

# **Performing an Aquatic Toxicity Assessment Under the Sustainable Futures Initiative**

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# Why Assess Toxicity

## Steps in Risk Assessment

- **Toxicity**
  - Identify potential adverse effects and concentrations (effect level) at which effects may occur.
- **Exposure**
  - How much of the chemical will the environment or human population be exposed to?
- **Risk Characterization**
  - Compare hazardous effect levels to potential exposure concentrations to evaluate potential risk.

# Aquatic Toxicity

## Why only aquatic toxicity?

- Most industrial releases go to freshwater
- Terrestrial and marine organisms are assessed only on a case-by-case basis depending on release

## Surrogate species

- Fish, aquatic invertebrates (daphnids), green algae
- Toxicity to these surrogate species used to predict toxicity to species in the aquatic environment

# Aquatic Toxicity (cont.)

- ✚ **Acute toxicity (short-term exposure)**
  - **Assessed using effect concentrations ( $LC_{50}/EC_{50}$ )**
- ✚ **Chronic toxicity (long-term exposure)**
  - **Assessed using chronic toxicity value (ChV) or No Effect Concentration (NOEC)**

# Standard Aquatic Toxicity Profile

| Endpoint                     | Measured or Predicted Value                     |
|------------------------------|---|
| Fish LC <sub>50</sub>        | <b>Experimental Data<br/>or<br/>ECOSAR Data</b> |
| Daphnid LC <sub>50</sub>     |   |
| Green algae EC <sub>50</sub> |   |
| Fish ChV                     |   |
| Daphnid ChV                  |   |
| Green algae ChV              |   |

# Category Data

## U.S. EPA Category Statements

- Provides toxicity patterns for 55 categories based on data for the chemical class
- Indicates if EPA has identified a particular concern for the chemical class
- If your chemical is not in one of the defined chemical classes that does not mean it won't have a concern.
- <http://www.epa.gov/opptintr/newchems/pubs/chemcat.htm>

# Using Measured Data to Assess Aquatic Toxicity

## + Measured (laboratory experimental) data

- Identify data on chemical substance or appropriate analog

## + Critical factors for appropriate analog

- Chemical class
- Log  $K_{ow}$ 
  - Often useful to have multiple analogs bracketing the Log  $K_{ow}$  of the chemical

# Publicly Available Ecotoxicity Databases

## ECOTOX

- Available on-line at <http://www.epa.gov/ecotox/>
- Populated with acute and chronic toxicity values
- Data are from the literature and may need validation

## TSCATS

- Available on-line at [http://www.syrres.com/esc/tscats\\_info.htm](http://www.syrres.com/esc/tscats_info.htm)
- Populated with toxicity studies

# Publicly Available Ecotoxicity Databases

## + HPV data

- <http://www.epa.gov/chemrtk/viewsrch.htm>

## + SIDS data

- <http://www.inchem.org/> click on Environmental Health Criteria  
<http://www.inchem.org/pages/ehc.html>

## + Hazardous Substances Data Bank (HSDB)

- <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>

## + General Internet search

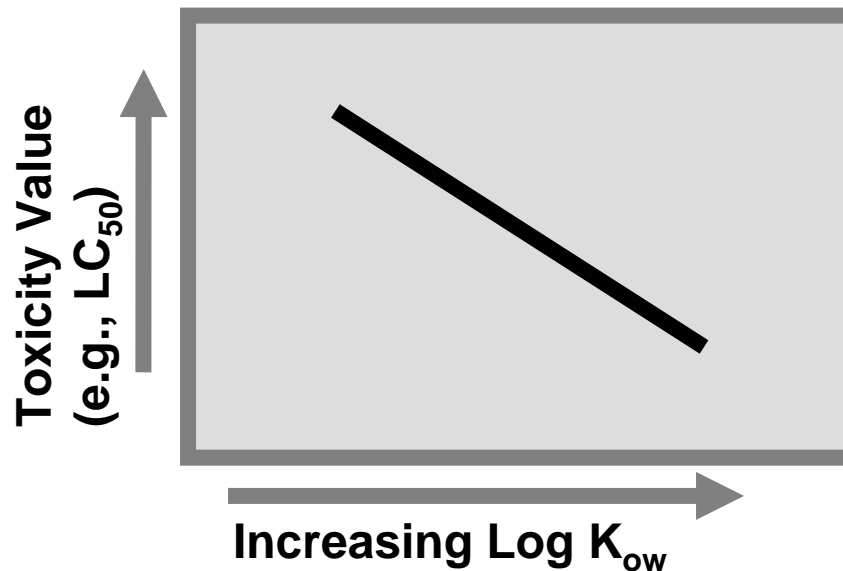
- 5 minutes per chemical

# Limitations of Using Measured Data

- ✚ **Not all endpoints available for the chemical of interest or an appropriate analog**
- ✚ **Assessing study quality (validity) is not always possible**
  - **Use data from multiple analogs if possible**
- ✚ **ChV is not usually reported**
  - **Geometric mean of NOEC and LOEC**
  - **NOEC can be used for assessment**

