value, if no minimum is provided. The Study Duration Comment "INCLUDES DURATION FROM ENDPOINT REGRESSION/EXTRAPOLATION, STUDY DURATION REPORTED AS XXX DAYS" is included.

Example 1: The Study Duration is reported as 20 days, but the author reports the ET50 as 15 (11-22) days. Report the Study Duration Minimum as 11 and Maximum as 22 days, Study Duration Comment: INCLUDES DURATION FROM ENDPOINT REGRESSION/EXTRAPOLATION, STUDY DURATION REPORTED AS 20 DAYS

Example 2: The Study Duration is reported as 10 - 20 days, but the author reports the ET50 as 15 (11-22) days. Report the Study Duration Minimum as 10 and Maximum as 22 days, Study Duration Comment: INCLUDES DURATION FROM ENDPOINT REGRESSION/EXTRAPOLATION, STUDY DURATION REPORTED AS 10-20 DAYS

When study duration values are extrapolated from a graph, the values can be approximated even when the mark does not appear on the graph. For example, if the value on the graph is in the middle of two marks, namely 10 and 14 days, the STUDY DURATION can be coded as “12 d” with a remark that it is from the graph in the Study Duration comment data field.

### Exposure Duration (EXPOSURE DURATION)

The exposure duration is a mandatory field for inclusion in the ECOTOX knowledgebase. In cases where the observation time is the only duration reported, it is assumed that the exposure duration is equivalent to the observation time. If the exposure duration is not reported, the paper is rejected. The period of time recorded in the EXPOSURE DURATION data field is the time of actual exposure to the chemical. (See ECOTOX Code Appendix – Appendix I for unit codes). If the application frequency is not reported, record ‘NR’.

In some cases a biological time is used, such as an exposure time reported as “until hatch”, “growing season” or “after the nth egg has been laid”. Use the code from Appendix I that best describes the author's words in duration units data field. ‘NA’ will be coded as the duration mean value for all biological time durations (e.g. EXPOSURE MEAN: NA, EXPOSURE UNIT: HT; corresponds to an until hatch duration), unless a numeric value is substituted (e.g. EXPOSURE MEAN: 2, EXPOSURE DOSE: HV; corresponds to a 2 harvests duration). However, references to time such as “observed at end of the study period” are not coded; such papers are rejected as having no exposure duration.

For injection, diet, topical and environmental exposures where the actual exposure is dependent on biological and environmental conditions, the exposure time is recorded as equivalent to the study time. This assumption is made to ensure consistency in data representation; it is not necessarily a true reflection of the exposure time.

When exposure duration values are extrapolated from a graph the values can be approximated even when the mark does not appear on the graph. For example, if the value on the graph is in the middle of two marks namely 10 and 14 days, the EXPOSURE DURATION can be coded as “12 d” with a remark that it is from the graph in the Exposure Duration comment data field.

**NOTE:** For any single test record, only one duration unit can be used for this field. If authors report a combination of durations and they use different units for the same test, one of the values is converted to the other and summed (use only whole or decimal numbers). For example, the author reports 28 day of exposure followed by 8 hours of behavior testing (while still being exposed), the duration would be coded as 28.33 days. The 8 hours is divided by 24 hours in a day to get 0.33 days.

Application Frequency (APP FREQ)

Report the frequency of the dose application in the APPLICATION FREQUENCY data field. Refer to ECOTOX Code Appendix – Appendix K for application frequency codes and units. If the exposure type is flow-through or continuous flow in lab studies, code “NA” as the frequency and "CON" as the unit in the APP FREQUENCY field.. If the exposure type for a lab study is static code “1” as the frequency and "X" as the unit in the APP FREQUENCY field. If an application frequency is not reported, record NR.

**Note:** The “X” in an application frequency unit represents the number that is written prior to the code.

Examples:

The author reports that the water was renewed every 48 hours. Code:

APP FREQ: 48 E X H

The author reports that the chemical was applied 3 times a month. Code:

APP FREQ: 3 X per MO

The author reports that the chemical was applied as a pulse dose of 3 hours every day. Code:

APP FREQ: 3 H per D

Control Type (CONTROL)

The type of test control(s) used in the study is reported in this field. Control information for the reported effect may be presented in the text, in a graph, or in table format. ECOTOX reviewers do not make assessments whether the controls were satisfactory or insufficient (e.g., were replicates run, did control organisms die), but simply document whether the author(s) present information that a control was used. When author’s state that controls were similar to treatment with the exception that no chemical was added, and within the same paragraph they describe using solvent in all treatments, a solvent control should be interpreted (refer to ECOTOX Code Appendix – Appendix M for control type codes and definitions).

When statistical comparisons are presented for multiple controls (e.g., statistics in relation to a standard control and statistics in relation to a solvent control), enter all control types used in the study in the CONTROL TYPE field and note the appropriate control type used for the coded statistics in MEASUREMENT REMARK. (e.g., stats based on solvent control) The data related to the solvent control is coded preferentially over the standard control data and should be noted as such.

When multiple controls are reported, enter all control types into the Control data field.

For plant field studies where both an untreated control and a weeded control are used, note that both ‘C’ and ‘O’ control types are used. For the weeds, the untreated control is designated as the ‘C’ control and O = HAND WEEDED is entered in the CONTROL COMMENTS data field. For the crop species, the hand weeded control is designated as the ‘C’ control and O = UNTREATED is entered in the CONTROL COMMENTS data field. These

controls are chosen as they more directly reflect the effect of the chemical on each species, eliminating the weed competition for the crop.

If a test is conducted where a radiolabeled chemical is added to all experimental units and the non-radiolabeled chemical is added to all but one (authors may call this the control), and the authors do not report a clean control (nothing added), the test is then coded with Z control (no control reported). See ECOREF 9212 for an example.

### Concentration Type (CONC TYPE)

The three forms of toxicants evaluated in ECOTOX are organic compounds, metals and inorganic non-metals. Each form can be identified as a concentration type code using the single letter abbreviation.

Organic compounds are defined by the pesticide terms, formulation (F) and active ingredient (A). For publications that report a chemical as a trade/formulation name, e.g. Captan, and note that the formulation contains 50% active ingredient but do not specify if the concentration that is reported is based on the formulation or active ingredient, code the Concentration Type as not reported (NR). If the paper uses the technical name, e.g. 1R-cis-Bifenthrin, and reports the purity as 77% but does not specify if the concentration is based on the active ingredient or total compound, code the Concentration Type as formulation (F).

ECOTOX encodes Concentration type (A) active ingredient:

- if the chemical Purity is  $\geq 85\%$
- if the chemical is obtained as a specific Grade or Standard (refer to ECOTOX Code Appendix - Appendix B Chemical Grade). Chemical grades and standards tend have a known and typically high purity ([www.ee.washington.edu/research/microtech/cam/PROCESSES/PDF%20FILES/ChemStorage.pdf](http://www.ee.washington.edu/research/microtech/cam/PROCESSES/PDF%20FILES/ChemStorage.pdf)).
- for specific formulation that have a high purity (refer to ECOTOX Code Appendix - Appendix C Chemical Formulations)

Metals are defined by the concentration types, total (T), dissolved (D), and labile/free (L); while ammonia or hydrogen sulfide compounds may have total concentrations (T) and/or un-ionized (U) concentrations. Organometals are compounds that contain covalent bonds between carbon atoms and metal atoms and are coded as total (T), dissolved (D), and labile/free (L) concentrations.

If multiple representative concentrations of a metal or inorganic non-metal are reported in the reference, both concentrations are included in the same ECOTOX record; i.e., both total and un-ionized concentrations are reported in the concentration field. If the author reports the ammonia concentrations as based on  $\text{NH}_4\text{-N}$  or  $\text{NH}_3\text{-N}$ , code CONC TYPE as "T" and "U", respectively in the same record and code the specific ion information in the ION fields.

Concentration Type is also linked to the Chemical Analysis Method (METHOD) field (see discussion below on Active Ingredient).

Concentration Types and Definitions

## Organic:

**FORMULATION (F):** Way in which basic pesticide (toxicant) is prepared for practical use (Ware, 1978). Generally reserved for commercial preparation prior to actual use and does not include the final dilution (Insect-Pest Management and Control, 1971) (e.g.; Baythroid, 2,4-D). Also included in this category are organic compounds with no pesticidal activity (e.g.; PCB, dioxin).

**ACTIVE INGREDIENT (A):** Chemical substance in a product that is responsible for the pesticidal (toxic) effect (Ware, 1978). Reported as "A" when the author refers to the concentration as active ingredient, active principle, active substance (A.S.), acid equivalent or various grades of reagents (i.e., Analytical, Reagent or Technical). When coding, a value in the publication may be reported as "AI kg/ha", "AE kg/ha", or "kg AI/ha"; in ECOTOX this type of value is reported as 'A' for CONC TYPE

If author reports both Formulation (F) and Active Ingredient (A) concentrations, the Active Ingredient (A) concentrations are coded.

**Note:** Information reported in the PURITY field does not necessarily determine whether concentration is A or F. In addition to the description above, using "A" as the concentration type occurs in situations such as the following:

- 1) Author states concentration of pesticide as "AI".
- 2) Author states %AI (PURITY) and reports measured concentration.
- 3) Author states measured concentration of a pesticide.

## Metal/Organometals/Inorganic metals:

**TOTAL (T):** The concentration of metals determined on an unfiltered sample after vigorous digestion, or the sum of the concentrations of metals in both dissolved and suspended fractions ( APHA et.al. 1992). Heavy metals and single elements (e.g. Na, Cl, Br) are coded as T.

**DISSOLVED (D):** Those constituents of an unacidified sample that pass through a 0.45 um membrane filter (e.g. soluble metal) (APHA et.al. 1992).

**LABILE (L):** The labile or free ion metal concentration determined by various analytical methods. When coding, the specific labile forms or complexes are not differentiated.

## Inorganic non-metals:

Concentrations of ammonia and hydrogen sulfide are reported in the literature in either the total or unionized form. Code the form as specified by the author. Ammonia may be reported as a variety of different forms, e.g., NH<sub>3</sub>, NH<sub>4</sub><sup>+</sup>, NH<sub>3</sub>-N, NH<sub>4</sub>OH, or NH<sub>4</sub>Cl. (US EPA 1979) The author must state whether the form is **T**otal or **U**nionized; **T** is the default for ammonia and hydrogen sulfide papers that do not state whether total or unionized concentrations are reported.

**TOTAL (T):** The dissociated, charged form of nitrogen or hydrogen related chemicals. This can take on numerous forms, e.g.; ammonium (NH<sub>4</sub><sup>+</sup>), nitrite (NO<sub>2</sub><sup>-</sup>), etc. (Rand and Petrocelli, 1985).T is the default for publications that do not state whether Total or Unionized concentrations are reported.

**UN-IONIZED (U):** The undissociated, uncharged form of ammonia or hydrogen sulfide. The ammonia molecule, NH<sub>3</sub>, is the unionized form. (In aqueous solution, ammonia assumes an equilibrium between NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup>.) The NH<sub>3</sub> is the toxic entity of the ammonia compound (Rand and Petrocelli, 1985).

Some aluminum compounds report many types of concentrations for aluminum; Total, Total Monomeric Inorganic, Reactive, Filterable, Dissolved, Labile, Non-Reactive, etc. In these cases, only code Total, Dissolved and Labile when specifically reported by the authors. If other types, e.g. Total Monomeric Inorganic, Reactive, Filterable, Non-Reactive, etc., appear in the paper, add CHEMICAL FRACTIONATION in the DOSE COMMENTS field on the Test Information page for each concentration and in the CONCENTRATION COMMENTS on the Results page for each result.

If speciation of the compound is reported, e.g.  $AlOH^+$ ,  $Al_2O_3^+$ , etc., add CHEMICAL SPECIATION to the OTHER EFFECTS COMMENTS.

See ECOTOX Code Appendix – Appendix A for additional information.

#### Test Type (STYPE)

Report the type of test set up for the toxicity study as reported by the author (e.g. ACUTE, CHRONIC, ELS (Early Life Stage), FLC (Full Life Cycle), Generational). See ECOTOX Code Appendix – Appendix GG for codes and definitions.

#### Test Location (LAB, NR, FIELDN, FIELDA,)

Enter the location or setting in which the experiment was conducted (see ECOTOX Code Appendix – Appendix H).

A natural field study (FieldN) is an experiment conducted outdoors in a natural setting. The selection of 'FieldN' will enable different parameters for field data to be coded (See Section 4 Field Test Parameters (Habitat tab) for further information).

An artificial field study (FieldA) is an outdoor study conducted in a simulated environment. Such studies include organisms isolated from their natural environment while still out of doors, e.g. concrete ponds, outdoor pots or livestock pens.

Laboratory tests (LAB) are conducted under indoor laboratory conditions.

If the location or setting cannot be determined from the publication, code as Not Reported (NR).

#### Test Method (TMETH)

Enter the name of the testing method used (or a modification of) for conducting the toxicity experiment (e.g. ASTM (American Society for Testing and Materials) or OECD (Organization for Economic Cooperation and Development)). If the author presents the method or guideline number and year (or it appears in the bibliography), enter this information in the Test Methods comments. See ECOTOX Code Appendix – Appendix HH for the codes and definitions.

If the author does not reference a standard test method, but does reference methods that were obtained from other authors, select Other Method (OM) and enter the author(s) and year published in the Test Method comment field.

#### Exposure Type (EXP TYP)

For the ECOTOX knowledgebase, the term 'exposure' is used to refer to the mechanism by which the toxicant was applied. Organisms are typically exposed to toxicants through diet, injection, topical or environmental routes. On occasion, an exposure may be through multiple routes (e.g., such as topical and oral).

Some exposures could be coded a variety of ways. For example, exposure as an aerial spray to a field plot could be coded either as a spray application or as exposure through multiple routes, e.g. topical (through skin) and diet (from consumption of exposed vegetation) for animals, or topical (through leaves) and environmental (root uptake) for plants. Within the ECOTOX knowledgebase, this instance is coded as a spray application. Multiple exposure route coding is applicable when the organism is exposed through two *independent* applications, for example, a contaminated diet *and* topical application for animals or contaminated soil *and* leaf spray for plants. In this scenario, 'MU' would be entered into the EXPOSURE TYPE data field and a remark ("FD and TP" or "PR and FS") would be noted in the Exposure Type comments section.

When coding, report the specific exposure type, e. g., for an intercutaneous injection, code as IC (intercutaneous). If an exposure type is not reported, code as Not Reported (NR). Refer to ECOTOX Code Appendix – Appendix J for exposure type codes.

If a species is implanted with a chemical capsule or time release pellet, code as the closest injecting type and note "Implant" in the Exposure Type notes field.

The use of "Pulse" as an exposure type requires special care. ECOTOX define a pulse exposure as chemical added to exposure system with clean media being added in between chemical exposures. For example, the author reports a pulse exposure of chemical in the water for 24 hours followed by clean water for 48 hours , then chemical added again for 24 hours. Authors will use the word "pulse" as adding chemical to the system but to be coded as Pulse, it must be as described above.

#### Media Type (MEDIA TYPE)

Enter the type of exposure media, (e.g., natural or artificial soil, fresh or saline water), filter paper, etc.), in the EXPOSURE MEDIA data field using codes presented in ECOTOX Code Appendix – Appendix L.

For terrestrial studies, if an aqueous exposure is conducted in pore water from a specific soil, report the soil parameters in the soil characteristics fields (pH, CEC, OM, etc.). If the bottom of the experimental chamber is covered with sand and then topped with filter paper, an EXPERIMENTAL DESIGN (EDES) remark should be made that sand was used in the chamber, but MEDIA should be FLT.

