6 Estimating Aquatic Toxicity Using ECOSAR

ECOSAR (Ecological Structure Activity Relationships) predicts the potential toxicity of industrial chemicals to organisms living in the water body to which the chemicals are discharged. The aquatic organisms are surrogates for the aquatic food web. This chapter provides a brief overview of ECOSAR. The reader is encouraged to review the extensive information that is available in the ECOSAR Help within the model itself. The latest version of ECOSAR can be downloaded at no cost from EPA, OPPT New Chemicals Program web site at http://www.epa.gov/oppt/newchems/tools/21ecosar.htm.

6.1 How Does ECOSAR Predict Aquatic Toxicity?

The model uses measured data to predict toxicity of chemicals lacking data by using Structure Activity Relationships (SARs) and Quantitative Structure Activity Relationships (QSARs) that estimate a chemical's acute (short-term) toxicity and, when data are available, chronic (long-term or delayed) toxicity. ECOSAR contains a library of chemical class-based QSARs for predicting aquatic toxicity along with an expert decision tree for selecting the appropriate chemical class.

QSARs include acute and chronic toxicity endpoints for (1) fish, (2) aquatic invertebrates (*Daphnia*), and (3) aquatic plants (green algae). These organisms are surrogate species representing the aquatic food web. Endpoints for acute and chronic toxicity to these three organisms form the standard EPA New Chemicals Program ecotoxicity profile. Limited QSARs have been developed for salt water and terrestrial species like earthworms when those data have been available, but the major focus of ECOSAR is freshwater species.

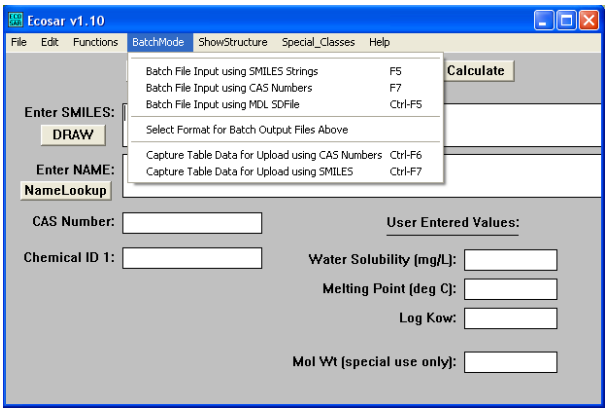
When data become available QSARs are validated / updated and additional QSARs can be developed. Some of the data used to develop QSARs is Confidential Business Information that was submitted as new chemical notices under TSCA section 5 (discussed in Chapter 1 of this document). The data itself is

CBI but can be used to develop QSARs that can be made publicly available through methods like ECOSAR.

6.1.1 Understanding ECOSAR Classes

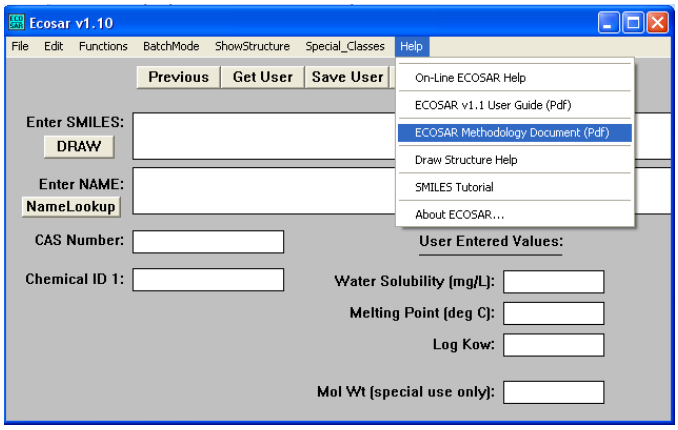
– Chemicals That Can Be Evaluated With ECOSAR

ECOSAR was designed and developed to evaluate organic chemicals with discrete structures. Inorganic or organometallic chemicals should not be profiled. Polymers and chemicals with a molecular weight greater than 1,000 should not be profiled using ECOSAR because these large chemicals were not included in the training set of chemicals. However, many polymers may be made up of dimers, trimers, and oligomers that have a molecular weight of less than 1,000. These smaller molecules *can* be evaluated with ECOSAR. If the chemical to be profiled is a mixture and there are discrete structures are available for each component in the mixture the components can be run through the model separately. ECOSAR does have a "batch" mode (left) to evaluate multiple chemicals with discrete structures. ECOSAR does not account for the unique physical properties of nanomaterials which may have novel mechanisms of toxicity.



As explained in detail in the ECOSAR Technical Reference Manual (available in the Help menu of ECOSAR, shown in the image below), ECOSAR predicts toxicity values for three general types of chemicals:

1. Neutral Organics – Neutral organic chemicals are nonionizable and nonreactive and act by inducing narcosis in a manner similar to that of general anesthesia. This general narcosis is referred to as *baseline toxicity*. The classes of chemicals that are known to present general narcosis include: alcohols, aliphatic hydrocarbons, alkyl halides, aromatic hydrocarbons, aryl halides, cyanates, disulfides, ethers, ketones, and sulfides.



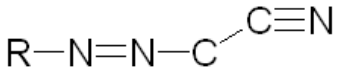
2. Organic Chemicals with Excess Toxicity – Some types of organic chemicals have a more specific mode of toxicity beyond the baseline toxicity due to the presence of reactive functional groups. Chemical classes which exhibit excess toxicity include: acrylates, aldehydes, anilines, aziridines, benzotriazoles, epoxides, esters, methacrylates, and phenols. Separate QSARs have been developed for several chemical classes with excess toxicity to at least one or more organisms. Some organisms are more sensitive to certain classes of compounds than others such as the herbicide-like chemicals that present significant toxicity only to green algae. As a result the designation of "excess toxicity" may not pertain to all aquatic organisms. A full list of the current classes of chemicals with excess toxicity programmed within ECOSAR is in Appendix 1 of the 2011 ECOSAR Technical Reference Manual (the "Methodology Document"), available in the Help menu (shown in the image above).

3. Surfactant (Surface-Active) Organic Chemicals - A surfactant has a hydrophobic component and a hydrophilic component and works by greatly reducing the surface tension of water. Surfactants do not dissolve in water but they form micelles which are dispersed aggregates of the surfactant with the hydrophobic component on the inside and the hydrophilic component on the outside. Many different types of chemicals have surfactant properties and there is no sharp distinction between those that do and those that don't. Generally, a compound with a polar functional group (the hydrophilic component) such as carboxylate or sulfonate, with a long (> 10 carbon) non-polar chain (the hydrophobic component) can be considered a surfactant. Surfactants are used in detergents, wetting agents, and emulsifiers. Within ECOSAR, the surfactants are grouped by total charge. These four general divisions are anionic (net negative charge), cationic (net positive charge), nonionic (neutral), and amphoteric (has both negative and positive charges) surfactants. The QSARs for surfactants can be linear or parabolic and the toxicity is often related to the size (number of carbons) of the hydrophobic component or the number of repeating hydrophilic components (i.e., ethoxylates).

