Predicting the Toxicities of Chemicals to Aquatic Animal Species

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List of Figures

List of Tables

Glossary

Adverse Outcome Pathway: A conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome at a biological level of organization relevant to risk assessment (Ankley *et al*, 2010).

Estimation, point: The statistical derivation of a best value of a parameter from an existing set of empirical data that are subject to random variation using a model of the relationship between the data and the parameter.

Prediction, point: The statistical derivation of an expected value of a random variable using a model of the relationship between an existing set of empirical data and a new value of the unknown random variable.

Mechanism of action: A complete and detailed understanding of each and every step in the sequence of events that leads to a toxic outcome, which includes detailed knowledge of the causal and temporal relationships among all the steps leading to a specific effect (Ankley *et al*, 2010).

Mode of action: A common set of biochemical, physiological, or behavioral responses that characterize an adverse biological response where major, but not necessarily all, linkages between a direct initiating event and an adverse outcome are understood (Ankley *et al*, 2010).

Non-Target Species: Organisms other than those that the pesticide is intended to kill / effect (*e.g.*, for an insecticides all species other than insects are considered non-target species).

Pest organism: The organism a pesticide is intended to kill or effect (*e.g.*, for an insecticide the target species are insects).

Predictive methods: Methods that can be used to obtain a predicted toxicity value when an experimentally determined toxicity value is not available for a taxon.

Structure-Activity Relationship: Methods that relate structural features of molecules to either biological or physico-chemical activity.

Qualitative Structure-Activity Relationship (SAR): Methods that relate structural features of molecules to an activity in a qualitative manner. Qualitative SARs are derived from non-continuous data (e.g., "yes" or "no" data such as structure is similar or not). They do not provide a point estimate.

Quantitative Structure-Activity Relationship (QSAR): Methods that relate structural features of molecules to an activity in a quantitative manner. QSARs are derived from continuous data (e.g., test results on toxic potency), providing a point estimate.

Tools: A computational application capable of calculating toxicity by applying model algorithms.

Abbreviations

ACE	Acute-to-Chronic Estimation
ACR	Acute-Chronic Ratio
ALT	Accelerated Life Testing
ALSV	Aquatic Life Screening Value
AOP	Adverse Outcome Pathway
ASTER	Assessment Tools for the Evaluation of Risk
ASTM	American Society for Testing and Materials
ALWQC	Aquatic Life Water Quality Criteria
CBI	Confidential Business Information
CMC	Criterion Continuous Concentration
CCC	Criterion Continuous Concentration
CWA	Clean Water Act
ECOSAR	Ecological Structure-Activity Relationships
EU	European Union
FA	Factor Analysis
FACR	Final Acute Chronic Ratio
FAV	Final Acute Value
FCV	Final Chronic Value
FDDCA	Federal Food, Drug, and Cosmetics Act
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
GEOMEAN	Geometric Mean
HPV	High Production Volume
ICE	Interspecies Correlation Estimation
INCHI	IUPAC International Chemical Identifier
IPSC	International Program on Chemical Safety
LOEC	Lowest Observable Effect Concentration
Log P	Log of the octanol/water partition coefficient
LRA	Linear Regression Analysis
LSER	Linear Solvation Free Energy Relationship
MATC	Maximum Allowable Test Concentration; (geometric mean of NOEC and LOEC)
MDRs	Minimum Data Requirements
MOA	Mode of Action
MPA	Multi-factor Probit Analysis
MSE	Mean square error
NAWQC	National Ambient Water Quality Criteria
NOEC	No Observable Effect Concentration
NRC	National Research Council
OCSPP	Office of Chemical Safety and Pollution Prevention (formerly Office of Prevention, Pesticides, and Toxic Substances [OPPTS])
OECD	Office of Economic Cooperation and Development
OW	Office of Water
OPP	Office of Pesticides

OPPT	Office of Pollution Prevention and Toxics
ORD	Office of Research and Development
PCA	Principle Component Analysis
PMN	Pre-Manufacturing Notification
PNN	Probabilistic Neural Network
QSAR	Quantitative Structure-Activity Relationship
(Q)SAR	Denotes either qualitative or quantitative Structure-Activity Relationship
QSPR	Quantitative Structure Property Relationship
RA	Risk Assessment Tools: Software and Users Guide
REACH	Registration, Evaluation, Authorisation, and Restriction of Chemicals
SAR	Structure-Activity Relationship (qualitative)
SMAV	Species Mean Acute Value
SMILES	Simplified Molecular Input Line Entry System
SSD	Species Sensitivity Distribution
TCE	Time-Concentration Event
Te	Toxicity Effect Ratio
TSCA	Toxic Substances Control Act
USEPA	U.S. Environmental Protection Agency
UVCB	Unknown or Variable composition, Complex reaction products or Biological
WOE	Weight-of-evidence

1 Executive Summary

The purpose of this white paper is to present an overview of predictive methods that may be useful to U.S. Environmental Protection Agency (USEPA) risk assessors along with States, Regional and Tribal risk assessors in extrapolating data for estimating a level of adverse effect (toxicity) of pesticide active ingredients and degradates to aquatic animals. The predictive methods discussed in this document may be used to derive surrogate values that can then be incorporated into approaches for developing aquatic life screening values (ALSVs) for chemicals that have small data sets; see white paper "*Exploration of Methods for Characterizing Effects of Chemical Stressors to Aquatic Animals*." These predictive methods will be integrated into a suite of tools for use by both Office of Water (OW) and the Office of Pesticide Programs (OPP) when insufficient acceptable chemical-specific data are not available from submitted data and the open literature to meet effects assessment data requirements established by the offices. These predicted values are intended for use in deriving ALSVs and supplementing data submitted under FIFRA 40 CFR Part 158 Subpart G (CFR 2010), or when additional data are required to reduce uncertainty in OPP's ecological effects assessments. Although the focus of this white paper is on predictive methods and/or tools that predict toxicity data specifically for pesticides, the predictive methods described in this paper may have broader applicability to other chemicals that are not pesticides.

Although many of the predictive methods (e.g. QSAR) discussed in this paper have been used by USEPA to predict the potential toxicity of chemicals where measured data are not routinely available, their use has been inconsistently applied. Even where predictive methods have served as a source of information on a compound, their utility in providing data that can be used qualitatively versus quantitatively is limited to the predictive capacity of the models in a weight-of-evidence (WOE) approach. This approach considers the predictive capacity of the model for chemicals with known physical/biological characteristics as compared to similarly structured compounds where such characteristics are unknown.

The predictive methods discussed in this white paper include quantitative/qualitative structure-activity relationships ((Q)SARs), read-across/bridging, Office of Economic Cooperation and Development (OECD)/USEPA chemical categories and/or mode of action, interspecies correlation estimation (ICE) models, acute-chronic ratios (ACR), and time-concentration effect (TCE) models. Documentation needed to substantiate the use of these predictive methods will be provided in both screening-level assessments intended to identify data gaps and prioritize testing needs as well as more refined assessments.

