OPERATION MANUAL
for the
ECOlogical Structure-Activity Relationship Model (ECOSAR) Class Program

ESTIMATING TOXICITY OF INDUSTRIAL CHEMICALS TO AQUATIC ORGANISMS USING THE ECOSAR (ECOLOGICAL STRUCTURE ACTIVITY RELATIONSHIP) CLASS PROGRAM

MS-Windows Version 2.0

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DISCLAIMER

This document has been reviewed and approved for publication by the Risk Assessment Division of the Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency (U.S. EPA/OPPT). Approval does not signify that the contents necessarily reflect the views and policies of all Offices/Divisions in the Environmental Protection Agency, nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

The ECOlogical Structure-Activity Relationship Model (ECOSAR) model and underlying methodology presented in this document have been developed over a period of more than 25 years by U.S. EPA/OPPT, EPA contractors, and/or others in the scientific and technical community to screen chemicals in the absence of data. U.S. EPA/OPPT has made this screening-level model, along with many other tools, available to industry and other stakeholders in the hopes that use of the models in the early stages of research and development or prior to submission of notifications to the Agency, will result in safer chemicals entering commerce.

Other chemical screening methodologies have been developed and are in use by other Agencies, chemical companies and other stakeholders. EPA recognizes that other models are available and that these models can also be of value in chemical assessment efforts. Models provide estimations with an inherent degree of uncertainty and therefore, valid measured data are always preferred over estimated data. If no measured or analog data are available, models such as the ECOSAR Class Program may be used to predict toxicity values that can be used to indicate which chemicals may need further testing or characterization.

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1. Introduction

The ECOlogical Structure-Activity Relationship Model (ECOSAR) Class Program is a computerized version of the ecotoxicity analysis procedures as currently practiced by the Office of Pollution Prevention and Toxics (OPPT) when data are lacking for risk assessment development. Quantitative structure-activity relationships (QSARs) have been used by the U.S. Environmental Protection Agency (EPA) since 1981 to predict the aquatic toxicity of new industrial chemicals in the absence of test data. ECOSAR has been developed within the regulatory constraints of the Toxic Substances Control Act (TSCA) and is a pragmatic approach to QSAR as opposed to a theoretical approach.

The QSARs presented in this program were developed by classification/sub-classification of chemicals based on similarity of structure and similarity in measured effect levels from aquatic toxicity data. When available, modes-of-action have been integrated into the classification scheme to substantiate trends seen in available data. Although acute and chronic toxicity of chemicals to fish (both fresh and saltwater), aquatic invertebrates (freshwater daphnids and saltwater mysids), and algae (freshwater green algae and saltwater algae) has been the focus of QSAR development, additional QSARs for certain chemical classes were developed for other organisms (e.g., earthworms, sea urchins, and *Lemna gibba*). QSAR development for chemical classes is based on measured test data submitted by industry under TSCA or collected from publicly available sources. To date, 709 QSARs have been developed for over 111 organic chemical classes, along with additional QSARs for surfactants, polymers, and dyes that can be obtained through the "Special Classes" menu. 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) and have been made public, when permitted, in the ECOSAR Equation Documents located in the 'Help' menu under the help icon,  . For more information on QSAR development, see the ECOSAR Methodology Document within the 'Help' menu.

The ECOSAR program is designed for the expert user. Specifically, users are expected to have some knowledge of environmental toxicology and organic chemistry, and are expected to use this knowledge to determine appropriate classifications when multiple classes are identified for a given chemical. Users are also expected to determine whether the use of ECOSAR to predict aquatic

toxicity is more suitable than an analog approach. ECOSAR is menu-driven and contains various help functions to assist the user. Users cannot change any of the equations or data stored within the program or accidently erase any important information. The following pages show how to install, access, and use the ECOSAR Class Program. If users have any questions or comments on the ECOSAR program, or find any errors, please contact:

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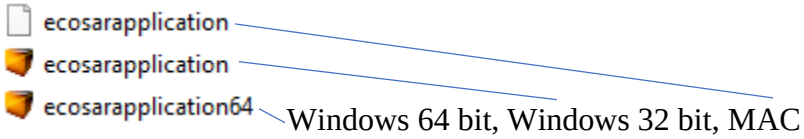
2. Computer-Software Requirements

The ECOSAR Class Program is designed for use on PC and Mac devices running Microsoft Windows (including Vista, 7, and higher), iOS, or OS X. The program may also work for various mobile and Unix platforms; however, it was not specifically optimized for those environments. ECOSAR is designed to run as a multi-tasking program (e.g., running ECOSAR batch-mode runs in the background while running another program in the foreground) and, thus, batch-modes can be run in the background until they are completed.

Although a mouse/touch-pad or other pointing device[1] is not required, it is highly recommended. If a mouse/touch-pad is not available, knowledge of the following keys is necessary: **Enter:** pressing the Enter (Return) key will cause the program to run if sufficient data are entered into the Chemical Input field; and **Tab** or **Shift-Tab:** changes entry fields (see Appendix B, Summary of Function Keys). ECOSAR v2.0 requires approximately 176 MB of hard disk space for the un-zipped package; the executable takes up 1,458, 474, and 3 KB for the 64-bit, 32-bit, and Mac compatible executables, respectively. Included in the package is the Estimation Program Interface (EPI) EPI_Unified Database (a database of over 110,000 Simplified Molecular Input Line Entry System [SMILES] notations indexed by Chemical Abstracts Service [CAS] number for program retrieval), which requires 84,655 KB of disk space.

3. Installing the ECOSAR Class Program

Users can download the ECOSAR Class Program for free from the EPA's website at: http://www.epa.gov/oppt/newchems/tools/21ecosar.htm. ECOSAR is a self-extracting file. Once it is unzipped to the C: drive, desktop, or wherever the user wants to install the program, the user can access the executable one of a few ways. The most direct route is to open the ECOSAR_v2.0 file, where it was unzipped to, and click the "ecosarapplication" folder. From there, select the "ecosar" .bat file, the ECOSAR_v2.0_32 shortcut, or the ECOSAR_v2.0_64 shortcut. If the user would prefer to assign a shortcut of their own, they can go into the "bin" file and select the "ecosarapplication" file compatible with their system (see below).

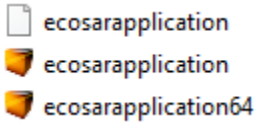


4. Starting the ECOSAR Class Program

The ECOSAR Class Program is started by clicking the "ecosar" .bat file, the ECOSAR_v2.0_32 shortcut, the ECOSAR_v2.0_64 shortcut, or any other shortcut that they have established.

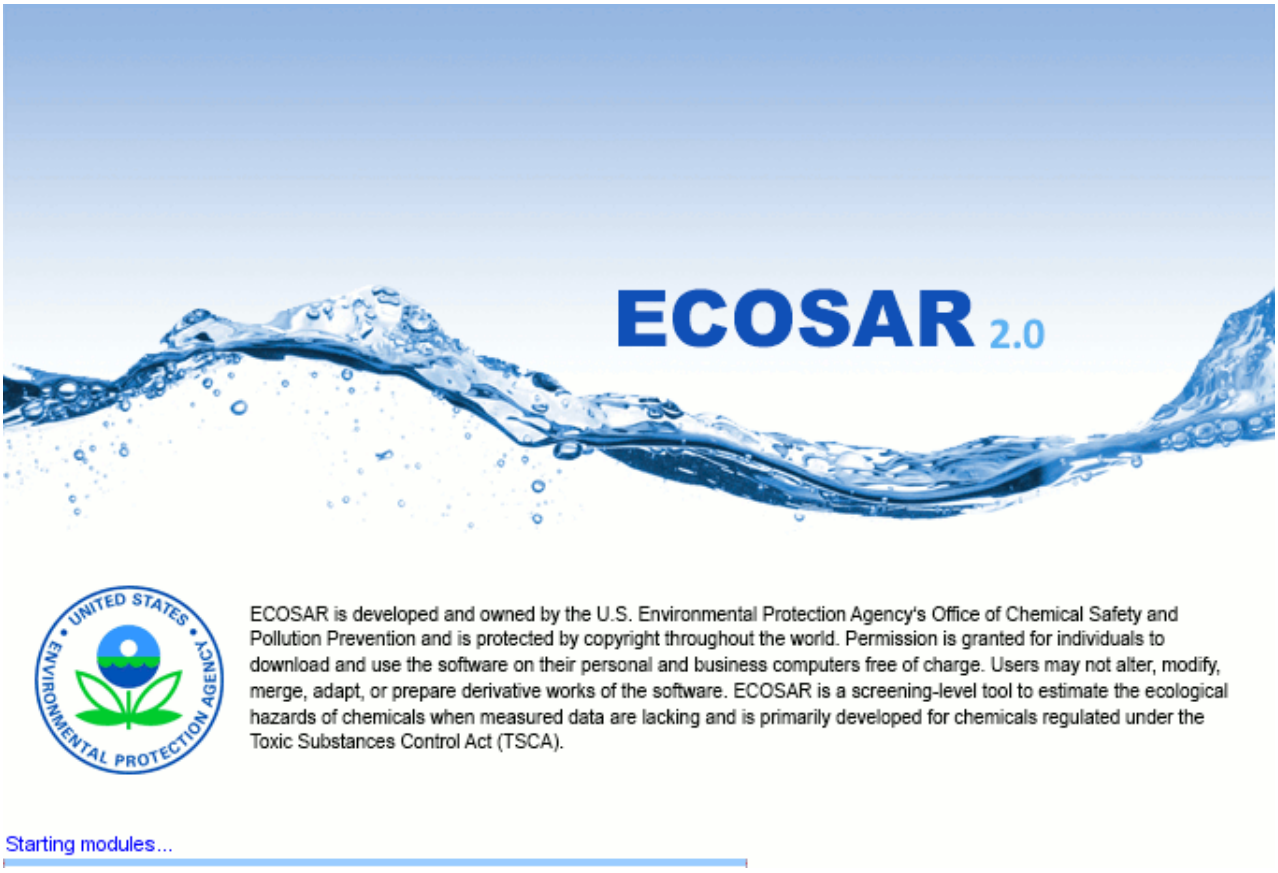
[1] Program developers have not optimized this interface for touch-screens to act as another type of pointing device and, thus, do not know whether the ECOSAR program will function properly on tablet-type devices.

The user can also run this program from the “bin” file by selecting the “ecosarapplication” file compatible with their system, seen previously.