– ECOSAR Classes

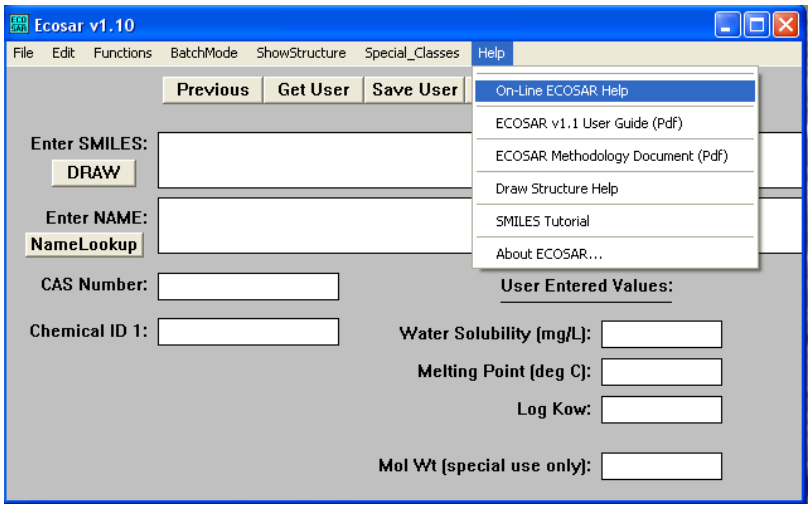
ECOSAR utilizes a combination of biology (mechanism) and chemistry (structural similarity principles) to organize the universe of chemicals into classes and estimate toxicity. Classes in ECOSAR are identified initially by associating data trends with molecular features. When a group of chemicals with similar toxicity profiles and structural features (fragments) can be identified, a class can be described. Currently there are more than 120 classes in ECOSAR.

An example of a class is Azonitriles which are identified by the base fragment shown on the right. The R group must be an alkyl, olefinic, acetylenic or aromatic carbon.



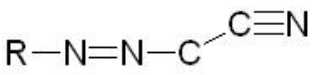
Azonitriles structure must meet the following criteria:

1. A nitrile group (carbon triple-bonded to nitrogen; SMILES of C#N) is attached to an alkyl carbon which also has an azo attachment (-N=N-).
2. The alkyl carbon between the azo and nitrile groups can have other attachments (no current exclusions).



– Documentation on ECOSAR Classes

ECOSAR is currently programmed to identify over 120 chemical classes and has more than 600 QSARs for numerous endpoints and organisms. The ECOSAR Help Menu (right) provides access to the *Class Definition Documents* (in the On-Line ECOSAR Help). These documents outline the structural definitions for each ECOSAR class, including "exceptions" to the rules. These "fragment definitions" comprise the expert decision tree component of ECOSAR used to identify the appropriate chemical class or classes for a particular molecule.



QSAR Equation Documents (also referred to as Technical Reference Sheets) are also provided for each class to help users understand how to properly use the model and interpret the results. Each QSAR Equation Document contains the following important information listed below.

- Data tables for training set of chemicals for that particular QSAR,
- QSAR equations,
- Number of chemicals in each training set, and
- Limitations of each QSAR.

For more detailed information see the On-Line ECOSAR Help, Content, click on "QSAR Class Reference Documents".

6.1.2 Developing QSAR Equations Using Measured Data

The QSARs in ECOSAR are based on linear mathematical relationships between the predicted log Kow and the corresponding log of the measured toxicity (mmol/L) for the specific training set of chemicals, which vary for each QSAR. The toxicity studies from which data are extracted are listed in the QSAR Equation Documents, expect when the data used were considered confidential business information (CBI). Data undergo an extensive validation before being included in the model. Measured data must meet certain criteria to be considered valid, including:

- toxicity is measured at pH 7 (neutral),
- Total Organic Carbon (TOC) < 2 mg/L,
- moderate water hardness (150 mg/L CaCO3), and
- active ingredient adjusted to, or measured at 100%.

Measured data on standard test species (as identified in OPPTS guidelines for aquatic toxicity testing at http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts) are preferred for developing the training sets. These species (fish, aquatic invertebrates, and aquatic plants) which comprise several genera, as well as families of organisms, are intended to represent general trophic levels of aquatic food webs. After collecting and validating the training set data for each QSAR, regression techniques are applied to the data set to derive mathematical relationships correlating log Kow and toxicity. The resulting equation from this regression analysis is provided in the QSAR Equation Documents along with a graphical representation of the data set.

Most resulting regression equations are linear and relate predicted log Kow to measured value, for example here is the equation for the Fish 96hr LC50 QSAR for acrylates:

log LC50 (mM/L) = -1.46 - 0.18 log Kow

The number of chemicals in each specific training set does vary. For example, the neutral organic 96-hour fish LC50 QSAR was based on data for > 300 chemicals, but the haloketone fish 96-hour LC50 QSAR was based on only 5 data points. The difference is due to the lack of aquatic toxicity data and knowledge base for many of the classes with excess toxicity.

6.1.3 Use of Acute-to-Chronic Ratios (ACRs)

Acute-to-Chronic Ratios (ACRs) are used to derive an endpoint value when that endpoint is missing because there are no measured data or suitable analog with which to derive a QSAR equation. ACRs can be applied directly to a measured value to determine the corresponding acute or chronic value. ACRs can also be used to derive QSAR equations within a chemical class when the corresponding empirically derived QSAR equation and ACR for that class is available.

Generally accepted Acute-to-Chronic Ratios are shown in the table below. The ACR method is used in U.S. EPA New Chemicals Program based on comparison of EC50/ChV for neutral organics and other classes (ChV equals geometric mean of NOEC and LOEC).

Class	Fish	Daphnid	Green Algae
Neutral Organics	10	10	4
Classes with Excess Toxicity	10	10	4
Polycationic Surfactants	18	14	4
Nonionic Surfactants	5	5	4
Anionic Surfactants	6.5	6.5	4

6.2 Evaluation and Validation Studies of ECOSAR

Any QSAR model should provide information on the performance of the model so that users can determine if the model predictions are reliable for their chemical of interest. Performance information should include the training set chemicals, data and data quality, methods for selecting variables, and what statistical methods were used to develop the QSAR. As described in section 6.A.1 of this document, ECOSAR provides users with a QSAR Equation Document for each QSAR. Included in the QSAR Equation Document are internal performance measures such as coefficient of determination (r2) and all descriptor values. Please remember that it is not possible for EPA to assemble and release *all* of the information regarding internal performance of ECOSAR because some of the data are Confidential Business Information (CBI) submitted under TSCA. When CBI data were used in the development of a QSAR, this is noted in the QSAR Equation Document (also referred to as the Technical Reference Sheet). Chemical identity (name, structure, CAS Registry Number) of these chemicals is masked.

6.2.1 External Evaluations of ECOSAR

Objective external evaluations of the predictive capabilities of a QSAR model are always desirable. Evaluation studies most often use chemicals not included in the development of the model (the training set) and compare the experimental data with the estimated values for the chemicals.

However it is important to understand the specific context surrounding the development and use of any model. Regulatory agencies often use preliminary classification criteria (ranges of values indicating high-moderate-low concerns) to make decisions on the potential toxicity of chemicals and may not actually require the use of the experimental data or estimated values themselves. EPA uses screening-level models like ECOSAR to prioritize chemicals in order to identify chemicals and situations that present lower toxicity and risk so these can be dropped from further review, allowing EPA to focus on the more problematic chemicals.

This regulatory context of ECOSAR should be considered when evaluating predictive accuracy. For example, in the regulatory context a “moderate” concern level for acute toxicity ranges from 1 to 100 mg/L. As a result a prediction of 5 mg/L and one of 50 mg/L would be considered “equivalent” because both values would lead the assessor to the same conclusion – that the chemical will likely be a moderate concern for acute toxicity. This differs from a more traditional statistical approach in which 5 mg/L does not equal 50 mg/L.

Evaluation and validation studies conducted on ECOSAR are listed below and the full citations are at the end of this chapter.

6.2.2 External Reviews of ECOSAR

External Peer Review
An independent peer review of ECOSAR was conducted as part of the development of the Organization for Economic Cooperation and Development's (OECD) guidance, The Principles for Establishing the Status of Development and Validation of (Quantitative) Structure-Activity Relationships [(Q)SARs] (OECD, 2004a).

Participation in US-European Union Validation Exercise
EPA and the European Union conducted a large-scale verification study of ECOSAR to compare SAR predictions with the results of data from testing. That study (OECD 1994; U.S.EPA 1994) found ECOSAR to be accurate 60-90% of the time depending on the endpoint assessed.