2 Introduction

In April 2009, EPA developed a document entitled "*Toward a Common Effects Characterization Methodology Scoping Document*" (hereafter referred to as the Scoping Document). The Scoping Document provided background for a proposal to develop a common effects characterization methodology for use in ecological assessments of chemicals (*e.g.*, pesticides) by EPA to meet the mandates of the Clean Water Act (CWA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The resulting framework would integrate the Agency's aquatic effects characterization methods and provide a common basis for achieving the water quality protection goals established under the CWA and FIFRA statutes. The effort focuses on data-limited situations where methods have not been clearly articulated for using limited taxa-specific data to characterize effects to aquatic communities composed of multiple taxa (vertebrate and invertebrate aquatic animals and vascular and nonvascular aquatic plants). Once sufficiently validated and vetted, the methods could then be used by state, local and Tribal water management agencies to interpret aquatic ecological risks associated with chemical exposure information, *e.g.*, monitoring data.

The proposed approaches articulated in the Scoping Document were subsequently discussed at stakeholder meetings in various regions throughout the country in January 2010. Since that time, three white papers, including this paper on "*Predicting the Toxicity of Chemicals to Aquatic Animal Species*", have been developed. The second white paper,"*Exploration of Methods for Characterizing Effects of Chemical Stressors to Aquatic Animals,*" explores possible means through which limited data sets could be either enhanced using predictive tools or accommodated using adjustment factors to characterize the lower range of sensitivities to specific chemicals that may exist in an aquatic community. Additionally, a third white paper, "*Deriving Plant Aquatic Life Screening Values for Pesticide Effects,*" has been developed exploring the uncertainties associated with and methods for characterizing effects to the aquatic vascular and nonvascular plant community from chemical exposure. . The tools and methods discussed in these papers are intended to compensate for limited data in describing potential effects of specific chemicals on aquatic animal and plant communities and would provide regulators with a means of deriving advisory values that will ensure the protection of the aquatic environment.

The three white papers are intended to discuss uncertainties associated with extrapolating limited taxa-specific data to predict effects on aquatic communities and to provide recommendations on methods for either enhancing and/or accommodating limiting data to enhance their ability to better represent species sensitivities across animal and plant taxa. This paper on predictive tools provides a broad overview of available tools that may be used to enhance and/or compliment limited data sets. Outputs (predictions) from the tools discussed in this paper are then used in the paper on methods for characterizing effects to explore how, either through assessment factors or species sensitivity distributions, values may be derived that can be considered representative of potentially sensitive species within the aquatic environment.

Under FIFRA, the Office of Pesticide Programs (OPP) receives test data for use in their risk assessments based on the guideline requirements specified in 40 CFR Part 158; however, the submitted data may not cover the aquatic taxonomic diversity as defined by the 1985 Guidelines minimum data requirements (MDRs). Although both OPP and the Office of Water (OW) consider data from the open literature, many pesticides have a smaller data set than that required to develop 304(a) aquatic life water quality criteria (ALWQC) using the established approach (Stephan *et al*. 1985, herein referred to as "1985 Guidelines"). OPP and OW are examining the extent to which they will leverage modeling approaches to predict the hazard of pesticides to aquatic organisms for which data is lacking, thus building upon existing methodologies for conducting risk assessments (Stephan et al., 1985; USEPA, 2007). The goal is to develop a well grounded, science-based, and informed process to develop surrogate values that will be used in an effects assessment methodology that provides consistency between OPP and OW approaches. In addition, these predictive techniques could also be used to characterize uncertainties in registrant-submitted data, thereby identifying potential gaps for future work; or possibly to reduce data requirements, where there is sufficient concordance of information, thereby reducing animal testing and associated costs. This effort would make greater use of existing data and available predictive methods in the decision making process. This is consistent with recommendations from the National Research Council (NRC) of the National Academies of Science recommendations in their report entitled "Toxicity Testing in the Twenty-first Century: A Vision and a Strategy" (NRC, 2007).

The use of modeling techniques to predict the potential toxicity of chemicals where empirical data are not available is not unprecedented in the field of risk assessment. USEPA has a long history of using structure-activity approaches under the Toxic Substances Control Act (TSCA) (Auer *et al*., 1990; Comber *et al.*, 2003; van Leeuwen *et al.*, 2009). Under Section 5 of TSCA (15 U.S.C. 2601–2692), USEPA's Office of Pollution Prevention and Toxics (OPPT) must provide a risk assessment of new chemicals within 90 days of receipt of a pre-manufacturing notification (PMN) (Nabholz *et al*., 1997). The PMN includes chemical structure, intended use, anticipated production volume and release information, and any available exposure or effects data (Nabholz *et al*., 1997). Although OPPT receives up to 2,000 PMNs annually, 65% include no test data (van Leeuwen *et al*., 2009), and only about 5% include ecotoxicity data (Zeeman *et al*., 1995). Because of these constraints, OPPT has leveraged both qualitative (SAR) and quantitative structure-activity relationships (QSAR) (Auer *et al.*, 1990; Nabholz *et al*., 1997; van Leeuwen *et al*., 2009) to address various risk assessment issues. Independent assessments of predictive methods such as EcoSAR and ASTER attest to the reliability of these models for predicting toxicity for non-specific modes of action (MOAs), and, with limited success, predicting toxicity for more specific MOAs such as reactive mechanisms (Moore et al., 2003; Reuschenbach *et al*., 2008). QSAR models have also been used throughout OPP, although infrequently. Use of QSAR models within OPP, specifically in ecological effects assessments, is typically based on the WOE approach where the predictive capacity of the models is a critical factor for determining whether the output may be used quantitatively or qualitatively. Examples of how various predictive methods have been used in OPP to predict toxicity to pesticide degradates, is provided in **Appendix A**. Additionally, OPP has identified positive attributes and desired qualities for models used in regulatory decision making, such as peer review from the scientific community, transparency, and model availability to the public (http://www.epa.gov/oppefed1/models/water/model_attributes.htm .)

This white paper describes various predictive methods, their history and background associated with development and use, and the importance of category approaches and mode of action in the application of these models. In addition, this white paper addresses criteria to be used in assessing the appropriateness of various modeling approaches, the state-of-the-art of existing applications / predictive methods, and how these predictive methods can be used to fill data gaps and address risk assessment uncertainties relevant to OPP and OW.

