

Unify Toxicity Module

ECOTOXicology Knowledgebase System

ECOTOX Coding Guidelines

Prepared for:

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A. OVERVIEW OF ECOTOX	1
1. Introduction to Review of Literature	1
2. Literature Reviewer Training.....	1
Training Sequence.....	1
Measures of Competency for Trained Reviewers	3
Steps in the Quality Assurance Process	3
3. General Coding Information.....	4
Overview.....	4
Coding Practices.....	5
B. ECOTOX CODING GUIDELINES.....	7
Quality Assurance Parameters	7
QA Date/Initials	8
Publication Reference Number, Author, Year	8
Total Tests	8
Reviewer/Date.....	8
Test Number (TESTID)	8
1. Test Chemical Parameters (Chemical Information Tab)	10
Chemical Name (TEST) and CAS Number (CAS #)	13
In a Mixture?	16
Chemical Grade (GRADE)	16
Chemical Purity (PURITY)	16
Chemical Formulation (FORM)	16
Chemical Comments (CHEMICAL COMMENT).....	16
Radiolabel (RADIOLABEL)	17
Chemical Carriers	17
2. Test Organism Parameters (Species Information Tab).....	19
Species (LATIN NAME/SPECIES #)	19
Habitat (HAB).....	20
Organism Source (ORGANISM SOURCE)	20
Lifestage (LIFESTAGE)	21
Age (AGE) and Age Unit (AGE UNIT).....	22
If the information is not reported in the publication, record the organism's age as 'NR'. Initial Weight (WTINT).....	22
Gender (SEX).....	22
Organism Characteristics (ORGANISM CHARACTERISTICS)	22

3. Test Information Parameters (Test Information Tab)	24
Exposure and Study Durations	24
Study Duration (STUDY DURATION)	26
Exposure Duration (EXPOSURE DURATION)	26
Application Frequency (APP FREQ)	27
Control Type (CONTROL).....	28
Concentration Type (CONC TYPE).....	28
Concentration Types and Definitions	29
Study Type (STUDY TYPE)	30
Test Type (STYPE)	31
Test Location (LAB, NR, FIELDN, FIELDA,)	31
Test Method (TMETH)	31
Exposure Type (EXP TYP)	31
Media Type (MEDIA TYPE)	32
Number of Doses (DNUM)	32
3a. Doses (DOSES).....	32
Dose Number	33
Control Type	33
Concentration Type.....	33
Doses 33	
Organism Number.....	35
Experimental Design (EXP DESIGN).....	35
Other Effects (OTHER EFCT)	37
Additional Comments	37
4. Field Test Parameters (Habitat tab)	37
Sub-habitat (HABITAT CODE).....	37
Sub-Habitat Comment (HABITAT COMMENT).....	38
Geographic Code (GEOGRAPHIC CODE)	38
Geographic Code Comment (GEOGRAPHIC LOCATION).....	38
Longitude/Latitude (LATITUDE/LONGITUDE)	38
Substrate (SUBSTRATE).....	38
Substrate Comment (SUBSTRATE REMARK)	38
Application Type (APP TYPE).....	39
Application Date (APP DATE)	39

Application Season (APP SEASON).....	39
Chemical Half-life (HALF-LIFE).....	39
Application Rate (AP RATE)(Water Studies Only)	39
Water Depth (DEPTH) (Water Studies Only)	40
5. Result Parameters (Result Information tab).....	40
Sample Number and Units (SAMPN)	40
Observed Duration (OBS TIME).....	41
Results Coding Hierarchy	42
Endpoint (ENDPOINT)	45
Trend (TREND).....	51
Effect (EFFECT).....	51
Effect Hierarchy.....	52
Response Site (RESPONSE SITE).....	53
Combining Response Site.....	53
Measurement (EFFECT MEASUREMENT)	53
Effect % (EFFECT PERCENT)	55
Combining EFFECT PERCENT.....	56
Projects (This data field is not encoded by reviewers)	56
5a. Concentration Parameters	57
Concentration Type (CONC TYPE).....	57
Ionic Fraction (ION).....	57
Effect Concentration (CONC).....	57
5b. Bioconcentration Value (BCF)	59
Statistical Significance (SIG)	61
Combining of Statistics.....	64
Significance Level (LEVEL).....	65
Combining of Level	66
Chemical Analysis Method (M/U/Z).....	66
Assigned Endpoint (ASSIGNED ENDPOINT)	68
Species Weight Final (WTAT)	69
Intake Rate (INTAKE)	69
Test Result Examples	69
Percent Lipid (LD)	71
Dry or Wet Weight and Percent (DW)	71

Steady State (STST)	71
6. Media Characteristic Parameters tab	72
Aquatic Studies Media Parameters	73
Terrestrial Studies Media Parameters	75
7. Dose Response tab (not currently coded).....	76
8. Companion Endpoint(s) tab	77
9. Paper Reviewing completion steps	78
9. Paper Reviewing Quality Assurance (RQA) completion steps.....	79
10. References	81
Attachment 1. ECOTOX Replicate Review Form.....	82

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). Sources such as author communications and referencing another work to obtain one of the five criteria is allowed for specific risk assessment/criteria projects (e.g. EcoSSL or CAD) and must be specified by the EPA Database Coordinator.

2. Literature Reviewer Training

Training Sequence

The training sequence 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 consultation with other ECOTOX knowledgebase personnel.

The scope of the six month intensive training period encompasses the following areas:

- endpoint toxicity test review (one month);
- effect only toxicity test review (two months);
- bioconcentration study review (one month);
- field study review (one month); and in-depth training within the areas listed above (one month).

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>);
- Fundamentals of Aquatic Toxicology (Rand (Ed), 1995);
- American Society for Testing and Methods; (ASTM, 1996);
- Selected toxicity literature publications

The reviewer initially reviews the ECOTOX Standard Operating Procedures: Coding Guidelines, applicable publications listed in the reference section for each of the databases, applicable US EPA Standard Evaluation Procedures and ASTM 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).

The secondary emphasis is to develop the ability to distinguish between exposure types (lethal, sublethal, bioconcentration). The reviewer is trained to recognize whether standard methods are reported for test methodologies and for the test endpoint. The reviewer is also trained to identify tests which are not applicable to ECOTOX.

Once the general introductory materials are read, the standard training guidelines introduce the reviewer to each category of toxicity literature. Information specific to areas of acute, chronic and bioconcentration literature is discussed in subsequent sections of this chapter. 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 sequence reviewing 20 publications per month. This amount increases until a level of 35 publications per month is attained. The average time estimated per review at the beginning of the training sequence is 1.5 hours per publication. The time should

decrease to one hour per publication. A part time reviewer's training expectations will be decreased accordingly.

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, during the 10 percent replicate review process, and through consultation with publications in the field of aquatic toxicology. As part of this process, consistency and concurrence between the document abstractors is attained.