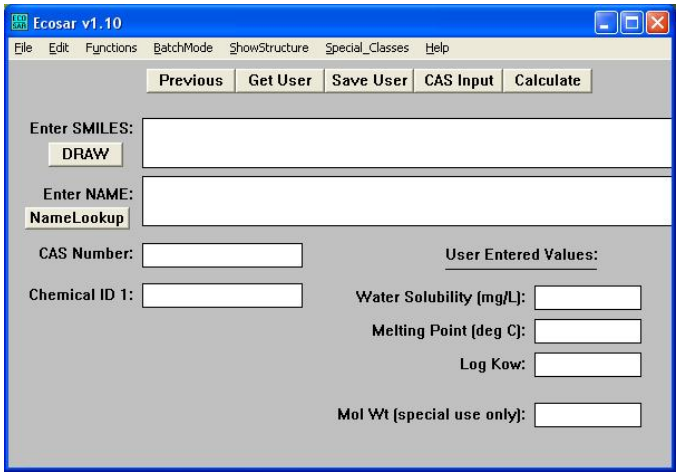
International Collaboration in Development of Effective Predictive Tools
ECOSAR was included in OECD's Report on the Regulatory Uses and Applications in OECD Member Countries of (Q)SAR Models in the Assessment of New and Existing Chemicals (OECD, 2006). Subsequently, the OECD asked EPA to include ECOSAR into the OECD QSAR Application Toolbox, which was developed starting in 2006. Inclusion in the OECD toolbox requires specific documentation, validation and acceptability criteria and subjects ECOSAR to international use, review, providing a means for receiving additional and on-going input for improvements. In an evaluation of a number of predictive tools used to profile chemicals and group them together based on similar toxicity, ECOSAR was the top performer (available at http://www.oecd.org/document/23/0,3343,en_2649_34379_33957015_1_1_1_1,00.html).

6.2.3 Peer-Reviewed Publications on ECOSAR (Listed at End of Chapter)

There have been numerous publications on the predictive accuracy of ECOSAR. Many of these Book Chapters / Reports, peer-reviewed Scientific Journal Articles, and Abstracts are listed at the end of this chapter.

6.3 Running ECOSAR

There are several important considerations to understand before running ECOSAR and these considerations are summarized in this section. Greater detail is provided in the Help menu of the model itself.



6.3.1 Model Inputs and Outputs

Model Inputs
The ECOSAR entry screen is shown on the right. The ECOSAR help menu "ECOSAR User Interface" section has detailed information on each item on the entry screen, and if you put your cursor in a data field and hit the F1 key a brief description of that field pops up.

A chemical can be entered into ECOSAR in several ways:

- Simplified Molecular Input Line Entry System (SMILES) notation (information on writing SMILES is in Appendix F of this document and in the SMILES Notation section of ECOSAR Help)
- CAS Registry Number or name if available in the look-up data bases
- Drawing program within ECOSAR
- A .mol file for single chemical entry, or .sdf file or string files to perform batch runs

If measured data are available for Water Solubility, Melting Point, and Log Kow, these values should be entered. Here are some important notes on these properties:

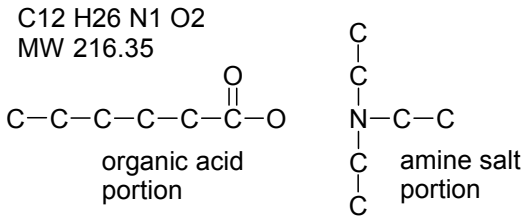
- **Log Kow**: If no log octanol-water partition coefficient value is entered by the user WSKOW will estimate the log Kow using KOWWIN.
- **WS**: If no Water Solubility is entered any experimental water solubility value from the built-in experimental database will be retrieved or it will be calculated from the log Kow value using WSKOW. The User Entered value is used in preference to an experimental database value which is used in preference to the estimated value.
- **MP**: Melting Point is used to calculate Water Solubility when a measured Water Solubility is not available. Entering Melting Point (in deg C) is optional however a measured value will generally result in a more accurate water solubility estimate.

6.3.2 Using the Mol Wt [Special Use Only] Function in ECOSAR

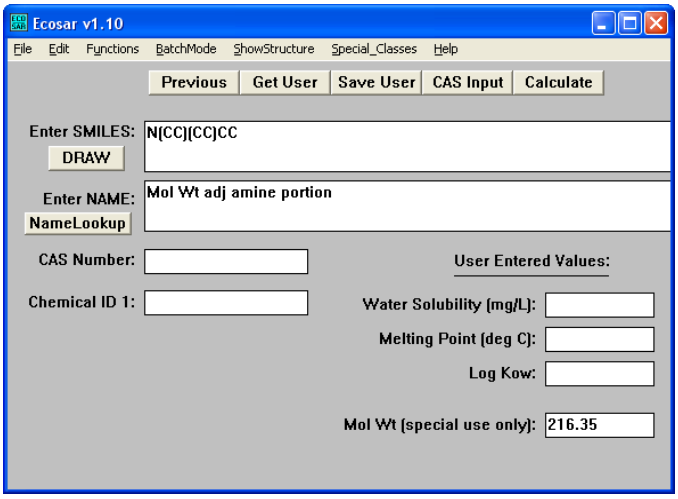
ECOSAR has a special use function that allows the user to do a molecular weight adjustment in order to evaluate the toxicity of a chemical whose toxicity is due to the presence of a specific moiety. Two examples below help explain how the **Molecular Weight (special use only)** function is used. The **Molecular Weight (special use only)** function is located in the lower right portion of ECOSAR data entry screen.

ECOSAR calculates endpoints initially in units of mmol / L (micromole per liter). In certain cases, usually with a complex salt, the toxicity is the result of a portion of the molecule and this moiety may have a different calculated MW than the molecule as a whole. Evaluating the entire structure will result in a less accurate prediction of toxicity. Using the **Molecular Weight (special use only)** function can aid in refining the toxicity estimate.

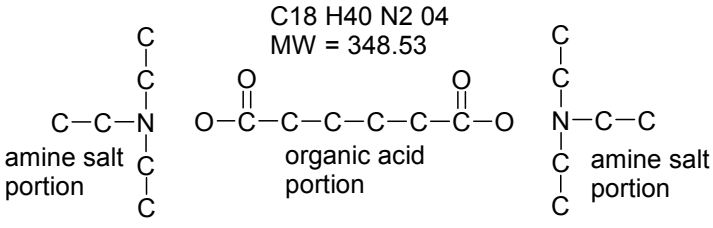
Example 1: You are evaluating a chemical that is an aliphatic amine salt of an organic acid (shown to the right). This substance may disassociate in aqueous media. You are concerned that the amine portion could have some toxicity. If you evaluate the complete molecule in ECOSAR the results indicate the chemical is a neutral with low toxicity. You will also get a structural alert telling you this chemical should be evaluated as a surfactant.



Enter the SMILES, draw, or otherwise input the amine structural unit / moiety as if it were a discrete chemical. Next, enter the molecular weight of the *entire* chemical (216.35) in the Mol. wt. (special use only) box. This is the molecular weight of the overall structure that contains one amine unit (as input). ECOSAR automatically adjusts for the MW input and will give you the predicted toxicity for the entire chemical based on the separate amine portion. The results indicate the chemical may have a moderate concern for acute and chronic exposures. You should remember to clear the Mol wt box before you evaluate another chemical because ECOSAR doesn't automatically clear the box when a new structure is entered.



Example 2: In the example above, you evaluated the toxicity of the portion of the molecule that contains *one* amine. In some cases the molecule you are evaluating may have multiple amines. For example, when you run the neutral form of the diacid shown to the right in ECOSAR you get results indicating it is low toxicity and you get the structural alert telling you this chemical should be evaluated as a surfactant. In order to evaluate the toxicity of the amine portions, just like the example above, you run the amine portion as if it were a discrete chemical then enter *one half* the molecular weight of the entire chemical (348.53 / 2 = 174.265) in the Mol. wt. (special use only) box. This is the molecular weight of the subunit that contains only one amine.