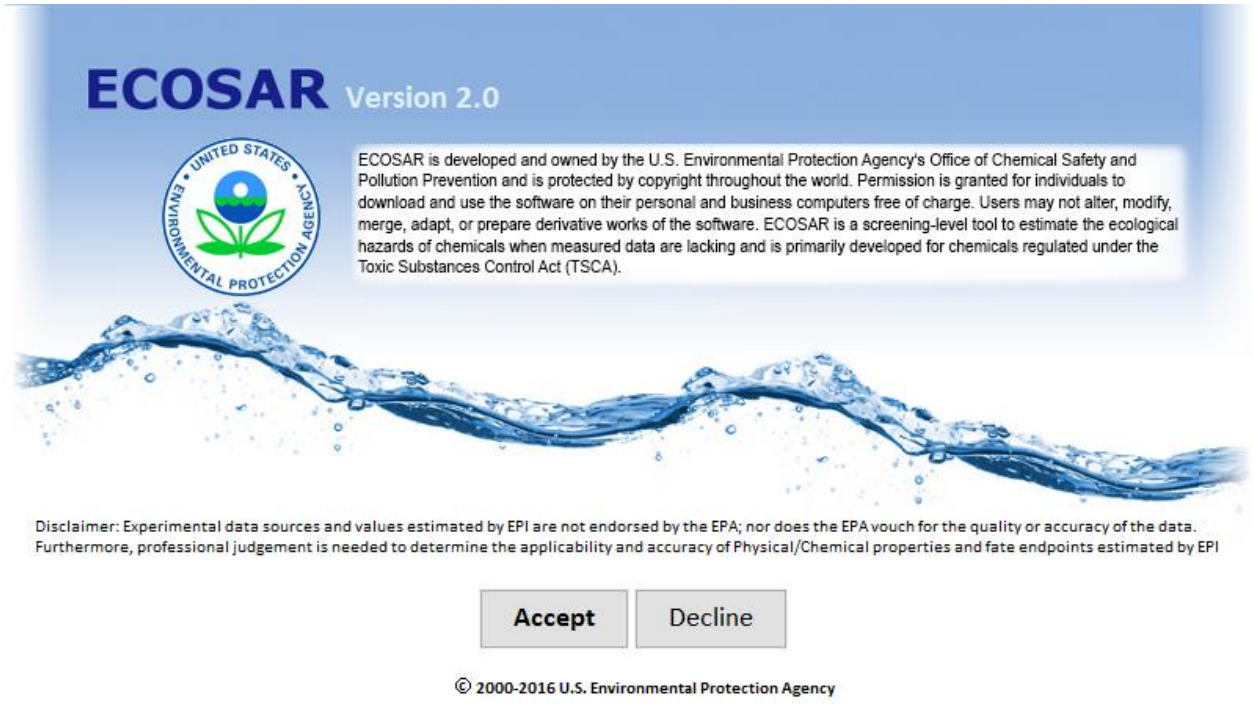


ECOSAR can be accessed through EPI Suite; however, the version associated with EPI Suite may not be the most recent version of ECOSAR. Version information can be obtained from the ‘Help’ menu by selecting ‘About ECOSAR.’ For additional information on starting Windows programs, consult your Windows documentation.

Once the ECOSAR program has been initiated, the following splash screen is displayed:



This will be replaced by the introduction screen where the user is asked to accept or decline the terms of service and the disclaimer.



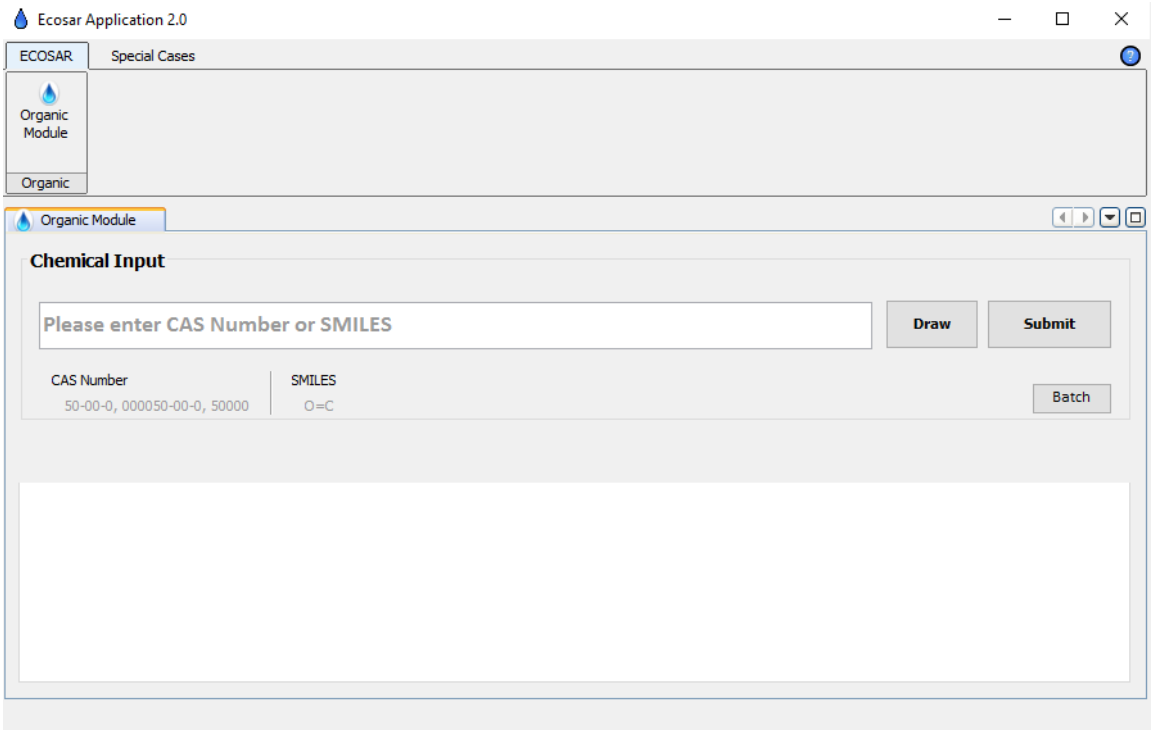
Users should note the language on the introduction screen regarding the need for professional judgment in determining the applicability and accuracy of the ecotoxicity endpoint values estimated by this program. Furthermore, inorganic and organometallic chemical classes (among others as noted in the help file) are currently outside the domain of ECOSAR v2.0. Clicking the 'Decline' button will cause the program to close.

After clicking 'Accept' on the above pictured introduction screen, the data entry screen for the Organic Module is displayed. The user may enter a CAS number, SMILES, or chemical name into the Chemical Input field in order to generate an ECOSAR prediction based on organic QSARs (described in Section 6). The user may also choose to draw a structure by clicking the 'Draw" button (described in Section 5.1.4, Entry Using the Drawing Tool) or enter multiple chemicals via the batch mode by clicking the 'Batch' button (described in Section 7, Batch Runs).

Certain user inputs, described in Section 6, can be entered, if needed, in the output tab for the generated prediction. QSAR predictions for surfactants, dyes, and polymers are generated from a separate data entry screen, which is described in Section 8. Supporting documentation for

ECOSAR can be obtained from the 'Help' menu and includes QSAR class definition and equation sheets (see), the ECOSAR v2.0 User's Guide, the ECOSAR Methodology Document, and tutorials for using the draw tool and for formulating SMILES structures.

Figure 1. Example Data Entry Screen



5. User Inputs for Generation Of ECOSAR Predictions

The information in Section 5 applies to the main data entry screen shown in Figure 1. The user is required to enter one of the three subsequently described data types into the Chemical Input field on the main data entry screen or draw a structure for a single chemical estimation. Data types accepted:

(1) **Enter SMILES:** Field for SMILES notation of the structure to be estimated. A maximum of 360 characters is allowed.

(2) **Enter Name:** Field for the name and/or description of the structure. A maximum of 120 characters is allowed.

(3) **CAS Number:** The CAS number.

5.1. Structure Entry

Calculations in ECOSAR from the main data entry screen require the chemical structure to be interpreted using SMILES notation. Briefly, a SMILES notation depicts a molecular structure as a two-dimensional picture. SMILES notations are comprised of atoms (designated by atomic symbols), bonds, parentheses (used to show branching), and numbers (used to designate ring opening and closing positions). Users unfamiliar with SMILES notations can consult a descriptive journal article (Weininger, 1988), the ECOSAR Class Program help file (accessed by selecting the  icon in the upper right corner of the program), http://www.daylight.com (Daylight Information Services), or http://www.epa.gov/ncct/dsstox/MoreonSMILES.html. An online SMILES translator is available from the National Cancer Institute (NCI) at http://cactus.nci.nih.gov/services/translate/ .

Seven different methods can be used to directly enter or retrieve the SMILES notation into the ECOSAR data entry screen:

(1) Direct entry by the user from the keyboard (see Section 5.1.1 for direct entry of special cases such as salts).
(2) Entry through the structure drawing tool (see Section 5.1.4).
(3) Importing of structures in MDL MOL file formats through the drawing tool (see Section 5.1.5).

The program can usually generate estimates for only one chemical at a time from this main screen and separate data entry is required for each chemical. If a chemical entry (name, SMILES) matches more than one entry, the user will be prompted to select from a menu of available chemical matches. It is possible to select more than one chemical from the disambiguation list, which will result in a multiple chemical analysis (see Figure 3 in Section 5.1.3).

Batch mode runs are possible and are described in detail in Section 7. Once a structure is entered, estimation for the entered structure is started by pressing the "Submit" button or hitting the “Enter” key.

5.1.1. Direct Entry

Direct entry requires knowledge of chemical structure and/or SMILES notation, as described above. For direct entry, a SMILES notation should be typed in the 'Chemical Input' field on the data entry screen (see Figure 1). A SMILES notation is considered terminated at the first blank space. Characters following the first blank space are ignored. Methodology for entering certain compounds is highlighted below.

(1) Organic anionic salts (e.g., haloacids conjugated with Na, K, Li): When entering an organic anionic salt, the conjugate cation (e.g., sodium, potassium, and lithium) should be surrounded with brackets (so, Na should be entered with brackets to be [Na]). ECOSAR will not run and will output an alert if a SMILES is entered incorrectly. If more than one salt form exists in the database, the disambiguation list will prompt the user to specify.

(2) Organic ammonium salts: Like the organic anionic salts described above, the conjugate salt (e.g., chloride ion) of organic ammonium salts can be directly entered. They can also be entered as amines, because at a neutral pH, ammonium moieties are reduced (e.g., loss of two hydrogens) to amines. Since QSARs are developed from test data using neutralized test solutions to replicate environmental conditions, ammonium salts should be entered into ECOSAR in the reduced amine form. Quaternary ammonium compounds (four carbons are bound to the nitrogen) are an exception since reduction (e.g., loss of hydrogens) at neutral pH is unlikely; however, these are classified as surfactants.

(3) Inorganics: If metals and some elements are included in entered SMILES structures, the user will receive a warning that the chemical should not be profiled. All metals should be bracketed in order to be considered correctly entered (e.g., [Zn], [Fe], [Ca]). Non-carbon elements considered in organic QSARs include oxygen, phosphate, sulfur, nitrogen, silicon, fluorine, bromine, chlorine, iodine, and hydrogen. Direct hydrogen entry in a SMILES notation is unnecessary for ECOSAR v2.0.

