Ecological Structure Activity Relationship (ECOSAR) Model for Predicting Toxicity

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Purpose of Performing Aquatic Toxicity Assessment

- **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 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)
- Ecotoxicity is one of the six basic tests which have generally been agreed to be necessary to understand the impact of high production volume chemicals
  - Less than 10% of HPV chemicals have all 6 tests

Standard Aquatic Toxicity Profile

Endpoint	Measured or Predicted Value
Fish LC_{50}	**Experimental Data Or ECOSAR Data**
Daphnid LC_{50}	
Green algae EC_{50}	
Fish ChV	
Daphnid ChV	
Green algae ChV	

Types of Data Used to Perform Toxicity Screen for Chemical Assessments

- Measured (laboratory experimental) data
  - Chemical substance or analog
    - 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

- 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.htm
    - Populated with toxicity studies

More Publicly Available Ecotoxicity Databases

- HPV data
  - http://www.epa.gov/chemrtk/
- SIDS data
  - http://www.inchem.org/ click on Environmental Health Criteria
  - http://www.inchem.org/pages/ehc.html
- Hazardous Substances Data Bank
  - http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB
- General Internet Search
  - 5 minutes per chemical

Types of Data Used to Perform Toxicity Screen for Chemical Assessments

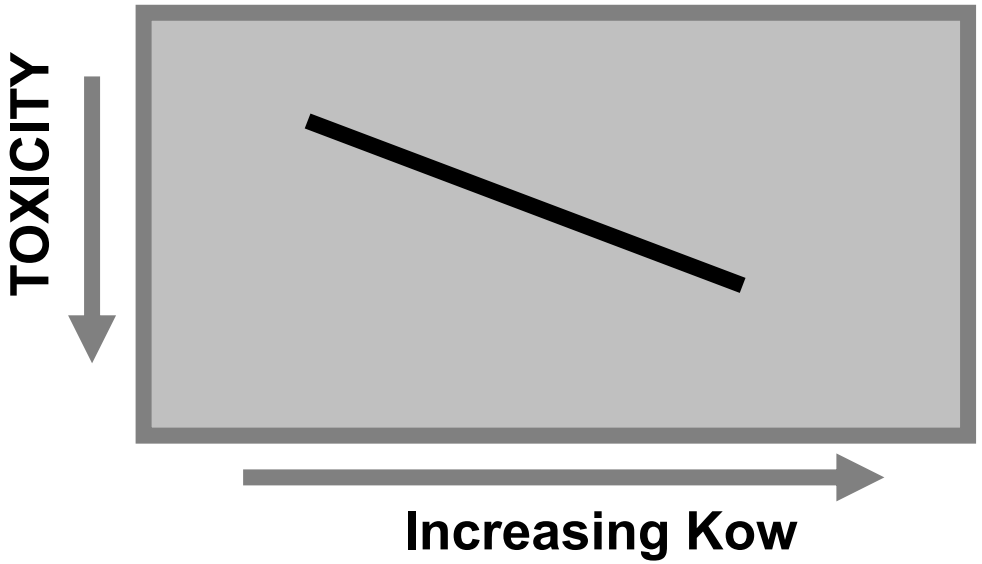
- Category Data
  - Indicates if EPA has identified a particular concern for the chemical class
  - U.S. EPA Category Statements
  - http://www.epa.gov/opptintr/newchems/pubs/chemcat.htm
    - 55 categories based on PMN data that have consistently been shown to induce toxic effects
    - Review articles
    - Pubmed searches
    - Pubmed can be searched on-line at http://www.ncbi.nlm.nih.gov

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

- Predicted data
  - Structure Activity Relationships (SARs)
  - Objective of SAR is to predict results of valid studies
    - Toxicity generally increases as K_{ow} increases until water solubility becomes too low
  - Use of SAR facilitates completion of data collection and review



Using SAR to Predict Aquatic Toxicity for Chemical Assessments

- ECOSAR
  - Computer program that uses chemical structure to predict toxicity of a chemical to aquatic organisms (SAR)
    - Acute and chronic toxicity endpoints
    - Fish, aquatic invertebrates, algae, and others
  - Available for free download at:
  - www.epa.gov/opptintr/newchems/tools/21ecosar.htm