# Using Predicted Data to Assess Aquatic Toxicity

- ✚ Use of SAR facilitates PMN review (1 hour per review)
- ✚ Structure Activity Relationships (SARs)
  - Toxicity generally increases as  $\log K_{ow}$  increases until water solubility becomes too low



# Using SAR to Predict Aquatic Toxicity for Sustainable Futures

## ECOSAR

- **Computer program using chemical structure to predict toxicity of a chemical to aquatic organisms (SAR)**
  - Acute and chronic toxicity endpoints
  - Fish, aquatic invertebrates, algae, and others
- **Download at no cost from:**  
<http://www.epa.gov/opptintr/newchems/tools/21ecosar.htm>

# How ECOSAR Predicts Toxicity

✚ Chemicals are grouped into classes. For most classes, regression equations relate *predicted*  $\log K_{ow}$  to toxicity








- Example SAR, acrylates

- $\log LC_{50} \text{ (mM/L)} = -1.46 - 0.18 \log K_{ow}$

✚ SARs are based on measured toxicity data

- Toxicity is predicted at pH 7, TOC < 2 mg/L, moderate water hardness (150 mg/L  $CaCO_3$ ), 100% active ingredient

# Chemical Divisions in ECOSAR

-  Neutral Organics
-  Classes with Excess Toxicity
-  Surfactants
-  Polymers\*
-  Dyes\*
-  Inorganics\*
-  Organometallics\*

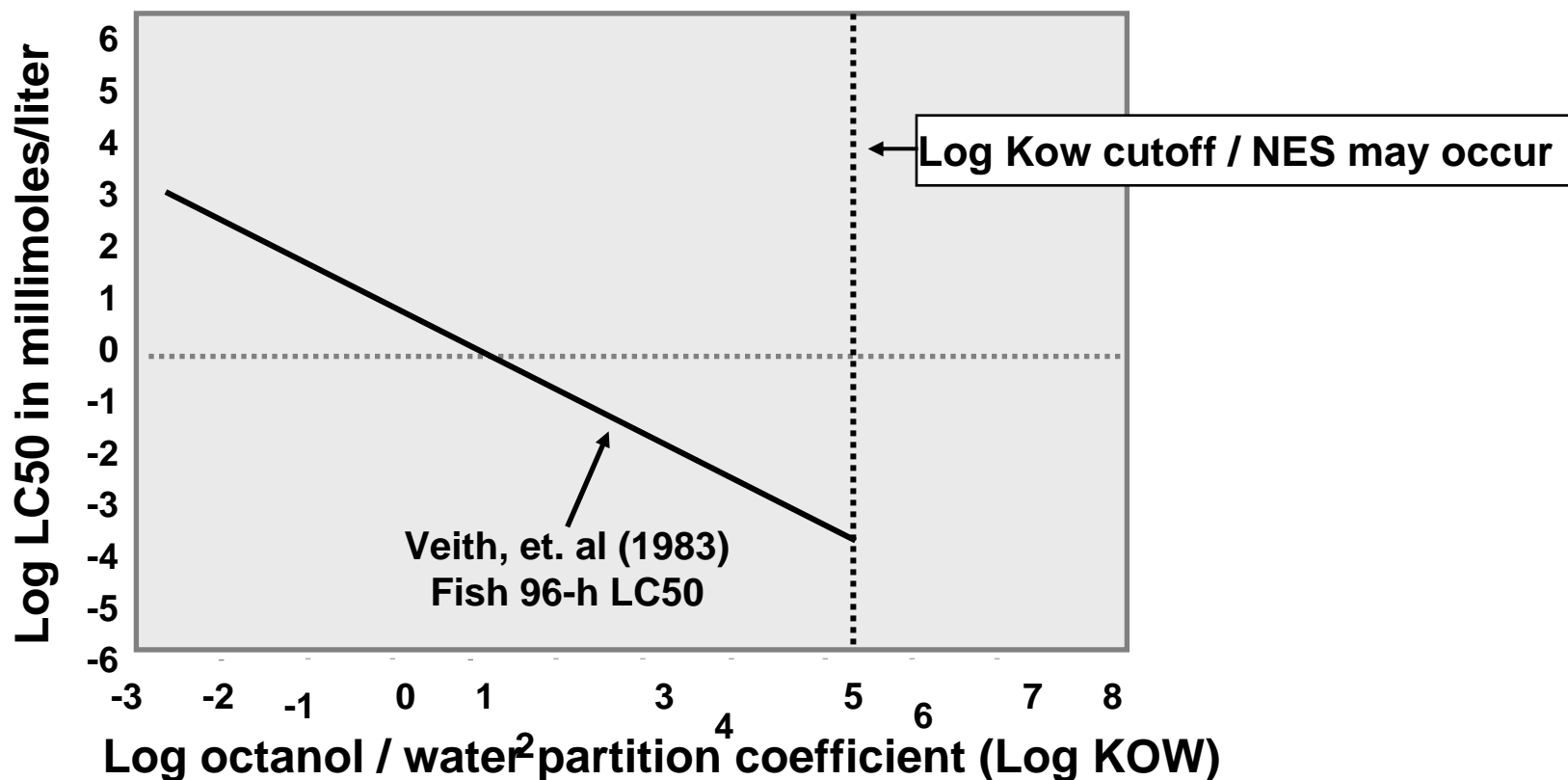
\* SARs for polymers, organometallics, or inorganics are not yet available, and only a limited number of dye SARs are available in the current version of ECOSAR