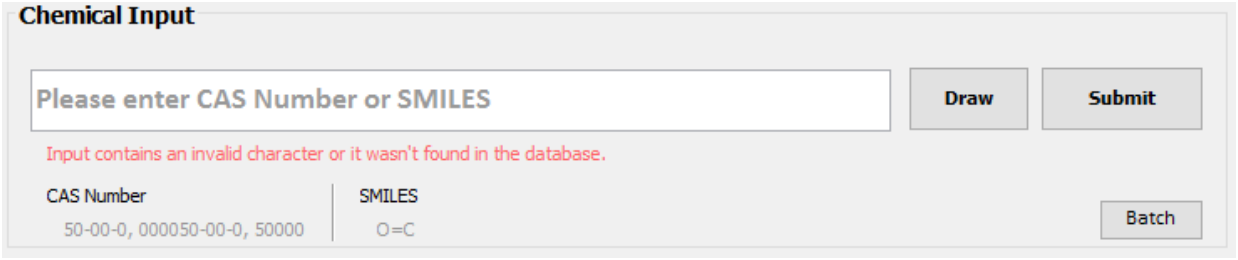
(4) Charged Chemicals: Charged species (e.g., [+] and [-] signs) can be entered directly into a SMILES notation for charged chemicals, but must also be surrounded by brackets. Azido compounds (commonly written as: $N^{+}=N^{-}$) must be written as '[N+]=[N-]' or can

be depicted as ‘N#N’ for the purposes of SMILES notation. Nitro compounds (commonly written as N^{+} (O^{-})=O) are written as ‘[N+][O-]=O’, as ‘N(=O)=O’, or ‘T’ for the purposes of SMILES notation.

5.1.2. CAS Number Entry

In previous versions, CAS numbers were retrieved through the “CAS Number Database” by selecting the ‘CAS Input’ button beneath the CAS number field on the data entry screen. This is not necessary in ECOSAR v2.0. SMILES, CAS number, and Chemical name are entered into the ‘Chemical Input’ entry field. If the CAS number is incorrect or invalid, an alert will appear below the “Chemical Input” field (see Figure 2). The program accepts CAS numbers with or without hyphens. The user should check that the structure output matches the entered CAS number.

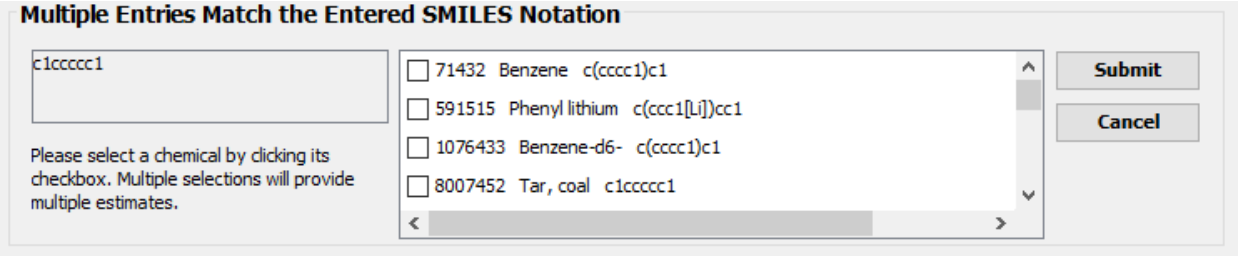
Figure 2. Entry Screen Error



5.1.3. Chemical Name Entry

In previous versions, SMILES notations were retrieved from the Name Look-Up Database. This is not necessary in ECOSAR v2.0. SMILES, CAS number, and Chemical name are entered into the ‘Chemical Input’ entry field. If the Chemical name is incorrect or invalid, an alert will appear below the “Chemical Input” field (see Figure 2). The program accepts chemical names regardless of capitalization. If more than one entry matches the chemical name, the disambiguation list will appear (see Figure 3). The user should check that the structure matches the chemical name entered.

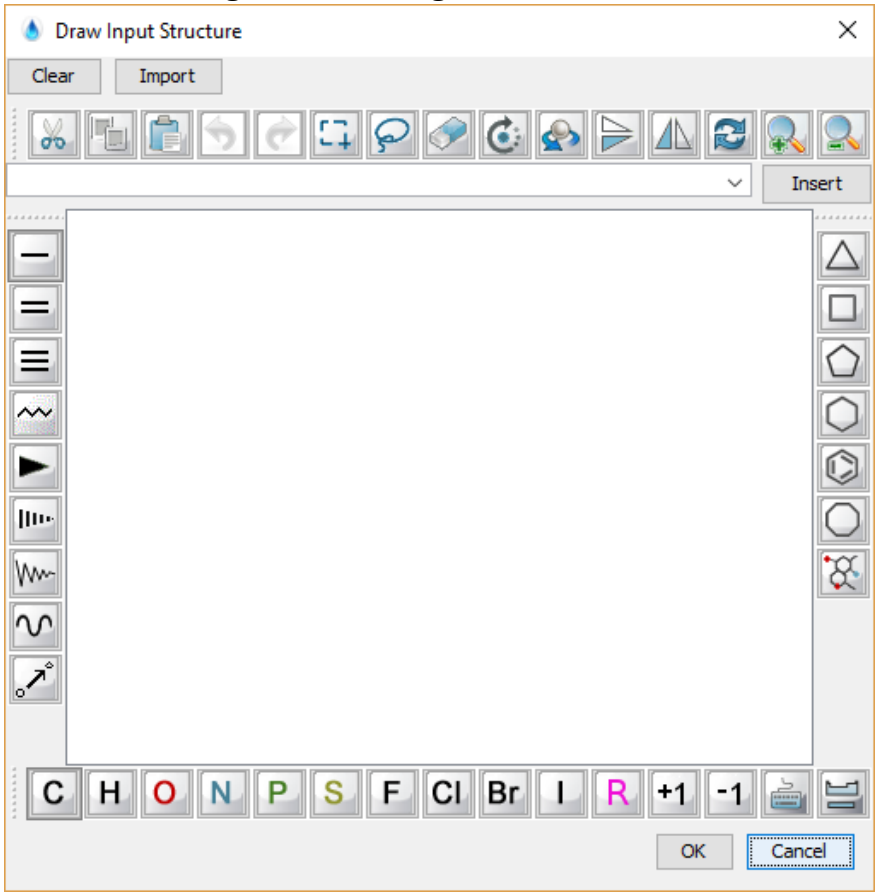
Figure 3. Disambiguation List



5.1.4. Entry Using The Drawing Tool

ECOSAR v2.0 now leverages the third-party package, jchempaint, for its structure drawing module that allows users to draw chemical structures and modify structures to generate SMILES notations for direct entry into the ECOSAR program. To initiate the module, select the 'Draw' button on the main data entry screen. The Blank Drawing Module Window is shown in Figure 4.

Figure 4. Drawing Module Window

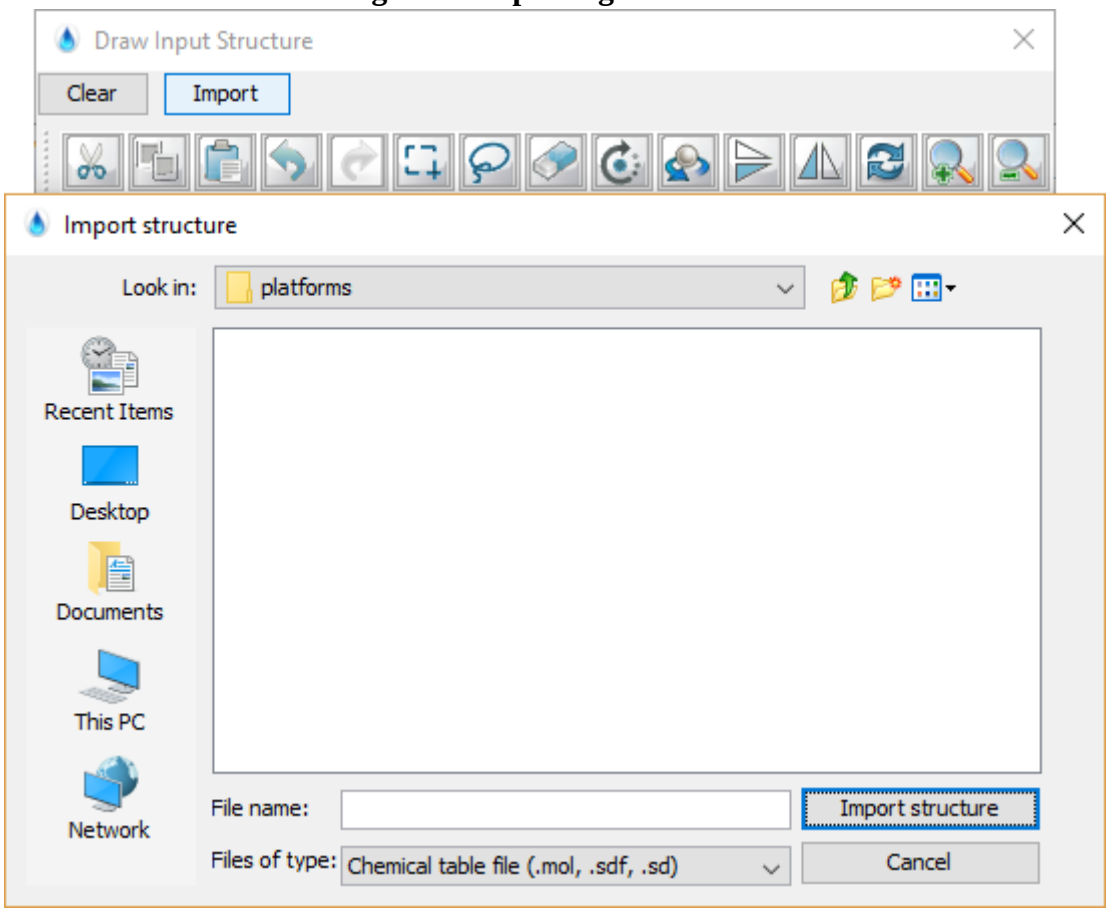


A tutorial is provided in the ‘Help’ menu for drawing structures with the Drawing Module (select Draw Structure Help). Once a structure is entered, the ‘OK’ button should be selected. The corresponding SMILES structure of the depicted chemical will be transferred to the ‘Chemical Input” field on the Data Entry screen and the drawing window will be shut. Pressing the ‘Cancel’ Button exits the Drawing Module with no transfer of SMILES and closes the Drawing Module.

5.1.5. Importing Structures

ECOSAR v2.0 has an "import" features that allows MDL MOL file formats to be imported directly into ECOSAR. The "import" feature is accessed from the ‘Drawing Module Window’ via the ‘Import’ button shown in Figures 4 and 5.