The ten percent replicate review process assures data integrity and promotes routine evaluation of coding practices. Through this training process, strengths and weaknesses in the data abstractor's expertise are identified and specific programs are established to enhance expertise where needed. Such programs include consultation with ECOTOX staff, toxicology publications and the EPA Database Manager, as needed. Evaluation of replicate reviews, which is performed on 10% of all coded references, is used to flag and correct any major discrepancies between replicates. In addition a screening of all completed entries to ensure consistency and completeness prior to data entry is required. Parameters routinely screened include water chemistry, test organism descriptors, calculated endpoints and total test numbers.

Steps in the Quality Assurance Process

1. Ten percent of the reviewed articles from each abstractor are randomly identified by the data coordinator. Information concerning the number of publications is entered into a file, maintained on the data coordinator's computer. The spreadsheet tracks the QA process and calculates the percent of the publications subjected to quality assurance for each reviewer (Table 1). The original reviewer's entry printout for the chosen publication is placed in the "Double Review and QA" file folder maintained by the data coordinator. An "ECOTOX 10% Tracking Form" sheet is maintained in the folder and filled out as articles are received (Attachment 1.). The spreadsheet file is also updated.

Table 1. ECOTOX 10% TRACKING SHEET EXAMPLE

Date Rec	Doc #	Tot Rec'd	# QAed	2nd Rev	2nd Comp	Coord	Completed
11/30/93	5342	8	1	JACKY	11/30/93	ANNE	01/15/94
12/14/93	6808	10	1	AMY			

2. The publication is given to a second reviewer for independent review. After completion of the second review, the data coordinator gives the copies of entry sheets and paper to the EPA Database Manager who compares both reviewer's entry printouts, documents the differences (if any) between reviewers, archives the information on the "ECOTOX 10% Replicate Review" form (Attachment 1.), then returns the form to the reviewers for comment. The reviewers note discrepancies by either agreeing with the EPA Database Manager's comments or expressing their differing opinions on the form. After the replicate review form is returned to the EPA Database Manager, discussions are held with both reviewers to resolve any remaining differences. Discrepancies due to differences in interpretation are resolved by the EPA Database Manager. Errors caused by incomplete Coding Guideline documentation are identified and modifications are made to the document.
3. Upon completion of the review process, the data coordinator checks to make sure the original reviewer's entry printouts contains the correct data, notes completion date on the "ECOTOX 10% Tracking Form". The ECOTOX 10% Replicate Review forms are filed with the double review entry printouts in the ECOTOX Reviewed files.

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 the Toxicity database.
- 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). When there is a discrepancy between data presented in the text or table and data presented in a graph, the paper is discussed with other reviewing staff and if the issue cannot be resolved the paper is forwarded to the EPA Database Manager for a final determination of which data point will be included in the knowledgebase.
- 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.
- The COMMENT field is a text field which contains additional information about a coding field. The COMMENT field is used when the information necessary for coding a field does not fit in the space provided. A complete list of comment identifiers used in public ECOTOX reports is documented in the appendices. When making a remark, use the appropriate codes from the ECOTOX code list. If a code does not exist for a certain chemical, enzyme, device, technique, etc., do not use the author's abbreviation. Write out the full name of the term and submit to the ECOTOX staff to determine if a new code should be created.
- 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.
- References that have data reported in the EPA Water Quality Criteria Documents (WQC) are coded into ECOTOX if it meets the ECOTOX criteria. If the data that is found in the WQC table does not meet the ECOTOX criteria, the data is not coded. However, the publication PDF is retained and a note is made in the bibliographic file stating that the paper was used in the WQC document, but the data was rejected and was not applicable to ECOTOX.
- 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.
- If a publication is missing pages, pages are cut off or illegible, determine if the information is critical for encoding. Print out a copy of the reference citation and write in the Order Notes field, a brief description regarding the incomplete status of the publication. For missing data that is critical to encoding the data, denote "critical for coding", and the person to forward the re-ordered paper to after updated information is received. If data are not critical, write "not critical for coding" on a printout of the reference citation and continue to code the paper. For non-critical incomplete publications, the information will be updated in UNIFY, but reordering the publication will be a lower priority.
- 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.
- Abstractors 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.
- Textual information is used over all other data, unless the value is noted in another section of the paper. Then, the most frequent value is encoded.
- 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.
- If during the coding process the reviewer needs to create additional diagrams or spreadsheets to aid in the correct interpretation of the paper, this information can be added as an attachment in the References module, if substantial, after the quality assurance procedure is completed. For example, many recalculations of the Result Concentrations needed to be made for a paper. The reviewer entered these values on a spreadsheet. This spreadsheet was used for quality assurance and was attached as an electronic copy attachment to the reference after the quality assurance procedure had been completed. To add an attachment to a reference, query the ECOREF # in the References screen, choose the "plus sign" next to Attachment at the top of the screen, add the attachment via the browse button, add a brief note stating what the attachment was used for and choose the Save Attachment button.

B. ECOTOX CODING GUIDELINES

Quality Assurance Parameters

Note that all of the Quality assurance parameters are automatically updated by the Toxicity module and are found in the Paper Status data fields at the top right section of the entry screen.

<input type="checkbox"/> Data Maintenance	Record History (Modified)	<input checked="" type="checkbox"/> Citation QAed	Unreviewed	Edit Paper Status
Attachment	Created by: csuomi on 03/22/2013 Modified by: csuomi on 04/29/2013 Citation QAed by: csuomi on 03/29/2013	PAPER STATUS: Returned (En Route)	Checkout: 03/28/2013	
		DATA STATUS: REVIEWED - UNIFY - COMPLETE	Checkin:	
		DUPLICATE STATUS: No duplicates	Last Returned: 04/29/2013	
		Recall	To Recall	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				
<i>Efficacy of Some Insecticides on Field Populations of Culex pipiens (Linnaeus) from Egypt by Emtithal, A.E.S., and A.E.B. Thanaa, 2012</i>				

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
4	Benzene, Invert, Egg
5	Benzene, Invert, Larvae
6	Benzene, Invert, Adult
7	Toluene, Fish, Egg
8	Toluene, Fish, Juvenile
9	Toluene, Fish, Adult
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)

ECOTOX is catalogued by the toxicant tested using the Chemical Abstracts Service (CAS) registry number. If a CAS registry number is not available through standard sources the toxicity data cannot be included in ECOTOX. Additional toxicants not included in ECOTOX are complex effluents, chemical mixtures and water/soil chemistry effects (e.g., pH) unless caused by a single toxicant (see Doses, Concentration and Media Characteristics for specifics on coding).

Chemical mixtures may be interpreted broadly. For example, a pesticide formulation is a mixture of two active ingredients, each ingredient may have a separate CAS number. If the formulation has a single unique CAS number, the chemical reported for ECOTOX is the formulation. If two different active ingredients are mixed together in a tank then applied as a toxicant, the data are not coded, as this is a mixture.

If the exposure is based on two metal compounds but the effect is based on one ion, e.g., copper sulfate and copper chloride and Cu is the toxicant, code copper as the test chemical and report the two compounds in chemical CHARACTERISTICS.