For aquatic studies, freshwater (FW) tests include 1) laboratory tests conducted in freshwater, reconstituted water, distilled water, or tap water or 2) field tests where the organism habitat is exclusively freshwater. If a salinity value of <4 0/00 is reported and the paper does not specify whether it is fresh or saltwater, it will be coded as a freshwater test.

Saltwater (SW) tests include 1) laboratory tests conducted in natural or artificial seawater, brackish water, or estuarine water or 2) field tests where the organism habitat is exclusively saline.

If a media type is not reported, the habitat where the species predominates is used. For example, *Daphnia magna* are a freshwater organism and would be coded as freshwater Media Type. An internet search may be conducted to locate the habitat/media type.

### Media Comments

Enter the media description that is used in the study. This includes the culture media for in Vitro studies or the soil or aquatic media for aquatic and terrestrial studies. Include commercial or scientific names and any additional description of the composition of the media.

For Example:

- NATURAL SPRING WATER AMENDED WITH SODIUM BICARBONATE
- CECIL SILT LOAM
- CHARCOAL-DECHLORINATED BLACKSBURG TAP WATER
- DUTCH STANDARD WATER, CANTON AND SLOOF, 1982
- TIFTON LOAM, PEAT AND SAND, 1:1:1 V/V/V
- INSTANT OCEAN
- MIRACLE-GRO MOISTURE CONTROL POTTING MIX
- ASTM HARD WATER

### Number of Doses (DNUM)

Enter the total number of exposure doses, including the controls, for each independent test design. If number of exposures is not reported, e.g. in a publication reporting only calculated endpoints such as LC50s, code the field as 'NR'. Do not include number of replicates in the total number of doses. Do not include the Historical, Baseline or Positive controls in the total number of doses.

### **3a. Doses (DOSES)**

(Table within the Test Information Tab)

There are multiple data fields that comprise a Dose Record; Dose Number, Control Type, Concentration Type Dose and Unit Organism Number and Dose Comments.

To Enter New Doses, select the Add Dose Link.

### Dose Number

Dose Number is auto generated by the Toxicity Module. Doses are entered by Control first then in order of lowest to highest. The Dose numbers can be edited, if needed by selecting the Reorder Dose selection.

### Control Type

Enter the Control Type used as needed. Only Controls that were entered above will appear in the dropdown. The Historical, Baseline or Positive controls are not used for this field as they are not included in the total number of doses.

Concentration Type

Concentration Type is generated by the Toxicity Module base on the entry made above. If multiple Concentration Types are present (based on entries above) multiple rows will appear for population.

Doses

Enter the concentrations and the units used for the toxicity test in the Mean, Min, and Max data fields.

If the author reports values for some of the doses, e.g. 4 out of 7 doses, code the doses presented and note 'ALL DOSES ARE NOT REPORTED' in the DOSE COMMENT data field.

If a background concentration is reported for the chemical being applied, report the background value in the control dose in the DOSE field.

Occasionally an author will report a concentration as a % or fraction of an LC50 value; e.g., either the sublethal concentration used was "10% of the 96-h LC50" or "1/10, 1/15 and 1/20 of the LC50". Such concentrations may be recalculated and used as the concentration tested if the original LC50 concentration is provided in the publication. Flag the recalculation in the paper so that the calculation may be QAed and document the recalculation in the margin or on a blank page of the publication. A comment of "RECALCULATED" in the DOSE COMMENT data field.

For terrestrial studies, if chemical concentrations (especially metals) are reported in terms of Total, Exchangeable, Water Soluble and Pore Water concentrations, Total is the concentration selected for entry into the DOSE data field. The other concentrations are reported as remarks.

For studies where a single chemical concentration is monitored as a water or soil chemistry parameter (e.g. sodium chloride reported as conductivity, calcium chloride reported as hardness), code the water or soil chemistry parameter in both the dose and the water chemistry parameter data fields.

When multiple dose/concentration units are reported (e.g., ppm and mg/kg body wt), code the unit that represents the more accurate dose the organism receives (e.g., mg/kg body wt is more accurate than ppm).

If an author reports concentrations in two different units, select the unit that is more easily converted to ECOTOX standardized units (see below). If a molar and non-molar unit are reported, always use the non-molar concentration. For example, the author reports both ug/L and uM, code the ug/L concentration as ECOTOX can convert this to a standardized unit. Similarly, if a gavage exposure is reported as ppm or mg/kg bdwt, code the mg/kg bdwt as this is the gavage conversion.

ECOTOX Standard units for Water exposure are ug/L; Injection, Oral or Gavage are milligrams per kilogram body weight (mg/kg bdwt); and Diet (i.e. Diet, Food, Drink, Choice, Gestation, Lactation) or Topical are parts per million (ppm).

If the author conducts a diet study and reports the daily and cumulative doses, the daily dose is coded and the cumulative dose reported as a comment, i.e. CUMULATIVE

DOSE ALSO REPORTED . This comment is also made in the RESULT CONCENTRATION COMMENT field

If the doses/concentrations are reported as both measured and unmeasured values but ALL analysis is based on the unmeasured values, code U in the CHEMICAL ANALYSIS field, the unmeasured values and note “MEASURED CONCENTRATIONS ALSO REPORTED” in the OTHER EFFECTS field.

If the author reports that the doses/concentrations were measured but as they were within a certain percentage of the unmeasured (nominal) values only the nominal values were reported in the paper, a comment is made noting this i.e. MEASURED VALUE WITHIN X PERCENT OF NOMINAL VALUE.

Example: Author states: Results showed that the measured concentrations varied generally less than 5 % from the nominal concentrations, therefore all calculations were based on nominal concentrations.

Code: Nominal values in DOSES with a Dose value Comment: MEASURED VALUE WITHIN 5 PERCENT OF NOMINAL VALUE. A “Z” is coded in the CHEMICAL ANALYSIS METHOD field

Another way this is commonly reported is as a percentage of the intended value. This comment is coded as: MEASURED VALUE WAS X PERCENT OF NOMINAL VALUE

Example: Author states: Results showed that the measured concentrations were 97.5 % of the intended concentration, therefore all calculations were based on nominal concentrations.

Code: Nominal values in DOSES with a Dose value Comment: MEASURED VALUE WAS 97.5 PERCENT OF NOMINAL VALUE. A “Z” is coded in the CHEMICAL ANALYSIS METHOD field.

Additionally, if the author states that they have analytically validated the concentrations and that no differences or no significant differences were found between nominal and measured concentrations were found but they do not report whether the values reported are the nominal or the measured values, the author reported text is used as the comment and the Chemical Analysis Method is coded as Z .

Example: Author states: Analytical validation of the concentrations used in the experiments was achieved by HPLC. No significant differences between nominal and effective concentrations was found.

Code: Nominal values in DOSES with a Dose value Comment: NO SIGNIFICANT DIFFERENCES BETWEEN NOMINAL AND EFFECTIVE CONCENTRATIONS. A “Z” is coded in the CHEMICAL ANALYSIS METHOD field

If the author reports the dose values as a “TIME WEIGHTED AVERAGE”, this is coded in the Comments field of the DOSE value.

Organism Number

Enter the number of organisms that were exposed at each dose. Do not sum all replicate values.

Experimental Design (EXP DESIGN)

This field is used to code additional study information.

- If the number of replicates is reported, it is captured in the EXP DESIGN field as “X REPLICATES” (e.g. 3 REPLICATES).
- For laboratory studies, enter the laboratory where the study is conducted, e.g. GREENHOUSE AT LOUISIANA AGRICULTURAL EXPERIMENT STATION, BATON ROUGE, LA. For field tests, report the application timing, e.g. pre-emergence, post-emergence, etc.
- If an organism is pre-exposed to another chemical and this is the only information that can be coded, the chemical that is associated with the observed effect is coded and “preexposure with X” is noted
- Note the exposure system dimensions (e.g. pond or lake depth, cage or enclosure size), type of artificial substrate and physical or chemical water chemistry parameters.

EXP DESIGN: 3 HA POLYETHYLENE LINED POND

EXP DESIGN: 4 x 4 M CAGE

EXP DESIGN: SEDIMENT

EXP DESIGN: INSTANT OCEAN

EXP DESIGN: SUSPENDED MESH BAGS

EXP DESIGN: 3 REPLICATES

- If the author reports that a precipitate was formed during the addition of the chemical or during the exposure period, code “precipitate formed”
- In field studies when the paper reports using standard agricultural practices such as the application of chemicals to the plots for weed, fungus and/or insect control and no effects are reported, then a comment is added to the EXP DESIGN field listing the chemicals used or reporting the number of chemicals used in the format of:

“CHEMICAL NAMES OR GROUPING” APPLIED, NO EFFECT REPORTED”

Examples:

DIFLUFENICAN, ISOPROTURON AND CHLORMEQUAT APPLIED, NO EFFECT REPORTED

## 10 PESTICIDES APPLIED, NO EFFECT REPORTED

Prior to 10/2014 “MAINTENANCE CHEMICALS USED” was used in EXP DESIGN to represent this practice.

- Additional information about media and test chambers is coded if one of the purposes of the study is to compare results observed under differing test conditions (e.g., pH, temp, humic acid, sediment) were used in the study, enter the changing parameter, e.g. 9.8 mg/L OM. If one of the purposes of the study is to compare experimental effects (pH, temp, sex) in addition to toxicant effects, report the additional effects in the OTHER EFCT field. (Refer to ECOTOX ECOTOX Code Appendix – Appendix V for a list of keywords)

Example: OTHER EFCT: pH efct

### Other Effects (OTHER EFCT)

Comments regarding other toxicity tests or effects reported in the publication that do not meet ECOTOX minimum data requirements are coded in this field. A keyword list (see ECOTOX Appendix V) for common terms is used as a guideline to assist the reviewer. The effect or endpoint codes are used when appropriate. The reviewer should maintain a list of new keywords and periodically submit this list to the EPA Database Manager. Commas separate each distinct term.

OTHER EFCT: UPTAKE, ELIMINATION, FATE

OTHER EFCT: TOXICITY SYMPTOMS, DIET STUDY

OTHER EFCT: MIXTURE, EFFLUENT, METABOLITES

If other chemicals are tested as a mixture with the test chemical, the keyword “mixture” is coded in the OTHER EFCT field.

When water chemistry effects (temperature, salinity, pH) are tested in conjunction with chemical toxicity, a Remark is coded in OTHER EFCT to reflect this type of interaction.

OTHER EFCT: SALINITY EFFECTS

### Additional Comments

The Additional Comments data field contains historical comments from both the aquatic and terrestrial databases. No entries are made in the Additional Comments data field. If records are completed for data maintenance, the comments in the Additional Comments data field will be moved to the corresponding remark data field.

## **4. Field Test Parameters (Habitat tab)**

(Invoked when Test Location of ‘FieldN’ selected)

**Figure 5. Toxicity Module Habitat Information Tab**

Sub-habitat (HABITAT CODE)

Select the one-letter code (see ECOTOX Code Appendix – Appendix X:) used to describe the habitat (e.g., Lacustrine, Riverine or Grasslands). If the author does not provide any information about the habitat, NR is coded.

Sub-Habitat Comment (HABITAT COMMENT)

The descriptor field is used to record any additional information of the author's description of the water body or terrestrial area, e.g. brackish marsh, oligotrophic lake, plastic tub, polyethylene lined enclosure, wheat crop.

Geographic Code (GEOGRAPHIC CODE)

This field will contain the state, province or country name of the test site along with the Geo code. If the test site is not reported, an "NR" is coded. (ECOTOX Code Appendix – Appendix BB contains a listing of country, region, province names and associated Geo code.)

Geographic Code Comment (GEOGRAPHIC LOCATION)

Water body, city, county or relevant site information is coded. (see ECOTOX Code Appendix – Appendix AA for field location abbreviations.)

Longitude/Latitude (LATITUDE/LONGITUDE)

If reported by the author the latitude and longitude are recorded. The "~" sign replaces the "°" sign in data entry. If not reported, NR is recorded.

An example of a longitude/latitude location (MED, Duluth, MN) is listed below:

Latitude: 46~50'51" N  
Longitude: 92~11'12" W

An example of a ranged longitude/latitude location is listed below:

Latitude: 52~30'-53~30' N  
Longitude: 107~30'-106~30' W

An example of a longitude/latitude location in degrees decimal minutes is listed below:

Latitude: 40~26.767' N  
Longitude: 79~58.933' W

Substrate (SUBSTRATE)

The bottom substrate is recorded as a two letter code by using the SUBSTRATE codes listed in ECOTOX Code Appendix – Appendix Y. If there are no applicable codes,

record as the author states in the literature. If a substrate is not reported, NR is recorded. A mixture of sediment types is coded as "MX" and should also include text for the most prevalent soil type(s) in the mixture.

Differentiate between organic and mineral soil/sediment by recording O for organic (leaves, detritus, debris) and M for mineral. Report % organic matter, if reported in literature.

Application Type (APP TYPE)

This code will contain the method of application of the chemical. Application type codes are located in ECOTOX Code Appendix – Appendix J.6.

For instances where the reviewer is unsure whether the chemical was applied directly to the water body by pumping, pouring, metering, etc., "DA" (Direct Application) will be coded.

Application Date (APP DATE)

The application date is the time of initial exposure. The format is MM-DD-YYYY, e.g. 12-01-1993. If one or more parts of the date is not reported, code the letter of the date that is missing (e.g. 12-DD-1993, MM-DD-dd-1993, 06-15-YYYY). If more than one initial date is reported (e.g. more than one pond exposed), record the dates as a REMARK. If one pond is exposed multiple times, only report the first application date and note #x in frequency. If the application date is not reported, NR is recorded.

If the data for multiple years are combined into one test, code the earliest application date in the APPLICATION DATE field and note the other durations as a REMARK.

Application Season (APP SEASON)

This field is used ONLY if no application date is given by the author but the author does specify a season. This field contains the season of initial application of the chemical. A list of application seasons with dates and ECOTOX codes is presented below:

Code	Season
WI	Jan-March
SP	April-June
SU	July-Sept
AU	Oct-Dec
NR	Not Reported

Water Depth (DEPTH) (Aquatic Studies Only)

Water depth value and unit are coded for the study site, as reported by the author. The software will convert the depth to a metric unit. "NR" is coded in the DEPTH field if the author does not report the water depth at the study site. If the author only reports the water depth of the entire system or the depth at which experimental units (i.e., cages) are suspended, "NR" is coded, and depth information is included in the EXP DESIGN field. (See ECOTOX Code Appendix – Appendix Z for valid unit codes)



Observed Duration (OBS TIME)

Observed duration is coded using the units reported in the publication (See ECOTOX Code Appendix – Appendix I for Unit Codes). Time information may be extracted from a figure. When observed duration values are extrapolated from a graph the values can be approximated even when the mark does not appear on the graph. For example, if the value on the graph is in the middle of two marks namely 10 and 14 days, the OBS TIME can be coded as “12 d” with a remark that it is from the graph: Observation Duration Comment data field “from graph”.

For a fluctuating or intermittent dosing (P) experiment, the total test time is recorded in the OBS TIME field with the exposure times and intervals between dosages reported in the APPLICATION FREQUENCY field.

Example: The author reports that the organisms were dosed with three pulses that were 45 minutes each in a 24 hour period. The test was run for 48 hours.

OBS TIME:48 H APP FREQ: 3 UNIT: X, 45 MI for 24 H

When an observation duration is not directly linked to a response, the observed duration is reported as the full range of time, e.g. "during a 10 week period" is coded as "0-10 wk" or if the observed response is for a portion of the exposure duration, i.e., from day 2 through 10 wk, then code as 2-70 d.