# Neutral Organics

- ✚ Non-electrolytic and biologically non-reactive compounds**
- ✚ Act as anesthetics or narcotics**
- ✚ Includes alcohols, ketones, ethers, alkyl halides, aryl halides, aromatic hydrocarbons, aliphatic hydrocarbons, many cyanates, sulfides, and disulfides.**

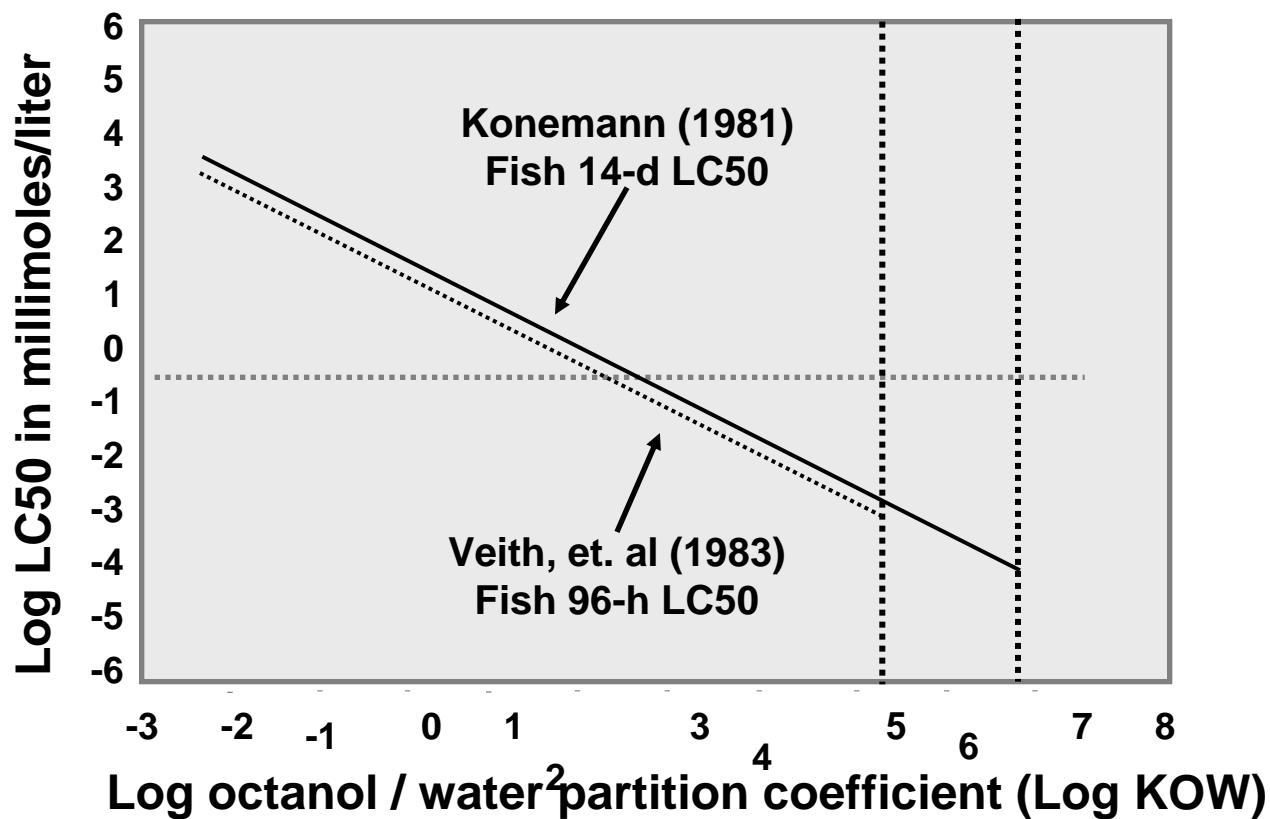
# Neutral Organics SAR Method

## Estimating Fish Toxicity of Neutral Organic Chemicals

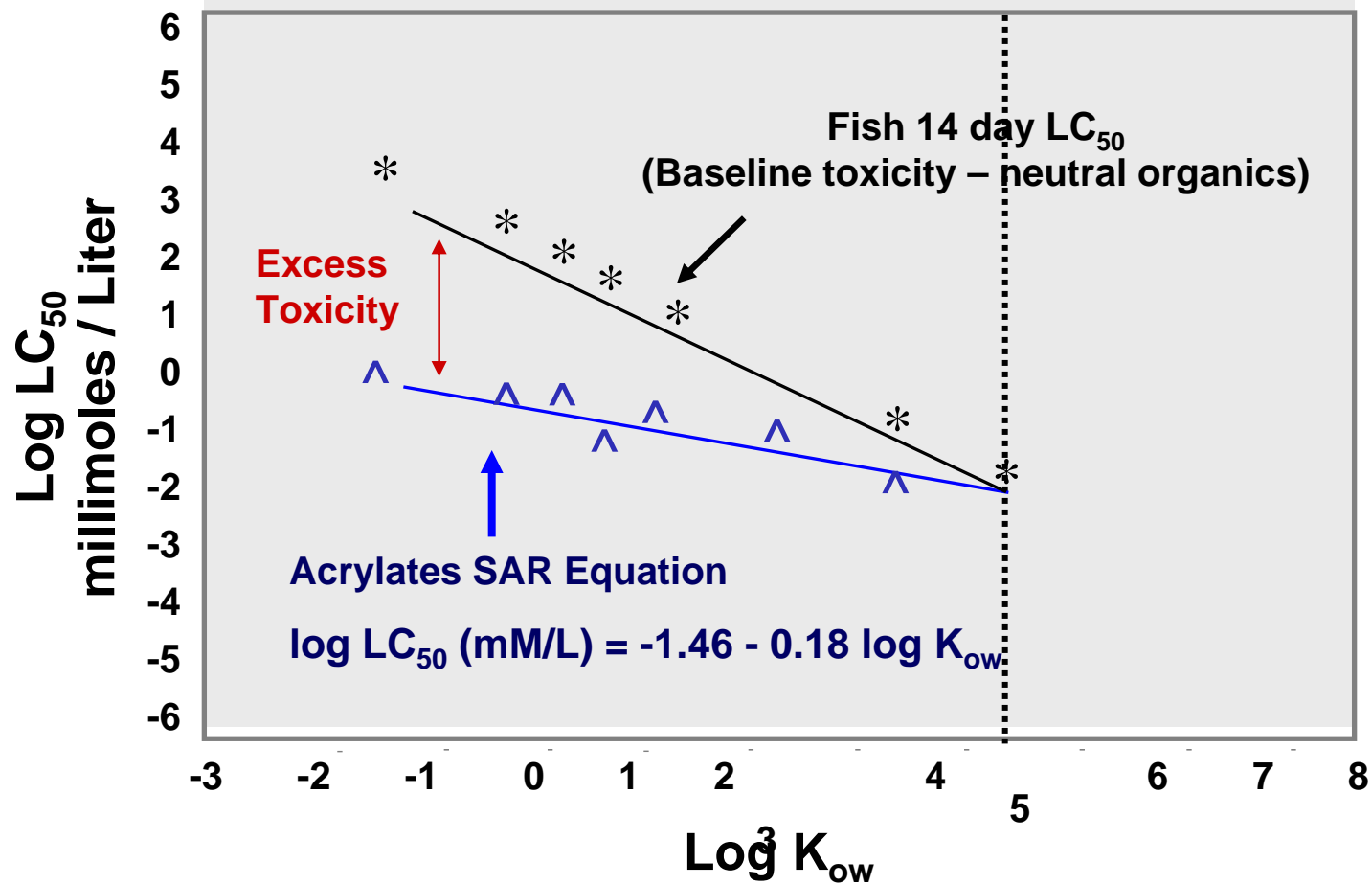


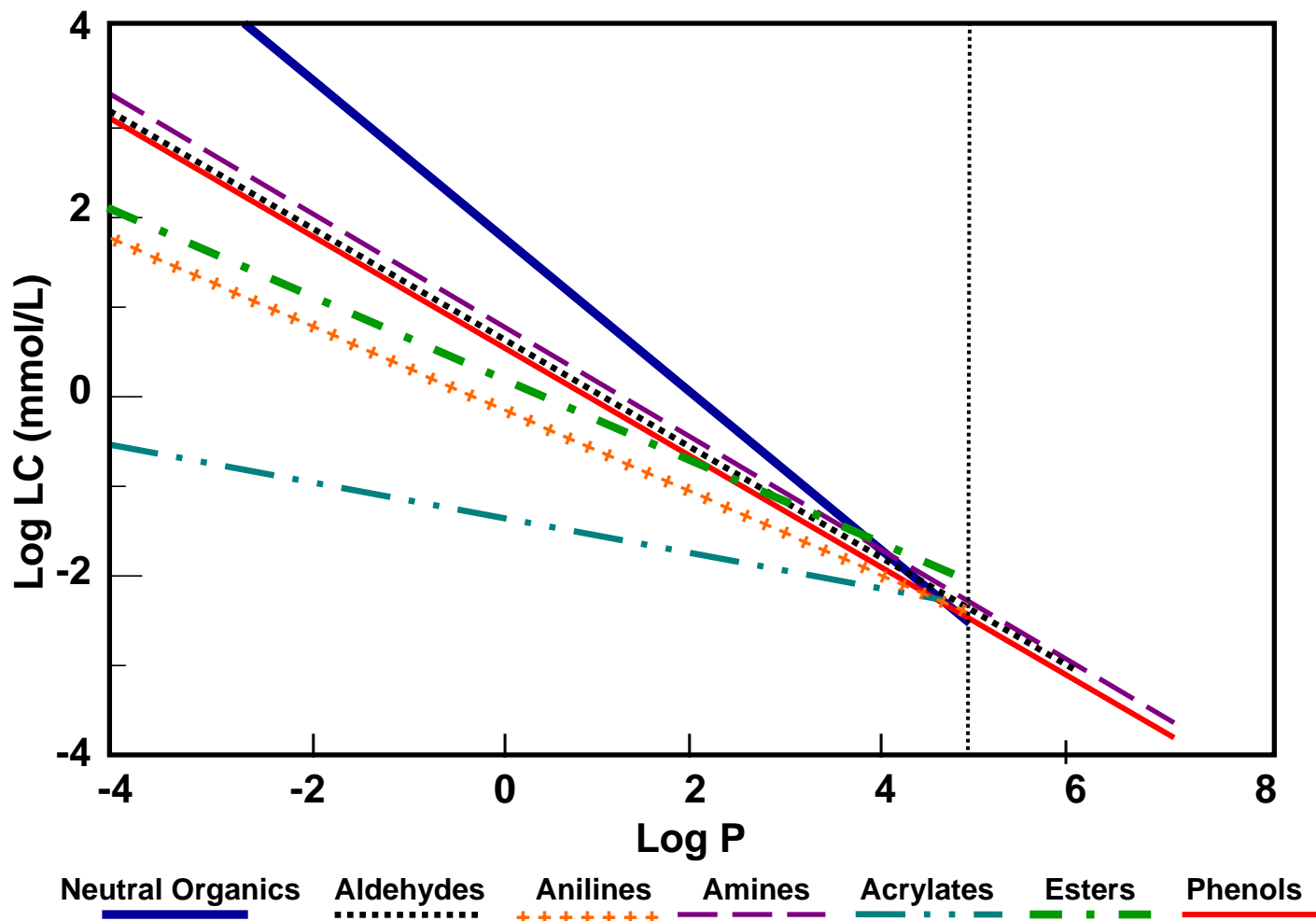
# Neutral Organics SAR

Comparison of the Konemann vs. Veith SARs  
for Estimating Fish Toxicity of Neutral Organic Chemicals



# Classes with Excess Toxicity





**Figure 1. Octanol-Water Partition Coefficient (log P) Cut-Offs and Predicted Magnitude of Fish Acute Toxicity (expressed as median lethal concentration,  $LC_{50}$ ) for Several Chemical Classes Using Equations from: Clements, R.G., Nabholz, J.V., *ECOSAR: A Computer Program for Estimating the Ecotoxicity of Industrial Chemicals Based on Structure Activity Relationships*, U.S. EPA, OPPT (7403), Technical Publication, 748-R-93-002, 1994.**