ECOSAR results show a moderate concern for the diacid. If the molecule were to contain three amines, enter one third the molecular weight of the entire molecule in the Mol. wt. (special use only) box, etc.

Model Results
The results page of ECOSAR provides Acute and Chronic toxicity values in mg/L for fish, invertebrate (Daphnids), and green algae. Acute Toxicity (short-term exposure) is assessed using Lethal or Effect Concentrations (LC/EC) values. The preferred values are EC50 or LC50. Chronic Toxicity (long-term exposure) is assessed using Chronic (ChV) values. The ChV is defined as the geometric mean between lowest observed effect level (LOEC) and no observed effect level (NOEC) from the study. If LOEC not available, a NOEC can be used alone. Here is the standard aquatic toxicity profile used by EPA for freshwater species (mg/L or ppm):

Acute Effects:	Chronic Effects:
Fish 96 hr LC50	Fish ChV
Daphnid 48 hr LC50	Daphnid ChV
Algae 72 hr or 96 hr EC50	Algae ChV

The SAR Chemical Class is also provided in the results. ECOSAR will identify multiple chemical classes if the query chemical has fragments that match structural descriptions of those classes. It is possible that portions of the query chemical can fit multiple classes. A case study evaluating the chemical prallethrin (CAS RN 23031-36-9) is provided at the end of this chapter as an example of a chemical identified by ECOSAR as having multiple chemical classes. The case study describes steps in selecting the most appropriate chemical class for the chemical evaluated.

The Log Kow cutoff values (described below) for the SARs used are provided so that the user can determine if the values are reliable for the chemical evaluated. If the chemical is not soluble enough to reach effects concentrations (referred to as "No Effects at Saturation or NES") this is also indicated.

Saving the Results
Results can be printed when displayed. You can save results by clicking on "Save Results" which will create a ".dat" file that can be opened using MSWord or WordPerfect. Output can also be copied (click on "Copy") through the Windows Clipboard. Structures can be saved as an ISIS ".skc" file or through the Windows Clipboard. Further explanations are in "Help" on the Results page.

6.3.3 Important Notes on the Proper Use of ECOSAR

ECOSAR users should have an understanding of organic chemistry, aquatic toxicology, and SARs. Interpretation of the model output requires an understanding of the model design and users are strongly encouraged to **read the ECOSAR Methodology Document contained within the ECOSAR Help Menu**. The Help Menu has greater detail on these considerations briefly described here.

Please remember that ECOSAR was developed for use by EPA scientists who are most familiar with proper use of the method. Currently ECOSAR will provide results for numerous types of chemicals entered and it is the responsibility of the user to determine proper applicability of the method.

As previously stated, ECOSAR was designed for and developed using data on *organic* chemicals, and as a result the following types of chemicals should *not* be profiled using ECOSAR:

- Inorganic or organometallic chemicals are not represented in the training sets used to develop ECOSAR.
- Polymers and chemicals with MW >1,000 also are not represented in the training sets used to develop ECOSAR. However, if the polymer is may be made up of dimers, trimers, and oligomers that have a molecular weight of less than 1,000 these smaller molecules can be run through the model.

- Mixtures: ECOSAR requires a discrete structure be entered so mixtures can't be profiled unless discrete representative structures of each constituent can be identified. Each substance can be run through the model separately. However this method does not account for synergistic effects.
- Nanomaterials: ECOSAR does not account for the unique physical properties of nanomaterials which may contribute to novel mechanisms of toxicity for this class of chemicals.

Log Kow Cutoffs: The limits of each QSAR must be understood if the results are to be interpreted properly. In general, when the log Kow is ≤ 5.0 for fish and daphnid, or ≤ 6.4 for green algae, ECOSAR provides reliable estimates for acute effects. If the log Kow exceeds those limits, empirical data indicate that the decreased solubility of these lipophilic chemicals results in "no effects at saturation" during a 48-hour to 96-hour test. For chronic exposures, the log Kow cut off is 8.0 or greater (indicating a poorly soluble chemical) and "no effects at saturation" are expected in saturated solutions even with long-term exposures. The user should always review these limits to determine when "no effects at saturation" may be expected for a chemical.

Water Solubility Considerations: For chemicals that are solids it is important to compare the toxicity estimates with the water solubility using the following decision logic:

Is the effect concentration below the water solubility of the solid chemical?
YES – Base the toxicity concern on the effect concentration
NO – Is the effect concentration ≥ 10x above the water solubility of the chemical?
YES – No effects at saturation (NES) (low concern)
NO – May have NES or effects may occur

Special Classes: Evaluating these special classes in ECOSAR can be problematic:

- Simple Salts: Use predicted log Kow for free acid or corresponding conjugate base for salts
- Larger Organic Salts: more difficult assessment
- Chemicals that rapidly hydrolyze ($t_{1/2}$ <1 hour at 20 deg C, pH 7)
- Chemicals that undergo rapid photolysis, oxidation, or pyrolysis asess degradation products

Surfactants and Dyes: Some classes do not use log Kow in SAR predictions. For example, anionic surfactant SARs are typically non-linear and describe the relationship between the hydrophilic portion and hydrophobic portion of the chemical. An example is the anionic surfactants SAR:

log LC50 (mg/L) = [(avg. no. of carbons -16)^2 - 10.643] ÷ 12.9346

6.4 Interpreting ECOSAR Results

6.4.1 ECOSAR v1.1 Results for the Sample Chemical Isodecyl Acrylate

```
SMILES : O=C(C=C)OCCCCCCCC(C)C
CHEM   : Isodecyl acrylate; 2-Propenoic acid, isodecyl ester
CAS Num: 001330-61-6
ChemID1:
MOL FOR: C13 H24 O2
MOL WT : 212.34
Log Kow: 5.074    (EPISuite Kowwin Estimate)
Log Kow:          (User Entered)
Log Kow:          (PhysProp DB exp value - for comparison only)
Melt Pt:          (User Entered for Wat Sol estimate)
Wat Sol: 1.753    (mg/L, EPISuite WSKowwin Estimate)
Wat Sol:          (User Entered)
Wat Sol:          (PhysProp DB exp value)

Values used in ECOSAR Estimation
--------------------------------
Log Kow: 5.074    (EPISuite Kowwin Estimate)
```

```
Wat Sol: 1.753    (mg/L, EPISuite WSKowwin Estimate)

ECOSAR v1.1 Training Set Data - No Data Available
----------------------------
ECOSAR v1.1 Class-specific Estimations - Acrylates
-------------------------------------
                                                    Predicted
ECOSAR Class  Organism      Duration  End Pt   mg/L (ppm)
============  ============  ========  ======   ==========
Acrylates    : Fish           96-hr     LC50        0.555
Acrylates    : Daphnid        48-hr     LC50        0.731
Acrylates    : Green Algae    96-hr     EC50        0.520
Acrylates    : Fish                     ChV     9.55e-005
Acrylates    : Daphnid                  ChV         0.010 !
Acrylates    : Green Algae              ChV         0.091
Acrylates    : Fish (SW)      96-hr     LC50        0.440
Acrylates    : Mysid (SW)     96-hr     LC50        0.041
Acrylates    : Fish (SW)                ChV         0.022 !
Acrylates    : Mysid (SW)               ChV     6.26e-005 !
============  ============  ========  ======   ==========
Neutral Organic SAR: Fish      96-hr     LC50        0.302
(Baseline Toxicity): Daphnid   48-hr     LC50        0.287
                   : Green Algae 96-hr   EC50        0.271
                   : Fish                ChV         0.041
                   : Daphnid             ChV         0.049
                   : Green Algae         ChV         0.271
 Note:  * = asterisk designates: Chemical may not be soluble
        enough to measure this predicted effect.

 Note:  ! = exclamation designates: The toxicity value was
estimated through application of acute-to-chronic ratios per methods
outlined in the ECOSAR Technical Reference Manual posted on the ECOSAR webpage.

Acrylates:                Baseline Toxicity SAR Limitations:
---------                 ---------------------------------
Max LogKow: 5.0 (LC50)    Max LogKow: 5.0 (Fish 96-hr LC50; Daphnid LC50)
Max LogKow: 6.4 (EC50)    Max LogKow: 6.4 (Green Algae EC50)
Max LogKow: 8.0 (ChV)     Max LogKow: 8.0 (ChV)
```

6.4.2 Interpreting ECOSAR Results for Isodecyl Acrylate

Was the Query Chemical in the QSAR Training Sets?
The results on the previous page show that this acrylate was not in the training set for these QSARs.