For delayed effects, report the duration of exposure to the toxicant only in the Exposure Duration field. The observation time is recorded but will be greater than the Exposure duration. (See ECOTOX Code Appendix – Appendix I for valid duration units)

If within a measurement the author reports data points over a range of durations and only data from one or some of the durations are statistically analyzed, data from each statistically analyzed date is coded and a note about the non-analyzed dates is coded in the OBSERVED DURATION COMMENT field, namely: OTHER DURATIONS REPORTED BUT NOT STATISTICALLY ANALYZED.

For example:

Mortality data points are reported by the author at 1, 2, 4 and 8 weeks, however only the 8 week data is statistically analyzed. A Result record(s) is coded for Mortality at 8 weeks and the following note is coded in the OBSERVED DURATION COMMENT field: “OTHER DURATIONS REPORTED BUT NOT STATISTICALLY ANALYZED”.

If the duration is referenced from a WQC (Water Quality Criteria) document, code this as reported in the WQC document, and note that the duration is based on a comment in the paper and how it was coded in the WQC document.

For generational studies, report results only for lifestages that are directly exposed to the toxicant.

Example 1: Parent exposed - Offspring in clean water.

Code effects on parents and code “generational study” in OTHER EFCT.

Example 2: Parents exposed - Offspring exposed

Code the effects on both parents and offspring. Code the initial lifestage of organisms tested in the ORGANISM CHARACTERISTICS field and code the lifestage of the organism being measured in the Sample Number and Unit field

(SAMPN). The observed duration for the offspring is coded at the time the parents were exposed plus the time the offspring were exposed.

When coding the endpoints LTxx or ETxx, which are based on the time it takes to get a XX% response, code the associated effect response time and not the total exposure time in the observed duration field.

Example 1: A test with algae is run for 4 hours, but it takes 41 minutes for the "T1/2" duration which is the time of PSII half-inactivation. The author reported T1/2 endpoint is similar to an ET50 endpoint, therefore a reviewer assigned ET50 is coded.

Code: Assigned Endpoint: R      Endpoint: ET50    Effect: PHY    Measurement: PSII  
Observed Duration: 41 mi

Example 2: A 96 hour test is run with fathead minnows and reports mortality. The author reports the LT50 value at 10 ug/l, 20 ug/l and 30 ug/l as 3.5 days, 2 day and 1 day, respectively.

Code:  
Assigned Endpoint: P    Endpoint: LT50                      Effect: MOR    Measurement: MORT  
Observed Duration: 3.5 d                      Concentration: 10 ug/l  
Assigned Endpoint: P    Endpoint: LT50                      Effect: MOR    Measurement: MORT  
Observed Duration: 2 d                      Concentration: 20 ug/l  
Assigned Endpoint: P    Endpoint: LT50                      Effect: MOR    Measurement: MORT  
Observed Duration: 1 d                      Concentration: 30 ug/l

**Note:** For any single test record, only one duration unit can be used for this field. If authors report a combination of durations and they use different units for the same test, one of the values is converted to the other and summed (use only whole or decimal numbers). For example, the author reports 28 day of exposure followed by 8 hours of behavior testing, the duration would be coded as 28.33 days. The 8 hours is divided by 24 hours in a day to get 0.33 days.

**Note:** In test scenarios where incubation times are reported, e.g. enzyme fixation assays, be careful to report the toxicant exposure time *not* the assay incubation time.

**Note:** In test scenarios that involve generational studies, the observation duration times are reported from the time the parents were exposed. For example the parents were exposed for 10 months prior to mating, and the progeny was born 2 months later, the observation duration for both the adult REP PROG effect and for the juvenile DVP ABNL is 12 months. The exposure duration would be the same for both - 10 months. The only difference between the two effects is in the sample unit. The sample unit for the adult effect would be 'AD' and for the juveniles it would be 'F1'.

*For specific projects (e.g. EFED), only the most sensitive endpoint is coded (see Specific Project Coding section, Most Sensitive Endpoints), if data for additional durations are reported but not coded, add an Observation Duration comment "ADDITIONAL DURATIONS REPORTED".*

### Results Coding Hierarchy

Toxicity test results for the ECOTOX knowledgebase are represented by a combination of the ENDPOINT, TREND, EFFECT, RESP SITE, REMARKS, MEASURE, EFCT%, SIG, LEVEL, CONC and BCF fields. Toxicity test results for ECOTOX are primarily reported for observations taken during the chemical exposure; however, when results are reported *only* for the period of time after the exposure (moved to clean water), i.e. recovery or delayed effects, this type of result is noted by using a "~" in conjunction with the endpoint/effect code, e.g. ~MOR for a delayed mortality effect.

ECOTOX codes data from papers in the following manner:

1. All author reported endpoints (from graphs, text and/or tables) will be encoded
2. NOEC/LOEC data points from statistically analyzed data at all measurements (i.e., author does not clearly identify these as NOEC/LOEC, but sig/nosig designations have been made), durations and response sites will be coded. These will be noted as "Reviewer Assigned"

**[Note:** Positive/beneficial effects (e.g. increase in growth over control, decrease in injury over control, increase in abundance over control) that are statistically significant are coded as NOEL/NOECs. A remark in the MEASUREMENT REMARK field must state that the effect was a statistically significant increase or decrease over control, whichever is applicable. For example, MEASUREMENT REMARK: statistically significant increase over control]

3. NR-ZERO and NR-LETH will be coded for aquatic and terrestrial studies following procedures outlined in the current ECOTOX coding guidelines
4. Data that are not statistically analyzed will be coded as a one line data marker, ranging all concentrations, durations and response sites per effect, unless there is another measurement within the same effect group that has been statistically analyzed. In this case, a note about the non-analyzed data is coded in the MEASUREMENT REMARK of the analyzed data.

For example:

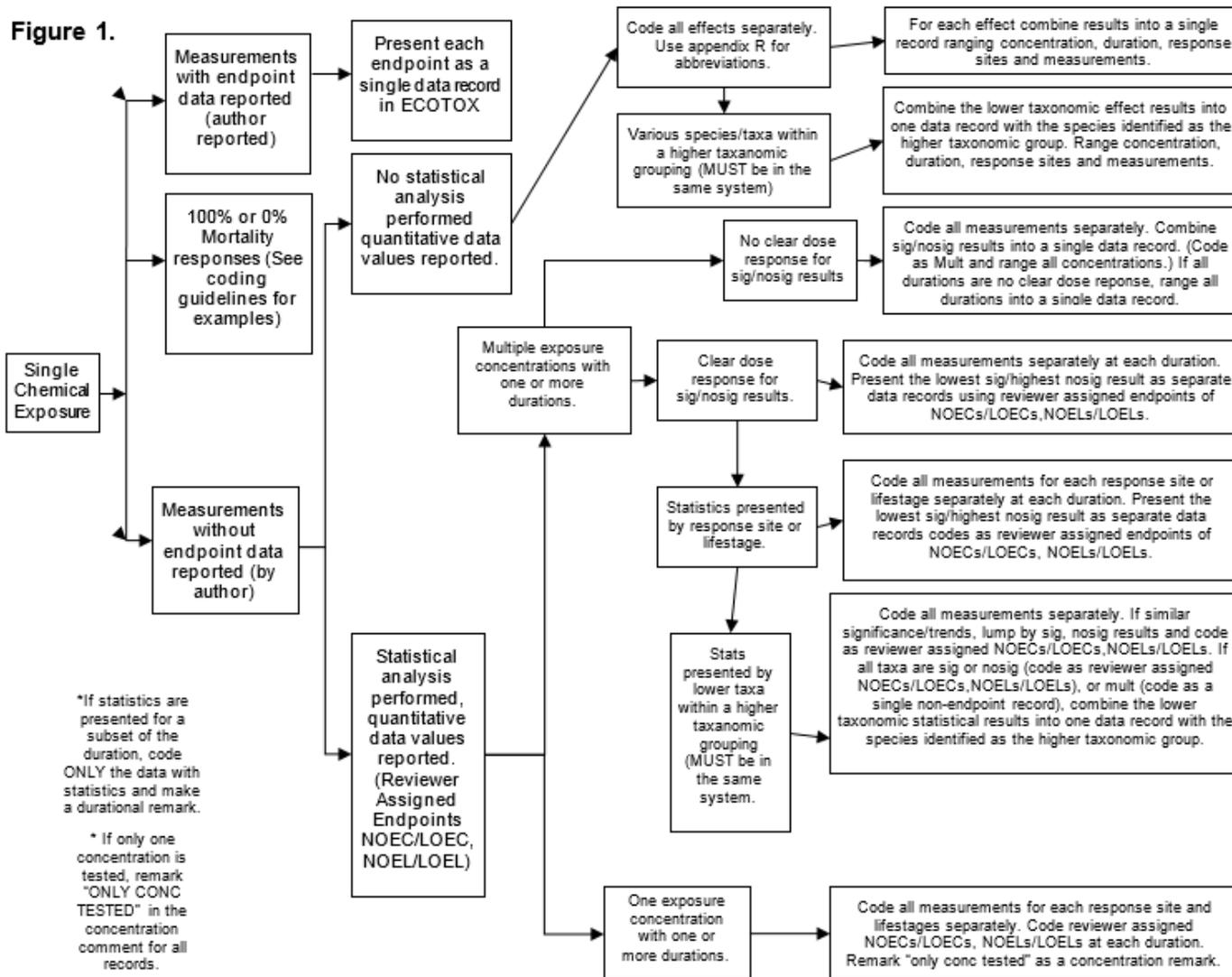
Length, Weight and Condition Factor (which are all measurements under the Effect group GRO are reported by the author, however only the Length data is statistically analyzed. A Result record(s) is coded for the Length data and the following note is coded in the MEASUREMENT REMARKS field: "WEIGHT AND CONDITION FACTOR REPORTED BUT NOT STATISTICALLY ANALYZED".

5. Data that are graphed and not statistically analyzed or data that is qualitative will be coded as a one line data marker, ranging all concentrations, durations and response sites per effect.
6. If the data encoded by a special project are not encompassed by the rules listed above (e.g. data from special projects that used the 10% change to assign endpoints), the data will be coded as follows:
  - For data reviewed by a special project prior to ECOTOX coding, not currently in the special projects database, the special project coder will note that a 10% endpoint was coded.
  - For data already in a Special project database, ECOTOX will extract a file of endpoints (noted by 10% text) in the endpoint comments (TRV) or statistic field (PCB) and examine the data encoded by the special project

For both of these cases, ECOTOX will add an entry (non-endpoint) to the knowledgebase noting that a of 10% change occurred at the specific duration or concentration in the remarks field (e.g., 10% TRV).

Figure 1. Shows the hierarchy of coding tests in ECOTOX.

Figure 1.



Coding Hierarchy

Coding Hierarchy

Endpoints always require a discrete record. For data not reporting an endpoint, at least one separate record is coded for each effect from either a unique experimental design or within one design scenario.

Food chain effects are coded for organisms throughout the chain if there is an exposure concentration reported at each level. For example, an algae is exposed to a series of concentrations and the residue in the organism is reported. The author then feeds the algae to the fish, reports the amount of chemical in the algae fed to the fish and measures growth of fish. Both the algae and the fish tests can be coded as there is a concentration and an effect for each species. In addition, a comment of FOOD CHAIN EFFECTS in the OTHER EFCT field for each of the tests coded is made..

Endpoint, Effect, Measurement and Statistics sections have further description and examples.

The following sections provide a brief description for each of these fields, followed by guidance for coding information from the publication for each of the fields.

#### Endpoint (ENDPOINT)

For the purposes of ECOTOX, an endpoint is the quantification of an observed effect obtained through statistics or other means of calculation for the express purpose of comparing equivalent effects (e.g., LC50). ECOTOX Code Appendix – Appendix T identifies and defines the ECOTOX endpoint codes. The endpoint field will be coded as NR if the author does not report or define an endpoint or there is no companion data point. All endpoints reported in the paper are coded.

Endpoint information is coded into ECOTOX if it is reported by the author, if the author's definition of the effect is equal to ECOTOX endpoint definitions, or if the data point is a companion endpoint to a LOEC, NOEC and/or MATC. “Companion endpoints” are endpoints assigned by the reviewer when the statistical results follow a clear concentration-response pattern and the author reports a NOEC, LOEC or MATC but fails to report the “companion endpoint”. For example, when an author reports a NOEC and does not specifically define the lowest statistically significant effective concentration as a “LOEC”, the data point is coded as a LOEC in ECOTOX by the reviewer. Similarly, for reported LOECs without NOECs, NOEC/ LOECs without MATCs and MATCs without NOEC/LOECs.

**Note:** For terrestrial studies, and endpoints are reported for soil and pore water, code two separate endpoints for the soil and pore water endpoints. Add a Concentration remark “soil conc“ or “pore water conc“, respectively.

**Note:** Positive/beneficial effects (e.g. increase in growth over control, decrease in injury over control, increase in abundance over control) that are statistically significant are

coded as NOEL/NOECs. A remark in the comments field must state that the effect was a statistically significant increase or decrease over control, whichever is applicable. For example, MEASUREMENT REMARK: statistically significant increase over control

If an author reports a NOEC and/or LOEC endpoint for one of the measurements in the text but does not specify the endpoints for the other measurements which show statistical data points within the same graph or table, the reviewer may extrapolate the SIG and NOSIG points for the other measurements as LOEC and NOEC using the R designation in the ASSIGNED ENDPT field. The reviewer may not extrapolate data from other tables or graphs. In studies where measurements are reported with statistical significance but the author does not report a NOEC and/or LOEC for any of the measurements, the reviewer does not code a NOEC and/or LOEC.

Example: The text contains the following statement: “The NOEC and LOEC for length were found to be 10 ug/l and 20 ug/l, respectively.” A table reports weight data as well as weight and mortality data which is statistically analyzed.

Conc (ug/l)	Length (cm)	Weight (mg)	Mortality (%)	* Sig at p<0.05
Control	21	30	0	
5	22	30	5	
10	20	24*	5	
20	15*	20*	8	
30	13*	18*	25*	

Code:

Endpoint Assigned	Endpoint	Effect	Measurement	Conc	Signif	Level
P	NOEC	GRO	LGTH	10 ug/l	NOSIG	p<0.05
P	LOEC	GRO	LGTH	20 ug/l	SIG	p<0.05
R	NOEC	GRO	WGHT	5 ug/l	NOSIG	p<0.05
R	LOEC	GRO	WGHT	10 ug/l	SIG	p<0.05
R	NOEC	MOR	MORT	20 ug/l	NOSIG	p<0.05
R	LOEC	MOR	MORT	30 ug/l	SIG	p<0.05

On occasion, authors will report LC50 information in the methods section of their publication, without reporting any accompanying test procedure information. These test results are coded if enough information is provided by the authors to verify that the value(s) were not published elsewhere, and that the study meets all five minimal criteria for acceptance. The term "Methods LC50" is coded in the ENDPOINT REMARK.

If replicate tests resulting in a number of endpoints, (e.g. LC50s), are conducted, each LC50 must be reported on an independent line, even though the chemical, species, duration and effect are the same. Mean results are not coded if individual results are reported,

Example: Rep 1 - LC50 = 23 ug/L Code only Rep 1 and Rep 2.

Rep 2 - LC50 = 25 ug/L

Mean - LC50 = 24 ug/L

If a data set is evaluated using more than one statistical analysis all resulting endpoints are coded separately (e.g. 2 LC50s for same data using probit and Spearman-Kärber will be coded as two separate data records; report statistical method in ENDPOINT REMARK). Additionally, note “statistical comparison” in the OTHER EFCT field (see OTHER EFCT section for more information).