If herbicides/fungicides/insecticides are applied as standard weed/fungus/insect control to both control and treatment groups prior to the application of the toxicant, the toxicant effects are coded and the application of the herbicide/fungicide/insecticide is noted in the EXPERIMENTAL DESIGN data field (see Experimental Design (EXP DESIGN) for formatting). If the chemicals are applied at the same time, this IS considered a mixture.

For toxicity tests with Nanoparticles, the paper may report that it contains a surface coating. For example, “Cadmium selenium (CdSe) quantum dots....coated with mercaptopropionic acid (MPA)..” Coatings are used to improve stability and dispersibility of the nanoparticle (Vasiliev, 2009). However, in some instances, the paper may report it as having a toxic effect. If the paper does not mention a toxic effect of the coating, the chemical will be reviewed as a single chemical with a comment on the coating in the Chemical Characteristics data field. For the example above, CdSe would be reviewed as Cadmium selenide with the comment “Coated with Mercaptopropionic acid”. If the paper were to report a toxic coating effect, it should be treated as a mixture and be sent to chemical verification staff if needed (as Cadmium selenide-mercaptopropionic acid mixture).

A similar example for lifestage studies occurs when the parental generation is exposed to one chemical prior to the gestational or post-gestational exposure of another chemical to the offspring produced, the parental data is coded until the second chemical exposure and the offspring data is coded only for the second chemical exposure. For the offspring records, it is noted in the Experimental Design field that the parents were exposed to a chemical prior to the gestational or post-gestational exposure.

Formulated or technical grade chemicals that report other chemicals as impurities are not considered mixtures.

Species exposed to more than one chemical during an exposure period are not necessarily considered mixtures. In some cases, the first chemical applied is used to treat a deficiency, disease, parasite, etc. to ensure more healthy test organisms or may be used to induce a biological response prior to a chemical exposure. When such a

treatment is described by the authors, encode the beneficial or inducing treatment as an Experimental Design comment and encode the toxic effects of the other chemical(s) for ECOTOX.

For example, the authors state that “Grasses in all experimental sites were treated in the seedling stage of growth with bromoxynil for the control of broad-leafed weeds. No other herbicides were applied before the treatments with dicamba or propham”. Encode “ BROMOXYNIL APPLIED, NO EFFECT REPORTED” in the Experimental Design comment area on the Test tab of the Toxicity module. Encode the dicamba and propham toxicity data for ECOTOX.

Another 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

Nutrient Copper	20 ug/l	40 ug/l
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%

B. Effect of copper and nutrient on plant growth

Nutrient Copper	0 ug/l	10 ug/l	20 ug/l	30 ug/l	40 ug/l
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 (CO₂) or ozone (O₃) 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(H₂PO₄)₂ 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 .

Purpose	CE Number	Name	Molecular Weight	Grade	Purity	Formulation	Radiolabel	Characteristics
Carrier	84175	Ethanol	46.069	NR	NR	NR	NR	NR

Figure 2. Toxicity Module Chemical Information Tab

Chemical Name (TEST) and CAS Number (CAS #)

The chemical name as it is reported in the publication is selected from the entries entered into the Skim module. Names will be populated to the Chemical Characteristics data display and ECOTOX Reviewers should confirm that the chemical entered at skimming is correct following the steps outlined below. If the name differs, the reviewer must return to the Skim screen and update skimming prior to proceeding with the review.

If several names (e.g., trade names, synonyms) are used, note the other names and formula in the Chemical Characteristics data field. In certain instances the author may modify the test chemical by neutralizing or by adding a salt. The chemical that is introduced into the organism is what should be coded as the Test Chemical. Example: Author reports purchasing hydrogen selenite CAS#7783008 which is neutralized with NaOH
Author reports administering sodium selenite CAS#10102188 to organism.
Code: Test Sodium selenite
CAS Number 10102188

To ensure the correct chemical has been assigned during the skim process complete the following:

If the authors report a product or catalog number, complete an internet search to confirm the chemical substance.

Check the Chemical module for the correct chemical name.

- If more than one CAS Number is retrieved, check the molecular formula
- If molecular formula is presented, match the molecular formula to a formula in EcoChem for the given name. [Note: Check the abstract, methods, footnotes, table footnotes, and the Acknowledgments section for this information]

- If molecular formula is not presented, do one of the following:
 - If a generic CAS number is available for the chemical, ensure that the generic compound has been skimmed to the paper
 - If a generic CAS number is not available, check to see if the metal has a common valence (see following table), e.g. copper chloride (the +2 valence is most common), assign the CAS Number for the most common valence for that chemical name.
 - For metal compounds that do not have a more common valence (see following table) and a generic CAS number is not available for the chemical name, assign the parent metal form, e.g. Arsenic, if arsenate is the chemical reported by the authors.
 - For metal or nonmetal chemicals that do not fit these rules, refer the chemical to the Chemical verification staff for further investigation.
 - If the chemical in the paper is reported as a Trade name, check to see if an alternate name is presented in the paper to ensure correct chemical assignment. For example, the trade name Horizon is associated with three chemical active ingredients in the literature; Fenoxaprop-p-ethyl, Clodinafop-propargyl and Tebuconazole. Select the Horizon associated with the correct active ingredient. If no active ingredient is reported, forward to the chemical verification staff for additional verification.
 - If a hydrated form of a chemical is used in the paper, record the hydrated form as reported by the author in the CHEMICAL CHARACTERISTICS field. Also include the molecular formula if reported. However the CAS Number for the non-hydrated form is used for reporting the chemical and is entered as such in the skimming module (see the example below). However, if the paper reports a formulated product that is a hydrated compound (e.g. Borax which has a molecular formula of $B_4Na_2O_7 \cdot 10H_2O$), the paper should be skimmed/coded to the CAS number for the hydrated form rather than to the non-hydrated form as there is no cue for review staff that the compound is hydrated. If the paper were to report Sodium borate decahydrate instead (a synonym for Borax) then it would be coded to the non-hydrated form following the guidelines for hydrates mentioned above.