Figure 5. Importing Structures

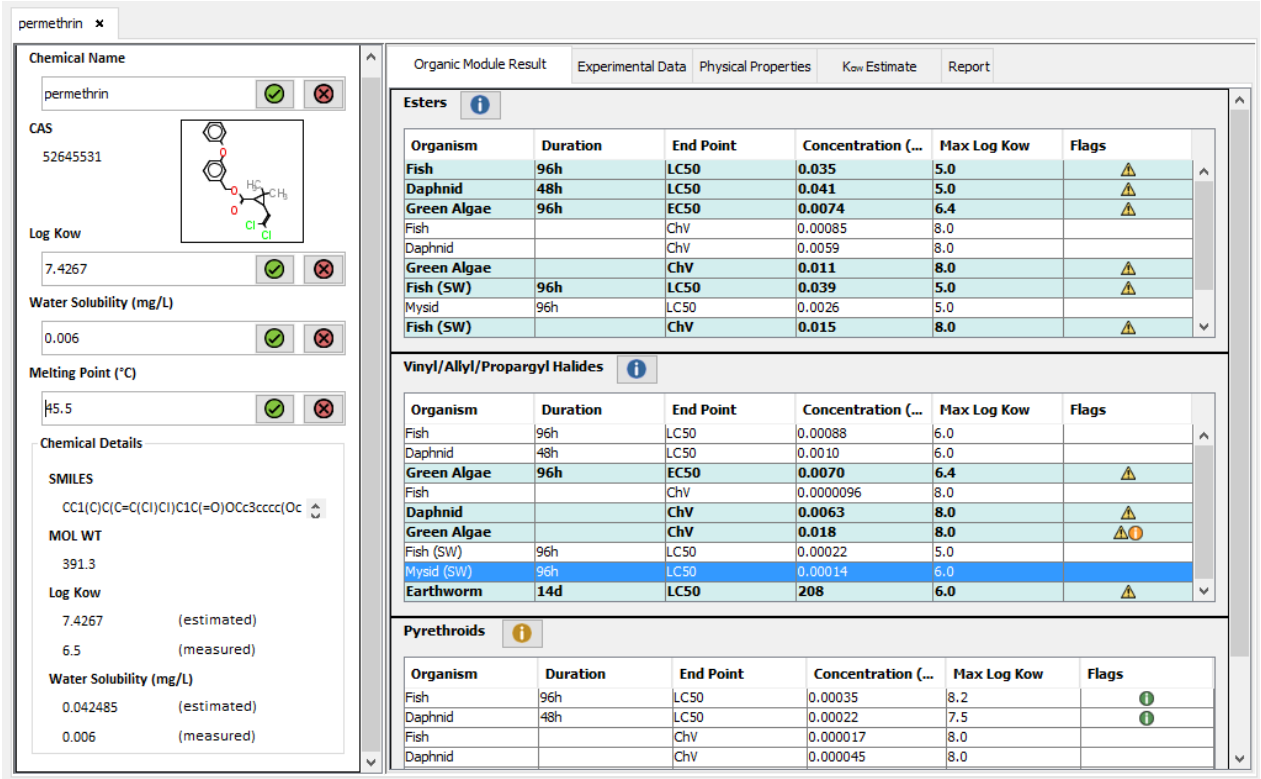


Imported structures are converted to SMILES notations and placed in the ‘Chemical Input’ entry field. ECOSAR filters the conversion to make the SMILES notation as compatible as possible with ECOSAR. However, some converted SMILES notations (especially SMILES with charged ions) will require some user modification before ECOSAR can estimate the structure.

6. Results Window

Generally, the Results Window (see Figures 6 and 7) provides the chemical structure (clicking on the small picture of the chemical structure opens a separate window with an Expanded Structure View), chemical attributes, measured training set data used in QSAR development for the query chemical, results of ECOSAR Class Program's estimations, and information specific to the interpretation of the QSAR results. Each of these is described in subsequent sections.

Figure 6. Results Window Panels



Organic Module Result | Experimental Data | Physical Properties | Kow Estimate | Report

Organism	Duration	End Point	Concentration (mg/L)	Reference
Daphnid	48h	LC50	4.0E-5	OPP Pesticides Ecotoxicit...
Daphnid		ChV	6.0E-5	OPP Pesticides Ecotoxicit...
Mysid	96h	LC50	7.0E-5	OPP Pesticides Ecotoxicit...
Daphnid	48h	LC50	6.0E-4	OPP Pesticides Ecotoxicit...
Fish	96h	LC50	0.00252	OPP Pesticides Ecotoxicit...
Fish	96h	LC50	0.0061	OPP Pesticides Ecotoxicit...
Daphnid	48h	LC50	0.0072	OPP Pesticides Ecotoxicit...
Fish	96h	LC50	0.0098	OPP Pesticides Ecotoxicit...
Fish	96h	LC50	0.01	OPP Pesticides Ecotoxicit...
Green Algae (SW)	72/96h	EC50	0.06	Walsh et al., 1980; EC50...
Green Algae (SW)	72/96h	EC50	0.09	OPP Pesticides Ecotoxicit...

Organic Module Result | Experimental Data | Physical Properties | Kow Estimate | Report

Water Solubility	
Water Solubility (mg/L)	6.00E-03
Water Solubility Temperature (C)	20
Water Solubility Type	EXP
Water Solubility References	USDA PESTICIDE PROPERTIES DATABASE

Log Kow	
Log Kow	6.50
Log Kow Temperature (C)	
Log Kow Type	EXP
Log Kow References	HANSCH,C ET AL. (1995)

Vapor Pressure	
Vapor Pressure (mmHg)	2.18E-08
Vapor Pressure Temperature (C)	25
Vapor Pressure Type	EXP
Vapor Pressure References	USDA PESTICIDE PROPERTIES DATABASE

pKa	
pKa	
pKa Temperature (C)	
pKa Type	
pKa References	

Henry's Law Constant	
Henry Law Constant ((atm*m^3)/mol)	1.87E-06
Henry Law Constant Temperature (C)	25
Henry Law Constant Type	EST
Henry Law Constant References	VP/WSOL

Organic Module Result | Experimental Data | Physical Properties | Kow Estimate | Report

Value	Value Type	Number	Coefficient	Total Coefficient
Fragment	-CH3 [aliphatic car...	2	0.547	1.095
Fragment	-CH2- [aliphatic car...	1	0.491	0.491
Fragment	-CH [aliphatic carb...	2	0.361	0.723
Fragment	=CH- or =C< [olefinc ...	2	0.384	0.767
Fragment	-CL [chlorine, olefi...	2	0.492	0.985
Fragment	Aromatic Carbon ...	12	0.294	3.528
Fragment	-O- [aliphatic O, tw...	1	0.292	0.292
Fragment	-C(=O)O [ester, aliph...	1	-0.951	-0.951
Fragment	-tert Carbon [3 or m...	1	0.268	0.268
Constant	Equation Constant ...	0	0	0.229

Notes

Log Kow

7.427

Figure 7. User Entry Panel

Chemical Name

permethrin

CAS

52645531

Log Kow

7.4267

Water Solubility (mg/L)

0.006

Melting Point (°C)

45.5

Chemical Details

SMILES

CC1(C)C(C=C(Cl)Cl)C1C(=O)OCc3cccc(Oc

MOL WT

391.3

Log Kow

7.4267	(estimated)
6.5	(measured)

Water Solubility (mg/L)

0.042485	(estimated)
0.006	(measured)

The Results Panel can simultaneously hold the output of multiple different evaluations in separate output tabs, which can be closed or re-ordered within the panel. Each tab does not need to be removed or closed before running another chemical in the program; the Results Panel will be updated automatically. Additionally, the left side of each output tab is occupied by the User Entry Panel (see Figure 7).

The following behaviors are available in the Results tab:

Copy: All tables can be copied and pasted from any of the subtabs by first selecting all the items in the table that the user wishes to copy, and then using the keyboard shortcut to copy the data (Ctrl + c in Windows, Command + c MAC). This command copies the results as shown (minus the rectangle enclosing the estimate) to the Windows clipboard. The table can then be pasted wherever the user would like to paste it either using the 'Paste' function in a spreadsheet, word processing, or other program, or using the keyboard shortcut (Ctrl + v Windows, Command-V MAC).

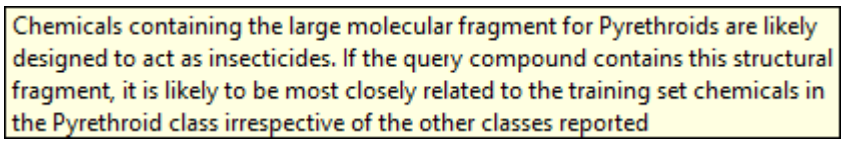
Close tab: All results tabs can be closed by clicking the small 'x' in the upper right corner of the tab, just to the right of the tab name.

6.1. Output Tabs

The Results Window Panels hold the following subtabs in the for the respective chemical (see Figure 6).

(1) Organic Module Result: This subtab holds the results of ECOSAR Class Program's estimations. This includes the class-specific Max Log K_{ow} cutoff as well as any flags or alerts associated with the values produced. Flags or alerts include: No Effect at Saturation ⚠, Acute to chronic ratio estimation used, and inexact Log K_{ow} cutoff. Additionally, Class definitions are available from the information buttons to the right of the class name .

Yellow information buttons indicate classes of special toxicological significance  . Mouse over the button for more information regarding these classes.



(2) Experimental Data: This subtab holds the measured training set data used in QSAR development for the query chemical, when available. If measured data used in the training set are considered to be TSCA confidential business information (CBI), these data are not shown in this section. Since data collection for the ECOSAR program began in the early 1980s, some data incorporated into ECOSAR came from data sheets that did not clearly identify the reference; however, extensive efforts have identified most references.

(3) Physical Properties: This subtab holds the physical-chemical properties for the evaluated molecule when available from the Physical Properties (PhysProp) Database.

(4) K_{ow} Estimate: This subtab shows the values used to arrive at the K_{ow} Estimate used in the calculation of the ECOSAR Class Program's toxicity endpoint estimations. This value is preferentially used in the estimated effect levels and can be changed by changes to the left User Entry Panel (see Figure 7).

6.2. Chemical Attributes

The Results Tab provides the attributes of the query chemical (see Figure 7). The SMILES structure used to predict the effect levels is depicted next to the corresponding CAS number.

The field with the molecular weight ('MOL WT') is determined from the SMILES notation used for ECOSAR predictions. Chemical Name and CAS number ('CAS') appear first with a picture of the structure. The Chemical Name field is editable.

Log K_{ow}, Water Solubility, and Melting Point are all editable. Chemical Details holds the SMILES, Molecular Weight ('MOL WT'), and estimated and measured values available for Log K_{ow} and Water Solubility. Log K_{ow} preferentially relies on the estimated value, whereas Water Solubility incorporates the measured value from PhysProp when available. For water solubility, units of

measurement are always milligrams per liter (mg/L). If the user enters a melting point, the value will display next to the 'Melting Point' field; otherwise, the field will import a value from PhysProp, when available.

6.2.1. Entering Measured Data for Variables

In previous versions of ECOSAR, User Entered Variables were entered on the main entry screen. They are now handled in the left input panel post-estimation in ECOSAR v2.0. Entering Values causes the estimations to be recalculated immediately and enables users to provide data for certain parameters that will be used in place of ECOSAR-predicted variables and/or values retrieved from PhysProp. These variables are the log K_{ow} value, the water solubility value, and the melting point value (see Figure 7).