### Specific Project Coding

**For Specific Project Coding (e.g. the EFED project)** encoding may follow slightly different procedures based on the habitat and species. The following is an outline of the differences:

If the paper is **Aquatic** – Code in full (all chemicals, all species, all data)

If the paper is **Terrestrial - AVIAN, BEES DOM, DOMA, HERP, MAMMAL or RODE** - Code all chemicals, most sensitive endpoints only

If the paper is **Terrestrial – FUNGI, INSECT, INVERT, Plant** – Code the chemical of concern only, most sensitive endpoints only

For chemicals with where target species are **PLANTS** (chemical use of Herbicide, Plant growth regulator and Defoliant) or **INSECTS** (chemical use of Insecticide, Insect growth regulator, Insect Repellent, Fumigant) data is not coded for agricultural, weed or pest species. For a list of species not encoded, see Appendix B: Plant and Insect species not Coded in the EFED Chemical Verification, Literature Searching and Application of EFED Criteria.

### “Most Sensitive Endpoints” means:

Data is coded for only the chemicals prioritized on the EFED Schedule, provided by the EPA. This includes all species reported in the paper that have endpoint data and only the most sensitive endpoint for each Effect Type. For example, if an author reports multiple endpoints for the Effect Type (ENZ), and there are three different enzyme Effect Measurements to choose, only one enzyme measurement with the most sensitive endpoint is coded. All author reported endpoints are coded (e.g. EC50, LC50 and LC25).

Endpoints of NOEC/LOEC are only reported as “NOEC” and “LOEC” if the author specifically reports this terminology.

If the author does not explicitly identify a NOEC/LOEC or a NOAEL/LOAEL (see Appendix T of the ECOTOX Code Appendix located at <https://cfpub.epa.gov/ecotox/blackbox/help/codeappendix.pdf>), NOAELs and LOAELs are determined by the reviewer by analyzing the author reported statistical analysis. The NOAEL is the highest tested concentration having no

statistically significant adverse effect and the LOAEL is the lowest tested concentration having a statistically significant adverse effect. (Rand, 1995).

#### Reviewer Identification of the NOAEL and/or LOAEL

The Reviewer may be required to review each toxicological study and to identify No Observed Adverse Effect Level (NOAEL) and/or Lowest Observed Adverse Effect Level (LOAEL) values.

It is important to note that the NOAEL and LOAEL are endpoint specific. For example, the selected LOAEL for a growth endpoint may be 5.7 Kg/ha whereas the LOAEL for a population endpoint may be 2.3 Kg/ha.

Publications or documents which report studies of interest to the regulatory community may identify a NOAEL and a LOAEL, only a NOAEL, or only a LOAEL. Many publications, particularly those reporting basic toxicological research, do not identify NOAEL or LOAEL values. In these cases, the Reviewer must determine whether there are sufficient data available to determine NOAEL and/or LOAEL values.

The process of identifying a NOAEL and/or LOAEL begins by determining whether statistical analysis of the data was performed. This may be obvious from presentation of data in summary tables. However, the Reviewer should carefully examine the text in the methods section, text of the results section, and footnotes in data tables for information on statistical analysis and results. In cases where no statistically significant results were observed, the only indication that statistical analysis was performed may be a description provided in the methods section. If an appropriate statistical analysis has been performed, the Reviewer applies the general rules below to identify NOAEL and/or LOAEL values. The general rules for determining each LOAEL/NOAEL and their exposure durations are as follows:

- The Reviewer identifies a NOAEL when there are no statistically significant differences.
- There are five experimental design scenarios possible when identifying a LOAEL:
  1. If one result is reported (single concentration and single duration) and the difference at that only data point is significant, then the reviewer codes a LOAEL at that duration and concentration.
  2. When there are multiple durations reported for one concentration and the organisms are dosed one time only (acute exposure), the first significant duration is chosen as the LOAEL, even if the data turns non-significant at later durations (See Examples A and B).

Example A – Acute Exposure – Multiple Durations/One Concentration

Doses\Duration	Day 1	Day 2	Day 3	Day 4	Day 5	NOAEL/LOAEL
10 mg/kg	no	no	sig	sig	no	Day 3 LOAEL 10mg/kg

Example B – Acute Exposure – Multiple Durations/One Concentration

Doses\Duration	Day 1	Day 2	Day 3	Day 4	Day 5	NOAEL/LOAEL
10 mg/kg	no	sig	no	sig	no	Day 2 LOAEL 10 mg/kg

- When there are multiple durations reported for the same concentration and the animals are dosed continuously, the LOAEL is only coded when the trend goes from non-significant to significant and does not change back at a later duration (See Example C and D).

Example C – Continuous Exposure – Multiple Durations/One Concentration

Doses\Duration	Day 1	Day 2	Day 3	Day 4	Day 5	NOAEL/LOAEL
10 mg/kg/d	No	no	sig	sig	sig	Day 3 LOAEL 10mg/kg/d

Example D – Continuous Exposure – Multiple Durations/One Concentration

Doses\Duration	Day 1	Day 2	Day 3	Day 4	Day 5	NOAEL/LOAEL
10 mg/kg/d	No	no	sig	sig	no	Day 3 LOAEL 10mg/kg/d

- When data are reported at multiple concentrations but only at a single duration, the reviewer assigns a LOAEL at the lowest, significant concentration (See Example E). Because multiple concentrations are reported in this case, companion NOAELs (at the largest concentration without a significant adverse effect) may be coded.

Example E – One Duration/Multiple Concentrations

Doses\Duration	Day 1	NOAEL/LOAEL
10 mg/kg/d	no	Day 1 NOAEL at 10 mg/kg/d, LOAEL at 20 mg/kg/d
20 mg/kg/d	sig	
30 mg/kg/d	sig	

- When the paper provides results at multiple durations and multiple concentrations, the reviewer first looks at the lowest concentration. If there is a significant, adverse effect (and a clear dose response), the shortest duration is coded. If there is no adverse effect at the lowest concentration, then the

reviewer proceeds to the next lowest concentration, and so on. When deciding between LOAEL endpoints at the shortest duration or lowest concentration, the lowest concentration takes precedence (See Example G)

Example G – Multiple Durations/Multiple Concentrations

Doses\Duration	Day 1	Day 2	Day 3	LOAEL/NOAEL
10 mg/kg/d	no	no	sig	Day 3 LOAEL at 10 mg/kg/d
20 mg/kg/d	no	sig	sig	
30 mg/kg/d	sig	sig	sig	

The following examples are provided to assist in properly assigning NOAEL and LOAEL values when multiple durations and multiple concentrations are reported and were taken from EcoSSL Guidance Documents 4-3, Standard Operating Procedures #4: Wildlife TRV literature review, data extraction and coding (<http://www.epa.gov/ecotox/ecossl/SOPs.htm>):

Example 1

Duration\Doses	10 mg/kg/d	20 mg/kg/d	30 mg/kg/d	NOAEL/LOAEL
1 week	no	no	no	2 week LOAEL 10
2 weeks	sig	sig	sig	
3 weeks	no	no	no	

Example 2

Duration\Doses	10 mg/kg/d	20 mg/kg/d	30 mg/kg/d	NOAEL/LOAEL
1 week	no	no	sig	1 week NOAEL 20; LOAEL 30
2 weeks	no	sig	no	
3 weeks	no	no	sig	

Example 3

Duration\Doses	10 mg/kg/d	20 mg/kg/d	30 mg/kg/d	NOAEL/LOAEL
1 week	no	sig	no	2 week NOAEL 20; LOAEL 30
2 weeks	no	no	sig	
3 weeks	no	sig	no	

Example 4

Duration\Doses	10 mg/kg/d	20 mg/kg/d	30 mg/kg/d	NOAEL/LOAEL
1 week	sig	no	no	3 week NOAEL 20; LOAEL 30
2 weeks	no	sig	no	
3 weeks	no	no	sig	

Example 5

Duration\Doses	10 mg/kg/d	20 mg/kg/d	30 mg/kg/d	NOAEL/LOAEL
1 week	no	no	sig	1 week
2 weeks	no	sig	no	NOAEL 20;
3 weeks	sig	no	no	LOAEL 30

Example 6

Duration\Doses	10 mg/kg/d	20 mg/kg/d	30 mg/kg/d	NOAEL/LOAEL
1 week	no	no	no	3 week
2 weeks	no	no	no	NOAEL 30
3 weeks	no	no	no	

Example 7

Duration\Doses	10 mg/kg/d	20 mg/kg/d	30 mg/kg/d	NOAEL/LOAEL
1 week	no	sig	no	3 week
2 weeks	no	sig	no	NOAEL 30
3 weeks	no	no	no	

- When selecting among multiple Effect Measures for the Same Effect type, the Effect Measure resulting in the lowest concentration LOAEL is coded, provided a clear dose response relationship is evident. For example, if a population abundance (POP/ABND) LOAEL of 0.50 kg/ha and (POP/BMAS) biomass LOAEL of 0.75 kg/ha were both reported, the more sensitive LOAEL of 0.50 kg/ha would be reported as the endpoint and Biomass would be recorded in the Endpoint Comment field. If both Effect Measure LOAELs are at the same concentration, then the LOAEL at the shorter duration is coded. If the LOAELs are at the same concentration and duration, the Effect Measure LOAEL with the smaller p-value is coded. If the LOAEL concentrations, durations, and p-values are all the same for multiple Effect Measures, then the reviewer chooses one LOAEL to represent the Effect Measures.

If there are multiple Effect Measures for the Same Effect Type, but they are from distinctly different experiments, each Effect Measure would be coded. For example, Exp1 has a POP/ABND endpoint, Exp2 has a POP/BMAS endpoint, and Exp3 has a POP/ABND endpoint, all three endpoints would be coded for the same paper.

## Endpoint Notes

If the author does not actually state that the value is an LD50 but states that “concentration x is the dose estimated to be lethal to 50% of the test organisms” and refers to statistical methods to estimate 50% lethality, the reviewer should code this as an LD50 endpoint because the author *defines* the LD50.

The ECOTOX knowledgebase recognizes and codes “companion endpoints”; for ECOTOX such endpoints are defined as statistically significant endpoints that neighbor an author-defined NOEC or LOEC.

When a publication reports an author defined LOEC and NOEC for a non-monotonical response pattern (i.e., lower concentration significant and at least one higher concentration not significant); code the LOEC/NOEC reported by the authors, but note TREND as ‘CHG’ to flag non-standard results.

In the ECOTOX knowledgebase, the occurrence of no mortality (0%) or complete mortality (100%) is treated as an endpoint. The endpoints NR-LETH and NR-ZERO will always be coded for mortality effects of 100% mortality and 0% mortality, respectively. If an author reports 0% or 100% survival, ECOTOX will encode these data respectively, as follows:

Endpoint: NR-LETH Trend:INC Effect:MOR Measurement: MORT and Effect % 100

Endpoint: NR-ZERO Trend:NEF Effect:MOR Measurement: MORT and Effect % 0

If for a laboratory test exposure testing for mortality and the authors report “all fish died”, code as NR-LETH and 100% mortality; however, for a field exposure, unless conducted in an enclosure of some type, it is difficult to assume that truly 100% of the fish are known to be dead; therefore, the field exposure report of “all fish died” is coded POP, DEC, ABND, and EFCT% is not coded. The term “nil” is defined as “naught or nothing”, therefore, when used by an author, it will be assumed to mean 0% mortality and coded as NR-ZERO.

Coding NR-LETH and NR-ZERO from statements is only completed when the object of the study is to test for mortality. For instances where the object of the study is for non-mortality data, e.g. biochemical effects, and the author reports no organisms died during the study, an NR-ZERO data point is not encoded.

The 100% mortality data point at the lowest concentration/ shortest duration is coded. Similarly, the 0% mortality data point at the highest concentration/ longest duration is coded. In contrast to other endpoints, the additional mortality effects are coded along with the NR-LETH and NR-ZERO endpoint data. For example:

Mortality Table 1				
ug/L	24 H	48 H	72 H	96 H
1	0	0	0	<b>0 NR-ZERO</b>
2	5	17	30	35
3	25	40	65	90
4	<b>100 NR-LETH</b>	100	100	100

A) LC50s reported in publication, code

LC50s as reported

NR-LETH:4 ug/L at 24 hr

NR-ZERO:1 ug/L at 96 hr

B) LC50s not reported in publication, code

NR-LETH:4 ug/L at 24 hr

NR-ZERO:1 ug/L at 96 hr

MOR:2-3 ug/L at 24-96 hr EFCT%:5-90

Mortality Table 2				
ug/L	24 H	48 H	72 H	96 H
1	0	0	<b>0 NR-ZERO</b>	11
2	20	25	38	72
3	45	60	67	90
4	90	<b>100 NR-LETH</b>	100	100

A) LC50s reported in publication, code

LC50's as reported

NR-LETH:4 ug/L at 48 hr EFCT%: 100

NR-ZERO:1 ug/L at 72 hr EFCT%: 0

B) LC50s not reported in publication, code

NR-LETH:4 ug/L at 48 hr EFCT%: 100

NR-ZERO:1 ug/L at 72 hr EFCT%: 0

MOR1-4 ug/L at 24-96 hr EFCT%: 0-100

Mortality Table 3				
ug/L	24 H	48 H	72 H	96 H
1	<b>0 START RANGE</b>	0	7	13
2	0	28	45	60
3	38	44	67	100
4	38	60	100	<b>100 END RANGE</b>

A) LC50s reported in publication, code

LC50's as reported

B) LC50s not reported in publication, code

MOR1-4 ug/L at 24-96 hr EFCT% 0-100

Mortality Table 4				
ug/L	24 H	48 H	72 H	96 H
1	<b>0 START RANGE</b>	0	0	0

Mortality Table 4				
ug/L	24 H	48 H	72 H	96 H
2	0	0	7	13
3	0	28	45	60
4	38	44	67	100
5	38	60	100	100
6	100	100	100	<b>100 END RANGE</b>

A) LC50s reported in publication, code

LC50's as reported

B) LC50s not reported in publication, code

MOR1-6 ug/l at 24-96 h, EFCT% 0-100

If the author reports an endpoint such as TLM, TL50, chronic value (ChV) or any terms with equivalent definitions that define endpoints such as those listed in ECOTOX Code Appendix – Appendix T, the equivalent ECOTOX endpoint is coded in the ENDPOINT field.

If the author reports an endpoint that is a delayed exposure effect (~xxx), the delayed effect endpoint is coded if no similar exposure endpoint has been coded. A specific exception is gut clearance prior to tissue analysis; e.g., “after the exposure the organisms were placed in clean water for 10 hours to allow the organism to clear the stomach contents”. This type of clearance is distinguished from depuration and is not coded as a delayed effect.

#### Trend (TREND)

The observed or measured response trend as compared to the control is coded when reported or graphically displayed.

When assigning a trend to a record, it should reflect the measurement which may or may not reflect the effect. For example, when authors report a decrease in survival; the effect is reported as MOR, and the trend is associated with the measurement; i.e., decrease in survival.