How ECOSAR Predicts Toxicity

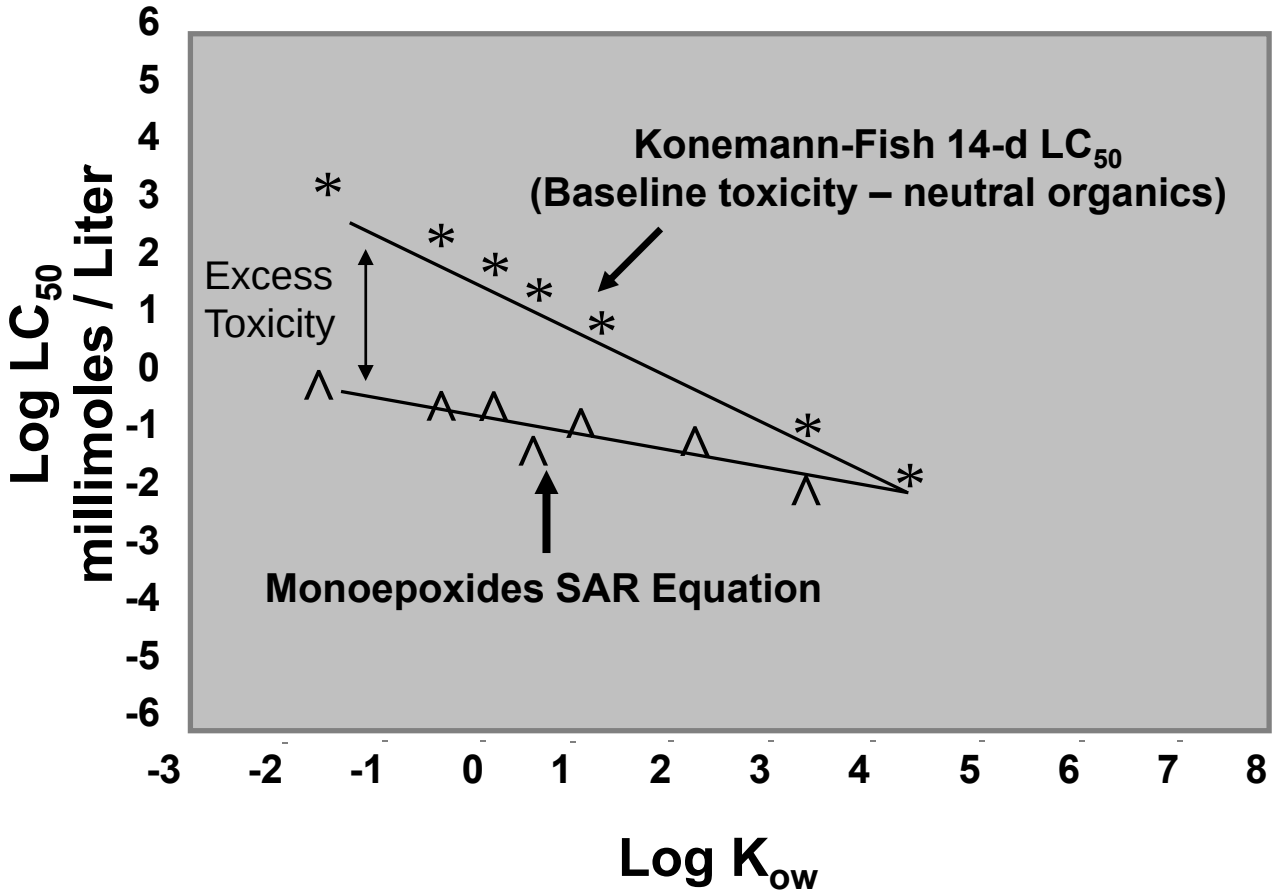
- Chemicals are grouped into classes. For each class, regression equations are used to relate ***predicted*** K_{ow} to toxicity
  - Example SAR, monoepoxides
    - 48-Hour Daphnid LC50:

      Log LC_{50} (mM/L) = 0.036 - 0.567 log K_{ow}
- SARs are based on measured toxicity data
  - Toxicity is predicted at pH 7, TOC < 2%, moderate water hardness (150 mg/L $CaCO_3$), 100% active ingredient

How the Model is Developed and Expanded

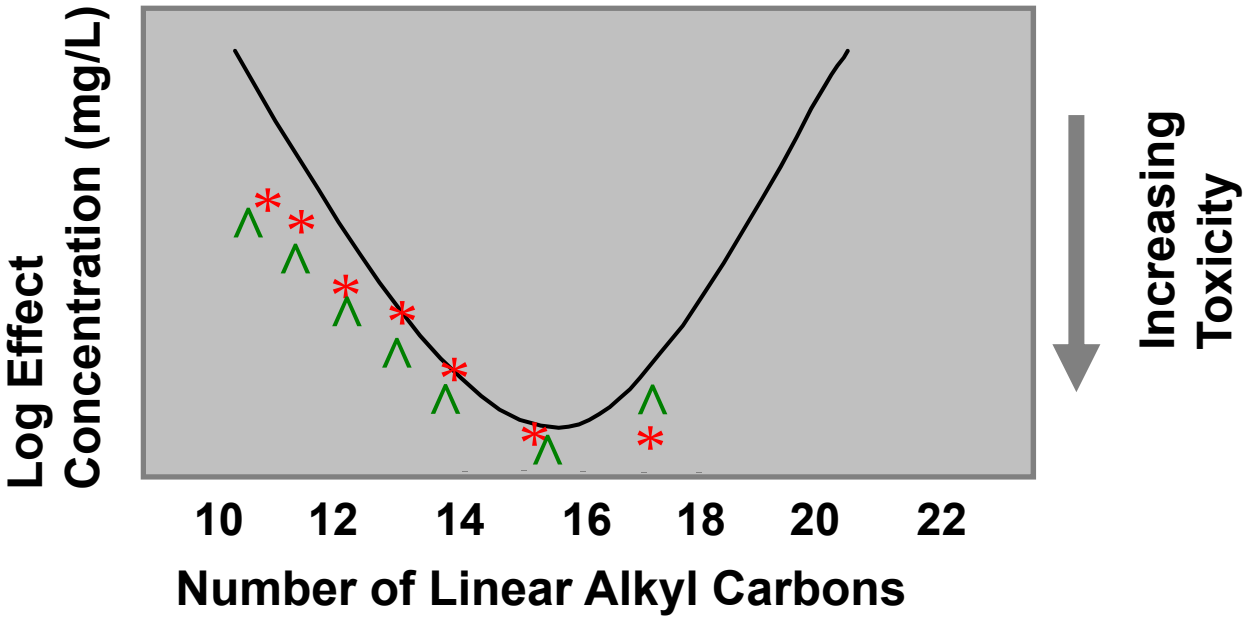
- Existing data are collected, and the relationships are turned into the equations used to predict toxicity (SAR)
- When additional data are available, the predicted values are compared to the new analytical data (such as HPV data)
- The new data are used to adjust the SAR regression values to improve the model

Monoepoxides
Fish 14-d LC_{50} QSAR



Other Types of SAR Equations In ECOSAR

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



Chemical Divisions in ECOSAR

- Organometallics*
- Inorganics*
- Dyes*
- Polymers*
- Surfactants
- Classes with excess toxicity
- Neutral Organics

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

Selected ECOSAR Chemical Classes with Excess Toxicity

40 chemical classes programmed in ECOSAR

ECOSAR identifies SAR classes

- 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

Assessing Chemicals Not Yet Programmed in ECOSAR

- Use analog data to satisfy the toxicity profile
- Use interpolation methods to estimate acute toxicity endpoints from chronic toxicity endpoints and vice-versa
  - http://www.epa.gov/oppt/newchems/pubs/sustainable/guidance06-06.pdf
- ECOSAR Technical Reference Manual contains valuable information on polymers, dyes, inorganics, and organometallics
  - http://www.epa.gov/opptintr/newchems/tools/sarman.pdf

Assessing Chemical Not Yet Programmed in ECOSAR (cont.)