Example:

Chemical cited in paper - Copper Sulfate ($CuSO_4 \cdot 5H_2O$)

Code:

Chemical Copper sulfate ($CuSO_4$)

Chemical Characteristics $CuSO_4 \cdot 5H_2O$

Elements and Common Valences

(from Periodic Table of the Elements, Sargent-Welch, 1980 cat. # S-18806)

Element	Symbol	Common Valence	Element	Symbol	Common Valence
Actinium	Ac	3	Molybdenum	Mo	6
Aluminum	Al	3	Neodymium	Nd	3
Americium	Am	3	Neptunium	Np	5
Antimony	Sb	3	Nickel	Ni	2
Arsenic	As	3	Niobium	Nb	5
Astatine	At	-1	Nitrogen	N	3
Barium	Ba	2	Osmium	Os	4
Berkelium	Bk	3	Oxygen	O	-2
Beryllium	Be	2	Palladium	Pd	2
Bismuth	Bi	3	Phosphorus	P	5
Boron	Be	3	Platinum	Pt	4
Bromine	Br	-1	Plutonium	Pu	4
Cadmium	Cd	2	Polonium	Po	4
Calcium	Ca	2	Potassium	K	1
Californium	Cf	3	Praseodymium	Pr	3,4
Carbon	C	4	Promethium	Pm	3
Cerium	Ce	3	Protactinium	Pa	5
Cesium	Cs	1	Radium	Ra	2
Chlorine	Cl	-1	Rhenium	Re	7
Chromium	Cr	3	Rhodium	Rh	3
Cobalt	Co	2	Rubidium	Rb	1
Copper	Cu	2	Ruthenium	Ru	3,4
Curium	Cm	3	Samarium	Sm	3
Dysprosium	Dy	3	Scandium	Sc	3
Erbium	Er	3	Selenium	Se	-4
Europium	Eu	3	Silicon	Si	4
Fluorine	F	-1	Silver	Ag	1
Francium	Fr	1	Sodium	Na	1
Gadolinium	Gd	3	Strontium	Sr	2
Gallium	Ga	3	Sulfur	S	6
Germanium	Ge	4	Tantalum	Ta	5
Gold	Au	3	Technetium	Tc	7
Hafnium	Hf	4	Tellurium	Te	-4
Holmium	Ho	3	Terbium	Tb	3
Hydrogen	H	1	Thallium	Tl	1
Indium	In	3	Thorium	Th	4
Iodine	I	-1	Thulium	Tm	3
Iridium	Ir	4	Tin	Sn	4
Iron	Fe	3	Titanium	Ti	4
Lanthanum	La	3	Tungsten	W	6
Lead	Pb	2	Uranium	U	6
Lithium	Li	1	Vanadium	V	5
Lutetium	Lu	3	Ytterbium	Yb	3
Magnesium	Mg	2	Yttrium	Y	3
Manganese	Mn	2	Zinc	Zn	2
Mercury	Hg	2	Zirconium	Zr	4

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. MIXTURE is still reported in the Other Effects comment filed. If it is not part of a mixture, no selection is made.

Chemical Grade (GRADE)

Enter relevant chemical grade information in the GRADE field. (refer to ECOTOX Code Appendix - Appendix B). If the author does not report the chemical grade information, enter Not Reported 'NR'.

Chemical 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. PU for purity would be entered into the FORMULATION data field (see CHEMICAL FORMULATION).

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).

Chemical Formulation (FORM)

Enter the chemical formulation code for the chemical reported. If there is more than one formulation code enter the code most closely related to the chemical purity, and enter the rest in the CHARACTERISTICS field. (refer to ECOTOX Code Appendix - Appendix C) If the author does not report chemical formulation information, enter 'NR'.

When authors report impurities in a formulated product (pesticide or organic compound), it will not be treated as a mixture, but rather as a discrete chemical.

The chemical formulation (FORM) is only coded when explicitly identified by the authors as the form (e.g., commercial) or when presented in a standard format (e.g., 25EC).

Chemical Comments (CHEMICAL COMMENT)

Enter relevant and specific chemical information, such as trade names, common names, isomers, or extra formulation codes, but do not code lot numbers or product numbers (usually taken from chemical catalogs).

For NANOparticle papers, report particle size (usually diameter), surface area and particle shape description (e.g. nano rods) in the CHEMICAL COMMENT field. If other information, e.g. Hydrodynamic size and Zeta potential is reported, "OTHER PARTICLE PARAMETERS REPORTED" is noted in the CHEMICAL COMMENT.

If a metal salt is tested but the author does not report which salt was used and only reports the metal name, code SALT TESTED in the CHEMICAL COMMENT.

For example: The only information reported by the author: “Copper, salt was used at a concentration of 10 mg/l.” Code SALT TESTED in the CHEMICAL COMMENT.

When coding the Chemical Grade, Chemical Purity, Chemical Formulation and Chemical Comment 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 Chemical Comment 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
- Atrazine 70WP, code Grade = NR, Purity = 70, Formulation = WP and Chem Char = NR
- Diuron 4EC, code Grade = NR, Purity = NR, Formulation = EC and Chem Char = 4EC
- Provado 1.6F containing 17.4% active ingredient, code Grade = NR, Purity = 17.4, Formulation = FF and Chem Char = 1.6F
- Dimethoate, 25.3% Active Ingredient, code Grade = NR, Purity = 25.3, Formulation = NR and Chem Char = NR

Radiolabel (RADIOLABEL)

If a radiolabeled chemical is tested, enter the isotope, according to the ECOTOX Code Appendix - Appendix D, in the RADIOLABEL field. When the specific isotope is not reported, the RADIOLABEL remark 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 unlabelled” in the CHEMICAL CHARACTERISTICS comment field.

If a test is conducted where a radiolabelled chemical is added to all experimental units and the non-radiolabelled 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.

Chemical Carriers

If a solvent carrier or Positive Control is used in the test, the Chemical Carrier fields are coded with the CHEMICAL CARRIERS, PURPOSE, GRADE, PURITY, FORM, RADIOLABEL and CHARACTERISTICS.

Occasionally two or more separate carriers or solvents are used. If the publication reports the ratio, include this information in the CHARACTERISTICS field for carriers or

solvents. If the carrier or solvent is used for different test chemicals but the use is not specifically described in the publication, code "as needed" in the CHARACTERISTICS field.

If the publication reports data for multiple chemicals and reports the use of multiple solvents but does not report which solvent is used for which chemical, code all solvents for all chemicals and remark "1 of X solvents used" in the CHARACTERISTICS field.

Example: 10 pesticides tested, acetone and methanol used as solvents but it is not stated which solvent is used for each chemical, code:

CHEMICAL CARRIERS: ACETONE CHARACTERISTICS: 1 of 2 solvents used

CHEMICAL CARRIERS: METHANOL CHARACTERISTICS: 1 of 2 solvents used

If an author reports the concentration of the solvent used during the preparation of the stock solution and reports the concentration of solvent that the organism received, the concentration that the organism received is reported in the solvent CHARACTERISTICS field. If the concentration reported for the solvent in the control differs from the concentration dosed to the organism, then the concentration that the organism received is reported in the solvent CHARACTERISTICS field.

Example: The solvent used is Acetone at < 0.1 %

CHARACTERISTICS: ACETONE, less than 0.1 percent

Buffers used to control the pH of the test are not coded. Acids or bases that are added to change the pH of a solution in order to enable a metal to stay in solution are not coded. Dietary feed content is not coded.

If test results are presented on the effect of the solvent alone on the test organism (normally referred to as the solvent control), the solvent concentration is reported and there is a separate clean water control, the solvent data is coded as a test. If the solvent is used for more than one chemical and the solvent control data points are not the same for each test, all solvent tests are coded separately. If the data appears to be the same points (the author used the same solvent control for all chemicals) only one test is coded.