Are QSAR Cut-off Values Exceeded?
The log Kow of the chemical (5.074) just exceeds the log Kow cut-off for the fish LC50 QSAR which is 5.0 so we will use that estimation with caution. The log Kow cut-offs for the other QSARs are not exceeded.

Determine a Full Aquatic Toxicity Profile
ECOSAR did provide the full aquatic toxicity profile for isodecyl acrylate but the program did estimate the Daphnid ChV by applying the acute-to-chronic ratio (explained in section 6.A.3 of this document and in the Acrylate QSAR Equation Document) to the Daphnid acute value, as designated by the exclamation point "!" next to the Daphnid ChV.

Determining the Concern Concentration (CC)
The Concern Concentration (CC) or Concentration of Concern (COC) is the lowest ChV divided by an uncertainty factor (assessment or safety factor) of 10. In order to be conservative and because the uncertainty (or assessment) factor is one significant digit, the CC is rounded up to one significant digit e.g., a CC of 175 will be rounded up to 200. For the example chemical

Setting Aquatic Toxicity Concern Level
High Concern
Any Acute value <1 mg/L
Chronic < 0.1 mg/L
Moderate Concern
Lowest of the 3 is > 1 and < 100 mg/L
Chronic >0.1 and <10.0 mg/L
Low Concern
All 3 are > 100
Chronic > 10.0 mg/L
OR there are No Effects at Saturation (occurs when water solubility of the chemical is higher than an effect concentration) or the log Kow value exceeds QSAR cut-offs.

6.5 Entering ECOSAR Predictions into SF Worksheet

This is the section of the Sustainable Futures Summary Assessment Worksheet (below) where the aquatic toxicity estimations (on the previous page) should be entered. The reader is encouraged to refer back to the completed full Sustainable Futures Summary Assessment Worksheet for this sample chemical which is included in chapter 2 of this document. A blank worksheet is included in Appendix H.

ECOTOXICITY:	
ECOSAR Class	Acrylates
Acute Toxicity	
Fish LC50	0.56 mg/L (ECOSAR)
Daphnid LC50	0.73 mg/L (ECOSAR)
Green Algae EC50	0.52 mg/L (ECOSAR)
Chronic Toxicity	
Fish ChV	0.00009 mg/L (ECOSAR)
Daphnid ChV	0.10 mg/L (ECOSAR)
Green Algae ChV	0.09 mg/L (ECOSAR)
Hazard Concern for Aquatic Toxicity	High
Concern Concentration	1 ppb (see discussion)

6.6 Peer-Reviewed Publications Relating to Validation, Verification, and Performance

6.6.1 Book Chapters or Reports

1. OECD (Organization for Economic Cooperation and Development). (2006) Report on the Regulatory Uses and Applications in OECD Member Countries of (Quantitative) Structure- Activity Relationships [(Q)SAR] Models in the Assessment of New and Existing Chemicals. Organization for Economic Cooperation and Development, Paris; ENV/JM/MONO(2006)25.

2. Eriksson, L; Johansson, E; Wold S. (1997) Quantitative Structure-Activity Relationship Model Validation. In: Chen, F; Schuurmann, G; eds. Quantitative Structure-Activity Relationships in Environmental Sciences - VII. Pensacola, FL: SETAC Press, pp. 381-397.

3. OECD (Organization for Economic Cooperation and Development). (2004a) The Principles for Establishing the Status of Development and Validation of (Quantitative) Structure-Activity Relationships [(Q)SARs]. Organization for Economic Cooperation and Development, Paris; ENV/JM/TG(2004)27.

4. OECD (Organization for Economic Cooperation and Development). (2004b) Annex 6: ECOSAR. In: Annexes to the Report on the Principles for Establishing the Status of Development and Validation of (Quantitative) Structure-Activity Relationships [(Q)SARs]; ENV/JM/TG(2004)27/ANN.

5. OECD (Organization for Economic Cooperation and Development). (2004c) Comparison of SIDS Test Data with (Q)SAR Predictions for Acute Aquatic Toxicity, Biodegradability and Mutagenicity on Organic Chemicals Discussed at SIAM 11-18. Organization for Economic Cooperation and Development, Paris; ENV/JM/TG(2004)26.

6. Posthumus, R; Sloof, W. (2001) Implementation of QSARS in Ecotoxicological Risk Assessments. Research for Man and Environment/National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands; RIVM report 601516003.

7. Zeeman, M; Rodier, D; Nabholz, J. (1999) Ecological Risks of a New Industrial Chemical Under TSCA. In: Ecological Risk Assessment in the Federal Government. U.S. White House, National Science & Technology Council, Committee on Environment & Natural Resources (CENR), Washington, DC; CENR/5-99/001, pp. 2-1 to 2-30.

8. Kaiser, KL; Niculescu, S; Mckinnon ,M. (1997) On Simple Linear Regression, Multiple Linear Regression, and Elementary Probabilistic Neural Network with Gaussian Kernel's Performance in Modeling Toxicity Values to Fathead Minnow Based on Microtox Data, Octanol/Water Partition Coefficient, and Various Structural Descriptors for a 419-Compound Dataset. In: Chen, F; Schuurmann, G; eds. Quantitative Structure-Activity Relationships in Environmental Sciences-VII, Pensacola, FL: SETAC Press, pp. 285-297.

9. OECD (Organization for Economic Cooperation and Development). (1994) US EPA/EC Joint Project on the Evaluation of (Quantitative) Structure Activity Relationships (QSARS). OECD Environment Monographs No. 88. Organization for Economic Cooperation and Development, Paris, France; OECD/GD(94)28.

10. U.S. EPA (Environmental Protection Agency). (1994) US EPA/EC Joint Project on the Evaluation of (Quantitative) Structure Activity Relationships (QSARS). U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; EPA 743-R-94-001.

11. OECD (Organization for Economic Cooperation and Development). (1994) U.S. EPA/EC Joint Project on the Evaluation of (Quantitative) Structure Activity Relationships (QSARS). OECD Environmental Monographs No. 88. Organization for Economic Cooperation and Development, Paris, France; OECD/GD(94)28.

12. Lynch, DG; Macek, G; Nabholz, J; et al. (1994) Ecological Risk Assessment Case Study: Assessing the Ecological Risks of a New Chemical Under the Toxic Substances Control Act. In: A Review of Ecological Assessment Case Studies from a Risk Assessment Perspective, Volume II. Washington, DC: Risk Assessment Forum, Office of Research and Development, U.S. Environmental Protection Agency, pp. 1-1 to 1-B4.

13. Nabholz, JV; Clements, R; Zeeman, M; et al. (1993) Validation of Structure Activity Relationships used by the Office of Pollution Prevention and Toxics for the Environmental Hazard Assessment of Industrial Chemicals. In: Gorsuch J; Dwyer F; Ingersoll C, et al.; eds. Environmental Toxicology and Risk Assessment: 2nd Volume. Philadelphia: American Society for Testing and Materials, pp. 571-590.