# ECOSAR Chemical Classes with Excess Toxicity

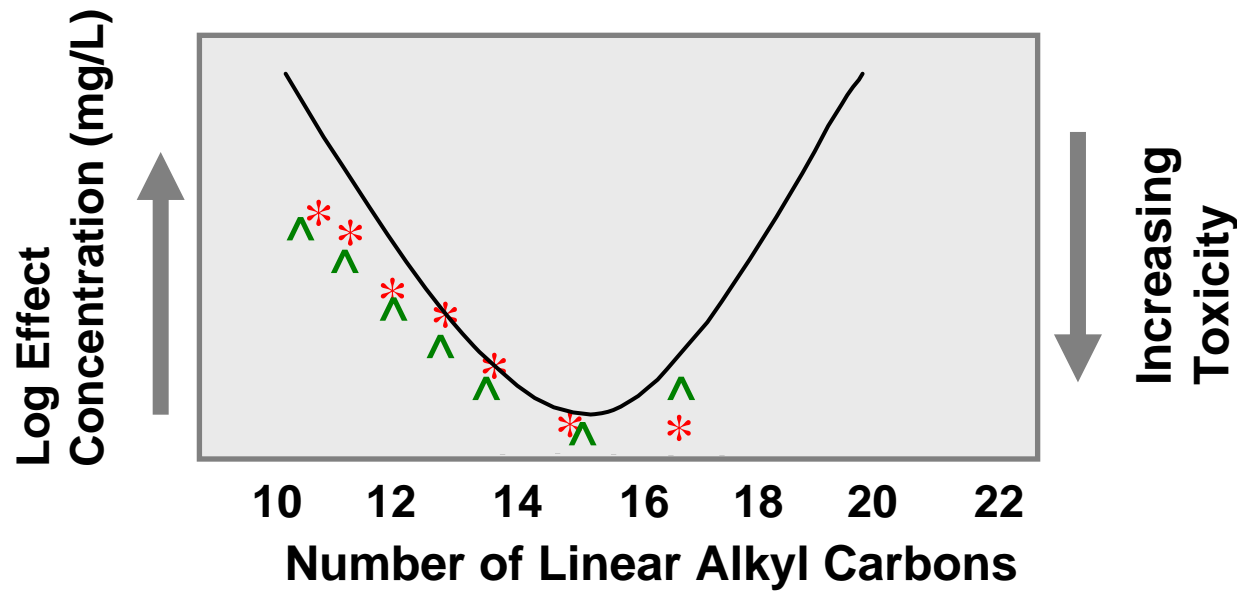
## 40 Chemical Classes

ECOSAR will identify appropriate SAR class

- ✚ Acid chlorides
- ✚ Acrylates
- ✚ Acrylates, methacrylates
- ✚ Alcohols, propargyl
- ✚ Aldehydes
- ✚ Amines, aliphatic
- ✚ Anilines
- ✚ Anilines, amino, meta or 1,3-substituted
- ✚ Anilines, amino, ortho or 1,2-substituted
- ✚ Anilines, amino, para, or 1,4-Substituted anilines, dinitroanilines
- ✚ Diazoniums, aromatic
- ✚ Epoxides, monoepoxides
- ✚ Epoxides, diepoxides
- ✚ Esters
- ✚ Esters, monoesters, aliphatic
- ✚ Esters, diesters, aliphatic
- ✚ Esters, phosphate
- ✚ Esters, phthalate
- ✚ Hydrazines