If an author states that all solvent was evaporated prior to the study or if a column coating procedure is described, the solvent is assumed to not be incorporated into the study and should not be coded. Enter "'X' Solvent evaporated" in the test chemical CHARACTERISTICS field, e.g. "acetone solvent evaporated".

For instances where a chemical carrier does not have a CAS number and it is determined by the chemical verification staff that it will never have a CAS number, the solvent will be entered in the CHARACTERISTICS field of the Chemical Name (TEST).

Example: The solvent used is National Sticker at 0.05 ul/l. There is no CAS#

CHARACTERISTICS: CARRIER NATIONAL STICKER, 0.05 UL/L

If an author presents results in which the test organism is exposed to multiple chemicals, it is important to determine if the two chemicals constitute a mixture or if one of the chemicals is being used as a carrier or solvent. A carrier is defined as an agent (other than water) in which the test chemical is mixed to make it miscible with the

dilution water before distribution to test chambers (Rand, 1995). If the other chemical is not a carrier, "MIXTURE" would be noted in OTHER EFCT.

Water should not be coded as a solvent. A solvent is defined as an agent (other than water) in which the test chemical is mixed to make it miscible with dilution water before distribution to test chambers. Solvents or carriers are used in toxicity tests where the concentrations of the test chemical are extremely low and a very small amount of test material must be added to the test chambers. (Rand, 1995)

2. Test Organism Parameters (Species Information Tab)

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 can not 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 4th instar larvae were used in the study. The following is coded:

LIFESTAGE: LV AGE: 4 AGE UNIT: ins

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

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 (eg., 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 alterization 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 effect 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.

3. Test Information Parameters (Test Information Tab)

Figure 4. Toxicity Module Test Information Tab

Exposure and Study Durations

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

	DURATION OF EXPERIMENT																
	Pre-Trt		Exposure					Observation									
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

Default Weaning and Gestation duration for Laboratory Species		
Avian Species	57 Species	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 radiolabelled chemical is added to all experimental units and the non-radiolabelled 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).

Since Technical Grade chemicals are typically 85-90% active ingredient (www.ee.washington.edu/research/microtech/cam/PROCESSES/PDF%20FILES/ChemStorage.pdf) and ECOTOX codes Technical Grade as active ingredient (A), code concentration type active ingredient (A) for all chemicals with a purity of 85% or greater.

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 NH₄-N or NH₃-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 (ie., 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, eg., NH₃, NH₄⁺, NH₃-N, NH₄OH, or NH₄Cl. (US EPA 1979) The author must state whether the form is **Total** or **Unionized**; **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 (NH₄⁺), nitrite (NO₂⁻), 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, NH₃, is the unionized form. (In aqueous solution, ammonia assumes an equilibrium between NH₃ and NH₄⁺.) The NH₃ 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⁺, Al₂O₃⁺, etc, add **CHEMICAL SPECIATION** to the **OTHER EFFECTS COMMENTS**.

See ECOTOX Code Appendix – Appendix A for additional information.

Study Type (STUDY TYPE)

For laboratory exposures where the intent of the study is to mimic field conditions in the laboratory, the study type is used to identify field simulation technique. For example, indoor mesocosm or microcosm studies should be noted as such in the STUDY TYPE field. If information about the study type is not reported, enter 'NR' (not reported). For laboratory studies completed in an aquarium, beaker, etc., the EXPOSURE TYPE field is used to report the study procedure.

For field exposures record the study type as reported by the author in the STUDY TYPE field. If information about the study type is not reported, enter 'NR' (not reported). The valid codes for this field are:

- ARTIFICIAL
- CHANNEL
- ENCLOSURE
- LITTORAL
- MESOCOSM
- MICROCOSM

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)). See ECOTOX Code Appendix – Appendix HH for the codes and definitions.

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, eg. 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 inbetween 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 ‰ 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. If a determination cannot be made regarding the use of either freshwater or saltwater, an NR (not reported) is recorded.

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 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.
- Enter information about the study media e.g. CECIL SILT LOAM, FW, DECHLORINATED WELL WATER FROM TARRYTOWN, NEW YORK, ASTM HARD WATER, etc. Tests with differing study media are coded as separate lines of data; it is not acceptable to combine tests by effect or study media variables across differing study media test scenarios.
- 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 efcts

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 (eg., 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.

Substrate Comment (SUBSTRATE REMARK)

Report the scientific name of the natural soil or commercial name of the artificial soil used in the study. If the scientific name is not included report the type of soil using the author's terminology, e.g., forest soil, sandy loam soil, arboreal coniferous soil.

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-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.

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

Chemical Half-life (HALF-LIFE)

Record the specific chemical half-life in water as measured by the author. If the half-life is referenced from another paper, code this field as NR. If information about the half-life is not reported, record NR.

Example: HALF-LIFE: 2d

Application Rate (AP RATE)(Water Studies Only)

For Field tests:

This field contains the application rate value and the units that the author reports. If an application rate is not reported by the author, record as NR. If an exposure concentration is not reported by author, the application rate must be reported. (See ECOTOX Code Appendix – Appendix N for application units)

For Lab tests:

If an author reports an application rate, the information is coded in the following format: in the Dose Remark data field “mean(min-max) unit”

Water Depth (DEPTH) (Water 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)

5. Result Parameters (Result Information tab)

The screenshot displays the 'Result Information' tab in a software application. At the top, there is a table with the following columns: ID, Chemical, Method, Species, Age, LifeStage, Study Type, Exposure Type, Test Location, #Results, Create Date, and Modified Date. Below this table, there are several input fields and dropdown menus for various parameters: Sample Size, Endpoint, Trend, Effect, Response Sites, Observed Duration, Concentration, Significance, Intake Rate, Dry Wet, Steady State, and Lite Eval Qk. A 'Concentrations' table is also present with columns for Concentration Type, Unit, and Concentration. The bottom right corner shows 'Page Number' and 'Reviewer Number' fields.

Sample Number and Units (SAMPN)

The sample number reflects the sample size (e.g., 10 embryos) that the observation or result value is based on at each exposure level. For endpoints based on calculations (e.g. LD50, EC50 etc.) rather than individual dose measurements, the sample number will be coded as 'NR'. Code 'NR' if no information about the observed sample has been reported.

Sample units correspond to the sample number; i.e., the unit on which the measurement or endpoint is based (see Ap ECOTOX Code Appendix – Appendix Q for applicable codes). Code 'NR' if the sample unit is not reported.

EXAMPLE: For a sample size of 190 eggs, the sample unit is eggs (EG); therefore, if the effect measurement is HTCH, and the observation response value is 90%, then 90% of 190 eggs hatched.

Note: For generational studies and measurements based on the progeny, note F1, F2, etc. in the sample units field.

Note: If a sample number is not provided, but a "unit" is, always enter the unit in the sample units field.