6.6.2 Scientific Journal Articles

14. Reuschenbach, P; Silvania, M; Dammannb, M; et al. (2008) ECOSAR Model Performance with a Large Test Set of Industrial Chemicals. Chemosphere 71(10):1986-1995.

15. Tunkel, J; Mayo, K; Austin, C; et al. (2005) Practical Considerations of the Use of Predictive Methods for Regulatory Purposes. Environ Sci Technol 39:2188-2199.

16. Öberg, T. (2004) A QSAR for Baseline Toxicity: Validation, Domain of Application, and Prediction. Chem Res Toxicol 7 (12):1630-1637.

17. Moore, D; Breton, R; MacDonald, D. (2003) A Comparison of Model Performance for Six QSAR Packages that Predict Acute Toxicity to Fish. Environ Toxicol Chem 22(8):1799-1809.

18. Cronin, M; Walker, J; Jaworska, J; et al. (2003) Use of QSARs in International Decision-Making Frameworks to Predict Ecologic Effects and Environmental Fate of Chemical Substances. Environ Health Perspect 111(10):1376-1390.

19. Hulzebos, EM; Posthumus, R. (2003) (Q)SARs: Gatekeepers Against Risk on Chemicals? SAR QSAR Environ Res 14: 285-316.

20. Kaiser, KL; Deardon J; Klein W; et al. (1999) Short Communication: A Note of Caution to Users of ECOSAR. Water Qual Res J Can 34:179-182.

Abstracts

21. Chun, J; Nabholz, J; Wilson, M. (2002) Comparison of Aquatic Toxicity Experimental Data with EPA/OPPT/SAR Prediction on PPG Polymers. Society of Environmental Toxicology and Chemistry Annual Meeting, Salt Lake City, UT.

22. Chun, J; Nabholz, J; Wilson, M. (2001) Comparison of Aquatic Toxicity Experimental Data with EPA/OPPT SAR Predictions on PPG Polymers. Society of Toxicology Annual Meeting, San Francisco, CA.

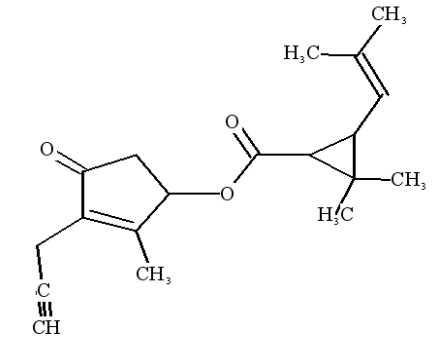
6.7 Selecting the Most Appropriate Chemical Class when Multiple Classes are Identified

The ECOSAR program will identify multiple chemical classes if the query chemical has fragments that match structural descriptions of those classes. It is possible that portions of the query chemical can fit multiple classes. The chemical Prallethrin (CAS RN 23031-36-9), shown below, is an example of a

chemical identified by ECOSAR as having multiple chemical classes. This case study describes steps in selecting the most appropriate chemical class for the chemical evaluated.

Prallethrin can be entered into ECOSAR using the CAS RN because the chemical is in the SMILECAS database incorporated into ECOSAR. The ECOSAR v1.1 results are shown below. Prallethrin is identified by ECOSAR as having fragments from four chemical classes: (1) Esters; (2) Vinyl/Allyl Ketones; (3) Vinyl/Allyl Esters; and (4) Pyrethroids. The results show that data for Prallethrin are in the training sets used to develop ECOSAR.

```
SMILES : CC(C)=CC1C(C)(C)C1C(=O)OC2C(C)=C(CC#C)C(=O)C2
CHEM   : Prallethrin
CAS Num: 023031-36-9
ChemID1:
MOL FOR: C19 H24 O3
MOL WT : 300.40
Log Kow: 4.880    (EPISuite Kowwin Estimate)
Log Kow:          (User Entered)
Log Kow: 4.49     (PhysProp DB exp value - for comparison only)
Melt Pt:          (User Entered for Wat Sol estimate)
Wat Sol: 1.788    (mg/L, EPISuite WSKowwin Estimate)
Wat Sol:          (User Entered)
Wat Sol: 8        (mg/L, PhysProp DB exp value)
```



Prallethrin

```
Values used in ECOSAR Estimation
--------------------------------
Log Kow: 4.880    (EPISuite Kowwin Estimate)
Wat Sol: 8        (mg/L, PhysProp DB exp value)

ECOSAR v1.1 Training Set Data
-----------------------------
```

CAS No	Organism	Duration	End Pt	Measured mg/L (ppm)	Ecosar Class	Reference
023031-36-9	Daphnid		ChV	0.00092	Pyrethroids	OPP Pesticides Ecotoxicity DB
023031-36-9	Daphnid	48-hr	LC50	0.0062	Pyrethroids	OPP Pesticides Ecotoxicity DB
023031-36-9	Fish	96-hr	LC50	0.022	Pyrethroids	OPP Pesticides Ecotoxicity DB
023031-36-9	Fish	96-hr	LC50	0.012	Pyrethroids	OPP Pesticides Ecotoxicity DB
023031-36-9	Fish (SW)	96-hr	LC50	0.026	Pyrethroids	OPP Pesticides Ecotoxicity DB
023031-36-9	Mysid	96-hr	LC50	0.003900	Pyrethroids	OPP Pesticides Ecotoxicity DB

```
ECOSAR v1.1 Class-specific Estimations
--------------------------------------
Esters
Vinyl/Allyl Ketones
Vinyl/Allyl Esters
Pyrethroids
```

ECOSAR Class		Organism	Duration	End Pt	mg/L (ppm)
Esters	:	Fish	96-hr	LC50	0.835
Esters	:	Fish	14-day	LC50	1.773
Esters	:	Daphnid	48-hr	LC50	1.275
Esters	:	Green Algae	96-hr	EC50	0.344
Esters	:	Fish		ChV	0.034
Esters	:	Daphnid		ChV	0.381
Esters	:	Green Algae		ChV	0.225
Esters	:	Fish (SW)	96-hr	LC50	1.065
Esters	:	Mysid	96-hr	LC50	0.253
Esters	:	Fish (SW)		ChV	0.256
Esters	:	Mysid (SW)		ChV	0.053
Esters	:	Earthworm	14-day	LC50	461.663 *
Vinyl/Allyl Ketones	:	Fish	96-hr	LC50	1.941
Vinyl/Allyl Ketones	:	Daphnid	48-hr	LC50	0.527
Vinyl/Allyl Ketones	:	Green Algae	96-hr	EC50	0.551
Vinyl/Allyl Ketones	:	Fish		ChV	0.171
Vinyl/Allyl Ketones	:	Daphnid		ChV	0.148 !
Vinyl/Allyl Ketones	:	Green Algae		ChV	0.599 !
Vinyl/Allyl Ketones	:	Fish (SW)	96-hr	LC50	0.907

Vinyl/Allyl Ketones	: Mysid (SW)	96-hr	LC50	0.085
Vinyl/Allyl Ketones	: Fish (SW)		ChV	2.651
Vinyl/Allyl Ketones	: Mysid (SW)		ChV	0.003 !
Vinyl/Allyl Esters	: Fish	96-hr	LC50	0.722
Vinyl/Allyl Esters	: Daphnid	48-hr	LC50	2.087
Vinyl/Allyl Esters	: Green Algae	96-hr	EC50	0.438
Vinyl/Allyl Esters	: Fish		ChV	0.004 !
Vinyl/Allyl Esters	: Daphnid		ChV	0.026 !
Vinyl/Allyl Esters	: Green Algae		ChV	0.116
Pyrethroids	: Fish	96-hr	LC50	0.005
Pyrethroids	: Daphnid	48-hr	LC50	0.004
Pyrethroids	: Fish		ChV	0.000212
Pyrethroids	: Daphnid		ChV	9.49e-005
Pyrethroids	: Fish (SW)	96-hr	LC50	0.017
Pyrethroids	: Mysid	96-hr	LC50	3.7e-005
Pyrethroids	: Fish (SW)		ChV	0.002
Pyrethroids	: Mysid		ChV	3.73e-006
Neutral Organic SAR (Baseline Toxicity)	: Fish	96-hr	LC50	0.639
	: Daphnid	48-hr	LC50	0.584
	: Green Algae	96-hr	EC50	0.551
	: Fish		ChV	0.085
	: Daphnid		ChV	0.097
	: Green Algae		ChV	0.501

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect.