6.2.2. Log K_{ow}

The log K_{ow} value is used in the calculation of the predicted effect level. If a user enters a log K_{ow} value in the User Entry Panel, that value will be updated immediately. In the absence of a user-entered log K_{ow} value, ECOSAR will automatically use the log K_{ow} value calculated from the KOWWIN program (available from EPI Suite). The variables used in calculating the estimated log K_{ow} can be found in the K_{ow} Estimate subtab in the Result tab. To minimize the potential effects of variability of measured log K_{ow} values due to factors such as variable study conditions, QSARs were developed from predicted log K_{ow} values, and it is often recommended that the predicted K_{ow} values (calculated and used by default in ECOSAR) be used in the model when there is uncertainty in the reliability of the available measured K_{ow} values (see the ECOSAR Methodology Document for further information).

6.2.3. Water Solubility

Water solubility values (mg/L) are compared with predicted effect levels in order to identify effect levels that exceed the limit of water solubility. If a user enters a water solubility value in the User Entry Panel, that value will be used to determine if the predicted effect level exceeds the water solubility. In the absence of a user-entered water solubility value, ECOSAR will use the measured

water solubility value retrieved from the Physprop Experimental Database (http://www.syrres.com/what-we-do/product.aspx?id=133). If no measured water solubility values are available, ECOSAR will calculate the water solubility value for all query compounds using the WSKOWWIN program available from EPI Suite. If available, a user-entered melting point (°C) will be used to calculate water solubility; otherwise, water solubility will be calculated without use of a melting point. Further details on the calculation of water solubility with WSKOWWIN are available from the help menu of EPI Suite and in the following document prepared for EPA (OPPT): Upgrade of PCGEMS Water Solubility Estimation Method (May 1994).

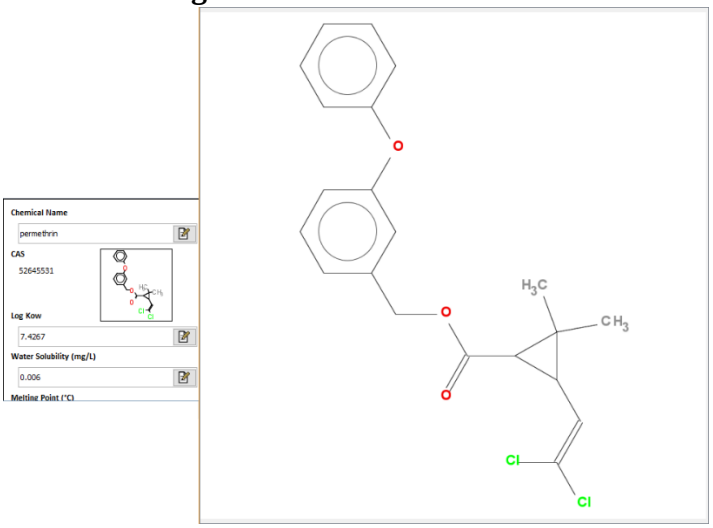
6.2.4. Melting Point

A user-entered melting point can be entered in the User Entry Panel. A user-entered melting point is used only in the calculation of water solubility. If a melting point is not entered, then the alternative method for water solubility calculation without a melting point value is used automatically by WSKOWWIN.

6.3. Structure Window

The Structure Window (see Figure 8) shows a 2-dimensional picture of the chemical structure. This can be found in the User Entry Panel. Clicking on the small picture of the chemical structure opens a separate window with an Expanded Structure View. The window shows the entire structure (it does not "clip" sections of the molecule). Some particularly large molecules (e.g., Vancomycin) have a hard time rendering in the Expanded Structure View. At times, the height or width of the window may need to be changed to give a better structure depiction. When results from the Results tab are printed, the accompanying structure is that of the smaller picture seen in the User Entry Panel (see Figure 7).

Figure 8. Structure Window



6.4. Experimental Data Tab

Training set data used to develop regression equations for QSAR class endpoints are presented in the training set data section of the results page (see Figure 6).

Data are not presented in any particular order. If measured data used in the training set are considered to be TSCA CBI, these data are not shown in this section. Since data collection for the ECOSAR program began in the early 1980s, some data incorporated into ECOSAR came from data sheets that did not clearly identify the reference; however, extensive efforts have identified most references. Data considered ‘supplemental’ or ‘adequate with restrictions’ may have been included in some training sets in the absence of better data. See the Technical Reference Manual (also referred to as the ECOSAR Methodology Document) for further information on data collection and selection methods.

6.5. Organic Module Result Tab

The mode of toxic action for most neutral organic chemicals is narcosis, and many types of chemical classes present toxicity to organisms via narcosis (i.e., ethers, alcohols, ketones). However, some organic chemical classes have been identified as having a more specific mode-of-toxicity. These are typically organics that are reactive and/or ionizable and exhibit excess

toxicity in addition to narcosis (i.e., acrylates, epoxides, anilines). In this version, the neutral organics ("baseline toxicity") class QSAR results do not appear unless it is the only class available for estimation. The baseline toxicity equations in the prior version ECOSAR v1.1 and in the current version ECOSAR v2.0 are based on data collected through 2010. (Please note that baseline toxicity equations in the previous ECOSAR v1.0 were based on data collected through 1999 and ECOSAR v0.99 was calculated from the 1981 Konemann Equation).

Two common notations reported for predicted effect levels are (⚠) used to designate predicted effect levels that exceed the water solubility limit and an exclamation (❗) used to designate effects levels predicted using an acute-to-chronic (ACR) ratio.

In the tables displaying the reported predicted effect levels, there is a column that indicates the maximum log K_{ow} cut-offs for each class ('Max Log Kow'). The K_{ow} cut-off values signify the point that a chemical is no longer particularly soluble and not likely to result in toxicity to the organism for the given duration. For chemicals with log K_{ow} values that exceed the limits, results are typically reported as "No Effects at Saturation" ⚠.

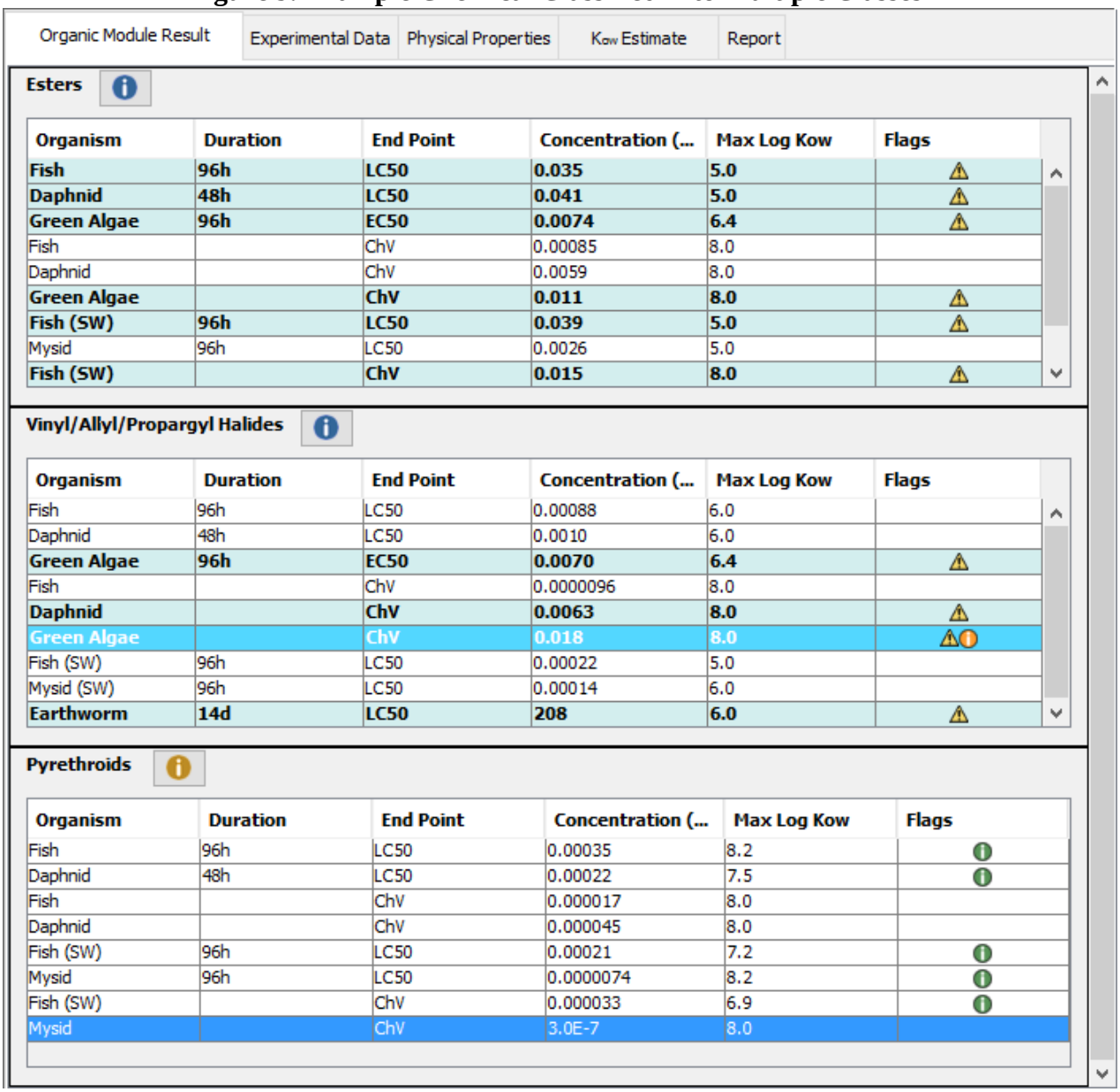
In general, log K_{ow} cut-offs are 5 for fish and daphnid acute endpoints, 6.4 for green algae 72/96-hour EC_{50} endpoints, and 8 for all chronic endpoints. However, when available training set data are more robust, attempts have been made to tailor log K_{ow} cut-offs to the specific QSAR. In some cases, log K_{ow} cut-offs have been depicted with a greater than sign (>). This cannot be rendered in the table format, so any place where the cutoff would be greater than the indicated number, the flag ℹ is used. This indicates that available data exhibited toxicity for class members having log K_{ow} values above the K_{ow} limit, but within the limit of water solubility. See the Technical Reference Manual (also referred to as the ECOSAR Methodology Document) for further discussion of log K_{ow} cut-offs.

6.6. Comprehensive Approach for Determining the Most Representative QSAR Class

The ECOSAR program may provide results for multiple classes if the entered structure contains the defined base-structure from each of those classes identified in the ECOSAR class definition

sheets. Figure 9 presents an example of a compound that fits into multiple chemical classes. The predictions section of the output is depicted in Figure 10 and described in the 'Selecting the Most Representative Class' section (see Section 6.6.4).