Note: A FieldN test scenario involves exposing plots or sample areas, in addition to specific test organisms. Usually the number of exposed organisms is unknown. The number of plots or sample areas is coded as '#/EU' (the number of experimental units)

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, ie., 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'.

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), ie. 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.

If a paper is being coded for the EFED project, it 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

“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). However if there are multiple durations reported for the same endpoint or multiple values for the same endpoint are reported, the most sensitive value is coded.

For further details on coding data for EFED projects see EFED Coding guidelines SOP.doc.

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, which ever 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	

Conc (ug/l)	Length (cm)	Weight (mg)	Mortality (%)	* Sig at p<0.05
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

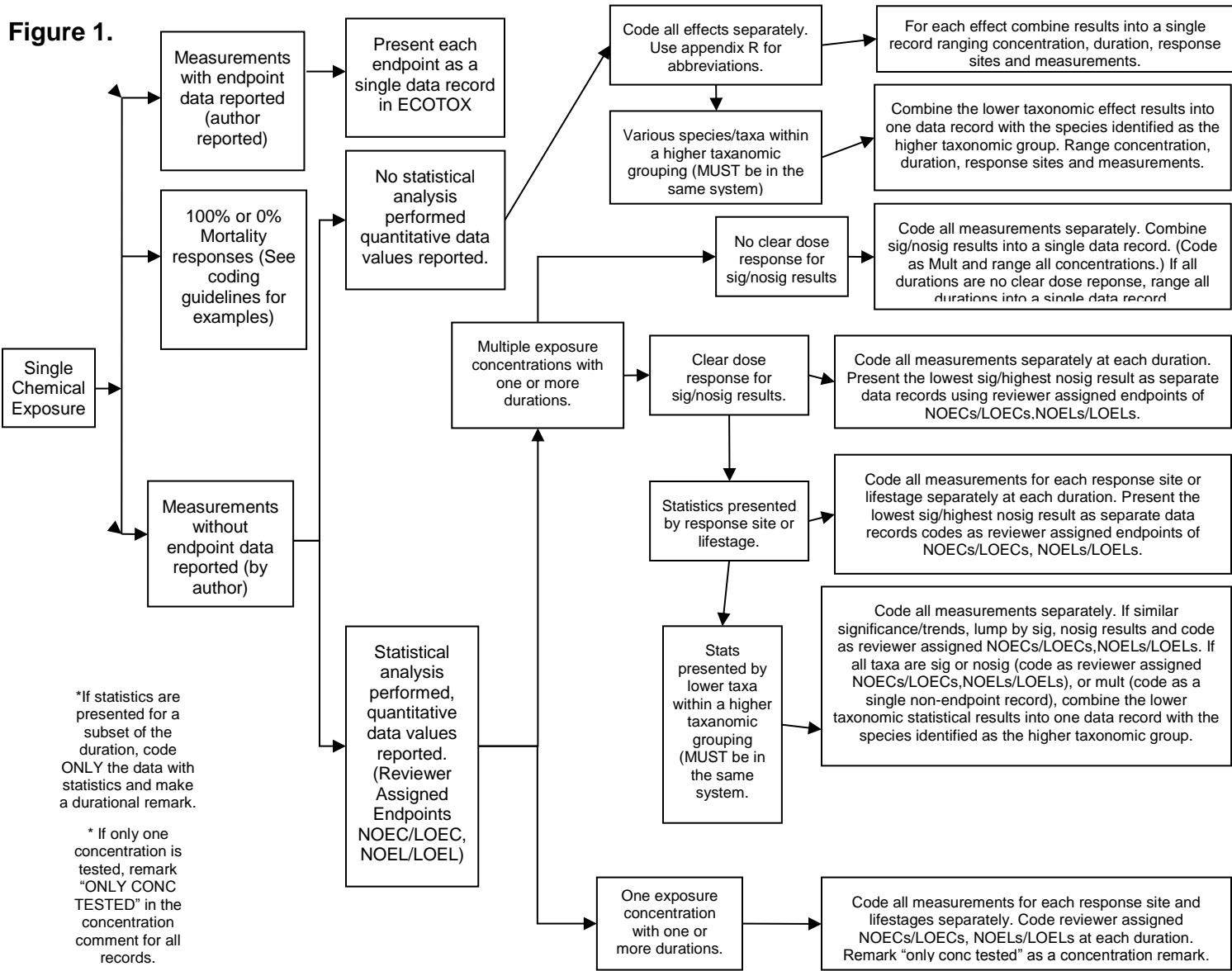
On occasion, authors will report LC50 information in the methods section of their publication, without reporting any accompanying test procedure information. These test results are coded if enough information is provided by the authors to verify that the value(s) were not published elsewhere, and that 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).

Figure 1.



Coding Hierarchy

*If statistics are presented for a subset of the duration, code ONLY the data with statistics and make a durational remark.

* If only one concentration is tested, remark "ONLY CONC TESTED" in the concentration comment for all records.

Coding Hierarchy

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	0 NR-ZERO
2	5	17	30	35
3	25	40	65	90
4	100 NR-LETH	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	0 NR-ZERO	11
2	20	25	38	72
3	45	60	67	90
4	90	100 NR-LETH	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	0 START RANGE	0	7	13
2	0	28	45	60
3	38	44	67	100
4	38	60	100	100 END RANGE

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	0 START RANGE	0	0	0
2	0	0	7	13
3	0	28	45	60
4	38	44	67	100
5	38	60	100	100
6	100	100	100	100 END RANGE

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 on broad groups of effects without specifying each individual effect. See Scientific Outreach Support for additional user support information.

The reported effect is interpreted to conform to the ECOTOX defined effects. If the effect is on the list of ECOTOX effects, use the ECOTOX effect code (see ECOTOX Code Appendix – Appendix S). If the author's effect is not in ECOTOX Code Appendix – Appendix S, but is similar to one already defined use the ECOTOX code which matches the definition and note the author's effect term in the MEASUREMENT REMARK field. If the author's effect appears to be a new effect code, discuss and forward to EPA Data Manager for approval.

Listed at the end of ECOTOX Code Appendix – Appendix R there are two special effect code conventions used in ECOTOX. The first is NOC (No effect code) used only for ENDPOINTS reported by the author as ns, e.g. “mortality and growth” and no specific effect code can be assigned. This code is used *only* when such effects cannot be separated into or reported as individual effects. The NOC code is rarely used and when used must be verified by one or more fully trained reviewers.

The second effect code convention is ~XXX to indicate that the result reported was observed after the exposure period ended and the organisms are observed in clean water, i.e., a delayed response. Within a publication, delayed response data is reported only if exposure period observations are not available for the same effect or endpoint. When delayed response data accompanies exposure period observations, the delayed response data is not coded but is recorded in OTHER EFCT as “recovery”.

NOTE: 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.

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.

Occasionally, effects describing a parasite-host relationship are coded in ECOTOX. For example, the effect on the host is typically coded as an IMM effect with the measurement code PRNF. The effect on the parasite is typically coded as a POP effect with the measurement code ABND.