Example: EFCT: MOR TREND: DEC MEASURE: SURV

The trend for BCF, LCxx, LTxx is coded as "inc", except for the effect SVC (shell valve closure) which is coded as "dec". The trend for ECxx, NOEC, LOEC, and MATC will be either "inc", "dec", "chg" or NR depending on the results of the test. In instances when a trend is non-monotonical code “chg”. The trend is noted as a two or three letter code:

<u>CODE:</u>	<u>TREND:</u>
INC	increase
DEC	decrease
NEF	no observed effect; e.g., when coding NR-ZERO the trend is NEF
CHG	no clear trend, results are variable (e.g. any combination of above trends listed)
NR	no trend reported <u>or</u> if no control response is reported then the trend is not able to be identified

Example: When a clear response, or lack thereof, is observed within an effect, it is coded as either INC, DEC, or NEF. The measurement used to evaluate the effect is reported in the MEASURE field, for example:

EFCT: GRO TREND: INC MEASURE: LGTH

When measurements do not report quantifiable data (see EFFECT MEASUREMENT section), data are combined into one record. If these data report multiple trends, code CHG in the TREND field and report the individual trends in MEASUREMENT\_REMARK field as in the following example:

Example: EFCT: HIS TREND: CHG MEASURE: EDMA,DISO,DEGN  
MEASUREMENT REMARK: inc EDMA, DISO, dec DEGN

#### Effect (EFFECT)

For ECOTOX knowledgebase purposes, a toxicological effect is the observation or measurement of a response resulting from the action of a chemical stressor (e.g., mortality). The ECOTOX knowledgebase internally categorizes all observed effects under at least one of eleven major effect group codes (Accumulation, Behavior, Biochemical, Cellular, Growth/Development, Lethal, Physiological, Population Community, Reproduction, Ecosystem and multiple groups). ECOTOX Code Appendix – Appendix R describes the major groups and associated effect definitions for each three letter code. The major effect groups are not used by reviewers; their purpose is to provide knowledgebase users the capability to search



If data for a number of tissues are presented along with statistical results report results for each tissue separately. If statistics are not presented, combine the results into one data record by entering each response site into the Response Site data field.

Combining Response Site

When the residue measured in one organ or tissue is further analyzed to indicate concentrations in cells or cellular fractions, a comment is placed in the Response REMARK field (e.g., "subcellular fraction" or "subcellular distribution").

If multiple tissues are being combined into one record, enter all of the individual tissues/organs are coded in the Response Site data field. If the response site does not have a response site code requested new code be added. When the response site is not reported, the field is coded as NR.

Measurement (EFFECT MEASUREMENT)

Generally, "measures" or "measurements" are variables used to aid in the interpretation of the degree of response to a toxicant by an organism. For example, measures of behavioral effects in ECOTOX include behavioral changes (BEH SWIM), changes in feeding activity (FDB FDNG), and stimulus avoidance (AVO STIM). ECOTOX Code Appendix – Appendix S lists the measurements currently used for each of the effects in the ECOTOX knowledgebase.

If more than one organism is measured in either an artificial (pot, cage, aquaria, etc.) or natural population (field, water body, etc.), reviewing staff will code the effect and measurement as POP BMAS (population biomass). For instances where there is an individual biomass (one organism, artificial or natural), reviewing staff will code GRO BMAS (growth biomass).

Note: Growth data reported on a part of an organism (i.e. weight or length of a specific organ/tissue/structure) is coded as MPH while growth data for the organism as a whole is coded as GRO. Roots and shoots of plants can regenerate and are considered a whole organism.

Each measurement for which an endpoint is reported in the text, tables, or graphs receives a separate data line. However, measurements that are not statistically analyzed, or are discussed in the text and do not report any numeric values may be combined into one record. If several measurements are combined, code each measurement code in the MEASURE field. See example 2 and 3 directly below.

For Gene Expression studies, authors will report measurements as either the genes that were expressed (e.g. Zgc:92628, Signal recognition particle 14 kDa, ATPase family – AAA domain containing 3A) or as a specific mRNA (e.g. CYPmRNA). Authors tend to use targeted techniques like Real-Time Quantative PCR or Protein Blotting, etc., and transcription processes like Microarray or Next Generation sequencing.

- In ECOTOX, if the author uses the targeted techniques, code each measurement separately. If the authors clearly identify a primer was used, note "PRIMER USED" in the Measurement Comment data field.
- If the author uses transcription processes, code a maximum of one NOEC and/or one LOEC or one NR record for all of the expressed genes. Add a comment in the Measurement Comment data field noting the number of genes expressed. If the numbers of up and down regulated genes are reported add the specific comment 'XXX GENES UPREGULATED AND XXX DOWNREGULATED.' If the author clearly identifies GEO or CEB Accession numbers, note "ACCESSION NUMBERS REPORTED" in the Measurement Comment data field.
- If the author uses both targeted and transcription processes in the paper, code the targeted data as noted above and report "GENE TRANSCRIPTION ANALYSIS ALSO REPORTED" in the Other Effects data field.

Example 1: Length and weight reported in table statistically analyzed.

CONC	0	10 ug/l	20 ug/l	30 ug/l	40ug/L
Length	-	nosig	nosig	sig	sig
Weight	-	nosig	sig	sig	sig

Code: Endpoint: NOEC EFCT: GRO MEASURE: LGTH CONC: 20ug/L - nosig  
 Endpoint: LOEC EFCT: GRO MEASURE: LGTH CONC: 30ug/L - sig  
  
 Endpoint: NOEC EFCT: GRO MEASURE: WGHT CONC: 10ug/L - nosig  
 Endpoint: LOEC EFCT: GRO MEASURE: WGHT CONC: 20ug/L - sig

Example 2: A decrease in length and weight reported on graph which is not statistically analyzed but values are given.

CONC (from graph) 0 10 ug/l 20 ug/l 30ug/L  
 Code: EFCT: GRO TREND: DEC MEASURE: WGHT,LGTH  
 CONC: 10-30ug/L

Example 3: Histology reported in text. At concentrations of 10 ug/l , 20 ug/l and 40 ug/l, Reports that liver had edema, swelling, and hypertrophy.

Code: EFCT: HIS MEASURE: EDMA, SWEL, HYPT CONC: 10-40 ug/l

More information on coding statistics is found in the statistics section of this SOP.

Many publications which report field data or laboratory microcosm studies present results for multiple species/taxonomic groups. The combining of results for species and taxonomic groups depends on whether statistics were applied to the data and whether a similar response is evident.

- If, within a higher taxonomic group (e.g., Algae), individual effects for several lower taxonomic groups are also presented (e.g., Bacillariophyta (diatoms), Chlorophycota (green algae), Pyrrophytophyta (dinoflagellates)) the data may be reported in a number of ways. Examples include:
  - The measurements within each group are statistically analyzed and are similar overall, i.e., INcreasing, DEcreasing or CHanging. Combine the results and code as:
 

Species: Algae      ORG CHARAC: 3 orders      EFFECT: POP MEASURE: ABND  
TREND: INC      SIG: SIG
  - The measurements within each group are statistically analyzed and differ from each other. Code each result as a separate line:
 

Species: Bacillariophyta      EFFECT: POP MEASURE: ABND      TREND: INC SIG: SIG  
Species: Chlorophycota      EFFECT: POP MEASURE: ABND      TREND: DEC SIG: SIG
  - If no statistical analysis has been reported, the results from the lower taxonomic groups can be combined into a single record representing the next highest representative taxonomic group.
 

Species: Algae      ORG CHAR: 3 orders      EFFECT: POP MEASURE: ABND  
TREND: CHG SIG: NR

For specific projects (e.g. EFED), only a single measurement is coded per effect (see Specific Project Coding section, Most Sensitive Endpoints), if data for additional Measurements are reported but not coded, add a Measurement comment "ADDITIONAL EFFECT REPORTED, e.g. ADDITIONAL BCM REPORTED".

Effect % (EFFECT PERCENT)

The EFFECT PERCENT field is used when the effect is reported as a percent change, e.g. percent of the total population or percent increase or decrease or if the percent response or raw data response is reported.

If the author reports the number dead (i.e., "5 of 20") do not recalculate as a percent.

- Example 1: 80% mortality      EFCT: MOR      TREND: INC      Effect Percent: 80  
MEASURE: MORT
- Example 2: 25% survival      EFCT: MOR      TREND: DEC      Effect Percent: 25  
MEASURE: SURV
- Example 3: 5 of 20 died      EFCT: MOR      TREND: INC      Effect Percent: NR  
MEASURE: MORT
- Example 4: 45% inc ATPase activity      EFCT: ENZ      TREND: INC  
Effect Percent: 45      Measure: ATPA
- Example 5: 53.33% Hematocrit      EFCT: BCM  
Effect Percent: 55.33      Measure: HMCT

If the percent effect is coded from a graph, code the percent values using a qualifier, i.e. <, >, or ~, using only the graphical intervals reported on the graph. Code, from graph, in the EFFECT PERCENT notes field. If the percent effect is graphed and is not clear enough to extrapolate, code "graphed" in EFFECT PERCENT notes field. If the effect percent is not reported, the field is coded as NR.

If the percent effect is presented as "xx% of the control, Code the percent in the field and code: of control in the EFFECT PERCENT notes field.

Example: GRO WGHT Dec 20-30% of the control  
EFCT: GRO      MEASURE: WGHT Effect Percent: NR 20-30      TREND: DEC  
Notes: of control

For NR-ZERO and NR-LETH endpoints, the EFFECT PERCENT field is coded as 0% and 100%, MORT respectively.

When no stats and effect % are presented at the highest concentration, the concentrations should be ranged and the EFFECT PERCENT should be presented as < the effect % from the paper.

Combining EFFECT PERCENT

When data for an effect are combined because a statistical analysis was not applied and/or a clear dose response was not observed, and several percent effect values are presented, there are two different ways to report data.

If the author reports the effect measurement on a single parameter, the effect percent is reported as a range.

Example 1:	30-75% mortality Effect Percent: 30-75	EFCT: MOR	MEASURE: MORT	TREND: INC
Example 2:	20-30% dec O2 consumption Effect Percent: 20-30	EFCT : PHY	MEASURE: OXYG	TREND: DEC

If the author reports the effect measurement on a multiple parameters, the effect percent is reported as 'NR'.

Projects (This data field is not encoded by reviewers)

The intent of this data field is to link results to an existing data set, i.e. CAD, TRV, EcoSSL, EFED, etc. This data field is NOT populated by reviewers, but rather by the person linking the data record to the existing data set or by the TOXICITY module when delivering the EFED data. It is used by ASTER to include evaluated data into the ASTER profile report.

**5a. Concentration Parameters**

(Table within the Results tab)

Concentration Type (CONC TYPE)

Concentration Type is populated from the entry made on the Test Information tab.

Ionic Fraction (ION)

For ionizing substances (e.g., metals, ammonia), report the dose as the ion if the concentration presented by the authors is reported as based on the ionic form of the compound (e.g., organotin as Sn). Code the appropriate ionic symbol in the ION field (see ECOTOX Code Appendix – Appendix O for Ion codes). If concentration is based on the total compound, code 'NR' in this field. For non-ionizing substances, code 'NR' in this field.

If the test chemical for a metal is reported as the elemental metal (i.e. mercury) code the ion (Hg) in the ION field.

Effect Concentration (CONC)

Report the effect concentration in the same units used by the author. If the author reports that the concentration is based on active ingredient or acid equivalent choose the unit preceded by AI or ae from the dropdown menu (e.g. AI mg/kg or ae mg/kg). Do not convert any units. (See ECOTOX Code Appendix – Appendix N for a list of concentration units). The confidence interval, fiducial limits, or range is recorded in the following format: **Mean (min to max)** if available and greater than zero, record confidence intervals with negative values in the Endpoint Comments "Author reports confidence limit as (min to max)". The media concentration is coded in this field, except for diet studies, where the concentration in the food is coded. If a test is run with two sources of chemical, such as diet and water, code the concentration of the diet in the CONC field, the EXP TYPE field as FD and code "WATER CONC RPT" in the Concentration REMARK field. If the concentration reported from spiked sediment studies is the pore or overlying water concentration, record the concentration in the CONC field, and code "PORE WATER FROM SPIKED SEDIMENT" or "OVERLYING WATER FROM SPIKED SEDIMENT" in the Concentration REMARK field and ensure that the Exposure Type is 'L' (leachate). If an author reports results in both the pore water concentration and the overlying water concentration both sets of results are coded with a remark noting which concentration it is in the Concentration REMARK field.

Measured concentration data are selected over nominal concentration results, except when the presentation of the results is more complete with the nominal concentration data vs. the measured data (i.e., lumping records vs. separate test results).

If the doses/concentrations are reported as both measured and unmeasured values but ALL analysis is based on the unmeasured values, code U in the CHEMICAL ANALYSIS field, the unmeasured values and note "MEASURED CONCENTRATIONS ALSO REPORTED" in the OTHER EFFECTS field.

If an author reports concentrations in two different units, select the unit that is more easily converted to ECOTOX standardized units (see below). If a molar and non-molar unit are reported, always use the non-molar concentration. For example, the author reports both ug/L and uM, code the ug/L concentration as ECOTOX can convert this to a standardized unit. Similarly, if a gavage exposure is reported as ppm or mg/kg bdwt, code the mg/kg bdwt as this is the gavage conversion.

ECOTOX Standard units for Water exposure are ug/L; Injection, Oral or Gavage are milligrams per kilogram body weight (mg/kg bdwt); and Diet (i.e. Diet, Food, Drink, Choice, Gestation, Lactation) or Topical are parts per million (ppm).

If the author conducts a diet study and reports the daily and cumulative doses, the daily dose is coded and the cumulative dose reported as a comment, i.e. CUMULATIVE DOSE ALSO REPORTED . This comment is also made in the DOSE COMMENT field

If the author reports the result concentration values as a "TIME WEIGHTED AVERAGE", this is coded in the Comments field of the CONCENTRATIONS value.

When coding numbers do not use commas. They can be mistaken for decimal points or numbers.

Code the mean and range of a stated concentration unless there are multiple test results being combined into one test record. In this case the lowest minimum concentration and the highest maximum concentration will be used.

Example 1: Endpoint reported as LC50 = 100 (50-150) ug/L  
Code: ENDPOINT: LC50 CONC: 100 (50-150) ug/L

Example 2: Effect (reported at 1 concentration tested with replicates)  
Rep1 = 10 (9-13) ug/L  
Rep2 = 11 (8-13) ug/L

Code: The lowest minimum value and the highest maximum value are coded.  
Conc: 8-13 ug/L

Example 3: Multiple concentrations reported with no statistics. Combine concentrations into one record coding the lowest minimum value and highest maximum value.

Nominal Conc.Measured Conc.

1 ug/L1.5 (1-2) ug/L

2 ug/L2.3 (2-3) ug/L

3 ug/L3.1 (2.5-3.3) ug/L

Code: Conc: 1 -3.3 ug/L

If the reported mean value does not fall within the reported range, confidence limits or interval, code the mean value in the Effect Concentration field. In the Concentration Comment field, code: "CL (or CI) reported as XX-XX" or "range reported as XX-XX", where XX-XX is the reported range, confidence interval or limit.

Occasionally an author will report a concentration as a % or fraction of an LC50 value; e.g., either the sublethal concentration used was "10% of the 96-h LC50" or "1/10, 1/15 and 1/20 of the LC50". Such concentrations may be recalculated and used as the effect concentration if the original LC50 concentration is provided in the publication. Flag the recalculation in the paper so that the calculation may be QA'd and document the recalculation in the margin or on a blank page of the publication. Put a slash next to the concentration value and note in the Concentration REMARK field: "Recalculated".

When concentrations are taken from a graph, put a slash next to the concentration value and note in the Concentration REMARK field: "from graph".

All reported concentrations are coded and identified as to whether the concentration is based on the active ingredient or formulation, or as the total, un-ionized or dissolved concentration, are identified (see CONC TYPE).