- ✚ Ketones, diketones, aliphatic

# Other Types of SAR Equations in ECOSAR

- ✚ Some classes do not use  $\log K_{ow}$  in SAR predictions
  - SARs for surfactants are based on the number of ethoxylate units and/or average carbon chain length
  - Anionic surfactants
    - $\log LC_{50} \text{ (mg/L)} = [(\text{avg. no. of carbons} - 16)^2 - 10.643] \div 12.9346$



# Assessing Chemicals Not Yet Programmed in ECOSAR

- ✚ Use analog data to satisfy the toxicity profile
- ✚ ECOSAR Technical Reference Manual contains valuable information on polymers, dyes, inorganics, and organometallics

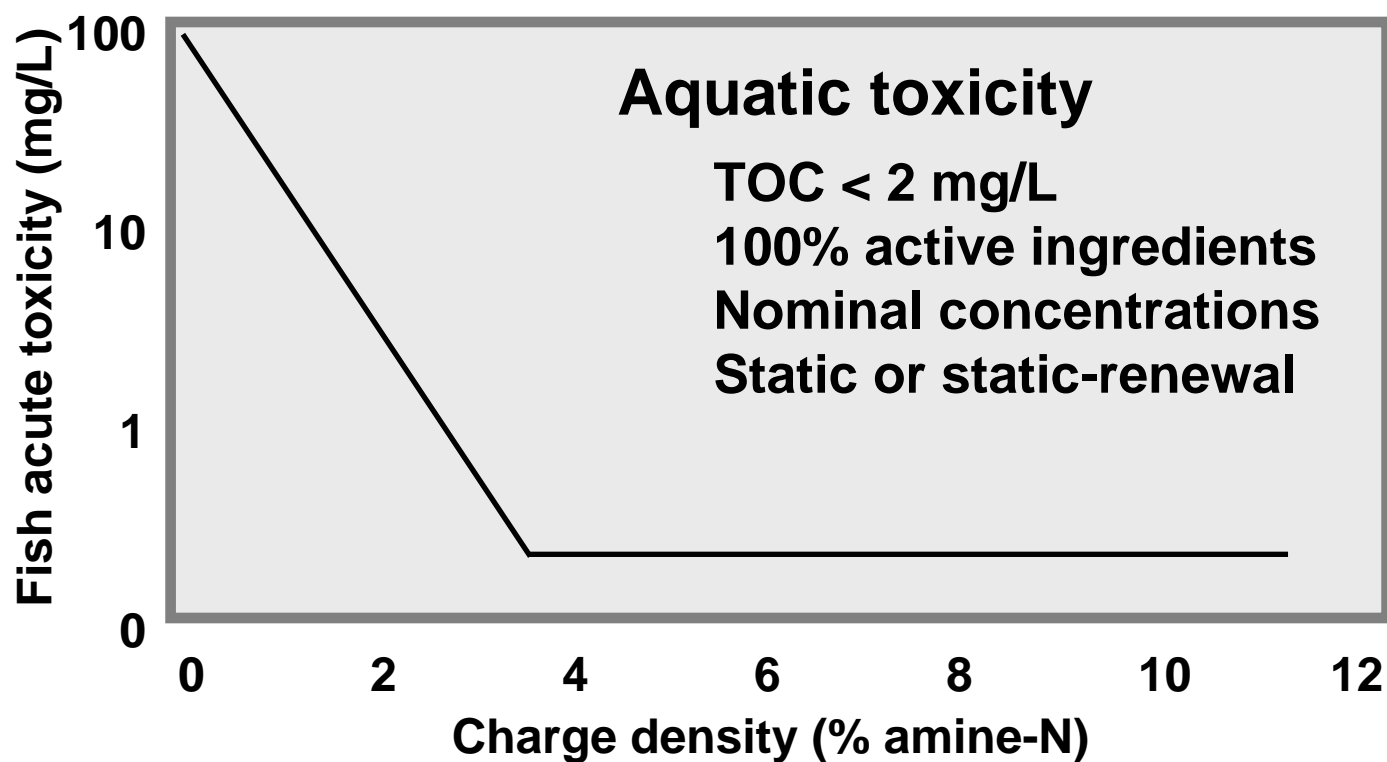
<http://www.epa.gov/opptintr/newchemicals/tools/sarman.pdf>