Effect Hierarchy

- A. If the author has defined an Endpoint for an effect, report the Endpoint as outlined in the preceding ENDPOINT HIERARCHY.
- B. When only effects are reported in the publication, no endpoints, code the concurrent effects (results reported concurrent with exposure to chemical) according to the abbreviations in ECOTOX Code Appendix – Appendix S. Code NR (not reported) in the ENDPOINT field.
 - i. If statistics are presented in a clear dose response, code the lowest significant effect as a LOEC and the highest nosig levels as a NOEC and appropriate p-values at all Durations, Measurements and Response Sites. Data must be monotonical at all durations to encode NOECs and LOECs.
 - ii. If statistics are presented and there is no clear dose response, code the ENDPOINT as NR, the significance as MULT and the appropriate value

for each Measurement and Response Site. The Durations and Concentrations are ranged.

- iii. If no statistics are used, or reported, combine the data by effect by coding a range for concentration and durations. all Measurements are encoded into the Measurement data field. Report as NR in the SIG and LEVEL fields. 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

- C. When the only effects that are reported are those subsequent to exposure, report these as delayed effects, noted with a ~ preceding the three-letter effect code, e.g. ~MOR. Follow the procedures outlined in Steps B i, ii, iii for reporting delayed effects.

Response Site (RESPONSE SITE)

A response site code is used to identify specific organ and tissue effect sites for residue, biochemical, cellular and/or physiological effect measurements. Response sites are used for the effect codes ACC, BCM, ENZ, HRM, CEL, GEN, HIS, INJ, IMM, PHY, GRO, and MPH effects and associated endpoints. The response site is coded as NR for the effect codes MOR, AVO, BEH, FDB, DVP, ITX, POP, REP and PRS. The two or three letter response site codes are listed in ECOTOX Code Appendix – Appendix U. It is acceptable to code a response site when tissues or organisms are pooled together for a measurement.

If a response site is not reported or not applicable, e.g. mortality, behavioral effects, code the site as Not Reported (NR).

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 the individual tissues/organs are coded in the Response Site data field. If the response site does not have a response site code request a 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, ie., 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 CHARAC: 3 orders EFFECT: POP MEASURE: ABND
 TREND: CHG SIG: NR

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, ie. <, >, 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	EFCT: MOR	MEASURE: MORT
	TREND: INC	Effect Percent: 30-75	
Example 2:	20-30% dec O2 consumption	EFCT : PHY	MEASURE: OXYG
	TREND: DEC	Effect Percent: 20-30	

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 (eg., 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 when available. 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⁺). 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 10⁸; often reported as 10⁸, 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 (C₁₂H₂₇ClSn)): 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.

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.

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, ie. 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 radiolabelled chemical is added to all experimental units and the non-radiolabelled 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.

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

mRNA Name	P VALUE	ENDPOINT ASSIGNED	ENDPOINT	ENDPOINT REMARK
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.

Conc	Control	1 ug/L	2 ug/L	3 ug/L	4 ug/L	5ug/L
Length	20 cm	35cm	36cm	21cm	15cm	14cm

	sig	sig	nosig	sig	sig P<0.05
Code: EFCT: GRO	MEASURE: LGTH	SIGNIF: MULT	LEVEL: p<0.05		
CONC: 1-5 ug/L	ENDPOINT: NR				

Example 2: 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); code the LOEC/NOEC reported by the authors, note TREND as 'CHG', and code SIG as 'MULT' to flag non-standard results.

Mortality	Control	10 ug/L	20 ug/L	30 ug/L	40 ug/L	50 ug/L
@ 24 hours		nosig	nosig	sig	nosig	sig

Author endpoints	NOEC	LOEC
------------------	------	------

Code:	ENDPT: NOEC	EFCT: MOR	MEASURE: MORT
	CONC: 20 ug/L	SIGNIF: MULT	TREND: CHG

	ENDPT: LOEC	EFCT: MOR	MEASURE: MORT
	CONC: 30 ug/L	SIGNIF: MULT	TREND: CHG

Note: The MULT code is not used to represent a combination of data which has been statistically analyzed with data that have not been analyzed. Code the statistical data over the non-statistically analyzed data. For example, in an instance where the exposure duration is 5 days, and the statistical results presented are for 4 days; code the statistical results, the observed duration as 4 days and enter the exposure duration as 5 days.

Significance Level (LEVEL)

The level of significance (e.g. test statistic) is coded when the author has reported statistical analysis in the test result. The terminology for significance level may be presented as: p =; p- or alpha value (A or α) ; χ^2 ; for t-test; % level. The terminology are equivalent and are generally in the range of p = 0.10 to p = 0.001.

The LEVEL 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. However, when the confidence level is other than 95%, the level is coded as reported.

If an author reports two levels of statistical significance (e.g. 0.05 and 0.01, 5% and 1%) for the same data point and the significance is different at the levels, the level at which the data is first statistically significant is coded. For example:

Premature Molting for 15 days is analyzed at 5% and 1%

Concentration (ug/L)	% premature molting	5% , 1% *	Code
control	0	d , C	LOEC at 5% significance at 1 ug/L
1	15.38	c, C	
30	66.67	b, B	
60	92.59	a, AB	
100	90.00	a, AB	
200	96.67	a, A	

* Same characters in a column indicate no significant difference

If an author reports two levels of statistical significance (e.g. 0.05 and 0.01, 5% and 1%) for the same data point and the significance is the same at both levels, the most sensitive level (e.g. 0.01) at which the data is first statistically significant is coded. For example:

Mortality for 9 days is analyzed at p=0.05 and p=0.01

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 **M**easured and **U**nmeasured (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 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 **U**nmeasured. When chemical measurements are conducted periodically throughout the exposure but the reported measurements are not correlated with the effects, code as **U**nmeasured. When chemical measurements are conducted periodically throughout the exposure and the effects are coordinated with the measurements, code as **M**easured.

Even if measured values are reported by the author to have deteriorated by the end of the exposure, the **Measured** 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

Species Weight Final (WTAT)

The body weight of the organism at the time of the observed effect response is reported. If multiple body weights are given throughout the test, report the weight at the time the response is reported. If a range of weight is given for the organisms, report the range of weights. Report the treatment group weight, if weights are given for both a treatment group and a control group.

Intake Rate (INTAKE)

Report the intake rate of the exposure media (not the contaminant) at the time of the result (or closest rate to that time) for diet studies (through food or drinking water) as reported by the author.