In certain cases, the ECOTOX concentration is routinely reported as some form of the test chemical. For metal salts, the concentration is generally expressed as ug ion/L (e.g., Hg<sup>+</sup>). Be sure to code the ION field with appropriate ion.

If the concentration is reported in exponential notation the number is written out (e.g., 1 x 10<sup>8</sup>; often reported as 10<sup>8</sup>, is coded as 100000000).

When the concentration is reported as the metal (e.g., Sn), but the chemical tested is identified as an organometallic (tributyltin chloride (C<sub>12</sub>H<sub>27</sub>ClSn)): Record the full name of the chemical tested in the TEST field, enter "T" in the CONC TYPE, report the concentration in the CONC field, and identify in the ion that the concentration is based on the metal component and report in the ION field.

For studies where a single chemical concentration is monitored as a water or soil chemistry parameter (e.g. sodium chloride reported as conductivity, calcium chloride reported as hardness), code the water or soil chemistry parameter in both the dose and the water chemistry parameter data fields.

If the author states that the control water was from a polluted source or was contaminated, 'contaminated controls' should be noted in the EXP DESIGN field. The concentration of chemical in the controls is not coded. If the author is reporting the chemical concentration of a control which is a background level, this information is not coded.

If the chemical used is an aged solution and the author is comparing the data to varying aged solutions (e.g. 2 d aged solution, 4 d aged solution and 6 d aged solution), all data are coded, "X d aged solution" is coded in the EXP DESIGN field, and "Aged Solution Efcts" is coded in the OTHER EFCT field.

If the author measures residual toxicity (e.g. leaves were collected from the field 10, 20, and 40 days after application of the chemical, brought into the laboratory and tested for toxicity) the reference is rejected as No Concentration. If there is data reported for zero (0) day residues, code the data using the concentration reported during application and add an Other Effects remark 'RESIDUAL TOXICITY'.

For field data, the water concentration may be reported as NR, if the application rate is reported (see AP RATE field). However, the concentration type (F,A,T,D,L,U) must still be coded in this field along with NR.

If a number of concentrations are reported to be tested but only data for some of those concentrations are reported due to high mortality, a comment is made stating this.

For example, the author reports testing 0, 10, 20, 30 and 40 ug/l but states that due to high mortality at the 40 ug/l concentration results at that level were not shown.

CONCENTRATION COMMENTS: DATA NOT REPORTED AT HIGHEST CONC DUE TO HIGH MORTALITY

## 5b. Bioconcentration Value (BCF)

(invoked when BCF/BCFD or BAF selected for Endpoint)

The Bioconcentration Factor (BCF) is a value, usually reported with units of L/kg, describing the degree to which a chemical can be concentrated in the tissues of an organism in the aquatic environment. At apparent equilibrium during the uptake phase of a bioconcentration test, the BCF is the concentration of a chemical in one or more tissues of the aquatic organism divided by the average exposure concentration in the water. The number is calculated by dividing the concentration of the exposure chemical found in the tissue by the concentration of the chemical found in the exposure water,

$$BCF = \frac{\text{g/kg chemical in organism tissue}}{\text{g/L chemical in H}_2\text{O}}$$

or it is calculated from a ratio of rate constants, if at steady state,

$$BCF = \frac{K1 (\text{uptake})}{K2 (\text{elimination})}$$

A bioconcentration endpoint is coded as either wet (or unknown) or as dry weight (BCF and BCFD, respectively). An accumulation (ACC) effect, measurement of RSDE, and the associated BCF value is coded in the BCF field. If the author does not calculate a BCF, the test is recorded as an ACC effect, measurement of RSDE, NR in the ENDPOINT field, and NR in the BCF field.

Report the BCF Unit as reported by the author. If the author does not state the unit, the reviewer locates the unit used for measuring the chemical in the organisms tissue and the unit measuring the chemical in the exposure media and determines the unit from the equation. The equation is written in the paper and is checked by the quality assurance reviewer for correct determination.

For example: The chemical in the organism is mg/kg and the chemical concentration in the media is mg/L. The equation is:

$$\frac{\text{mg/kg}}{\text{mg/L}} = \frac{\text{mg}}{\text{kg}} \times \frac{\text{L}}{\text{mg}} = \frac{\text{L}}{\text{kg}}$$

If a unit cannot be determined from the information in the paper or it is unitless, RA (ratio) is used for the unit.

If a BCF is reported for the parent compound and for a metabolite, record the parent compound BCF and note "metabolite BCF" in OTHER EFCT.

If the BCF is normalized for lipid, "lipid normalized" is reported in the ENDPOINT REMARK field.

ENDPOINT REMARK: lipid normalized

If an author reports more than one type of BCF, i.e. lipid normalized, regular, or radioactive equivalents, for the same data point; code lipid normalized over regular and regular over radioactive equivalents. The secondary analysis endpoint is reported in OTHER EFCT.

OTHER EFCT: radioactive equivalent BCF

For papers that report BCFs and provide Lethal Body Burden information, note “Lethal Body Burden” in OTHER EFCT. However, in a publication reporting only residue data as lethal body burdens code the effect as ACC, the measurement as RSDE, and report “Lethal Body Burden” in MEASUREMENT REMARK.

If an author indirectly measures the uptake of a chemical in an organism by measuring the loss of the chemical from the test media, the data are not coded. However, if the chemical is measured in the excrement (urine or feces) it is coded as RSDE in urine (UR) or feces (FC).

**NOTE:** BCFs of less than 1 (when converted to the standard unit of L/kg) and negative BCF values are suspect. The BCFs of less than 1, in the standard unit of L/kg, are coded as reported by the author and negative BCF values are coded as <1. If the value is <1 and not in the standard unit, forward to the EPA database coordinator for review and approval.

If a BCF is reported for an author reported “control” the test set up must be looked at closely. If the test is conducted where a radiolabeled chemical is added to all experimental units and the non-radiolabeled chemical is added to all but one (authors may call this the control), and the authors do not report a clean control (nothing added), the test is then coded with Z control (no control reported). See ECOREF 9212 for an example.

Specific Project Coding

For Specific Project Coding (e.g. the EFED project), if multiple concentrations, durations and/or tissues are reported with BCF or BAF values, the highest BCF or BAF value among the concentrations tested at the longest duration is coded.

Example: BCF values are reported for three durations, four concentrations and three tissues. The highest BCF value at the longest duration is coded in the case below: the Liver BCF of 700 at 60 days and 500 ug/l is coded, with 700 being recorded in the BCF Field. The Endpoint Comment would be: Intestine and Kidney BCFs at 40, 50, and 60 days and additional concentrations also reported.

Chemical Concentration	50 ug/L	125 ug/L	250 ug/L	500 ug/L
INTESTINE				
40 day BCF	30	75	76	82
50 day BCF	30	77	85	82
60 day BCF	65	85	87	90
KIDNEY				
40 day BCF	28	18	19	20
50 day BCF	35	18	21	21
60 day BCF	40	18	30	28
LIVER				
40 day BCF	180	390	420	400
50 day BCF	200	400	420	550
60 day BCF	350	580	450	700

Statistical Significance (SIG)

The statistical significance field is coded when the author has presented statistical analysis as compared to the controls in the test result.

The valid codes for this field are:

<u>CODE</u>	<u>DEFINITION</u>
SIG	Concentration(s) identified as significant (code lowest concentration in series of significant treatments)
ASIG	All toxicant concentrations significant (including single concentrations exposures)
NOSIG	Concentrations identified as not significant (code highest concentration in series of non-significant treatments)
ANOSIG	All toxicant concentrations not significant (including single concentrations exposures)
MULT	Combination of sig and nosig results; pattern of significance is non-monotonical, or results combined due coding practices.
NA	Not applicable (use for LC50, EC50, BCF, MATC, NR-LETH, AND NR-ZERO)
NR	Not reported

If statistics are presented in the publication, unless the authors state otherwise, assume that the exposure treatments were compared to the controls.

When statistical comparisons are presented for multiple controls (e.g., statistics in relation to a standard control and statistics in relation to a solvent control), code all controls in the CONTROL TYPE field and note the appropriate control type used for the coded statistics in MEASUREMENT REMARK. (e.g., “STATS TO V CONTROL“, “STATS TO C AND V CONTROL“, “STATS TO C CONTROL”) The data related to the solvent control is coded preferentially over the standard control data and should be noted as such in the MEASUREMENT REMARK field. If the author does not report which control the statistics are compared to, no remark is made.

Data are separated into individual records if statistics are based on concentrations and a clear dose response is shown. For example:

1) If only one concentration is presented and the response differs over time, code four records

Example 1:	Conc.	24h	48h	72h	96h
	1 ug/l	nosig	nosig	sig	sig

Code four Endpoints : 2 NOECs at 24 and 48 hours and 2 LOECs at 72 and 96 Hours at CONC: 1 ug/l  
SIGNIF for NOECs : ANOSIG, SIGNIF for LOECs: ASIG

2) If multiple concentrations and durations are presented. Code the statistics at each duration separately.

Example 2:	Conc.	24h	48h	72h	96h
	1 ug/l	nosig	nosig	nosig	sig
	2 ug/l	nosig	nosig	sig	sig
	3 ug/l	nosig	sig	sig	sig

Code: NOEC at 24 h 3 ug/L; NOEC/LOEC at 48h 2 ug/L 3 ug/L; NOEC/LOEC at 72 h 1 ug/L 2 ug/L;  
LOEC at 96 h 1 ug/L

Use the Companion Endpoint tab to mark for each NOSIG SIG companion endpoint group

3) If multiple concentrations and durations are presented and results are non-monotonic within each duration, code one record combining all concentrations all durations.

Example 3:	Conc.	24h	48h	72h
	1 ug/l	nosig	sig	nosig
	2 ug/l	sig	sig	sig
	3 ug/l	nosig	nosig	nosig

Code Conc: 1-3 ug/L, TIME: 24 h, SIGNIF: Mult    ENDPOINT: NR  
Conc: 1-3 ug/L, TIME: 48 h, SIGNIF: Mult    ENDPOINT: NR  
Conc: 1-3 ug/L, TIME: 72 h, SIGNIF: Mult    ENDPOINT: NR

The SIG field is coded as "NA" for records having an endpoint of MATC, LCxx, ECxx, LTxx, BCF, ETxx, ICxx, LDxx, LETC, BCFD, NR-LETH, and NR-ZERO. For effects without endpoints, code significance as author reports, or NR.

The significance for LOEC and NOEC must be coded as SIG and NOSIG respectively unless a publication reports a LOEC and NOEC for a non-monotonical response pattern (i.e., lower concentration significant and at least one higher concentration not significant); In this case code the LOEC/NOEC reported by the authors, but note TREND as 'CHG' and code SIG as 'MULT' to flag non-standard results.

The reviewer interprets hypotheses tests to determine a dose response endpoint. A significant clear dose result is coded as a LOEC; no significant dose result is coded as a NOEC. Only the highest NOEC (NOSIG) and the lowest LOEC (SIG) concentration is reported; unless all concentrations are SIG or all concentrations are NOSIG. In this instance, code the NOEC/LOEC as identified and note that the significance is ASIG (all significant) or ANOSIG (all not significant) respectively. If the significance level is reported, it is coded in the LEVEL field described below.

In cases where the author reports only a LOEC (SIG) or NOEC (NOSIG), code the companion data point. For example, if a stat sig "growth" is reported in the text and in the table sig is noted the reviewer should pick the nosig level and report this also.

If the author states that there is a statistically significant increase or decrease in an observed effect, whether or not they report the statistical method used, but does not report a significance level, code SIG or NOSIG and NR in LEVEL field.

If a table has a footnote defining \* values as significant as p<xx, it is acceptable for the reviewer to assume that data points without an asterisk are not significant.

If the author states there is a significant increase or decrease in an observed effect but does not say it is "statistically significant," code NR in SIG field.

- When the highest concentration and all lower concentrations tested show no significant response, code NOEC ANOSIG and the highest concentration in CONC field.

Example:	10 ug/L	NOSIG	P<0.05
	20 ug/L	NOSIG	P<0.05
	30 ug/L	NOSIG	P<0.05

Code: Endpoint: NOEC CONC: 30 ug/L    SIG: ANOSIG    LEVEL: P<0.05

- When the lowest concentration and all higher concentrations tested show a significant response, code LOEC ASIG and the lowest concentration in CONC field.

Example:        10 ug/L    SIG    P<0.05  
                   20 ug/L    SIG    P<0.05  
                   30 ug/L    SIG    P<0.05

Code:            Endpoint: LOEC CONC: 10ug/L    SIG: ASIG LEVEL: P<0.05

- If only one concentration is tested and statistics are performed, code ASIG or ANOSIG in stats and "only conc tested" as a CONC remark.

Example:        10 ug/L SIG    P<0.05

Code:            Endpoint: LOEC CONC: 10ug/L    ASIG        P<0.05    Conc REMARK: "only conc tested"

If an author reports p values but the level at which the data are considered significant is not reported by the author, use the conservative significance level of  $P \leq 0.05$  as a benchmark. The ENDPOINT ASSIGNED is coded as R and ENDPOINT REMARK is coded with "SIG ASSIGNED"

Example: The paper reports 9 unique mRNA measurements at one concentration and gives p values but does not report the level that is considered significant. Using a benchmark level of significance of  $p \leq 0.05$  the following endpoints are coded:

mRNA Name	P VALUE	ENDPOINT ASSIGNED	ENDPOINT	ENDPOINT REMARK
BHMT	0.57	R	NOEC	SIG ASSIGNED
ETIF	0.04	R	LOEC	SIG ASSIGNED
UCE	0.01	R	LOEC	SIG ASSIGNED
Tubulin	0.21	R	NOEC	SIG ASSIGNED
Trypsin	0.07	R	NOEC	SIG ASSIGNED
UBI	0.14	R	NOEC	SIG ASSIGNED
SAMS	0.32	R	NOEC	SIG ASSIGNED
Hcy a	0.72	R	NOEC	SIG ASSIGNED
Hcy b	0.31	R	NOEC	SIG ASSIGNED

If a paper presents statistically analyzed data using least square difference (LSD) analysis, determine the statistical significance by the following calculation:

Dose	Response
Control 1	2.4
Dose 2	4.6
Dose 3	4.8
Dose 4	6.7
LSD(0.05)	1.1

Subtract or add the LSD value (1.1) to the control value to get the lowest value that is significantly different. In this example, anything above 3.5 or below 1.3 is a significant value at the  $p=0.05$  level. Therefore, all doses are significantly different from the control because they are all greater than 3.5 (Pers. Comm., R. Regal, UMD Statistics Dept., 1999).

Combining of Statistics

If a measurement has no clear dose response as interpreted by the reviewer when statistics are reported, it is coded as multiple significance (MULT).

Example 1:        Five concentrations are tested and the two highest and two lowest show significance but the middle concentration does not, code MULT.

Growth table with length affected by copper.



Concentration (ug/L)	% Mortality	P=0.05 , p=0.01 *	Code
control	6.3	b , B	NOEC at p=0.01 at 25 ug/L, LOEC at p=0.01 at 50 ug/L
25	6.4	b, B	
50	24.6	a, A	
100	20.2	a, A	
150	32.2	a, A	

\* Same characters in a column indicate no significant difference

### Combining of Level

When a range of concentrations is coded, and there are multiple levels of significance reported, range the values.

Non-monotonical response was reported for data with concentrations of 15, 30 and 60 ug/L. At 15 ug/L the p-value was  $p < 0.05$  (sig), at 30 ug/L the p-value was  $p < 0.10$  (nosig) and at 60 ug/L the p-value was  $p < 0.001$  (sig).