3 General Overview of OPP and OW Data Requirements

USEPA's OPP and OW are undertaking a paradigm shift related to toxicity testing that moves towards a more efficient and refined risk assessment. The goal is to reduce the use of whole organism testing and the overall cost of the risk assessment process while maintaining the breadth and depth of data coverage (NRC, 2007; CFR 2008.). This paradigm shift makes greater use of available data where predictive relationships are sufficiently robust to support a risk assessment, moving from a guidelines-driven risk assessment, to one that targets whole organism testing requirements based on a WOE approach.

3.1 OPP Data Requirements

Within OPP, this process starts with problem formulation to identify what is known about the environmental fate and effects of a particular chemical and it's degradates and associated data gaps and uncertainties. Risk assessors either make conservative assumptions when data are not available or attempt to draw inferences from similarly structured compounds. While guideline data are typically submitted for parent compounds undergoing registration, a full complement of guideline studies are rarely available for degradates of potential concern; predictive modeling can enable degradates to be considered qualitatively. Further, while guideline studies provide data on freshwater and estuarine/marine fish and invertebrates, the number of studies for these taxa is limited. Most baseline datasets for chemicals submitted by a registrant are comprised of two freshwater fish and one freshwater invertebrate toxicity studies. Data for estuarine/marine species are typically confined to one estuarine/marine fish and two estuarine/marine invertebrates. Internationally, requirements for pesticide registration data are similar. For higher profile chemicals, a broader range of data may be available from both the registrant-submitted studies and open literature. However, for newer chemicals, open literature studies are oftentimes limited. Registration of pesticides in other jurisdictions often offers additional data.

OPP's aquatic risk assessments evaluate individual taxa and are based on the most sensitive vertebrate and invertebrate endpoints for freshwater and estuarine/marine species. Reliance on the most sensitive species is intended to be conservative and does not reflect intra- and inter-species variability. When sufficient data exist, assessments are refined using species sensitivity distributions (SSDs) to provide a qualitative description of the range of sensitivities within and between taxa. As such, OPP's risk conclusions have not typically relied on SSDs to quantitatively characterize the potential effects of pesticides (and degradates), but rather to

qualitatively characterize the conservatism of the most sensitive endpoint. With respect to degradate toxicity, OPP uses the best available information to determine whether degradates are more, less, or equally toxic as compared to the parent compound. In situations where degradates are more than or equally as toxic as the parent and/or toxicity data are not available for the degradates, OPP derives aquatic exposure estimates by using a total toxic residue approach. These approaches will be used to supplement the currently required test data from registrants, adopting a more integrated approach where validated models will be used along with empirical data in a WOE approach in the overall risk assessment process.

3.2 OW Data Requirements

OW does not have the statutory authority under the CWA to request specific toxicity tests and relies on open literature and data submitted by registrants in support of pesticide registrations under FIFRA to develop SSDs to support the development of ALWQC, as per the 1985 Guidelines. Similar to the challenges confronting OPP, OW may not have sufficient data to meet the 1985 Guidelines MDRs. However, in such circumstances, OW is currently constrained from moving forward to develop ALWQC. The 1985 Guidelines and the Aquatic Life Screening Values white paper describe the MDRs in detail, including a description of how the MDRs are used to generate either an ALWQC or an ALSV. In short, a freshwater Criterion Maximum Concentration (CMC) is derived from acute (*e.g.* LC_{50}) toxicity values using the following taxonomic groups:

- family Salmonidae
- family in Osteichthyes
- family in phylum Chordata
- planktonic crustacean
- benthic crustacean
- an insect
- family in phylum that does not include arthropods or chordata
- family in any order of insect or phylum not represented

A saltwater CMC may be derived when data are available for the following groups:

- two families in phylum Chordata
- family in not in Chordata or Arthropoda
- either Mysidae or Penaeidae family
- three other families not in the phylum Chordata
- any other family

The chronic ALWQC, or Criterion Continuous Concentration (CCC), may be derived when the following information is provided:

- eight chronic studies from the same taxa as described for the CMC; OR
- chronic studies from three families of aquatic animals for which acceptable acute data are

available and one being an acutely sensitive species (this approach utilizes acute to chronic ratios (ACRs) discussed in Section 4.4 of this white paper).

OW will explore the use of the techniques described in this white paper to fill gaps in taxonomic data to facilitate derivation of ALWQC. A critical component in migrating towards the use of predictive methods will be to identify those that provide reliable and relevant endpoint data and result in transparent and scientifically defensible effects assessments.

4 General Concept and Background of Predictive Methods

This section provides an overview of approaches, history of use, and technical basis for the predictive methods that are the focus of this paper; namely quantitative and/or qualitative structure-activity relationships ((Q)SARs), interspecies correlation estimation (ICE) models, acute-chronic ratios (ACRs), and time-concentrations event (TCE) models. Section 5 provides general descriptions of the applications through which these models are available. An important consideration in the selection of appropriate models is the mechanistic-basis of the toxic response. QSAR models can be developed using global models which cover a number of different mechanisms of action within a single equation or local models which predict for a more limited set of structurally-similar chemicals, with the assumption that they are all acting via a similar MOA. The linkage of the mechanism of action to the adverse outcome pathway (AOP), with a reasonable level of confidence, is an important step in developing causal linkages of chemical exposure to the observed apical endpoint response (OECD, 2007d). Therefore this section begins with an overview of terminology and historical background on approaches related to MOA.

4.1 Consideration of Mode of Action in Predictive Toxicology

Determining mode of action (MOA) for most substances is typically based on information related to potency, structural features, *in vitro* data, responses related to duration of exposure, and/or mixture effects, although approaches have been developed to help 'bin' chemicals into acute MOA (Verhaar *et al*., 1992; Russom *et al*., 1997). Pesticides are, by definition, substances designed specifically to affect an organism's biological processes, and generally there is a known molecular site of action within the pest organism. Gathering information related to the specific MOA to the pest organism, and how conserved the molecular site of action is across non-target species will be a critical step in leveraging (Q)SARs to fill data gaps. Therefore, an important requirement for use of mechanistic approaches is having an understanding of the biological processes for relevant species. Another important consideration is that non-target species may include many species, which taxonomically, would be considered targets, but by intended use are actually non-target species. For instance, an insecticide with a registered use on golf courses to reduce certain insect pests may appear in streams via run-off events, and thereby cause effects on aquatic insects that are not the intended target species. For the purposes of this document, aquatic organisms will be considered non-target species, with the exception of aquatic plants

when herbicides (e.g., algaecides) are the pesticide discussed and fish when piscicides are discussed.

The understanding of a chemical's mechanistic profile is insightful for any risk assessment process. Information regarding the key events that lead to an adverse outcome, as well as toxicokinetics, toxicodynamics, and metabolism provide a biological basis to support assumptions used within the WOE approach supporting a risk assessment. Chemical agents can be characterized by their mode or mechanism of action, and, if used with SAR/QSAR approaches, this pathway information can infer greater confidence in predicted adverse outcomes.