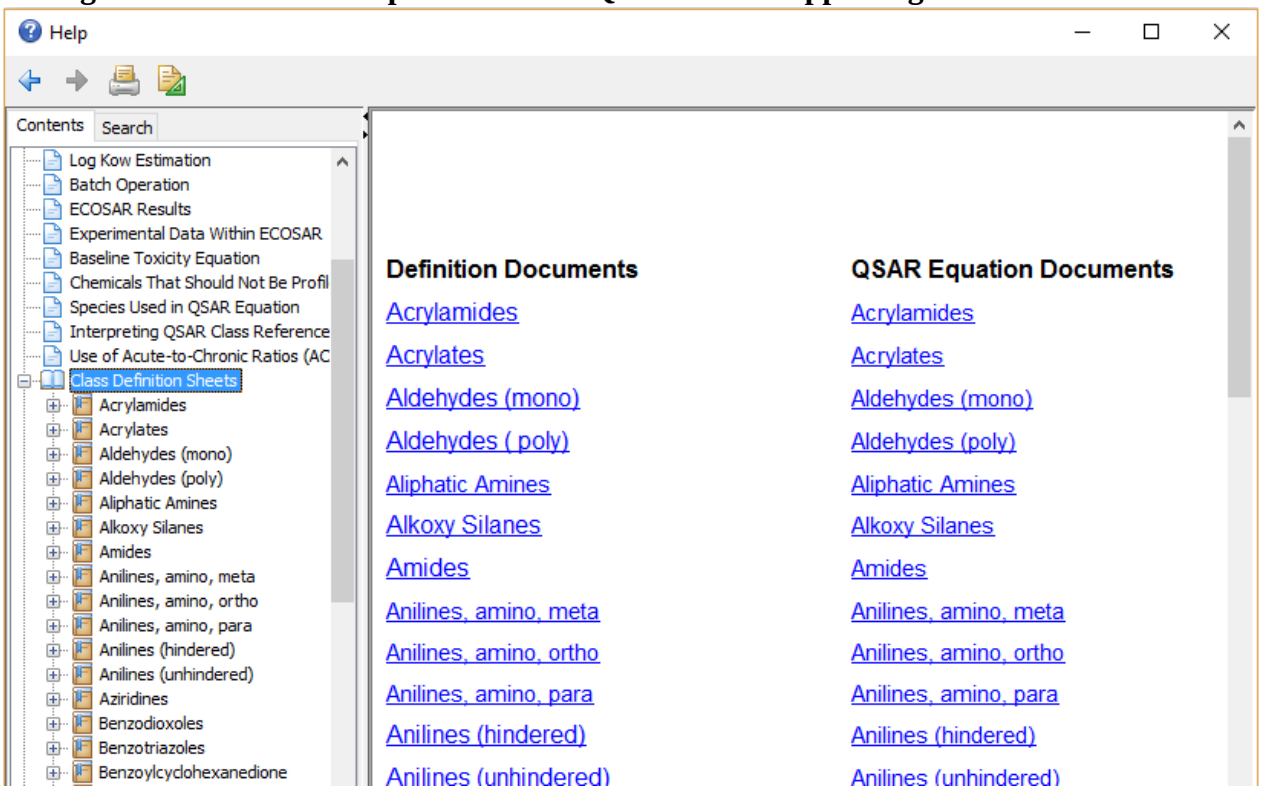
Figure 9. Example Chemical Classified Into Multiple Classes



When the program identifies multiple classes, the user must determine the most suitable class for estimating toxicity using knowledge of environmental toxicology, organic chemistry, and statistics. If available, measured data should be used over predicted data as long as the measured

data are considered adequate (determination of study adequacy is the responsibility of the user). Additionally, the Help menu ( ) contains 'Class Supporting Information' that includes definitions and equation documents for all QSARs (Figure 10). These documents enable the user to evaluate adequacy of predictions.

Figure 10. ECOSAR Help Screen With QSAR Class Supporting Information Shown



In the absence of adequate measured data, the traditional approach has been to use the most conservative effect level from any of the multiple classes listed. However, this is not always the best approach. The methods described in sections 6.6.1 through 6.6.4 provide useful guidance for eliminating classes that are not truly representative of the query compound or classes with insufficient data to fully support a regression equation. Considerations for class determination are described below and are correlated with the above example.

6.6.1. General Classes 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., haloimides). 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 in Figure 8, permethrin is identified as an ester, vinyl/allyl/propargyl halide, and pyrethroid.

Sub-classifications are created in ECOSAR when compounds with larger, more complex structural moieties (pyrethroids) are identified that exhibit toxicity levels that are unlike estimates for the more general classes (esters, vinyl/allyl/propargyl halides), even though those complex compounds may still contain those simple molecular features. In the example depicted in Figure 8, the general classes identified for permethrin would be esters and vinyl/allyl/ propargyl halides (relating to smaller functional groups contained within permethrin). The more specific sub-class is pyrethroids, which define a much larger part of the permethrin molecule. The first step to identifying the optimum prediction would be to compare the chemical class definition with the structural features of the query compound, permethrin. Figure 10 shows how to access the QSAR class supporting information that enables the user to assess structural features of the query compound. The user needs to determine how many molecular features of permethrin fit each class definition and whether that class is the most specific available in ECOSAR for the query chemical. The following is EPA's interpretation of the ECOSAR output for the example depicted in Figure 8.

(1) Esters: Permethrin unequivocally fits the esters definition, but this is a general class that addresses only one of the structural features of this compound.
(2) Vinyl/Allyl/Propargyl Halides: The vinyl/allyl/propargyl halide in permethrin is part of a terminal vinyl/allyl moiety. There have 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 permethrin should or should not be excluded from this class.
(3) Pyrethroids: Permethrin unequivocally fits the pyrethroids definition and literature resources consistently identify the compound as a pyrethroid pesticide for which the

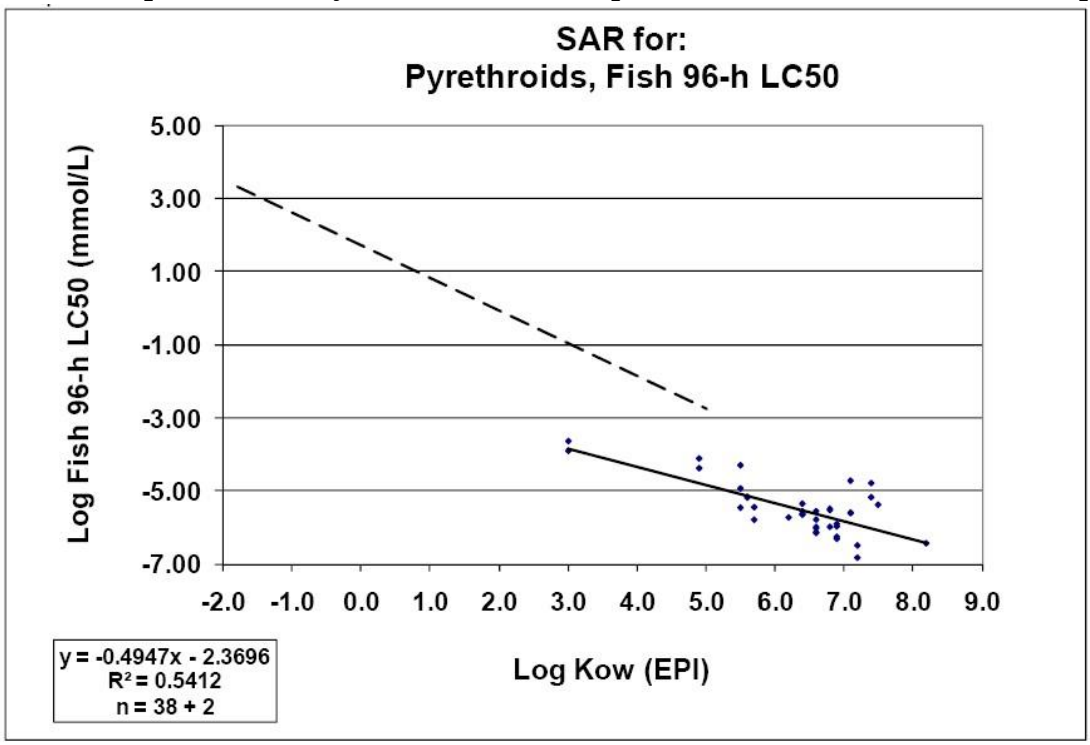
class is modeled. The structure features of the pyrethroid class are also more specific to the structural features of permethrin than to the features of the esters QSAR class.

The next step involves looking at the equation documents to determine the quality of the QSARs (see Sections 6.6.2 and 6.6.3).

6.6.2. Correlation of K_{ow} with Toxicity in QSAR Training Data Sets

For each developed QSAR, a graph (see Figure 11) is displayed in the ECOSAR Equation Document (see Figure 18 for access QSAR Supporting Documents including ECOSAR Equation Documents) along with a table of supporting data.

Figure 11. Graph from the Pyrethroids QSAR Equation Document for the F96 Endpoint



A coefficient of determination (r^2) is reported in both the depicted graph (a scatterplot) and the text within the ECOSAR Equation Documents. The coefficient of determination is a numeric representation of how much variation in one variable is directly related to the variation in another variable (e.g., endpoint effect level [mmol/L] vs. log K_{ow}). A correlation coefficient can be determined by taking the square root of the presented coefficient of determination. Users should consult the ECOSAR Equation Documents of identified classes to quantitatively determine correlation of variables within training data sets based on the coefficient 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.[2] However, a weak relationship does not necessarily indicate little or no correlation; if adequate data were scarce 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.

For simplicity, significance of the relationship of effect levels (mmol/L) vs. log K_{ow} values for each identified class will be determined for the fish 96-hour LC_{50} endpoint only.

(1) Esters: Pearson's Correlation Coefficient (r) is -0.88. Using 5% uncertainty (p = 0.05), the correlation between the F96-hour LC_{50} value (mmol/L) and the log K_{ow} value is statistically significant.

(2) Vinyl/Allyl/Propargyl Halide: - Pearson's Correlation Coefficient (r) is 0.30. Using 5% uncertainty (p = 0.05), the correlation between the F96-hour LC_{50} value (mmol/L) and the log K_{ow} value is not statistically significant.

(3) Pyrethroids: Pearson's Correlation Coefficient (r) is -0.74. Using 5% uncertainty (p = 0.05), the correlation between the F96-hour LC_{50} value (mmol/L) and the log K_{ow} value is statistically significant.

[2] 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).

6.6.3. Robustness and Distribution of Supporting Datasets for 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 classification or sub-classification 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 K_{ow} values of the available data for a given training set, the log K_{ow} value of the queried compound may be notably less than or greater than the minimum and maximum log K_{ow} values of the training set. Sometimes, data are distributed so that the regression line overlaps or crosses over the depicted neutral organic line (dashed line, see Figure 11), 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 ECOSAR Equation Documents of identified classes to visually determine correlation from the depicted graphs of each endpoint. For the above permethrin example, the following can be interpreted from the ECOSAR equation sheets.

(1) Esters: This QSAR 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.

(2) Vinyl/Allyl/Propargyl Halides: This QSAR may be used to estimate toxicity for vinyl/allyl/propargyl halides. The training set for this class contains 21 different chemicals in the training set.

(3) Pyrethroids: The log K_{ow} values for data points that are within the solubility limit range from 3 to 8.2. Thus, if the log K_{ow} 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 then the esters QSAR class.