CONC: 15 to 60 SIG: Mult LEVEL:  $p < 0.10$  to 0.001 ENDPOINT: NR

### Chemical Analysis Method (M/U/Z)

This parameter identifies whether quantitative analyses of the toxicant concentration in the test media were conducted and whether measured concentrations were used to report the results.

This field represents/defines the concentration which was used in reporting the endpoint or effect; publications may report **Measured** and **Unmeasured** (nominal) concentrations for one test scenario, use the code which represents whether the specific effect/endpoint concentration was measured or unmeasured. If both measured and unmeasured concentrations for the specific effect/endpoint are reported, record only the measured concentrations.

If authors report that the measured values are within a percentage of the nominal and only the nominal concentrations are reported, code the chemical analysis methods as '**Z**', signifying that the concentrations were analyzed.

For instances where the author only reports measured concentrations for some of the treatment (DOSE) levels but reports all unmeasured concentrations, code the unmeasured values for each treatment level so that the range of concentrations is consistent and monotonical. The measured concentration values are coded into the DOSE comments for the corresponding unmeasured value. Denote all of the CHEMICAL ANALYSIS METHOD as '**X**', signifying that a combination of measured and unmeasured values was reported.

If the doses/concentrations are reported as both measured and unmeasured values but ALL analysis is based on the unmeasured values, code U, the unmeasured values and note "MEASURED CONCENTRATIONS ALSO REPORTED" in the OTHER EFFECTS field.

If in a paper the author reports one test set up, reports both measured concentrations and unmeasured concentrations for all concentrations tested and reports some endpoints based on the measured values and some endpoints based on the unmeasured values, two separate tests are coded. One test reports the measured values in DOSES with the endpoints based on the measured values with M in CHEMICAL ANALYSIS METHOD. A second test is coded reporting the unmeasured values in DOSES with a Dose Comment "MEASURED VALUES ALSO REPORTED" with the endpoints based on the unmeasured values and U in CHEMICAL ANALYSIS METHOD. Note that if an endpoint is reported in both unmeasured and measured values it is coded as the measured value in the measured test. For example, if the LC50 is reported in both measured and unmeasured values, only the measured value result is coded.

For instances where authors report either unmeasured or measured test concentrations and report the results as the opposite analysis method, e.g. author reports nominal test concentrations and reports the ECxx result values based on measured concentrations, do not code the test concentrations. Add a comment in the Experimental Design data field MEASURED TEST CONCENTRATIONS REPORTED or UNMEASURED TEST CONCENTRATIONS REPORTED as needed.

When chemical measurements are conducted on stock solutions, but nominal concentrations are reported for effects or endpoints, code as **Unmeasured**. When chemical measurements are conducted periodically throughout the exposure but the reported measurements are not correlated with the effects, code as **Unmeasured**. When chemical measurements are conducted periodically throughout the exposure and the effects are coordinated with the measurements, code as **Measured**.

Even if measured values are reported by the author to have deteriorated by the end of the exposure, the **M**easured code should still be used. It is acceptable to assume that if the author used measured concentrations in residue analysis, that these measured values were carried over to calculate BCFs. (See ECOTOX Code Appendix – Appendix P for codes)

Assigned Endpoint (ASSIGNED ENDPOINT)

If the reviewer codes an endpoint which is not stated by the author in the paper, or interprets an author’s endpoint definition to be equivalent to the ECOTOX endpoint definition, an “**R**” for reviewer-assigned endpoint is entered into the field. In all cases except for NR-ZERO and NR-LETH, “by definition”, the endpoint the author reports or the description of the endpoint is coded in the ENDPOINT REMARK data field. However, if the reviewer codes an endpoint as the author states it in the paper, a “**P**” is coded for paper-assigned endpoint.

**Note:** An “R” is always coded for the endpoints NR-ZERO and NR-LETH, however no further information is coded in the ENDPOINT REMARK field.

Reviewers will follow these guidelines in assigning endpoints:

1. BY DEFINITION: If the author does not actually state that the value is an LD50 but states that “concentration x is the dose estimated to be lethal to 50% of the test organisms”, the reviewer should code this as an LD50 endpoint because the author defines the LD50. Such a designation is accompanied by noting “R” in the PR data field.
2. When the author provides text which identifies a value as the “highest tested concentration having no statistically significant adverse effect”, the reviewer should code this as a NOEL/NOEC; the “lowest tested concentration having a statistically significant adverse effect” is coded as a LOEL/LOEC. In both cases, the PR field will be coded as 'R' to reflect reviewer assignment of an endpoint. Because LOEL/NOEL values are assigned under very specific experimental and statistical conditions, ECOTOX reviewers will be assigning complementary NOELs or LOELs only when the author assigns either a LOEL or NOEL.
3. When the author provides statistical information, which designates concentrations as significantly different from the control, the reviewer will code this information as SIG or NOSIG. The reviewer will also report the level of significance in the LEVEL data p-value in the p-value field.
4. An author reports an Endpoint similar to one defined in the ECOTOX SOP.

Example 1. Author uses TLm for 50% mortality endpoint. Reviewer can code the endpoint as LC50 and puts “R” for reviewer assigned endpoint (code TLM in the ENDPOINT REMARK field).

5. Coding a companion endpoint

Example 2. Author reports NOEC in paper but does not include companion endpoint - LOEC. If reviewer can determine LOEC from data, an “R” is coded for the LOEC (code “by definition” in the ENDPOINT REMARK field) and a “P” is coded for the NOEC.

6. If the author presents biologically significant data which does not include statistical analysis because all of the animals died, the reviewer should not interpret this data as a non-monotonical response. Code the appropriate biologically significant endpoints as NOEC/LOEC values. For example:

Example 3

WHOLE BODY GROWTH	
CONTROL	139 G
10 UG/ML	138 G
20 UG/ML	136 G
30 UG/ML	100 G*
40 UG/ML	95 G*
50 UG/ML	ALL DEAD

\* = SIGNIFICANT AT 0.05

CODE: EFFECT:	GRO (GROWTH)	ENDPOINTS:	NOEC @ 20 UG/ML
	MEASURE: WGHT (WEIGHT)		LOEC @ 30 UG/ML

Example 4

% DEFORMITY	
CONTROL	0%
10 UG/ML	0%
20 UG/ML	0%
30 UG/ML	0%
40 UG/ML	100%
50 UG/ML	ALL DEAD

CODE: EFFECT:	DVP (DEVELOPMENT)	ENDPOINTS:	NOEC @ 30 UG/ML
	MEASURE: DFRM (DEFORMATION)		LOEC @ 40 UG/ML

Test Result Examples

1. If the author has defined an ENDPOINT and/or has reported a 0% and/or 100% mortality response, report the endpoint/mortality as outlined in the Endpoint Hierarchy. Select the appropriate effect as described below.

ENDPOINT REPORTED (NR-ZERO):

ENDPOINT: NR-ZERO	MEASURE: MORT	
TREND: NEF	EFCT%: 0	
EFFECT: MOR	SIG: NA	LEVEL: NA

If applicable, statistical results should appear in the SIG field, the level of significance should be reported in the LEVEL field, the percent effect should be presented in the EFCT% field, and the trend should be reported in the TREND field.

ENDPOINT REPORTED (LOEC):

ENDPOINT: LOEC	MEASURE: LGTH	
TREND: DEC	EFCT%: 20	
EFFECT: GRO	SIG: SIG	LEVEL: a<0.05

**Note:** For NOEC endpoints, NOSIG is coded in the SIG field. For LOEC endpoints, SIG is coded in the SIG field.

2. If the author-reported effect is a clear dose response result using statistical analysis, and the author does not identify an endpoint, select the appropriate effect from ECOTOX Code Appendix – Appendix S.

Clear dose response data where a statistically significant effect was observed, are represented by two data records. One data record represents the lowest concentration at which a statistically significant effect occurred. An Endpoint, LOEC is encoded and "SIG" is coded in the SIG field, the observed trend is coded in the TREND field, the percent effect is coded in the EFCT% field, and the level of significance is reported in the LEVEL field. The observed measurement is coded in the MEASURE field. Remarks on the effect are made in the EFFECT\_REMARK field.

CLEAR DOSE RESPONSE:

ENDPOINT: LOEC	SIGNIF: SIG	TREND: DEC
EFFECT: GRO	EFCT%: 20	LEVEL: a<0.05
MEASURE: LGTH		

The second data record represents the highest concentration at which no effect occurred. An Endpoint, NOEC is encoded and NOSIG is coded in the SIG field. If a percent effect is reported it is presented in the EFCT% field.

If the concentration identified as SIG is the lowest concentration reported or the concentration identified as NOSIG is the highest concentration reported, code the NOEC/LOEC at the highest/lowest concentration and the appropriate code (ASIG and ANOSIG) in the SIG field.

Standard methods recommend that when determining a NOEL/LOEL, at least three exposure concentrations be used (Menzer 1994 at 1406);. If the author uses only one exposure concentration AND assigns a NOEL/LOEL or SIG/NOSIG result, a Remark noting "only conc tested" will be coded.

3. If the author reported effect shows unclear dose response results, using statistical analysis, select the appropriate effect from ECOTOX Code Appendix – Appendix S.

When data have been statistically analyzed, and the results presented have significant effects in an unclear dose response pattern (e.g., significant effects at the high and low concentrations, and not significant at the middle concentration), "MULT" is coded in the SIG field to signify multiple significance. The level is coded with a full range of p-values (e.g. p<0.05-0.001).

UNCLEAR DOSE RESPONSE:

ENDPOINT: NR	MEASURE: ACHE	
TREND: CHG		
EFFECT: ENZ	SIG: MULT	LEVEL: P<0.05-0.001

4. If the author reports a descriptive or qualitative effect without statistical analysis, select the most appropriate effect from ECOTOX Code Appendix – Appendix S. One record is coded with a full range of exposure concentration and time. Code all measurements for the specific effect. The appropriate trend is coded in the TREND field. If these data report multiple trends, code CHG in the TREND field and report the individual trends in MEASUREMENT\_REMARK field. The percent effect over the concentration tested is reported in the EFCT% field. NR is coded in SIG and LEVEL fields.

NO STATISTICAL ANALYSIS:

ENDPOINT: NR

MEASURE: LESI, ATRS,  
DEGN, EDMA,EXCS

MEASUREMENT  
REMARK: INC LESI,  
ATRS, DEGN, EDMA,  
DEC EXCS

TREND: INC  
EFFECT: HIS

EFCT%: NR  
SIG: NR

LEVEL: NR

Percent Lipid (LD) (Accumulation only)

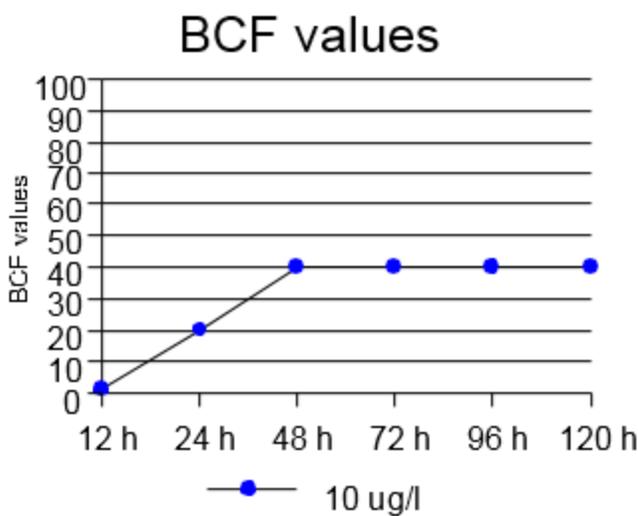
If percent lipid information is provided in the publication, record as a % value

Dry or Wet Weight

Record whether the residue/bioconcentration/bioaccumulation, growth, population or biochemical data are reported on a dry or wet weight basis , by selecting D, W or NR from the dropdown.

Steady State (STST) (Accumulation only)

Record whether the residue/bioconcentration/bioaccumulation data at the time of the result is at steady state or equilibrium by selecting Yes, No or NR from the dropdown. If the author does not report in the text that the data are at steady state or equilibrium but represents the data graphically, this information can be determined by the graphed data. If the residue or BCF data are presented in a graph and the data points flatten out and continue to flatten during the exposure phase, the point at which it flattens out is the steady state or equilibrium point. For example:



The steady state or equilibrium is reached at 48 hours. STST is coded as YES for the BCF values at 48, 72, 96 and 120 hours.

If the author does not report this information code as 'NR'

The steady state or equilibrium is reached at 48 hours. STST is coded as YES for the BCF values at 48, 72, 96 and 120 hours.

If the author does not report this information code NR.

Result Remarks

The Result Remarks data field contains historical comments from both the aquatic and terrestrial databases. No entries are made in the Result Remarks data field. If records are completed for data maintenance, the comments in the Result Remarks data field will be moved to the corresponding remark data field.

**6. Media Characteristic Parameters tab**

Parameter	Mean	Min	Max	Unit	Comments
Temperature	NR	NR	NR	NR	
Hardness	NR	NR	NR	NR	
Alkalinity	NR	NR	NR	NR	
Dissolved Oxygen	NR	NR	NR	NR	
pH	NR	NR	NR		
Salinity	NR	NR	NR	NR	
Conductivity	NR	NR	NR	NR	
Organic Carbon Type	NR				
Organic Carbon	NR	NR	NR	NR	
Humic Acid	NR	NR	NR	NR	
Sodium	NR	NR	NR	NR	
Chlorine	NR	NR	NR	NR	
Dissolved Inorganic Carbon	NR	NR	NR	NR	
Calcium	NR	NR	NR	NR	
Potassium	NR	NR	NR	NR	
Magnesium	NR	NR	NR	NR	
Sulfate	NR	NR	NR	NR	
Sulfur	NR	NR	NR	NR	

Figure 7. Media Characteristic tab

The following media/water chemistry parameters are included in ECOTOX, and are coded in appropriate fields.

These measured values pertain either to the test media/water chemistry or the dilution or culture media/water chemistry values. In the absence of test media/water chemistry parameters, it is acceptable to report the culture, holding tank, acclimation, control or dilution media/water, or pretest conditions denoted by an asterisk (\*) which is entered directly after the value (e.g. 8.6\* mg/L). Media/water chemistry parameters measured prior to or after the exposure period are coded only if test media/water chemistries are not reported in the publication. If the author reports the test conditions as “similar” to other methods in the paper, code test conditions as “NR”.

When media/water chemistries differ between samples (e.g., test chamber or water body), and results are obtained from only some of the samples, media/water chemistries should be reported for only those samples actually tested. The following are two examples on coding media/water chemistries:

Conc	Temperature (Celsius)	pH
1 ug/l	20.4 (20.1 - 20.7)	7.9 (7.5 - 8.3)
2 ug/l	20.5 (20.2 - 20.8)	8.0 (7.6 - 8.4)
3 ug/l	20.4 (20.0 - 20.8)	7.8 (7.7 - 7.9)

Example 1: Statistics are presented for Protein content and it is found to be significant at 2 and 3 ug/l. The data coded for Temperature and pH are for the specific concentration.

Code: Endpoint: LOEC Effect: BCM Measurement: PRCO Concentration: 2 ug/l Significance: SIG pH: 8.0 (7.6-8.4) Temperature: 20.5 (20.2-20.8)

Example 2: Statistics are presented for Glucose content and all concentrations are found to be not significant. The data coded for Temperature and pH are for the range of concentrations.