These mode of action-based techniques have permitted the development of mechanistically-based toxicity models such as those included in the ASTER and EcoSAR systems, and QSAR models published in the open literature (see Könemann, 1981; Veith et al, 1983; Russom et al., 1988; Schultz 1987). The development of expert systems that can assess the adverse effects of large numbers of chemicals in a computationally-efficient manner, and do so with an understanding of toxic mechanisms is essential considering the large number of chemicals in commerce for which no ecotoxicological data are available. Through the use of these modeling techniques, the acute toxicity of chemicals with non-specific MOAs, which are estimated to be approximately 70% of the discrete organic chemicals released into the environment, can be predicted with a high level of confidence (Bradbury et al., 2003).

4.1.1 Mode of Action vs. Mechanism of Action

The terms mode of action (MOA) and mechanism of action have been used interchangeably in the past, and have had conflicting definitions (Guyton *et al.*, 2008). For the purpose of this white paper, the terminology cited in the National Research Council report "Toxicity Testing in the 21st Century" is used. The term "mode of action" is defined as an understanding of selected key events and/or processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in a disease state or other adverse effect (NRC, 2007). A "key event" is an empirically observable precursor step that is itself a necessary element of the MOA or is a biologically based marker for such an element (NRC, 2007). A "mechanism of action" differs from MOA in that it contains a more detailed and complete understanding and description of each of the key events, often at the molecular level (NRC, 2007). The elucidation of the key events along the toxicity pathway for a particular toxic response in a biological system is a data rich determination. Significant information should be developed to ensure that a scientifically justifiable MOA underlies the process leading to the adverse outcome.

4.1.2 Adverse Outcome Pathway

The NRC report released in 2007 outlined a vision and strategic plan for toxicity testing in the context of emerging predictive methods for use in supplementing or replacing existing animal testing procedures (NRC,

2007). As part of this report, a toxicity pathway framework (Figure 1Figure 1. Abbreviated Toxicity Pathway (from NRC, 2007).

) was developed that forms the basis of discussions within the document. A toxicity pathway is defined as a cellular response that when sufficiently perturbed, and when the organism is unable to adapt, would lead to toxicity and/or disease (NRC, 2007). In order to ensure consideration of the "toxicity pathway" to identify typical apical endpoints observed in the whole organism, and, ultimately, to population responses, this concept was expanded to an Adverse Outcome Pathway (AOP) framework (see **Figure 2.** Adverse Outcome Pathway (modified from Ankley et al., 2010).

) (Ankley *et al*., 2010). The AOP provides a structure to organize collected data/information, with anchors at the molecular initiating event and the adverse outcome, describing interactions at the molecular target site, and the cascade of responses at the cellular, tissue, whole organism, and population levels. Based on the definitions provided in Section 4.1.1, a fully developed, mechanistically-based AOP describes a mechanism of action, while an AOP with information gaps and /or uncertainties could be described as a mode of action. In either case, the AOPs can serve to build the WOE required for a comprehensive risk assessment, and avoid confusion that may be related to use of the mode vs. mechanism of action terms. As discussed in Sections 4.1.3 and 7.4 the determination of the AOP involves a review of all aspects of the exposure and experimental design, since the selected AOP could differ depending on organism life stage, length of exposure, concentrations used, taxonomy, *etc*. Therefore the identification of alternate AOPs should be part of the process.

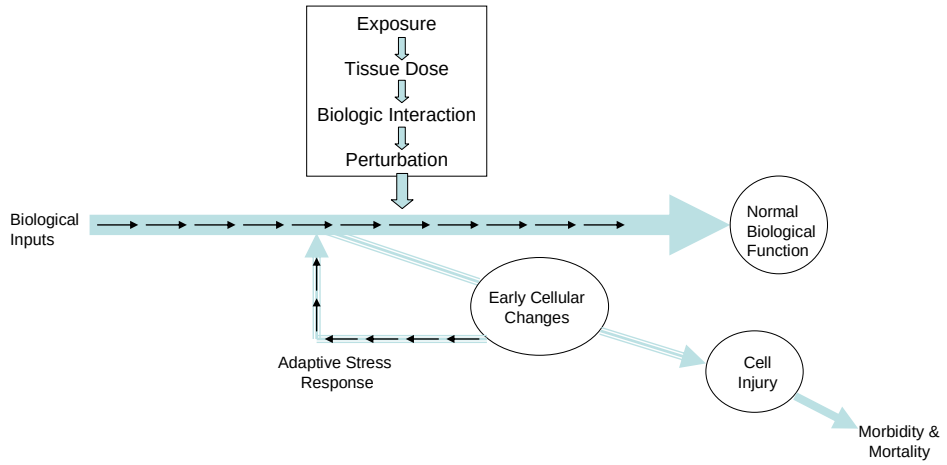

Figure 1. Abbreviated Toxicity Pathway (from NRC, 2007).

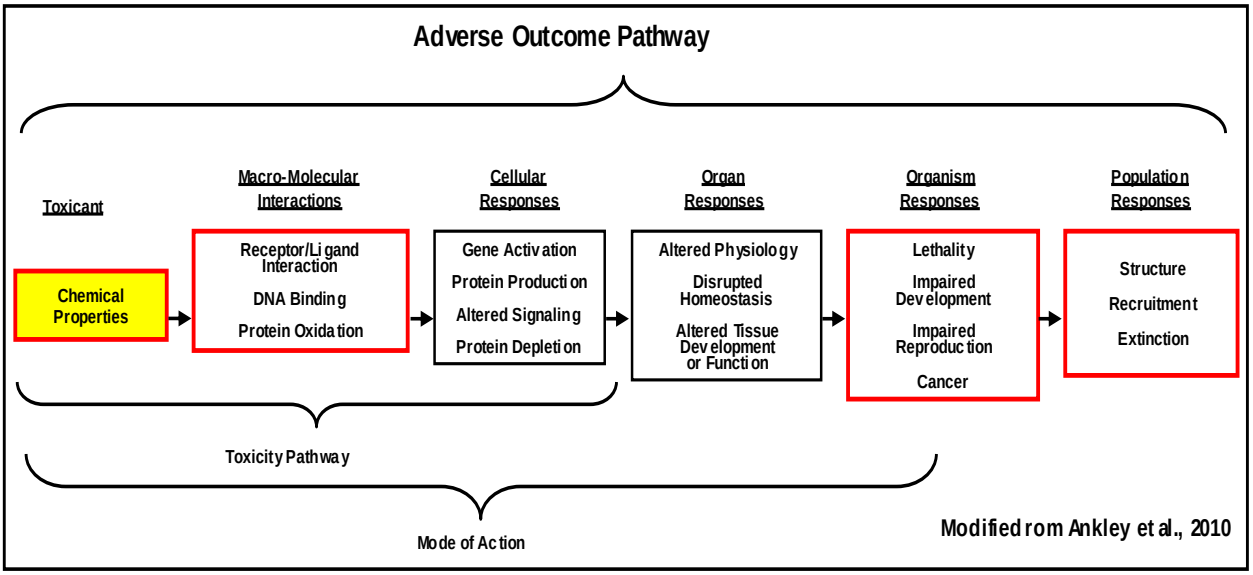

Figure 2. Adverse Outcome Pathway (modified from Ankley et al., 2010).