# Assessing Chemicals Not Yet Programmed in ECOSAR (Cont.)

## Polymers

- Neutral polymers usually have low toxicity concern
- Cationic and amphoteric polymer SARs are based on charge density
- Anionic polymer SARs based on distance between COOH or type of acid
- Additional Reference - Ecological Assessment of Polymers: Strategies for Product Stewardship and Regulatory Programs. John D. Hamilton (Editor), Roger Sutcliffe (Editor). ISBN: 0-471-28782-2

# Polycationic Polymers Polyamine Polymers



# Special Cases

## Salts

- Use predicted  $\log K_{ow}$  for free acid or base for salts

## Chemicals that rapidly hydrolyze ( $t_{1/2} < 1$ hour at 20°C, pH 7) and chemicals that undergo rapid photolysis, oxidation, or pyrolysis

- Assess each degradation product

# Running ECOSAR

## Before running ECOSAR

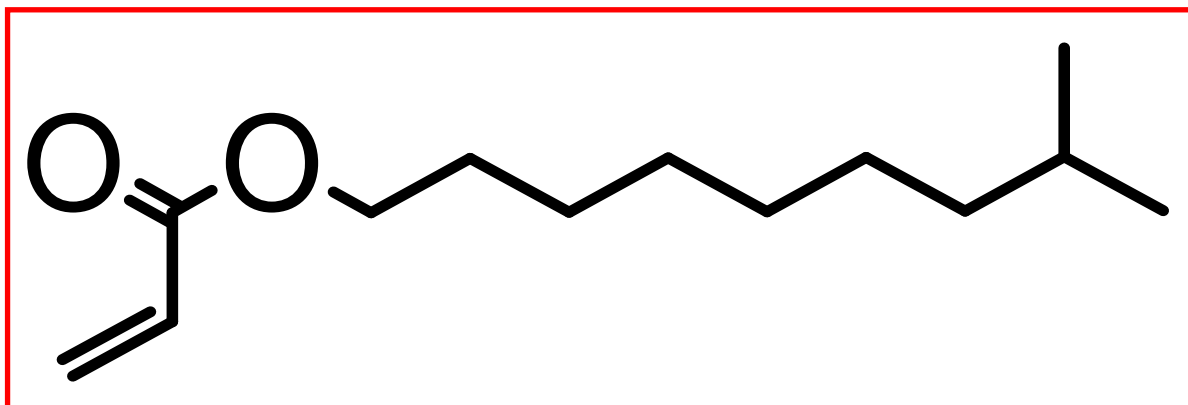
- **Consult the ECOSAR Technical Reference Manual**
  - Available for free download at <http://www.epa.gov/opptintr/newchemicals/tools/sarman.pdf>
  - May contain important information on SARs including SAR equation and statistics

## Start ECOSAR



# Example Chemical

## Isodecyl Acrylate



# ECOSAR – Chemical Hierarchy

**Initial Selection** [X]


The ECOSAR Chemical Hierarchy contains six divisions as shown below in the selection list. Select the chemical division you want to start with and press the "OK" button.

Inorganics, Organometallics, Polymers, Surfactants and Dyes are the "Special Classes". Access to the "Special Class" QSARs is also available from the ECOSAR Main Menu Bar.

Select:

- Inorganics
- Organometallics
- Polymers
- Dyes
- Surfactants.....
  - Anionic Surfactants
  - Cationic Surfactants
  - Nonionic Surfactants
  - Amphoteric Surfactants
- All Others

The "All Others" division requires a SMILES notation for evaluation. ECOSAR's default data entry screen (requiring a SMILES) applies to the "All Others" division. The correct QSAR class is determined from the SMILES.



# ECOSAR – Data Entry (cont.)

## Three ways to enter data:

- **Enter CAS number**
  - **Structure is automatically imported into ECOSAR if the CAS number is in the accompanying SMILECAS database of >120,000**
- **SMILES notation**
- **Import structure**
  - **Importable formats include:**
    - **Alchemy III MOL - HyperChem HIN – PCModel**
    - **Beilstein ROSDAL - MDL ISIS SKC - Softshell SCF**
    - **BioCAD Catalyst TPL - MDL MOL - SYBYL Line Notation - ChemDraw - SYBYL MOL2**

# ECOSAR – Data Entry

SMILECAS Database Retrieval

Enter CAS Number (with or without hyphens):

1330-61-6

OK

ECOSAR v0.99g

File Edit Functions BatchMode ShowStructure Special\_Classes Help

Previous Get User Save User CAS Input Calculate

Enter SMILES: O=C(C=C)OCCCCCCCC(C)C

Enter NAME: 2-Propenoic acid, isodecyl ester

CAS Number: 001330-61-6

Chemical ID 1:

Chemical ID 2:

Chemical ID 3:

Log Kow:

Measured Water Sol (mg/L):

Melting Point (deg C): -100

Measured Log Kow:

# ECOSAR – Results

SMILES : O=C(C=C)OCCCCCCCC(C)C

CHEM : 2-Propenoic acid, isodecyl ester

CAS Num: 001330-61-6

ChemID1:

ChemID2:

ChemID3:

MOL FOR: C13 H24 O2

MOL WT : 212.34

Log Kow: 5.07 (KowWin estimate)

Melt Pt: -100.00 deg C

Wat Sol: 2.222 mg/L (calculated)

ECOSAR v0.99g Class(es) Found

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Acrylates

# ECOSAR - Results

| ECOSAR Class                               | Organism | Predicted Duration | End Pt | mg/L (ppm) |
|--|----------|--------------------|--------|------------|
| =====                                      | =====    | =====              | =====  | =====      |
| Neutral Organic SAR<br>(Baseline Toxicity) | : Fish   | 14-day             | LC50   | 0.604      |

|           |               |        |      |        |
|-----------|---------------|--------|------|--------|
| Acrylates |               | 96-hr  | LC50 | 0.900† |
| Acrylates | : Fish        | 48-hr  | LC50 | 0.554† |
| Acrylates | : Daphnid     | 96-hr  |      | 0.066  |
| Acrylates | : Green Algae | 32-day | ChV  | 0.005  |
|           | : Fish        |        | EC50 |        |

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

**† Fish and daphnid acute toxicity log Kow cutoff: 5.0**

**Green algal EC50 toxicity log Kow cutoff: 6.4**

**Chronic toxicity log Kow cutoff: 8.0**

**MW cutoff: 1000**

# ECOSAR - Results

| ECOSAR Class                               | Organism      | Predicted<br>Duration | End Pt | mg/L (ppm) |
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| Acrylates                                  | : Fish        | 96-hr                 | LC50   | 0.900*     |
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| Acrylates                                  | : Daphnid     | 96-hr                 |        | 0.066      |
| Acrylates                                  | : Green Algae | 32-day                | ChV    | 0.005      |
|  | : Fish        |                       | EC50   |            |

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

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MW cutoff: 1000

# ECOSAR - Results

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| Acrylates                                  | : Daphnid     | 96-hr                 |        | 0.066              |
| Acrylates                                  | : Green Algae | 32-day                | ChV    | 0.005              |
|  | : Fish        |                       | EC50   |                    |

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# ECOSAR - Results

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|           |               |        |      |        |
|-----------|---------------|--------|------|--------|
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Chronic toxicity log Kow cutoff: 8.0

MW cutoff: 1000

# Interpreting Results

## ✚ What to do if predictions are made for multiple ECOSAR classes?

- Use lowest value for each endpoint

## ✚ Interpreting $\log K_{ow}$ cutoffs

- Expect no effects at saturation (NES) for liquid chemicals with  $\log K_{ow} > 5$  for acute tests for many chemical classes
- Expect no effects at saturation for liquid chemicals with  $\log K_{ow} > 8$  for chronic tests for many classes

# Interpreting Results (cont.)

- ✚ **Full standard aquatic toxicity profile includes 6 values:**
  - **3 acute values (1 for each surrogate species)**
  - **3 chronic values (1 for each surrogate species)**
- ✚ **Daphnid and Green Algae ChVs were not determined by ECOSAR for isodecyl acrylate**

# Interpreting Results (cont.)

- ✚ **Daphnid and algae ChV values can be calculated using acute-to-chronic ratios (ACRs)**
  - **The following ACRs are often appropriate:**
    - Fish ChV prediction = Fish LC<sub>50</sub>/10
    - Daphnid ChV prediction = Daphnid LC<sub>50</sub>/10
  - **A ratio of 4 is often used for Green algae**
    - Green algae ChV prediction = Green algae EC<sub>50</sub>/4
  
- ✚ **Support use of ACRs and algal toxicity ratios using analog or category data**

# Standard Environmental Toxicity Profile for Isodecyl Acrylate

| Endpoint                           | Predicted Value (mg/L) |
|------------------------------------|------------------------|
| 96-hr Fish LC <sub>50</sub>        | 0.900*                 |
| 48-hr Daphnid LC <sub>50</sub>     | 0.550*                 |
| 96-hr Green Algae EC <sub>50</sub> | 0.070                  |
| Fish ChV                           | 0.005                  |
| Daphnid ChV                        | 0.060                  |
| Green Algae ChV                    | 0.020                  |

\* The log K<sub>ow</sub> of the chemical (5.07) is *slightly* above the cutoff (5.0) for these effects; therefore, there may be no effects at saturation (NES) or effects may occur. Without supporting analog data, assume that effects may occur.

# ECOSAR – Assigning Toxicity Concern Levels

| Concern Level | Definition   |
|---------------|--|
| High          | Lowest acute toxicity value is < 1 mg/L (chronic value <0.1 mg/L)  |
| Moderate      | Lowest predicted acute value is between 1 and 100 mg/L (chronic value between 0.1 and 10 mg/L)   |
| Low           | Lowest predicted acute value is >100 mg/L (chronic value >10 mg/L); no effects predicted at saturation (below the solubility limit); or log $K_{ow}$ is greater than the cutoff of 8 |