- Polymer SARs based on charge (anionic, cationic, amphoteric, or neutral)
  - Cationic and amphoteric polymer SARs are based on charge density
  - Anionic polymer SARs based on distance between COOH or type of acid
  - Neutral polymers usually have low toxicity concern

- Reference for toxicity assessment of polymers
  - **Ecological Assessment Polymers: Strategies for Product Stewardship and Regulatory Programs**
    John D. Hamilton (Editor), Roger Sutcliffe (Editor)
    ISBN: 0-471-28782-2

Special Cases

- Salts
  - Use predicted K_{ow} for free acid or base for salts
- Chemicals that rapidly hydrolyze

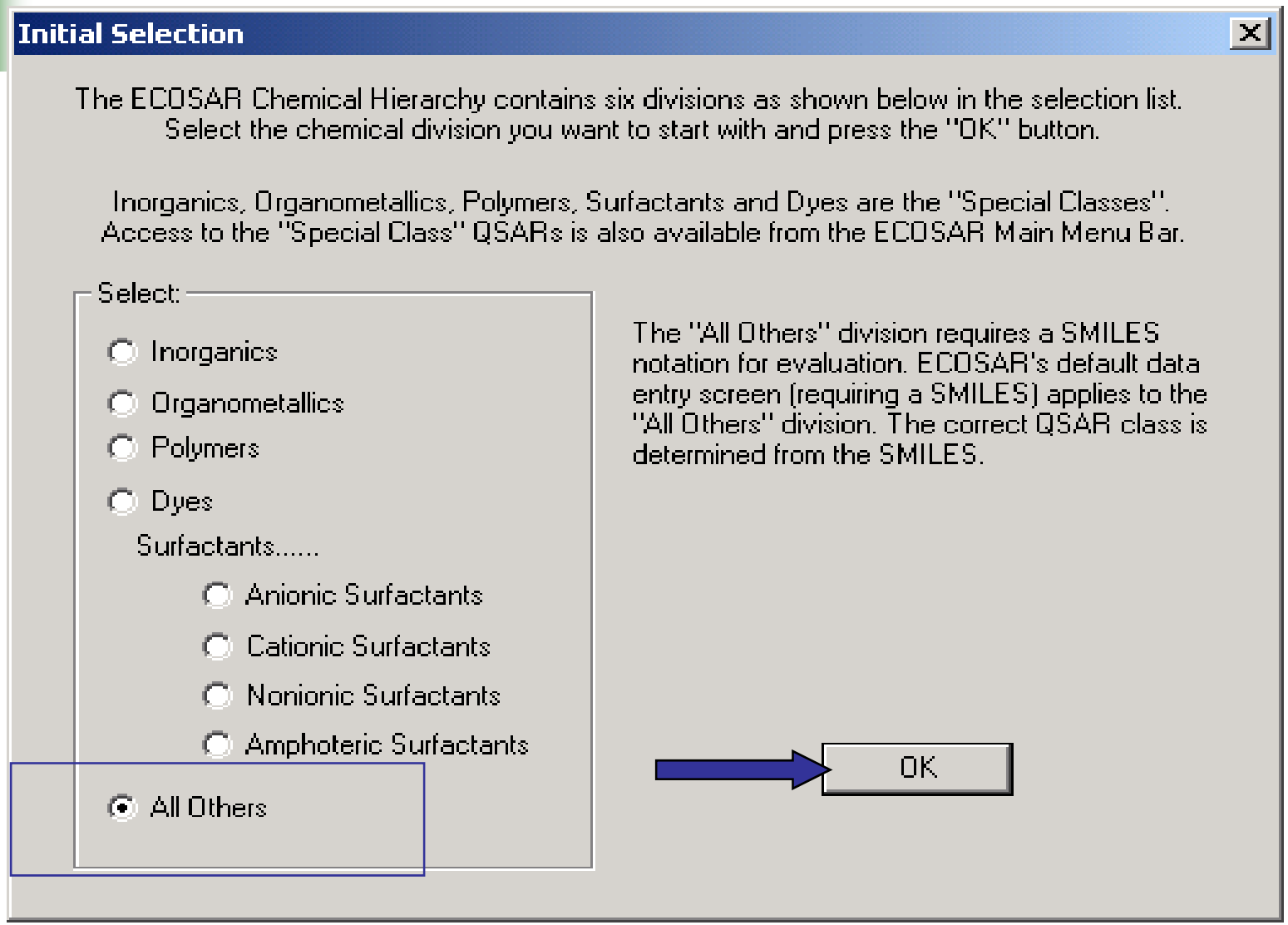
  ($t_{1/2}$ <1 hour at 20☞C, pH 7) and chemicals that undergo rapid photolysis, oxidation, or pyrolysis
  - Assess each degradation product