4.1.3 Methods of Classifying Substances for Use in AOPs

As mentioned in Section 4.1.1, the determination of an AOP for a substance is a data intensive process, which requires the building of WOE in support of the final determination. The International Program on Chemical Safety (IPCS), a joint program between the World Health Organization, International Labour Organization, and the United Nations Environmental Programme, has developed an approach based on the Bradford Hill criteria (Hill, 1965) to be used in cancer and non-cancer endpoint assessments, but they also apply to ecological endpoints (Boobis *et al*., 2008). The criteria for developing AOPs include the following:

1. Postulate a mode of action;
2. Identify key events and associated critical parameters,
3. Identify concordance of the dose-response relationships with key event, correlate dose dependency on increase in magnitude of key event;
4. Characterize the temporal association of toxic responses and key events;
5. Describe the WOE linking the key events including the strength, consistency, and specificity of association of key events and adverse outcome;
6. Describe biological plausibility and coherence of the AOP as it relates to:
    a. the molecular site of action,
    b. systems biology,
    c. relationship of the MOA and the observed adverse outcome,

    d. evidence from structural analogs,
    e. considerations related to organism life stage, sex and/or taxonomy, and
    f. consistency/adequacy of data in determining the AOP;
7. Discuss possible alternative AOP(s);
8. State fully and explicitly the uncertainties, inconsistencies, and data gaps associated with the determination of the AOP; and
9. Provide conclusions about the AOP.

The IPCS framework is intended as an analytical tool for organizing and judging whether the available data/information actually supports the postulated MOA, and subsequently can be used in the development of an AOP. The goal is a flexible and transparent process, but, as with any type of framework, the determination of whether the evidence is sufficient to make the final determination is based on the professional judgment as it relates to the context of how the information will be used.

Although reliable QSAR models existed in the 1980s to predict toxicity for non-polar narcotic substances (Könemann, 1981, Veith *et al*, 1983), methods on classifying untested substances into MOA groups had not yet been developed. In the 1990s, researchers in the Netherlands and the US developed schemes for grouping substances into general MOA categories (Verhaar *et al*., 1992; Russom *et al*., 1997). Although developed after the IPCS framework document, these approaches used WOE approaches and processes similar to those outlined in the Bradford Hill criteria (Hill, 1965).

The approach developed by Verhaar and co-workers placed chemicals into the following four categories (Verhaar *et al*., 1992):

- Inert chemicals: chemicals that are not reactive, and that do not interact with specific receptors within an organism.

- Less inert chemicals: chemicals that are not reactive, but are slightly more toxic than baseline toxicity due to hydrogen bond donor acidity.

- Reactive chemicals: chemicals that react unselectively with biomolecules, or substances that are bioactivated via metabolism.

- Specifically acting chemicals: chemicals that interact with receptor biomolecules.

These general classes were developed primarily using excess toxicity ratios (Veith *et al*., 1983, Lipnick *et al*.1987, Russom *et al*., 1988) and structural alerts gleaned from the literature, as well as empirical test results from an extensive toxicity database using the guppy (*Poecilia reticulate*) (Könemann, 1981). The excess toxicity ratio is used frequently to identify substances that are potentially acting differently then non-polar narcotics. As background, non-polar narcosis is also referred to as baseline toxicity; representing the minimal toxic response in a biological system. **Figure 3** below demonstrates how this baseline toxicity compares to water solubility for the fathead minnow. As lipophilicity increases, the toxicity approaches the water solubility in an acute exposure. The narcosis MOA is driven by hydrophobic interactions between chemicals

and biological membranes (Yamakura *et al*., 2001). Traditionally, the octanol/water partition coefficient (log P) has been used to represent this activity, and has been a readily calculated and fairly reliable parameter for use in predicting non-polar narcosis. An estimate of excess toxicity (Te) is calculated by using reliable QSAR models to predict the baseline acute toxicity (*e.g.*, Könemann, 1981; Veith *et al.*, 1983) and dividing this value by empirical data from acute toxicity tests (see **Eq.** 1). Verhaar found that inert chemicals have Te values around 1.0; less inert substances have Te ratios ranging from 5-10, and reactive and specifically acting chemicals have Te ratios of 10-10000 (Verhaar et al., 1992). Russom and coworkers found similar ranges in Te values for nonspecific and specific modes of action (Russom *et al.*, 1997; See **Figure 4**).

$$Te = LC50_{\text{baseline narcosis prediction}} / LC50_{\text{experimental}} \qquad \textbf{Eq. 1}$$

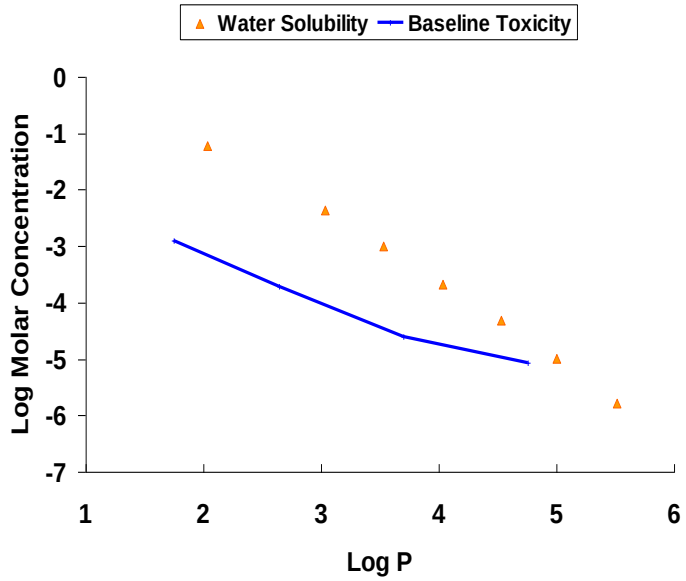

Figure 3. Plot of water solubility vs. baseline toxicity (Russom unpublished data).