6.6.4. Selecting the Most Representative Class

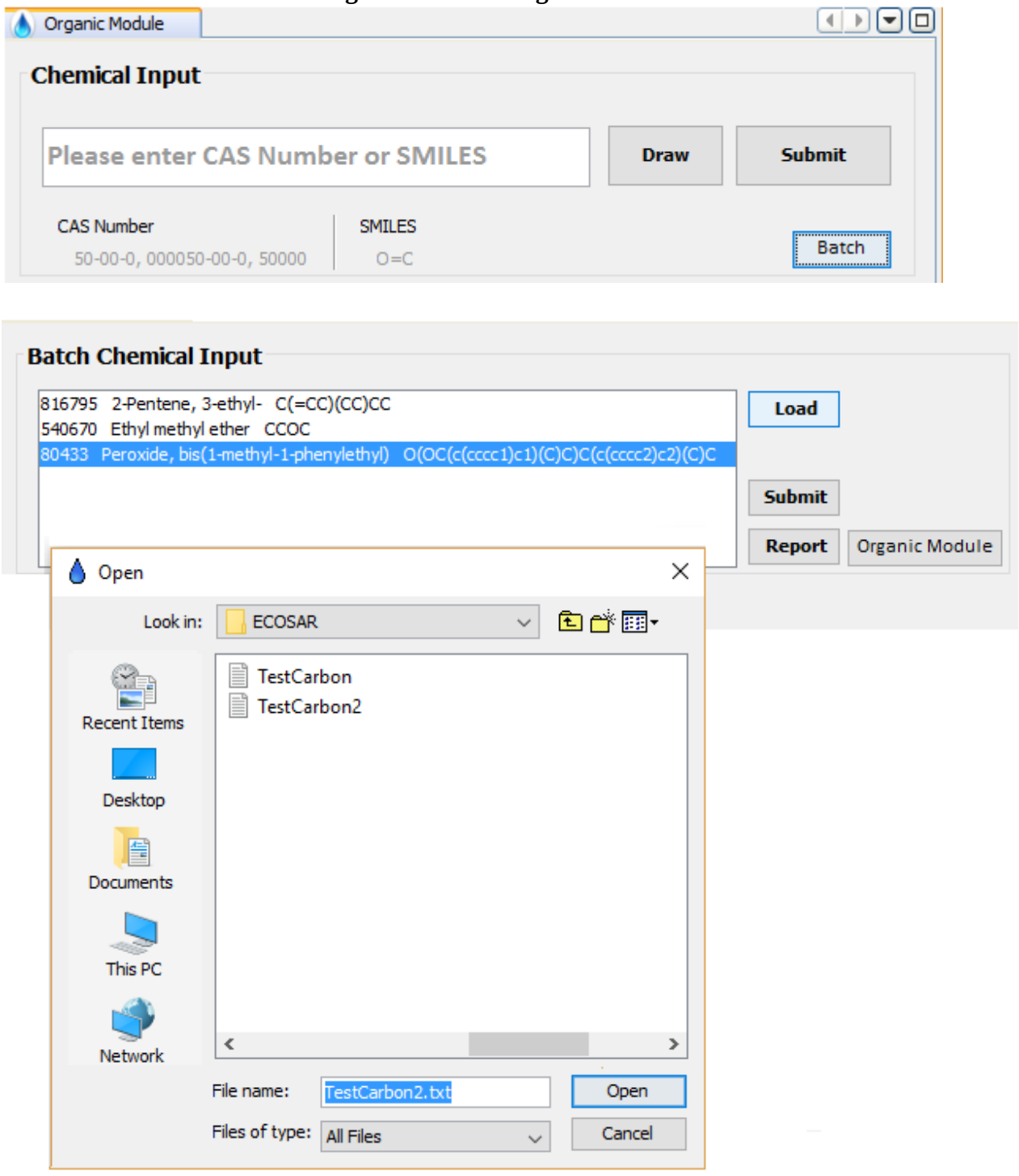
Traditionally, in the absence of adequate measured data, the most conservative effect level is used when predictions are identified from multiple classes. The methods described in Sections 6.6.1 through 6.6.3 are useful for identifying classes that are not representative of the query compound or classes with insufficient data to fully support the regression equation. In the above permethrin example output (see Figure 8), available information from Class Equation and Definition Documents could support a user's decision to exclude the esters and vinyl/allyl/propargyl halides predictions. The remaining class, pyrethroids, appears to be the most representative for the query compound and also results in the most conservative effect levels (see Figure 8).

There is no one standard method for selecting the most representative predictions and, often, the best approach would be to select the most conservative effect level until measured data become available. The model developers emphasize that each predicted profile should be accompanied with a discussion on the appropriateness of the estimates and a description of the identified uncertainties. The complexity of the discussion will vary depending on the expertise of the user and the significance of the chemical management decision being made.

7. Batch Runs

Batch runs are used to make multiple estimates from a single input file that contains multiple chemical identifiers. The ECOSAR Class Program can accept "batch inputs" from four different data types in input files (SMILES Strings, CAS numbers, chemical name, and MDL SD files) and can output the data in two different formats (standard text output as a single run report for specifically selected chemicals, and as an Excel file). Each input file must be in a specific format; otherwise, the batch run will fail. Program access to "batch-runs" (depicted in Figure 12) is available from the main Input Panel by pressing the 'Batch" button.

Figure 12. Accessing Batch Mode



7.1. Batch Input Files

Batch run inputs are initialized by pressing the ‘Load’ button which allows the user to upload external files. These files can include SMILES strings, CAS numbers, chemical names, and MDL SD files. The configuration of these files is described below.

7.1.1. SMILES, CAS, and Chemical Name Strings

Batch runs can be carried out with properly formatted input files. This is defined as a string format list in a plain text file (usually with a ".txt" file extension) containing a list of SMILES notations, CAS numbers, or chemical names. An example String Format follows:

```
CCCCO  Butanol c1ccccc1  Benzene
Fc1ccccc1  Fluorobenzene
CC(=O)C  Acetone
816795
540670 CCOC
000050-00-0
71-43-2
000050-02-2
```

7.1.2. MDL SD Files

SD files (Structure Definition files) are text files containing chemical structures (stored as MOL files) and other data that can be uploaded and generated by various commercial chemistry programs such as ISIS/Base, ChemFinder, and Accord for Excel. The following example is a section from an SD file:

```
 -ISIS-  04010908242D

 4  3  0  0  0  0  0  0  0  0  0999 V2000
   2.4667   -0.0833    0.0000 O   0  0  0  0  0  0  0  0  0  0  0  0
```

```
    2.4667   -0.9125    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
    1.7500   -1.3292    0.0000 H   0  0  0  0  0  0  0  0  0  0  0  0
    3.1833   -1.3292    0.0000 H   0  0  0  0  0  0  0  0  0  0  0  0
2         1  2  0  0  0  0
3         2  1  0  0  0  0
4         2  1  0  0  0  0
M  END
>  <CAS> (000050-00-0)
000050-00-0

>  <NAME> (000050-00-0)
FORMALDEHYDE

>  <Kow> (000050-00-0)
3.500000000000000e-001

$$$$
```

The various fields are delimited with "<" and ">" brackets. This formaldehyde example includes <CAS>, <NAME>, and <Kow> fields. To extract ID (Name) for an ECOSAR batch run, in the SD File Option Box, enter the field name exactly as it appears between the brackets.

7.2. Conducting Batch Runs

To conduct a batch run, a user must determine the input format of the query compounds (e.g., SMILES strings, CAS number list, chemical name, or MDL SD files) and decide how the results will be captured in the output. The format of the input query compounds is described in Section 7.1. Batch runs can capture results as (1) Set of Individual Reports and (2) Excel Table Data.

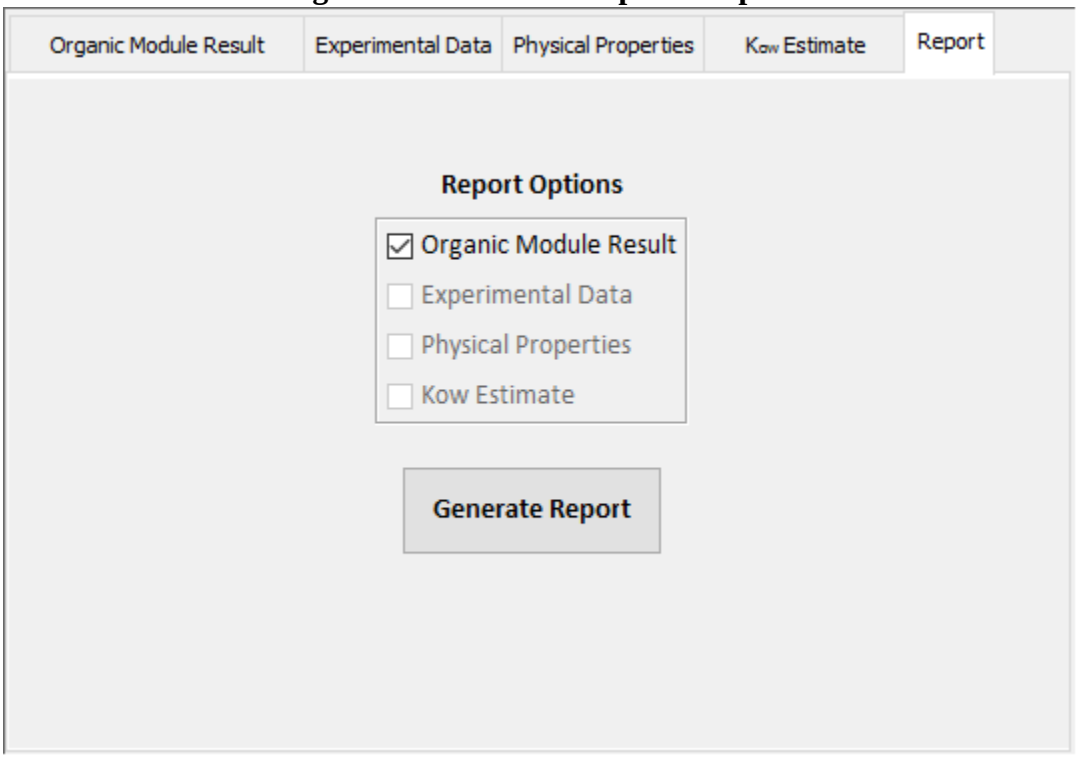
(1) Individual Reports are the same as the individual run reports and capture results for each compound the same as they would appear in the "Result Panel" (i.e., if each compound was estimated individually); these output files can be saved in a number of file outputs.

(2) Table Data Output captures both ECOSAR-predicted effect levels. Output varies slightly depending on whether data were input as SMILES strings or MDL SD files. Fields output in the Excel table include (1) a 'CAS' field that depicts an ID number for measured data, (2) chemical name, when available, (3) a 'SMILES' field that depicts the entered SMILES string, (4) an 'ECOSAR Class' field that lists the identified ECOSAR classes for input SMILES strings, (5) an 'Organism' field that identifies the organism for the predicted and measured effects, (6) a 'Duration' field that identifies the exposure duration for predicted and measured effects, (7) an 'End Point' field that identifies the endpoint (e.g., LC_{50}, EC_{50}, or ChV) for the effect level, (8) a 'Concentration (mg/L)' field that reports predicted effect levels, (9) Max Log K_{ow} gives the class and organism-specific cutoff value, (10) a 'Flags' field that identifies flags present for predicted effect levels, and (11) an 'Alert' field to indicate any further alerts.