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: $\alpha < 0.05$

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: $\alpha < 0.05$
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	EFCT%: NR	
EFFECT: HIS	SIG: NR	LEVEL: NR

Percent Lipid (LD)

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

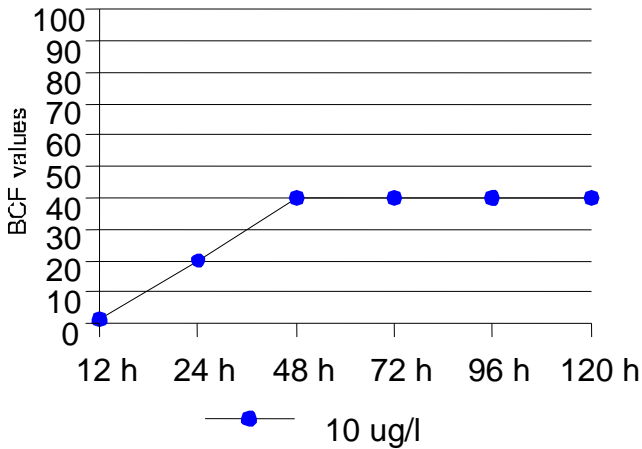
Dry or Wet Weight and Percent (DW)

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. If percent moisture is reported, record the percentage value. If the author does not report this information code as 'NR'.

Steady State (STST)

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:

BCF values



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

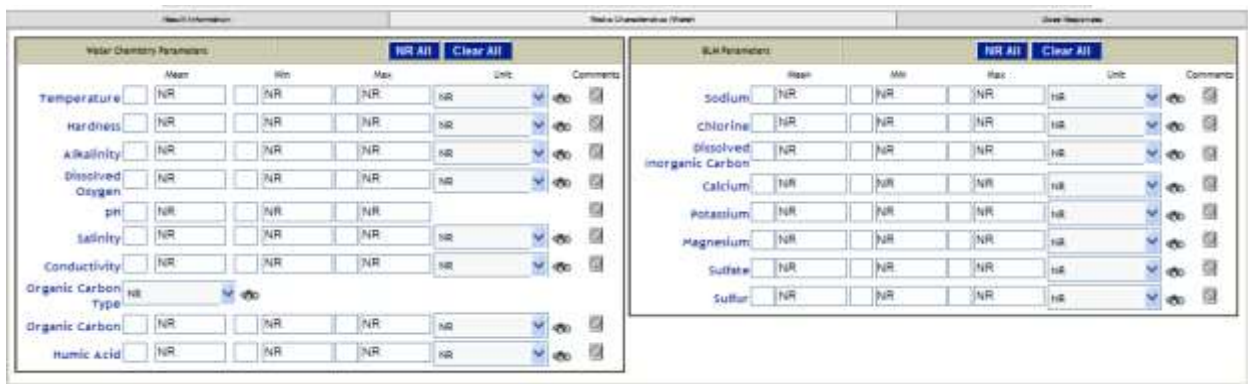


Figure 7. Media Characteristic tab

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 ₃	<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 ₃	<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

Field Name	Standard Unit	Abbreviation	Comment
Sulfate	mg/l	<u>SO4</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	<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".
Soil Texture Sand	%	<u>SA</u>	Report the sand texture of the soil as stated by the authors
Soil Texture Silt	%	<u>SI</u>	Report the silt texture of the soil as stated by the authors
Soil Texture Clay	%	<u>CL</u>	Report the clay texture of the soil as stated by the authors Clay may be reported as bentonite, kaolinite or montmorillonite.
CEC	uS/cm	<u>CEC</u>	Report cation exchange capacity and units (Refer to ECOTOX Code Appendix – Appendix FF for cation exchange capacity units) of the test media.
pH	--	<u>pH</u>	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 pHCaCl2), 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: http://ncsslabdatamart.sc.egov.usda.gov/querypage.aspx was be 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	mg / L	<u>OM</u>	If the authors report that a standard (see Attachment G

Field Name	Standard Unit	Abbreviation	Comment
Matter			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 http://ncsslabsdatamart.sc.egov.usda.gov/querypage.aspx 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	%	<u>MOIST</u>	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).

Dose Response data is not being coded at this time (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	0 mg/kg (HC) C	100 mg/kg (HC)	1000 mg/kg (HC)	10000 mg/kg (HC)	100000 mg/kg (HC)	Comment
03481	AD	Surv	NR	14 d	0 mg	0 mg	0 mg	0 mg	0 mg	
03482	AD	Surv	NR	14 d	0 mg	0 mg	0 mg	0 mg	0 mg	

ID	Dose (Conc. Type)	Dose Response	Stat. Method	Stat. Method Value	Sample Size	Significance	Significance Level
73465	0 mg/kg (HC) C	NR	SE	0.0	Mean	NR	NR
73466	100 mg/kg (HC)	NR	SE	0.0	Mean	NR	NR
73487	900 mg/kg (HC)	NR	SE	0.0	Mean	NR	NR
73488	1000 mg/kg (HC)	NR	SE	0.0	Mean	NR	NR

Figure 8. Dose Response tab

8. Companion Endpoint(s) tab
(only evoked 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	MA	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	NR	INC	LC20	MOR	MORT	NR	NA	21 d	F 234 (147 - 301) mg/kg d soil	135	5	04/22/2013	
2087263	NR	INC	LC50	MOR	MORT	NR	NA	21 d	F 362 (275 - 438) mg/kg d soil	135	6	04/22/2013	
2087264	NR	INC	LOEC (NOEC)	MOR	MORT	NR	SIG	21 d	F 389 mg/kg d soil	135	7	04/22/2013	
2087265	NR	INC	NOEC (LOEC)	MOR	MORT	NR	NOSIG	21 d	F 195 mg/kg d soil	135	8	04/22/2013	
2087266	NR	DEC	EC20	REP	FCND	NR	NA	42 d	F 88 (38 - 115) mg/kg d soil	135	9	04/22/2013	
2087267	NR	DEC	EC50	REP	FCND	NR	NA	42 d	F 133 (95 - 173) mg/kg d soil	135	10	04/22/2013	
2087268	NR	DEC	LOEC (NOEC)	REP	FCND	NR	SIG	42 d	F 194 mg/kg d soil	135	11	04/22/2013	
2087269	NR	DEC	NOEC (LOEC)	REP	FCND	NR	NOSIG	42 d	F 97 mg/kg d soil	135	12	04/22/2013	

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 the 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 review navigates to the References module, marks “do not have” and complete the skimming. 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.

9. 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.

10. References

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Attachment 1. ECOTOX Replicate Review Form

<input type="checkbox"/>	Replicate
<input type="checkbox"/>	Review
<input type="checkbox"/>	Complete

ECOTOX 10% REPLICATE REVIEW

Reference Number: _____

Section I

Coordinator Comments (Data abstraction discrepancies outlined):

Initials: _____ Date: _____

Section II

Second Data Abstractor Comments (Response to discrepancies):

Initials: _____ Date: _____

Section III

First Data Abstractor Comments (Response/Incorporation of modifications):

Initials: _____ Date: _____

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Section IV (If non-applicable, go to Section VI)

Coordinator Comments (Remaining data abstraction discrepancies outlined):

Further action required, see below:

Initials: _____ Date: _____

Section V (If Section IV is used, complete Section V)

First Data Abstractor Comments (Response/Incorporated modifications outlined):

Initials: _____ Date: _____

Section VI

Replicate review complete; no further action required.