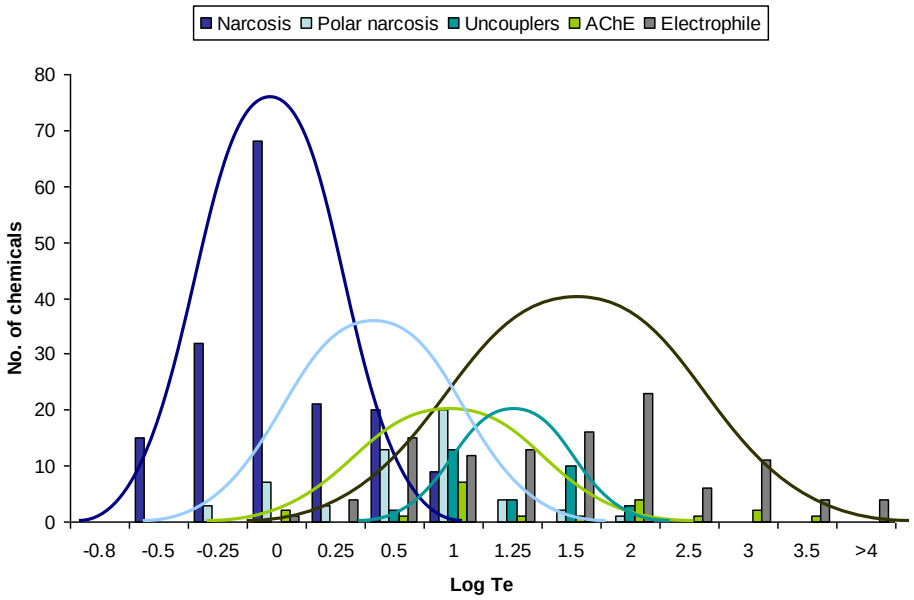

Figure 4. Number of chemicals by the logTe values (modified from Russom et al., 1997).

A workshop sponsored by USEPA in 1988 (Bradbury and Lipnick, 1990) provided a major contribution to chemical structure/property information used in both approaches. The workshop brought together experts in the field who described features of chemicals as they related to non-polar narcosis (Franks and Lieb, 1990), polar narcosis (Veith and Broderius, 1990), uncouplers of oxidative phosphorylation (Terada, 1990), electophilic, (Hermens, 1990; Carlson, 1990), carcinogenic (Kadlubar *et al*., 1990), redox cycling (Mason 1990), and various pesticidal MOAs (Coats, 1990; Duke, 1990; Fukuto 1990). Expanding on this workshop, Russom and coworkers outlined eight general MOAs, and defined structural fragments associated with seven of the MOAs (Russom et al., 1997). Respiratory inhibitors/blockers substructures were not defined due to the low number of chemicals available for assessment. MOA determination was based on the dose-response interpretation, Te ratios, mortality as it related to duration, and behavioral information gleaned from the fathead minnow data set (N=620) (Russom et al., 1997). Additionally, joint toxic action studies and toxicodynamic profiles conducted independently on a subset of the database, structural alerts identified from the 1988 workshop, and evidence on structural analogs found in the open literature were used in a WOE approach in assigning MOA (Russom et al., 1997).

It is important to note that there is no single list that identifies all existing MOAs and related AOPs (see Schmidt, 2009). Both the Verhaar and Russom approaches focused on industrial organic chemicals; however, these approaches did not include metals or many classes of pesticide active ingredients and pharmaceuticals. Both approaches used extensive data collections of acute lethality data for either the guppy (Könemann, 1981) or fathead minnow (Russom et al., 1997). Therefore, the assignment of a MOA using these approaches is specific

for fish during short exposure durations. **Appendix B** provides an overview of MOAs identified in the literature, with a special emphasis on pesticide active ingredients, and previous work done on industrial organics. It should be noted that this list is not intended to be comprehensive of all potential MOAs, but rather a starting point to build the WOE necessary to develop an AOP. Keeping in mind the Bradford Hill Criteria, risk assessors should be (1) identifying a potential AOP and associated key events; (2) determining whether the AOP is conserved across taxonomic hierarchy; *i.e.*, would the AOP be similar for non-target species; (3) investigating the applicability of specific AOPs to chronic exposure; (4) determining MOAs and AOPs for mixtures, and (5) determining whether QSARs are available for specific AOPs.

4.2 (Quantitative) Structure-Activity Relationships

The basic assumption of any structure-activity approach is that the chemical's structure imparts properties that relate directly to the chemical's activity, and assume that a group of chemicals that produce the same biological activity (*e.g.*, sodium ion modulators) have something similar about their chemistry (*e.g.*, pyrethroids). Structure-activity models include qualitative (SAR) and quantitative models (QSAR), with (Q)SAR referring to either qualitative or quantitative approaches. SAR models are based on non-continuous data, such as identifying active vs. inactive chemicals based on the presence or absence of specific structural features or properties (*e.g.* screening tools that identify whether a chemical will bind or not bind to a receptor). Examples of SAR approaches include prioritization and ranking of chemical lists (*e.g.*, Russom *et al.*, 2003; Schmieder *et al.*, 2003a,b) or data gap analysis using read-across approaches (*e.g.*, Hewitt *et al.*, 2010). QSAR models are based on continuous data and result in a quantitatively derived prediction of activity (e.g., EC50), related to a chemical, physical or structural property (e.g., the ER-BA endpoint which is a quantitative prediction of the relative binding affinity of compound X to the endocrine receptor). These relationships rely on information on many chemicals to predict the activity of a single chemical lacking data. The goal is to quantify 'structural similarity' imparting biological activity by defining structural analogs or chemical categories that may act 'similarly.' In the context that similar structures result in similar activity, it would then follow that an untested chemical that is similar in structure may produce the same activity.

(Q)SAR predictive methods can be grouped based on the type of extrapolation approach used. Although the focus of this white paper is chemical-to-chemical and species-to-species extrapolation techniques, it is important to acknowledge that other activity-to-activity extrapolations, such as predicting whole organism responses from *in vitro* test data and lab-to-field extrapolations, also exist but are not within the scope of this paper.

SAR approaches existed prior to TSCA's promulgation in 1976, and were primarily used in the drug and pesticide discovery and development arena. These techniques became critical to TSCA risk assessments (as mentioned in Section 2) due to imposed time constraints (90 days to complete a risk assessment) and the number of PMN reviews (up to 2,000 assessments/year) for new chemicals. SAR methods allowed EPA to maximize both efficiency and consistency in the evaluation of potential hazard. The use of QSARs by risk assessors in assessing potential toxic effects of organic chemicals on aquatic organisms evolved as computational efficiency and

toxicological understanding advanced, and has proved to be a scientifically-credible tool for use in predicting toxicity for structurally similar substances with little or no available empirical data. The development of SMILES (Weininger, 1988) as a means to identify structure information in a computer readable format, and the advancement of desktop computing, in the 1970's made (Q)SAR methods readily accessible to risk assessors outside of OPPT (Benfenati, 2007). The European Union (EU) under its REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals) legislation will be building on the efforts developed under TSCA to address the approximately 9,000 chemical safety dossiers that must be completed in December of 2010 (Royal Society of Chemistry, 2010). Many of these substances have data gaps and the use of (Q)SAR approaches will be critical in meeting the legislative deadlines (van Leeuwen *et al.*, 2009).