7.2.1. Obtaining Individual Report Outputs

In order to conduct batch runs yielding individual report outputs, select the required chemicals from the uploaded input file. Only chemicals that have successfully loaded or were formatted correctly from the input file will show up in this field. Hit the 'Submit' button and all the chemicals selected will run a full estimation. Following that, go to the Report subtab in the Results Tab and select the "Generate Report" button (see Figure 13).

Figure 13. Individual Report Outputs



7.2.2. Obtaining Table Data Outputs

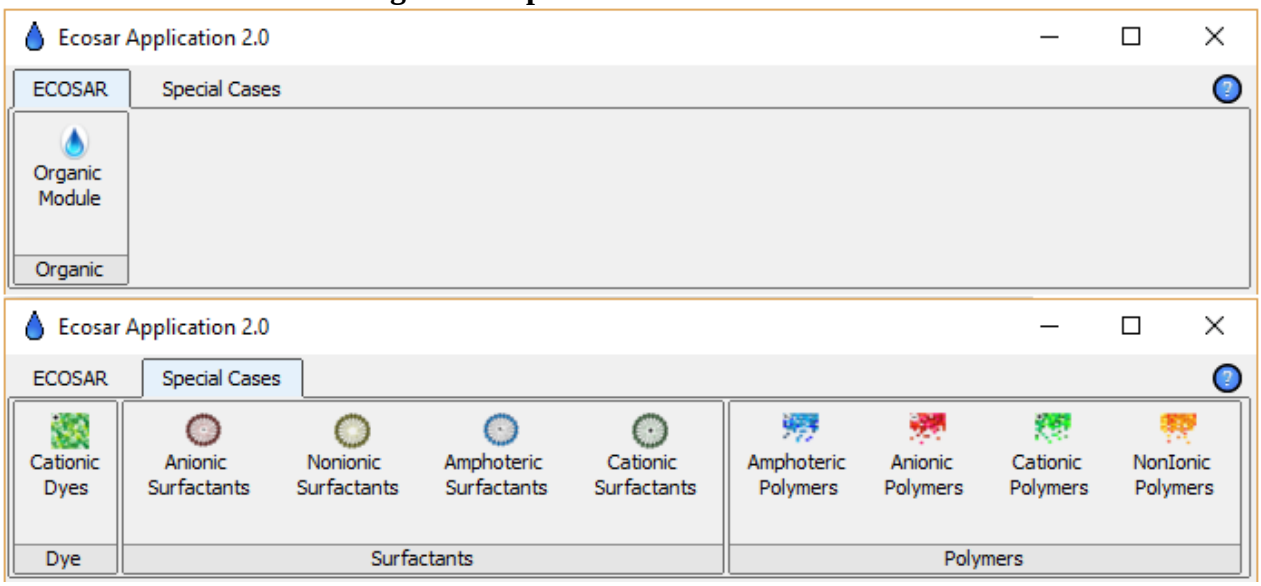
As an alternative to the Individual Report Outputs that is generated as described in Section 7.2.1, the “Report” button in the “BatchMode” entry area will generate results in a Microsoft Excel file, which can be converted into various other table formats.

8. Special Class Calculations

The ECOSAR Program has been developed primarily for the following scenario: (1) enter a SMILES, CAS number, or chemical name, (2) the ECOSAR program determines the appropriate ECOSAR class(es) from the SMILES notation, and (3) ECOSAR calculates the ecotoxicity QSARs using a log K_{ow} value. Several "Special Classes" of ECOSAR QSARs or classifications do not always use the log K_{ow} value or cannot be adequately classified from the SMILES notation. These "Special Classes" include dyes, polymers, and surfactants. QSARs are available for various anionic, cationic, nonionic, and amphoteric surfactants and polymers. Instead of the log K_{ow} value, these surfactant QSARs may utilize the number of ethoxylate units or the average length of the carbon chain. The polymer QSARs may utilize the percent amine nitrogen, the cation to anion

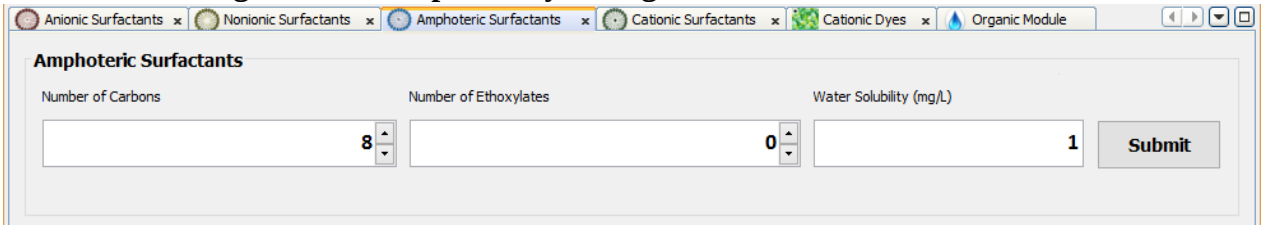
ratio, and the polymer type. Dye QSARs are also available for triphenylmethane dyes and ethoxylated triphenylmethane dyes, which utilize ethoxylate units for QSAR predications. These "Special Classes" are accessed from the Main Menu bar (see Figure 14).

Figure 14. Special Classes in ECOSAR



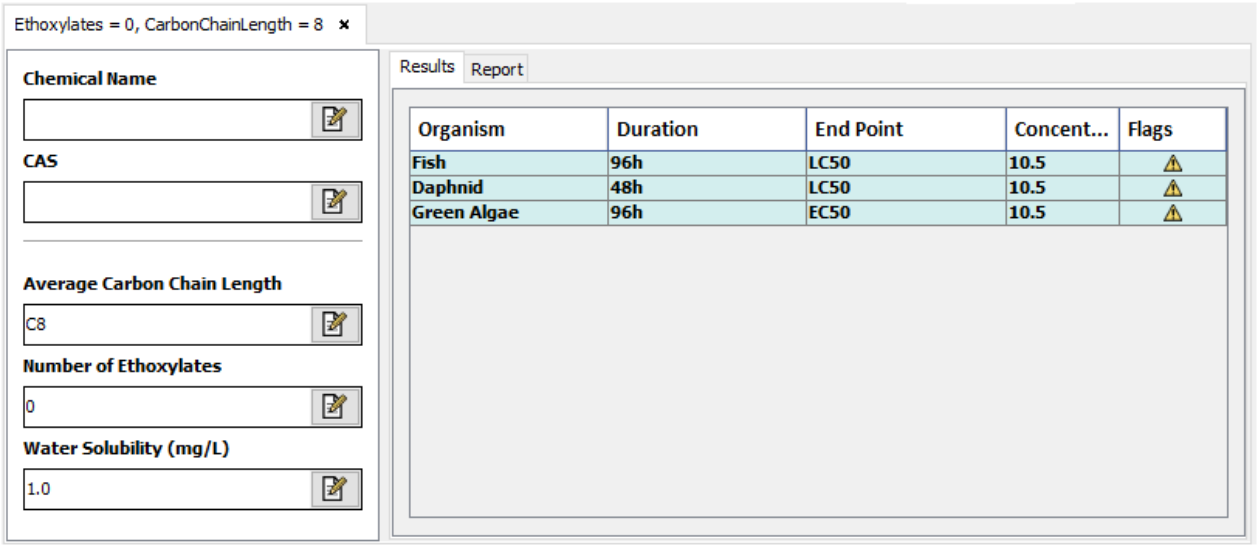
The Special Classes have their own data entry panels (see Figure 15).

Figure 15. Example Entry Dialog Box for Anionic Surfactants



The calculated results are placed in the same Results tab as results using SMILES notations (an example is illustrated in Figure 16).

Figure 16. Example Results Window for Anionic Surfactants



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Meylan, W.M. and P.H. Howard. 1994b. Validation of Water Solubility Estimation Methods Using Log K_{ow} for Application in PCGEMS & EPI (Sept 1994, Final Report). Prepared for Robert S. Boethling, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC; prepared by Syracuse Research Corporation, Environmental Science Center, Syracuse, NY 13210.

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Appendix A. Glossary of Terms and Abbreviations Associated with ECOSAR

ACR	Acute-To-Chronic Ratio
CAS	Chemical Abstracts Service
CBI	Confidential Business Information
ChV	Chronic Value. The geometric mean of the NOEC and LOEC.
EPA	U.S. Environmental Protection Agency
F96	Fish 96-Hour Endpoint
FChV	Fish Chronic Value
D48	Daphnid 48-Hour Endpoint
DChV	Daphnid Chronic Value
EC_{50}	Median Effect Concentration. A statistically derived concentration of a substance that can be expected to cause a specific effect (e.g., growth inhibition) in 50% of test animals. It is usually expressed as milligrams (mg) of substance per liter (L) water.
ECOSAR	ECOlogical Structure Activity Relationship
FW	Freshwater
GA72/96	Green Algae 72- or 96-Hour Endpoint
GAChV	Green Algae Chronic Value
GM	Geometric Mean
hr	Hour
K_{ow}	Octanol-Water Partition Coefficient
LC_{50}	Median Lethal Concentration. A statistically derived concentration of a substance that can be expected to cause death in 50% of test animals. It is usually expressed as milligrams (mg) of substance per liter (L) water.
Log	Logarithm; a base of 10 is used in ECOSAR calculations.
LOEC	Lowest Observed Effect Concentration. Lowest concentration of a substance that produced statistically significant effects.
MATC	Maximum Acceptable Toxicant Concentration. Value is equivalent to the ChV.
mg/L	Milligrams Per Liter
mmol/L	Millimoles Per Liter
n	The number of measured data points used to develop the QSAR equation sometimes followed by a '+' and then a number that represents the number of predicted data points and the number of measured data points above the limit of solubility.
NA	Not Applicable
N/A	Not Applicable
No	Number
NO	Neutral Organic

NOEC	No Observed Effect Concentration. Highest tested concentration of a substance that produced no statistically significant effects.
PPM	Parts Per Million
QSAR	Quantitative Structure Activity Relationship
r	Correlation Coefficient
r^2	Coefficient of Determination
SMILES	Simplified Molecular Input Line Entry System
SW	Saltwater
TSCA	Toxic Substances Control Act

Appendix B. Summary of Function Keys

Provided below is a summary of function keys that correspond to buttons and/or functions accessible from the ECOSAR data entry screen. These function keys can be used as alternatives to the buttons, which may require a mouse/touch pad to work.

F1: Pressing the F1 key accesses a help message for the individual field where the blinking cursor is located. General Help is available from "Help" on the Menu Bar at the top of the screen. It is a standard Windows help system; to access a specific help topic, simply click on the topic (or keyword) that is highlighted in green where the mouse pointer changes to a hand.

Enter: Pressing the Enter (Return) key sends the cursor to the next data entry field. If an entry has been made into the Chemical Input field (CAS, SMILES, name), pressing Enter will cause the estimate to run.

Tab or **Shift-Tab:** Changes entry fields.

Ctrl + Tab: Return to the Chemical Input field or other first entry field in a panel.