Running ECOSAR

- Before running ECOSAR
  - Consult the technical reference manual – Available for free download at http://www.epa.gov/oppt/newchems/sarman.pdf
    - May contain important information on SARs including SAR equation and statistics

Start ECOSAR

ECOSAR – Chemical Hierarchy

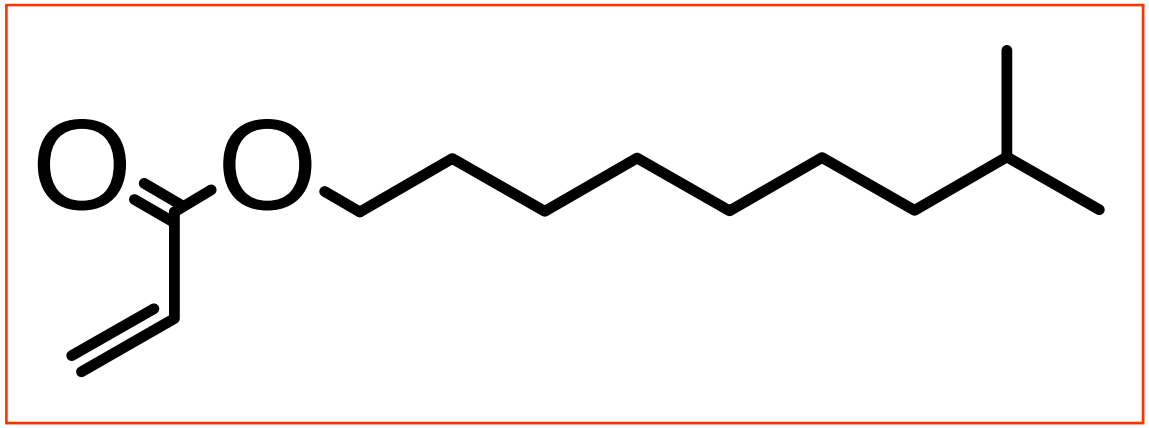


ECOSAR – Data Entry (Cont.)