Note: ! = exclamation designates: The toxicity value was estimated through application of acute-to-chronic ratios per methods outlined in the ECOSAR Technical Reference Manual posted on the ECOSAR webpage.

Esters:

Maximum LogKow: 5.0 (Fish 96-hr LC50; Daphnid LC50, Mysid LC50)
Maximum LogKow: 6.0 (Fish 14-day LC50; Earthworm LC50)
Maximum LogKow: 6.4 (Green Algae EC50)
Maximum LogKow: 8.0 (ChV)

Vinyl/Allyl Ketones:

Maximum LogKow: 5.0 (Fish 96-hr LC50; Daphnid LC50; Mysid LC50)
Maximum LogKow: 6.4 (Green Algae EC50)
Maximum LogKow: 8.0 (ChV)

Vinyl/Allyl Esters:

Maximum LogKow: 5.0 (LC50)
Maximum LogKow: 6.4 (EC50)
Maximum LogKow: 8.0 (ChV)

Pyrethroids:

Maximum LogKow: >8.2 (Fish, Mysid 96-hr LC50)
Maximum LogKow: >7.2 (Fish (SW) 96-hr LC50)
Maximum LogKow: >7.5 (Daphnid 48-hr LC50)
Maximum LogKow: 8.0 (Chronic Values)

Baseline Toxicity SAR Limitations:

Maximum LogKow: 5.0 (Fish 96-hr LC50; Daphnid LC50)
Maximum LogKow: 6.4 (Green Algae EC50)
Maximum LogKow: 8.0 (ChV)

There Are Measured Data For This Chemical

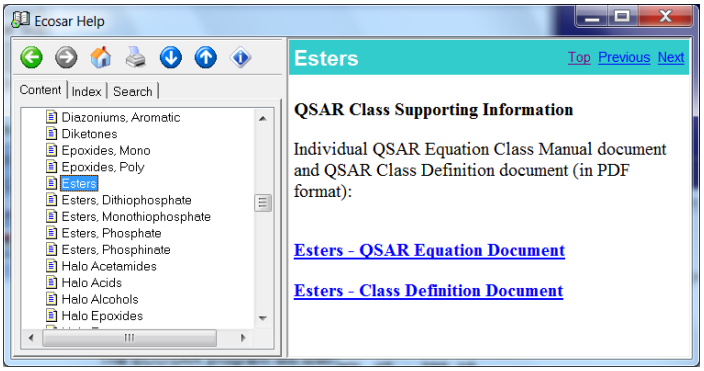
The first thing that you should notice on the results page is that measured data on this chemical from the EPA Office of Pesticide Programs were included in the training set for the Pyrethroids class. The data for Fish 96-hr

LC50, Daphnid 48-hr LC50 and Daphnid ChV, should be used instead of any estimated data. The remaining endpoints will be taken from the estimations to complete the aquatic toxicity profile.

Selecting the Most Appropriate Class

How do you determine which of the classes identified is the most appropriate? Traditionally the most conservative effect level of the multiple estimates for a given endpoint is selected when predictions are identified from multiple classes. However, this is not always the best approach.

The user should evaluate several critical elements to eliminate classes that are not truly representative of the query compound or classes with insufficient structural similarity to justify using the class. These elements are described in the steps below. The HELP menu in ECOSAR has QSAR equation and definition documents for all QSARs within each chemical class (the Ester QSAR example is shown here).



This documentation allows the user to evaluate adequacy of predictions. Considerations for each identified class are described below and are correlated with the example above.

Steps in Evaluating Appropriateness of Each Class Identified

1. Compare the definition of each chemical class with the evaluated compound.
2. For each class identified, evaluate the amount of variation in one variable as directly related to the variation in another variable, known as the coefficient of determination (r2). In ECOSAR the r2 is variation between the specific endpoint and the Log KOW.
3. For each class identified, evaluate the robustness and distribution of supporting datasets used to develop the QSAR classes.
4. Summarize and select the most appropriate class.

Step 1: Compare the definition of each chemical class with the evaluated compound.

General vs. Sub-classes: Some classes within ECOSAR are considered *general classes* and represent a simple molecular moiety (e.g., Esters, Aliphatic Amines, Phenols, Amides). Other *sub-classes* define more specific and complex molecular configurations (e.g. Nicotinoids, Pyrethroids) or define explicit molecular attachments to otherwise general classes (e.g., Haloamides). Depending on ECOSAR programming, predictions for the general classes as well as the more specific sub-classes may be displayed in the ECOSAR output. In the example depicted (shown above), Prallethrin is identified as an Ester, Vinyl/Allyl Ketone, Vinyl/Allyl Ester, and a Pyrethroid.

Sub-classifications are created in ECOSAR when compounds with larger, more complex structural moieties (pyrethroids) are identified that exhibit toxicity levels which are unlike estimates for the more general classes (esters, vinyl/allyl ketones, vinyl allyl esters), even though those complex compounds may still contain those simple molecular features. In the example described here, the general classes identified for prallethrin are esters, vinyl/allyl ketones, and vinyl/allyl esters (relating to smaller functional groups contained within prallethrin). The more specific sub-class is pyrethroids which define a much larger part of the prallethrin molecule.

The first step in finding the most appropriate class is reviewing the online QSAR documentation for each class identified and comparing the chemical class definition with the specific chemical evaluates. The user needs to determine how many molecular features of the query chemical can be found within the structure the class definition, how sufficient that particular molecular coverage is to create a toxicity profile, and whether that class is the most specific available in ECOSAR for the query chemical.

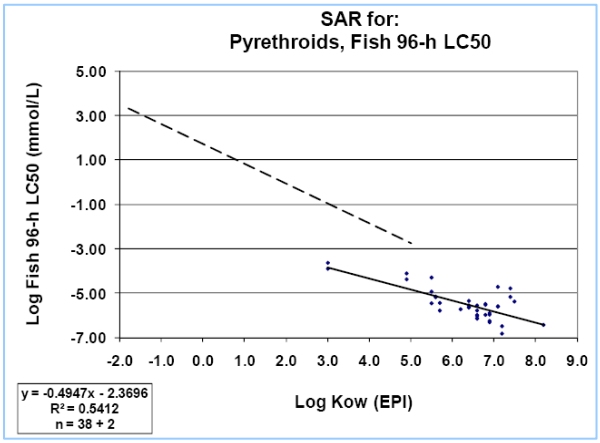
Here is a class-by-class evaluation comparing the QSAR class definitions with the structure of prallethrin:

1. Esters - the compound does fit the esters definition, however keep in mind this is a general class. In addition, the ester fragments present correspond to a small portion of the entire molecule and is not likely to be the only reactive site.
2. Vinyl/Allyl Ketones - the vinyl/allyl ketone in prallethrin is within a 5-carbon ring. Although there has been scientific discussions on whether to restrict vinyl/allyl classes to only terminal vinyl/allyl moieties, ECOSAR definitions for these classes have not yet been restricted due to uncertainty. Thus, the user must decide whether this class should be excluded. And like the Esters class, the vinyl/allyl ketone fragments present correspond to a small portion of the entire molecule and is not likely to be the only reactive site.
3. Vinyl/Allyl Esters - the allyl moiety is within a ring structure. As discussed for Vinyl/Allyl Ketones, some scientists indicate that the vinyl/allyl moiety must be terminal and/or cannot be within a ring. Again, the user must decide whether this class should be excluded.
4. Pyrethroids - the compound fits the pyrethroids definition and literature resources consistently identify the compound as a pyrethroid pesticide from which the class is modeled. The class is also more specific to the query compound than the esters QSAR class, and does cover a significant portion of the query chemical.