4.2.1 Chemical-Structure Methods

Critical to any (Q)SAR technique is clearly defining the chemical structure. Structures can be depicted in one-dimensional (1-D) formats (*e.g.*, molecular formula), 2-D formats (*e.g.*, Simplified Molecular Line Entry System (SMILES) (Weiniger, 1988) or InCHI™ code (Stein *et al*., 2003)), or 3-D formats (*e.g.*, structures that include geometry and spatial information). Since chemical structures are three-dimensional, they can have geometric isomers (*i.e.*, cis and trans or E and Z), optical isomers (*i.e.*, D/L, R/S, +/-) or display tautomerism, which typically exist in equilibrium within solutions. Chemicals can also be flexible, allowing rotation around single bonds. When there is more than one point of rotation, this can lead to a large number of conformers. In biological tissues, a chemical could exist as multiple conformers, which is an important consideration as it relates to activity within biological tissues (*i.e.*, adsorption and binding to receptors) (see Serifimova *et al*., 2002 for example). Many (Q)SAR approaches do not take into account the 3-D nature of chemical structures because the models were built using only the 1-D or 2-D structural information. This is changing as computational power is increasingly available at the desktop, and may be critical in some aspects of modeling pesticide activity, which may be dependent on the overall 3-D structure of the compound as opposed to the chemical's substructure.

Within the context of pesticides, a chemical active ingredient has a discrete structure, while a formulated product mixture has many chemical structures beyond that of the active ingredient. "Inerts", which are components of the formulated product that are not the active ingredient, include solvents, adjuvants, and other chemicals and are designed to improve the effectiveness of the active ingredient, but are not intended to affect the pest organism in the same way as the active ingredient alone (Weinhold, 2010). Inert ingredients may make up a significant portion of some formulated products. Within the context of this paper, the use of the terms "active ingredient" or "chemical" refer to a single discrete chemical structure and not a formulated product representing mixtures of chemicals. When developing (Q)SAR models, it is important to take into account whether the empirical data are based on the formulated product (eg., mixtures of structures or an active ingredient with a discrete, single structure).

4.2.2 Chemical Category Approach

The chemical category approach is an important tool in assessing chemicals with data gaps within USEPA's OPPT and in support of EU's REACH and OECD risk assessment activities. An analysis done by van der Jagt and coworkers on REACH data requirements found that large data gaps exist for substances with dossiers due in 2010 (van der Jagt *et al*., 2004). They estimate that REACH legislation will require the use of 3.9 million test animals to fulfill data requirements if alternative methods are not used, but that 72% of the testing needs do not have reliable or acceptable alternative methods available, specifically validated QSAR models for a wide range of chemical categories / AOPs, and acceptable *in vitro* test methods that can be used in risk assessments. An analysis of the Canadian Domestic Substances List, where four QSAR computational tools (i.e., ASTER, EcoSAR, PNN and TOPKAT) were evaluated, found that QSAR models worked well for non-specific AOPs (e.g., nonpolar narcosis), but were insufficient for risk assessment purposes for AOPs related to more specific mechanisms (Moore et al., 2003). At the same time, the EU and USEPA have a commitment to reduce the use of animal testing (NRC, 2007; Holmes *et al*., 2010). In the absence of validated QSAR models for some AOPs, risk assessors have been exploring the use of chemical category approaches to help fill these data gaps, while statistically-rigorous QSAR models and *in vitro* test methods are developed, validated, and approved for use within a risk assessment context (van Leeuwen *et al*., 2009). As with REACH efforts, the QSAR models specific to many pesticide active ingredients are not included in the existing (Q)SAR modeling applications, since the focus of model development was on industrial organics to meet needs under TSCA legislation. But, similar to REACH, data gaps may be filled using the chemical category approach, while efforts to develop validated and reliable QSAR models are underway.

A chemical category is defined as a group of substances with physicochemical, human health, or ecotoxicological attributes that are similar or follow a pattern as a result of structural similarity (OECD, 2007a). The "similarity" can be based on chemical structure/substructure (*e.g.*, common functional group or chemical class), properties including behavior in physical or biological process (*e.g.*, similar precursors or breakdown products), an incremental or constant change in potency (*e.g.*, increased carbon chain length), and/or the function / use of the substance (*e.g.*, detergents, fragrance) (OECD, 2007a). The OECD is holding a workshop in December of 2010 to explore the development of mechanistically-based chemical categories using AOPs (see OECD, 2009a). Chemicals within a category are not required to be similar in all these properties, and a substance can belong to more than one chemical category. The chemical category approach uses a WOE approach taking information from many tested chemicals and inferring information for an untested substance (see **Figure 5**). Each substance within a category is not tested, but rather data for select chemicals are used to make an effect assessment decision. The resulting information is used in a qualitative (*e.g.*, relative potency based on a read-across of empirical data) and a quantitative (*e.g.*, point-estimate from a QSAR model) manner (OECD, 2007c). Through this process, the category is used to fill data gaps (e.g., populate an MDR), and prioritize/rank substances to better inform the final effect assessment and testing strategies.

In order to be useful, a consistent approach for defining chemical categories has been developed (USEPA, 2002; OECD, 2007a; OECD, 2009b). The steps include grouping a series of chemicals based on a pattern or similarity within the group, gathering information on physicochemical

properties, fate, and ecological and human health effects for each chemical within the proposed category, evaluating the data for reliability, relevance and adequacy, using a read-across approach to identify potential data gaps, and finally, evaluating the data to determine if there is sufficient evidence to support the development of the chemical category. A critical aspect of reviewing data reliability, relevance, and adequacy is the endpoints of concern (e.g., LC50) and the associated test guidelines (e.g., OSCPP harmonized test guideline for fish acute toxicity). Examples of building categories can be found in Enoch (2010), Enoch *et al.*, (2009), USEPA (1999), and Worth and Patlewicz (2007).