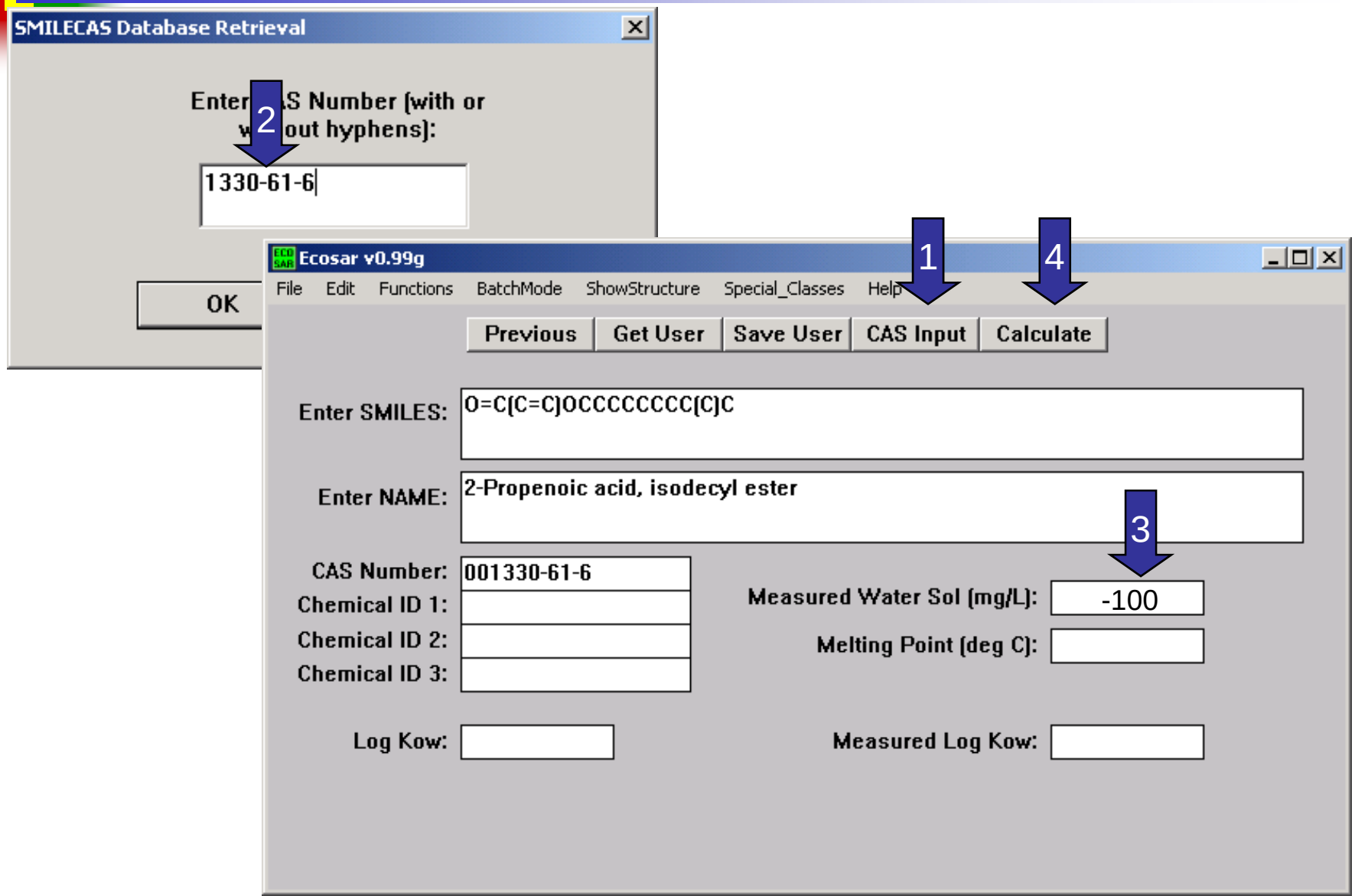
- Three ways to enter data:
  - Enter CAS number
    - Structure is imported into ECOSAR if the CAS number is in the accompanying SMILECAS database of >120,000
  - SMILES notation
  - Import Structure
    - Importable formats include:
      - Alchemy III MOL - HyperChem HIN - PCModel
      - Beilstein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

Example Chemical

Isodecyl acrylate



ECOSAR - Data Entry



SAR Equations for Acrylates Used by ECOSAR

- Acrylates:
  - Log 48-h LC_{50} = 0.00886 - 0.51136 log K_{ow} (Daphnids, mortality)
  - Log 96-h LC_{50} = -1.46 - 0.18 log K_{ow} (Fish, mortality)
  - Log ChV = -1.99 - 0.526 log K_{ow} (Fish chronic value; survival/growth)
  - Log 96-h EC_{50} = -1.02 - 0.49 log K_{ow} (Green Algae, growth)
- The values calculated by these equations are in units of millimoles/L

ECOSAR - Results

SMILES : O=C(C=C)OCCCCCCCCC(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

Acrylates

ECOSAR - Results (cont.)