The next two steps involve looking at the equation documents to determine the quality of the QSARs (see Sections 2 and 3).

Step 2: Evaluate the amount of variation in one variable as directly related to the variation in another variable, known as the coefficient of determination (r2).

The QSAR equation document for each class identified has graphs and the supporting data tables for each endpoint. The graph for the Pyrethroids Fish 96-hr LC50 SAR is shown on the right. The dashed line represents baseline toxicity and the solid line shows the trend for fish 96-hr LC50 data points for pyrethroids. A coefficient of determination (r2) is reported in both the graph (a scatter plot) and in the text of the QSAR Equation Document. The coefficient of determination is a numeric representation of how much variation in one variable is directly related to the variation in another variable for the training set (e.g., endpoint effect level (mmol/L) vs. Log Kow). A correlation coefficient can be determined by taking the square root of the presented coefficient of determination. Users should consult the QSAR equation documents of identified classes to quantitatively determine correlation of data sets based on the correlation of determination.



Depending on the user's knowledge and understanding of statistics, a level of significance can be determined for relationships observed in each QSAR class for each endpoint using a correlation coefficient derived from the presented coefficient of determination.[1]

Keep in mind that a weak relationship does not necessarily indicate little or no correlation; if little adequate data were available for a certain endpoint, low correlation may be a product of insufficient supporting data and/or may indicate that further sub-classification or reclassification is needed. A weak relationship can also indicate significant variation was observed in laboratory studies due to difficult-to-test substance or poor study design.

[1] This discussion is beyond the scope of this document. Since these methods are a simple correlation of two variables, there is an abundance of material for determining significance using Pearson's correlation coefficient including a publicly available educational document from the Smithsonian National Zoo (http://nationalzoo.si.edu/Education/ClassroomPartnerships/BioDivMonPro/TrainingCourseandManuals/trainingmanual/SA%203.pdf).

For simplicity, significance of the relationship of effect levels (mmol/L) vs. Log Kow values for each identified class will be evaluated for the Fish 96-hr LC50 endpoint only. This evaluation can be done for each endpoint.

1. Esters - Pearson's Correlation Coefficient (r) is -0.88. Using 5% uncertainty (p = 0.05), the correlation between the F96-hr LC50 value (mmol/L) and the Log Kow value is statistically significant.
2. Vinyl/Allyl Ketones - Pearson's Correlation Coefficient (r) is -0.84. Using 5% uncertainty (p = 0.05), the correlation between the F96-hr LC50 value (mmol/L) and the Log Kow value is statistically significant.
3. Vinyl/Allyl Esters - Pearson's Correlation Coefficient (r) is -0.44. Using 5% uncertainty (p = 0.05), the correlation between the F96-hr LC50 value (mmol/L) and the Log Kow value is *not* statistically significant.
4. Pyrethroids - Pearson's Correlation Coefficient (r) is -0.74. Using 5% uncertainty (p = 0.05), the correlation between the F96-hr LC50 value (mmol/L) and the Log Kow value is statistically significant.

Step 3: Evaluate the robustness and distribution of supporting data sets used to develop the QSAR classes.

The supporting data sets (training sets) used to derive QSARs within a chemical class range from the very large, e.g., neutral organics, to the very small, e.g., aromatic diazoniums. If a class or sub-class is supported by a large dataset that is well correlated, then strength of the association is increased and adequacy of the resulting regression equation is better substantiated. Additionally, depending on the range of Log Kow values of the available data for a given training set, the Log Kow value of the queried compound may be notably less than or greater than the minimum and maximum Log Kow values of the training set. Sometimes data are distributed so that the regression line overlaps or crosses over the depicted neutral organic line (dotted line), which may be an artifact of the training set data and/or may indicate that excess toxicity for that particular endpoint was not observed. These issues are not always apparent from the ECOSAR results output and may result in predictions that seem anomalous. Users should consult the QSAR Equation Documents of identified classes to visually determine correlation from the depicted graphs of each endpoint.

For our prallethrin example, the following conclusions can be made from the QSAR Equation Documents regarding robustness and distribution of the supporting data sets for these classes.

1. Esters - This SAR may be used to estimate toxicity for a variety of esters that include acetates (non-acids), benzoates, dicarboxylic aliphatics, and phthalates derived from aliphatic alcohols and phenol, and does indicate excess toxicity above baseline.
2. Vinyl/Allyl Ketones - The class-specific SAR equation is yielding less toxic values than estimated from baseline toxicity (neutral organics), as indicated in the text of the ECOSAR Equation document. This may be an artifact of the training data set, but may also indicate that the vinyl/allyl ketone moiety does not exhibit excess toxicity.
3. Vinyl/Allyl Esters - The training data set consists of 4 chemicals. All data are considered TSCA CBI and, thus, their identities are unknown. One of these data points appears to be an outlier, which may have contributed to low correlation.
4. Pyrethroids - The Log Kow values for data points that are within the Log Kow range from 3 to 8.2. Thus, if the Log Kow value of the query compound is much less than 3, there may be some uncertainty with the prediction. However, the pyrethroid QSAR class, which by definition contains an ester moiety, appears to exhibit much greater toxicity than any of the QSAR classes described above.

The conclusion is that the Pyrethroid class has statistical significance, greatest structural coverage of the molecule and predicts greatest toxicity.

Step 4: Summarize and select the most appropriate class.
In our prallethrin example output (see above), available information from QSAR Class Equation and QSAR Definition documents could support a user's decision to exclude the Esters, Vinyl/Allyl Ketones and Vinyl/Allyl Esters predictions. Of the remaining classes, the Pyrethroid class appears to be the most representative of the query compound and also results in the most conservative effect levels (see results above).

Complete the Aquatic Toxicity Profile
Measured data for the query chemical, prallethrin, CAS 23031-36-9, for Fish 96-hr LC50, Daphnid 48-hr LC50 and Daphnid ChV are available. Use the lower (more toxic) of the two values for Fish 96-hr LC50. You will use the prediction from the Pyrethroid class to fill in the other endpoint – Fish ChV.

Notice that there are no predictions for Green Algae in the Pyrethroid class set of QSARs, indicating that there were not enough Green Algae data to develop a QSAR. This data gap can be addressed by identifying a close analog or by doing acute and chronic toxicity testing on algae. It is important to realize that ECOSAR will not provide estimates for all chemicals.

Acute					
	Fish	96-hr	LC50	0.012	Measured
	Daphnid	48-hr	LC50	0.0062	Measured
	Green Algae	96-hr	EC50		(Data Gap)
Chronic					
	Fish		ChV	0.000212	Pyrethroid QSAR prediction
	Daphnid		ChV	0.00092	Measured
	Green Algae		ChV		(Data Gap)

6.8 Obtaining Additional Training Materials on ECOSAR

The training materials covering ECOSAR that were developed by EPA for use in the 3 day Sustainable Futures hands-on training sessions may provide you with additional information.

Check the Sustainable Futures web site at http://www.epa.gov/oppt/sf/meetings/train.htm#materials for information on how to get copies if the training materials. These materials are frequently updated and it was decided that the most effective way to provide up-to-date copies is provide a contact name to whom requests for the materials can be sent. For PDF copies of the presentations contact Kelly Mayo-Bean, U.S. EPA (mayo.kelly@epa.gov).