Code: Endpoint: NOEC Effect: BCM Measurement: GLUC Concentration:3 ug/l Significance: ANOSIG pH: 7.8 (7.7 – 7.9) Temperature: 20.4 (20.0 - 20.8)

When the author refers to the media/water chemistry values as approximate a "~" is coded in front of the value.

Graphed data are coded as a range or as “less than” or “greater than” values and the term “FROM GRAPH” is noted in the corresponding remark data field.

Media/water chemistry values should be coded as reported by the author.

For studies where a chemical concentration is monitored as a water or soil chemistry parameter (e.g. sodium chloride reported as conductivity, calcium chloride reported as hardness), code the water or soil chemistry parameter in both the dose and the water chemistry parameter data fields.

Aquatic Studies Media Parameters

(Invoked by selecting 'Water' from the Species Habitat data field)

Field Name	Standard Unit	Abbreviation	Comment
Temperature	C	<u>TEMP</u>	When temperatures are reported for incubation chambers or water baths, these temperatures are acceptable for reporting as test temperatures. Do not code temperatures noted as "room temperature".
Hardness	mg / L CaCO <sub>3</sub>	<u>HARD</u>	If the author only reports the terms "hard" or "soft", these terms are recorded. If the author reports a hardness value but does not identify a unit and/or refers to the value as "total", standard units are assumed and the value coded.
Alkalinity	mg / L CaCO <sub>3</sub>	<u>ALK</u>	If the author reports an alkalinity value but does not identify a unit and/or refers to the value as "total", standard units are assumed and the value coded.
Dissolved Oxygen	mg / L	<u>DO</u>	A "SAT" code is used for 100% saturation
pH	--	<u>pH</u>	pH range is between 1 and 14
Salinity	0/00	<u>SALIN</u>	Some authors report the unit as ppt. This is parts per thousand and should be coded as 0/00 not as parts per trillion ppt.
Conductivity	umhos / cm	<u>COND</u>	Report the conductivity and unit reported by the author
Organic Carbon	mg / L Carbon	<u>ORG C</u>	Organic carbon must designate (T=Total, P=Particulate, D=Dissolved); if more than one type of organic carbon is reported in the publication, record T in the field and the other values (P or D) as a Remark; if the value is reported as "organic carbon" without identifying type, assume the value is expressed as Total and report T. Sediment organic carbon values are not reported.
Humic Acid	%	<u>HA</u>	Report the Humic acid value and unit reported by the author
<u>Sodium</u>	mg/l	<u>Na</u>	Report the Sodium value and unit reported by the author
<u>Chlorine</u>	mg/l	<u>Cl</u>	Report the Chlorine value and unit reported by the author
Dissolved inorganic carbon	mg/l	<u>DIC</u>	Report the Dissolved inorganic carbon value and unit reported by the author
Calcium	mg/l	<u>Ca</u>	Report the Calcium value and unit reported by the author
Potassium	mg/l	<u>K</u>	Report the Potassium value and unit reported by the author
Magnesium	mg/l	<u>Mg</u>	Report the Magnesium value and unit reported by the author
Sulfate	mg/l	<u>SO<sub>4</sub></u>	Report the Sulfate value and unit reported by the author
Sulfur	mg/l	<u>S</u>	Report the Sulfur value and unit reported by the author

Terrestrial Studies Media Parameters

(Invoked by selecting 'Soil' from the Species Habitat data field)

Field Name	Standard Unit	Abbreviation	Comment
Temperature	C	TEMP	When temperatures are reported for incubation chambers or water baths, these temperatures are acceptable for reporting as test temperatures. Do not code temperatures noted as "room temperature".
Soil Texture Sand	%	SA	Report the sand texture of the soil as stated by the authors
Soil Texture Silt	%	SI	Report the silt texture of the soil as stated by the authors
Soil Texture Clay	%	CL	Report the clay texture of the soil as stated by the authors
CEC	uS/cm	CEC	Report cation exchange capacity and units (Refer to ECOTOX Code Appendix – Appendix FF for cation exchange capacity units) of the test media.
pH	--	pH	pH range is between 1 and 14  If the author specifies a measurement method for the pH value (e.g., that the pH value is measured by pHKCl or pHCaCl <sub>2</sub> ), code the pH value and identify the measurement method in the REMARKS field. If the authors report that a standard (see Attachment G for list of standard soils) or commercially available artificial soil is used, but do not present pH, use the pH reported in the standard test method referenced by the author.  Note that when coding plants and invertebrate papers for the TRV project and the pH of a specific soil type was not given in the publication, a search of the USDA/NRCS National Cooperative Soil Survey (USA) online site, at the following web address: <a href="http://ncsslabsdatamart.sc.egov.usda.gov/querypage.aspx">http://ncsslabsdatamart.sc.egov.usda.gov/querypage.aspx</a> was conducted for the Characterization Data of the specific soil series. If the pH was found, the range of the pH values for all soil depths in the pH data field was coded and a remark in the comments section "from USDA web source" was made.
Organic Matter	mg / L	OM	If the authors report that a standard (see Attachment G for list of standard soils) or commercially available artificial soil is used, but do not present organic matter content, use the organic matter content reported in the standard test method referenced by the author.  Note that when coding plants and invertebrate papers for the TRV project and the organic matter of a specific soil type was not given in the publication, a search of the USDA/NRCS National Cooperative Soil Survey (USA) online site, at the following web address <a href="http://ncsslabsdatamart.sc.egov.usda.gov/querypage.aspx">http://ncsslabsdatamart.sc.egov.usda.gov/querypage.aspx</a> was conducted for the Characterization Data of the specific soil series. If the organic matter was found, the organic matter values were ranged for all soil depths in the OM data field and a remark in the comments section "from USDA web source" was made.
Moisture	%	MOIST	Report the percent moisture as reported by the author.

For Terrestrial Non-Soil Studies, only the Temperature data fields will be invoked. If pH of a culturing media, e.g. oocyte rearing solution, is reported, encode the pH in the Experimental Design data field.

**7. Dose Response tab (not currently coded).**

Coding of Dose Response data stopped in 2009. This information is for historical reference.

Response data for each dose tested was reported by encoding each individual data point, e.g. percent mortality, number of offspring, amount of protein, etc.

Data was entered by selecting the Result record that was associated with the Dose Response data to be coded. The Add Dose Response button was chosen to enable the dose response screen entry fields.

Data was entered into fields in the same manner as listed in the sections above.

ID	Sample Unit	Measurement	Response Site	Duration	Dose Response						Collapse
					0 mg/kg (NC) C	100 mg/kg (NC)	500 mg/kg (NC)	1000 mg/kg (NC)	5000 mg/kg (NC)	10000 mg/kg (NC)	
82693	AD	SURV	NR	14 d	5 org	5 org	5 org	5 org	5 org	5 org	
82694	AD	SURV	NR	14 d	5 org	5 org	5 org	5 org	5 org	5 org	

Dose Response Information										
Sample Unit	Measurement	Response Site	Dose Response Unit	Duration	Comments					
AD	SURV	NR	org	14 d	Replicate 1					

ID	Dose (Conc Type)	Dose Response	Stat. Method	Stat. Method Value	Sample Size	Significance	Significance Level
73465	0 mg/kg (NC) C	5 NR NR	SE NR All	0.0	5 NR NR	NR	NR NR NR
73465	100 mg/kg (NC)	5 NR NR NR	SE NR All	0.0	5 NR NR NR	NR	NR NR NR NR
73467	500 mg/kg (NC)	5 NR NR NR	SE NR All	0.0	5 NR NR NR	NR	NR NR NR NR
73468	1000 mg/kg (NC)	5 NR NR NR	SE NR All	0.0	5 NR NR NR	NR	NR NR NR NR

Figure 8. Dose Response tab

### 8. Companion Endpoint(s) tab (only invoked if NOxx, LOxx or MATC coded)

ID	Chemical	Habitat	Species	Age	Lifestage	Study Type	Exposure Type	Test Location	#Results	Create Date	Modified Date	Collapse
2052205	118967 - 2,4,6-Trinitrotoluene	Soil	1499 - Enchytraeus albidus	NR	NA	NR	EN	LAB	8	04/22/2013	04/22/2013	

ID	Sample Number	Trend	Endpoint	Effect	Measurement (s)	Response Site(s)	Significance	Observed Duration	Concentration	Page Number	Reviewer Number	Modified Date	Collapse
2087262		INC	LC20	MOR	MORT	NR	NA	21 d	F 234 (147 - 301) mg/kg d soil	135	5	04/22/2013	
2087263		INC	LC50	MOR	MORT	NR	NA	21 d	F 362 (275 - 438) mg/kg d soil	135	6	04/22/2013	
2087264		INC	LOEC (NOEC)	MOR	MORT	NR	SIG	21 d	F 389 mg/kg d soil	135	7	04/22/2013	
2087265		INC	NOEC (LOEC)	MOR	MORT	NR	NOSIG	21 d	F 195 mg/kg d soil	135	8	04/22/2013	
2087266		DEC	EC20	REP	FCND	NR	NA	42 d	F 88 (38 - 115) mg/kg d soil	135	9	04/22/2013	
2087267		DEC	EC50	REP	FCND	NR	NA	42 d	F 133 (95 - 173) mg/kg d soil	135	10	04/22/2013	
2087268		DEC	LOEC (NOEC)	REP	FCND	NR	SIG	42 d	F 194 mg/kg d soil	135	11	04/22/2013	
2087269		DEC	NOEC (LOEC)	REP	FCND	NR	NOSIG	42 d	F 97 mg/kg d soil	135	12	04/22/2013	

Result Information		Media Characteristics (Soil)			Dose Response			Companion Endpoints	
ID	Sample Number	Trend	Endpoint	Effect	Measurement(s)	Response Site(s)	Significance	Observed Duration	
<input checked="" type="checkbox"/>	2087265	NR	INC	NOEC	MOR	MORT	NR	NOSIG	21 d
<input type="checkbox"/>	2087268	NR	DEC	LOEC	REP	FCND	NR	SIG	42 d
<input type="checkbox"/>	2087269	NR	DEC	NOEC	REP	FCND	NR	NOSIG	42 d

Figure 9. Companion Endpoint tab

This tab allows users to link NOxx, LOxx and MATC values together.

To link the endpoints together, select the result that is to be linked from the result information view. Next, on the Companion Endpoint tab, select the result (s) that should be grouped together by marking the check box in front of the result. The Toxicity Module will perform an immediate check to ensure that the results can be linked together. If there is an error, the Toxicity Module will report the error for immediate rectification.

Note that all results that are to be linked must have the same result information, i.e. Duration, Response Site, Measurement, etc. A maximum of three (3) results records can be linked together, a NOxx, LOxx and MATC.

Example 1: The NOEC, LOEC and MATCs are reported for Growth - Length, Growth - Weight and Mortality - Mortality, the Growth Length endpoints are companion endpoints group 1, the Growth Weight are companion endpoints group 2 and the Mortality Endpoints are companion endpoints group 3. The data is coded as:

Endpoint: NOEC Effect: GRO Measurement: LGTH

Endpoint: LOEC Effect: GRO Measurement: LGTH Endpoint: MATC Effect: GRO Measurement: LGTH

Endpoint: NOEC Effect: GRO Measurement: WGHT Endpoint: LOEC Effect: GRO Measurement: WGHT

Endpoint: MATC Effect: GRO Measurement: WGHT

Endpoint: NOEC Effect: MOR Measurement: MORT

Endpoint: LOEC Effect: MOR Measurement: MORT

Endpoint: MATC Effect: MOR Measurement: MORT

## 9. Paper Reviewing completion steps

Ensure all chemicals reported in the paper are addressed:

If the paper is fully coded (UNIFY COMPLETE), all chemicals including those that are added with a reject keyword must either have a CAS# or be archived. If this step has not been completed, the chemical name(s) is forward to chemical verification staff for verification.

Reviewer electronic quality assurance checks:

When the reviewing of a paper has been completed, the reviewer should conduct an Electronic QA by pressing the Electronic QA icon. A list of errors and a list of warning is generated. These must be addressed prior to completion.

In addition, it is helpful to open the coded data within the Excel icon and do a quick self-quality assurance of the information entered. In doing this, the reviewer can spot and fix potential errors prior to the Quality Assurance staff receiving the paper for final checking.

Marking the paper coded:

When the coding has been completed for the paper, the Reviewer selects the Reviewed icon and follows the prompts for guidance on the reviewing status of the paper. Choose the correct responses for the way the paper was coded and UNIFY will assign the status based on the answers. The review must have an understanding of the difference categories of reviewing and ensure that the status has been assigned correctly.

Attaching completed skimming sheet and checking citation information:

After all reviewing steps have been completed the completed skimming sheet is attached to the back of the paper.

Check the printed skimming sheet to ensure that it shows the correct status of each chemical listed.

Check the Citation information against the paper and checklist (title, author, pages, pdf name, Ecoref Number, exposure type, effect groups, species groups and status). All citation information needs to be accurate and correctly reflected on the cover page of the paper. For example, if FISH and DOMA are on the checklist, then they both need to be on the front of the paper as well as AT for the habitat.

## 10. Paper Reviewing Quality Assurance (RQA) completion steps

After a paper has been coded, it is forwarded to another experienced Reviewer for Quality Assurance. The QA procedure follows these general steps:

1. With paper in hand, the records are brought up in UNIFY by inputting the EcoReferences Number in the Ecoref Number search box on the References search screen.
2. Click on the Test or Result ID number and move between each of the screens or download the data to Excel for QA. Reviewers will have written test numbers (1, 2, 3, etc.) next to the endpoints coded in the paper that correspond to the test number they give each record. For each record, make sure you are following the information in the paper that corresponds to the endpoint coded.
3. First make sure that the chemical information matches what is reported in the paper (CAS number, name, purity, grade, etc.)
4. Make sure that the species Latin name matches the test organism in the paper. Check to see that the organism Body Weight, Lifestage, and Age information is reported correctly. If cultivars or varieties are reported in the Organism Comments, be sure that they are correctly matched with the endpoints chosen from the paper.
5. Check that the correct Control has been coded.
6. Confirm the correct Number of Concentrations tested, the Concentration Values, and Units are the same as reported in the paper.
7. Check the paper and evaluate whether the Reviewer coded the correct Concentration Type and Method of Chemical Analysis.

8. Quickly scan the water parameter fields (e.g. temperature, pH, Hardness) to see that they match the paper. Also quickly skim aquatic papers to make sure no water chemistry fields were missed in the coding.
9. If the paper is a field study, make sure the correct Field Study Information has been coded (e.g. Application Date, Substrate Code)
10. Closely examine the paper to be sure the correct Duration, Effect Measurement, and Response Site has been coded.
11. Check the endpoint coded and make sure the Reviewer has chosen the correct Endpoint and corresponding Concentration and Conc Type.
12. Make sure the reported p-value is correct and that all Comment fields have reported the necessary information.
13. Once all records have been QA'ed for a paper, skim the paper to make sure that no data is missing from coding.
14. If issues are found or when the QA'er disagrees with how a field was coded, an email to the Reviewer is generated noting issues. If the Reviewer agrees the changes are made and it is returned to the QA'er. If the Reviewer does not agree with the issues a reply email is sent back to the QA'er with the reasoning. If there are still issues, the QA'er should discuss the mistakes with the Reviewer and come to a conclusion. If the QA'er and Reviewer cannot decide on the correct course of action, the issue is brought to the task manager or another experienced reviewer for additional consultation. The third party will either make a decision on how the field should be coded or seek assistance from other experienced Reviewers.
15. When the QA'er deems the coding correct and complete they select the "RQAed" button to automatically approve all recently coded or modified records for a paper.
16. The paper is sent for return memo processing.

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