ECOSAR Class	Organism	Predicted Duration	End Pt	mg/L (ppm)
Neutral Organic SAR (Baseline Toxicity)	: Fish	14-day	LC50	0.604
Acrylates	: Fish	96-hr	LC50	0.900†
Acrylates	: Daphnid	48-hr	LC50	0.554†
Acrylates	: Green Algae	96-hr	EC50	0.066
Acrylates	: Fish	32-day	ChV	0.005

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

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 any liquid chemicals with log Kow >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

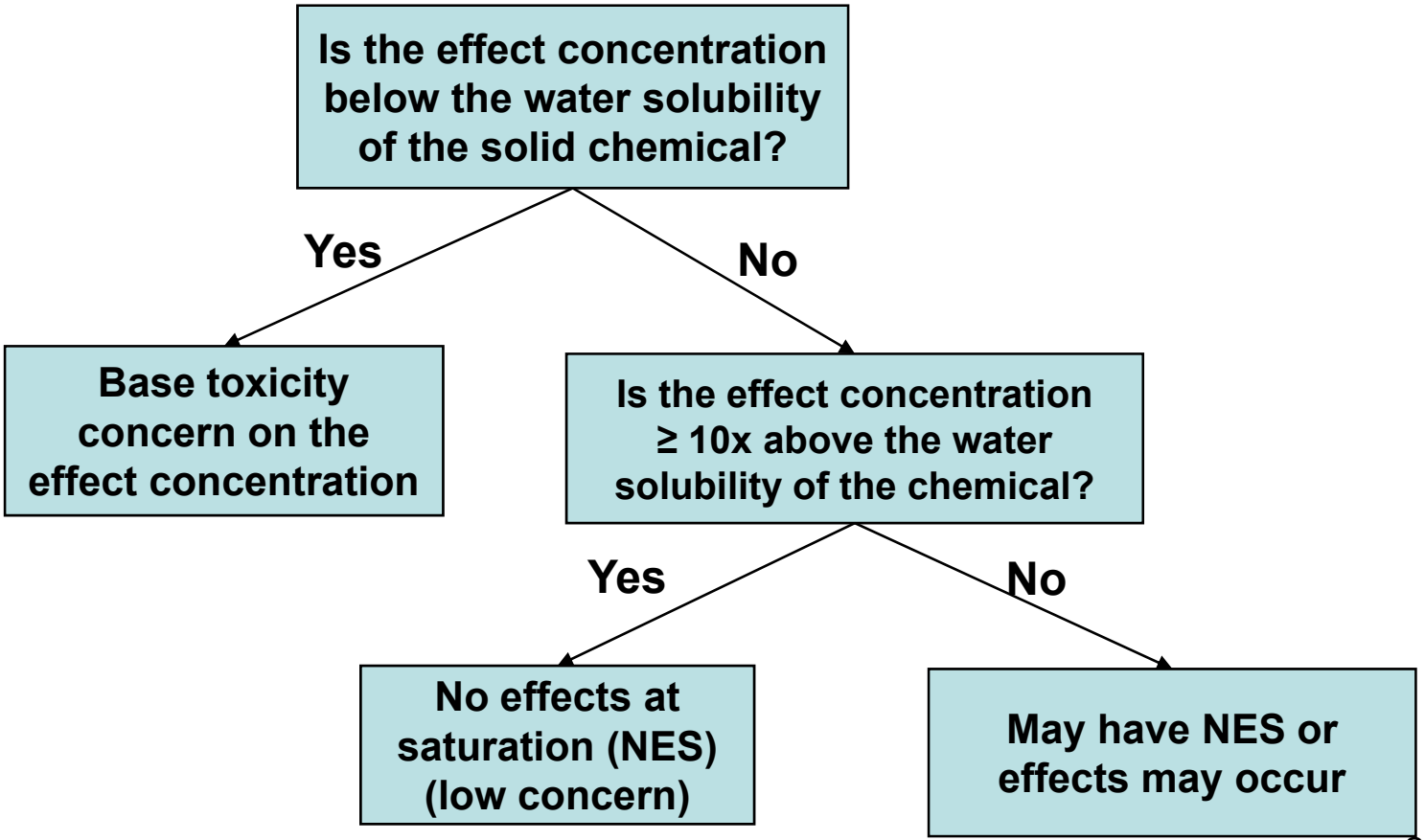
- Definitions
  - LC_{50} = Median concentration associated with 50% mortality
  - EC_{50} = Median concentration associated with effect on 50% of the organisms
    - LC_{50} and EC_{50} determined by statistical analysis
  - ChV = Chronic toxicity value
    - The ChV is the geometric mean of the NOEC and LOEC

Interpreting Results (Cont.)

- Baseline toxicity (neutral organic 96-hour or 14-day fish LC_{50})
  - Interpreting baseline toxicity
- 14-Day vs. 96-hr fish LC_{50}
  - No statistical difference between the intercept or slope
- Interpreting K_{ow} Cutoffs
  - Expect no effects at saturation (NES) for *liquid* chemicals with Log K_{ow} >5 for acute tests for many classes
    - Log K_{ow} of isodecyl acrylate is slightly above 5 so **may** get NES
  - Expect no effects at saturation for *liquid* chemicals with Log K_{ow} >8 for chronic tests for many classes

Interpreting Results (Cont.)

- Log K_{ow} cutoffs are not used for solid chemicals
- For solids, compare the effect concentration with solubility



Interpreting Results (Cont.)

- Full standard environmental toxicity profile includes 6 values:
  - 96-hour fish LC_{50}
  - 48-hour daphnid LC_{50}
  - 96-hour green algae EC_{50}
  - Fish, daphnid, and green algae ChV's
- Daphnid and green algae ChV's were not determined by ECOSAR for isodecyl acrylate
- Daphnid and algae ChV values can be calculated using acute to chronic ratios (based on analog data)
  - Daphnid ChV prediction = 48-hour $LC_{50}/10$
  - Green algae ChV prediction = 96-hour $EC_{50}/4$
- If appropriate acute to chronic ratios cannot be determined, higher assessment factors are applied to the effect concentrations
  - Generally results in a more conservative risk assessment

Standard Environmental Toxicity Profile for Isodecyl Acrylate

Endpoint	Predicted Value
96-hr fish LC_{50}	0.900*
48-hr daphnid LC_{50}	0.550*
96-hr green algae EC_{50}	0.070
Fish ChV	0.005
Daphnid ChV	0.06
Green algae ChV	0.02

* The log K_{ow} of the chemical (5.07) is at 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 predicted acute 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

ECOSAR Validation Study

- Test data from 462 chemicals were compared to the predicted toxicity values for acute and chronic toxicity for fish, daphnids, and green algae

- Predicted: measured toxicity ratios were calculated
  - Ratio of 1.0 = accurate prediction
  - Ratio < 1.0 = SARs were over predicting toxicity
  - Ratio > 1.0 = SARs were under predicting the toxicity.

Results of Validation Study (Nabholz, 1993)

- Algal chronic value ratio = 1.07
- Algae 96-hour EC_{50} ratio = 0.81
- Daphnid 48-hour LC_{50} ratio = 0.79
- Fish 96-hour LC_{50} ratio = 0.64
- Fish chronic value ratio = 0.24
- Work on validating the SARs is continuously ongoing in RAD

SAR/MPD Study (1990 – 1993)

- U.S. EPA used SAR to predict toxicity of new chemicals submitted to the EU via Minimum Premarket Datasets
  - http://www.epa.gov/opptintr/MPD-SAR/

MPD[1] / SAR

Predicted LC_{50} / Measured LC_{50}

HIGH QUALITY

	Scientific[2]	Regulatory[2]
TOTAL (224)	0.71	0.87
Fish (114)	0.76	0.96
Daphnids (105)	0.64	0.76
Green algae (5)	1.3	1.3

1 European Union Minimum Premarket Dataset

2 Regulatory dataset: a ratio of 1 was assigned when inequalities were consistent with each other; Scientific dataset: inequalities were treated as discrete values

Overall Conclusions of SAR/MPD Study

- SAR approach was largely successful in putting hazardous chemicals into review

- SAR limitations:
  - Eco toxicity (fish toxicity tended to be over predicted)
  - SAR would benefit from targeted testing, which is not allowed under TSCA

References

- You can find additional information about HPV program and EPA assessment models and methodologies in http://epa.gov/oppt/chemrtk/ and http://www.epa/gov/oppt/newchems/

- You can find out more about the use of ECOSAR as well as the other models by contacting us at CERM:

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