An approach for determining the utility of (Q)SARs is to construct a chemical category matrix table such as that depicted in **Figure 5**. The matrix consists of category members, in this case chemicals in each of the columns, and corresponding sets of properties (*e.g.*, octanol/water partition coefficient) and/or activities, (*e.g.*, effects data) represented in each of the rows. The solid dots represent properties/activities for which reliable data exist. The hollow dots represent acute values for untested species. As illustrated in **Figure 5**, data gap filling can be done using read-across from one tested chemical to an untested chemical. The observation of a trend (increasing, decreasing, or constant) in the experimental data for a given endpoint across chemicals can also be used as the basis for interpolation (when the value to be estimated is bracketed on either side by empirical data) and also extrapolation (when the value to be estimated is bracketed on only one side by empirical data). As depicted in **Figure 5**, using a combination of predictive methods, *i.e.*, SARs/read-across, extrapolation and interpolation, the matrix of properties/activities for chemicals under consideration can be rendered less uncertain by making greater use of existing data. While initially there may be many data gaps and considerable uncertainty, the existing data inform the understanding of how chemicals with little to no data may act. The extent to which the matrix of information can be used quantitatively versus qualitatively depends on the strength of the relationships being used to predict within and beyond the measured ranges. Below is an overview of approaches that can be used within these chemical category matrices.

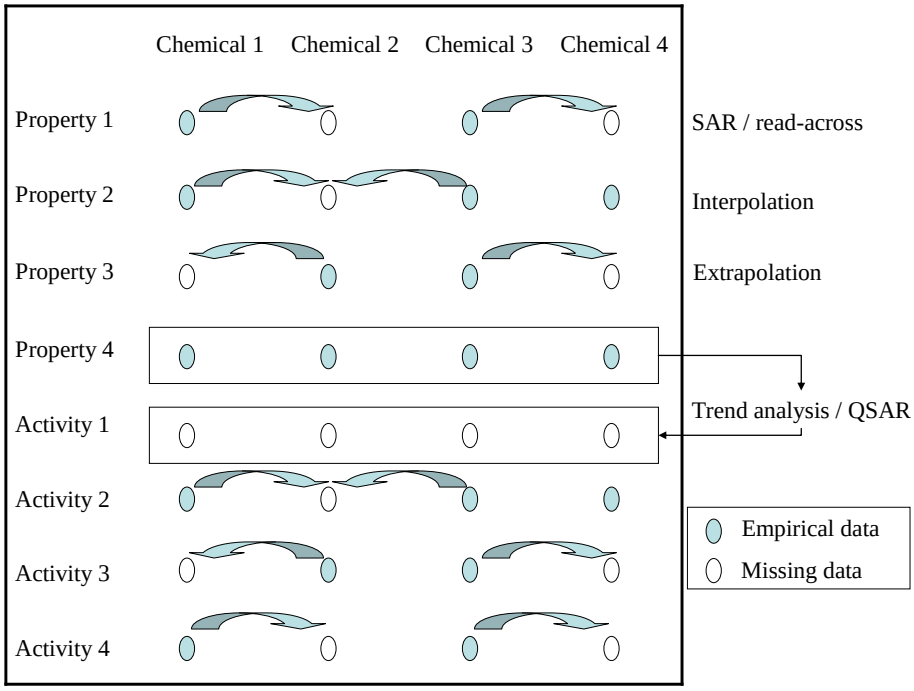

Figure 5. A chemical category is created to describe a series of "similar" pesticides. Data gaps within the data set are identified and filled based on information from data rich chemicals. (Modified from van Leeuwen et al, 2009).

Read-across / data bridging approach: Endpoint information for one chemical (the source chemical) is used to predict the same endpoint for another chemical (the target chemical), which is considered to be "similar" in some way (usually on the basis of structural similarity or on the basis of the same AOP). It may be performed in a qualitative or quantitative manner. (OECD, 2009b)

Interpolation is defined as the estimation of an intermediate term. The process of interpolation is depicted in **Figure 5** or filling data gaps relative to Property 2 and Activity 2 for Chemical 2. Interpolation is the estimation of a value for a chemical member using measured values from other members on "both sides" of that member within a defined chemical category. As such, the activity/properties of chemicals with structures that are intermediate [between] those where data exist can be predicted and bracketed.

Extrapolation is defined as the estimation of a value outside of a tabulated or observed range. Perhaps more to the point, it is a means by which to predict properties/activities, from what is known over a range of similar endpoints using measured data, to chemicals with no measured data outside that range. As such, extrapolation refers to estimation of a value for a member that is near or at the category boundary using measured values from internal category members. The process of extrapolation is depicted in **Figure 5** for filling data gaps relative to Property 3 and Activity 3 for Chemicals 1 and 4. Thus,

toxicity values could be predicted for chemicals that lie on either side of the range of information based on measured data.

QSAR / Trend Analysis: Within a chemical category, QSARs can be developed based on the trends in the empirical data. By plotting the activity and properties of chemicals with empirical data, a user can predict the chemicals with data gaps in activity based on predicted or measured property information. A key aspect to this approach is ensuring that the property is somehow related to the activity being modeled. Justification for use of property and activity data in predicting a particular activity for an unknown substance could include the applicability domain of the particular category developed, AOP of the chemicals under consideration, and commonality in substructures or break-down products.

Table 1 presents an example of a read-across for a series of pyrethroids, where the yellow boxes with "??" entries represent missing data points. Data from chemicals on either side of deltamethrin can be used to interpolate an LC50 value, or data for allethrin, dimethrin, resmethrin, and permethrin can be used to extrapolate a predicted value for bifenthrin. Using simple regression techniques and log P as the independent variable, the predicted toxicity of deltramethrin is 27 ug/L, and the predicted toxicity for bifenthrin is 7.9 ug/L. An important aspect of this category is that it is mechanistically-based, since all component chemicals modulate the sodium ion channel resulting in neurotoxicity. Another consideration is that all the test data are based on a standard test method (e.g. flow-through exposure; 96 hr duration, *etc*.). For the test data in **Table 1**, all studies are from either the USEPA's fathead minnow database (i.e., resmethrin and permethrin results; see Russom et al., 1997)) or from the US Geological Survey data set (i.e., allethrin and dimethrin; see Mayer and Ellersieck, 1986), with all following the ASTM (American Society for Testing and Materials) guidelines for fish acute toxicity testing (ASTM 2007). But differences exist in test procedures. The LC50s for resmethrin and permethrin are based on measured concentrations of the pesticide, while the LC50s for allethrin and dimethrin are based on nominal concentrations. Measured chemical concentrations may be critical for some extrapolations (i.e., for volatile substances), but may not be critical for these higher log P chemicals. These types of detailed analysis will need to be conducted when using read-across approaches.

Table 1. An example read-across for pyrethroids. Ordered by the physical chemical property LogP, which describes the partitioning aspect of the toxicity.

Parameter	ALLETHRIN	DIMETHRIN	DELTAMETHRIN	RESMETHRIN	PERMETHRIN	BIFENTHRIN
Fathead minnow LC50 values (ug/L)	53.0	62.0	??	6.16	16.0	??
Log P	5.52	6.57	7.02	7.11	7.61	8.15

4.2.3 Analog Approaches

There is a long history of the use of analogue selection techniques in which data associated with structurally similar chemicals are used to predict risk of chemicals where no data are available (*e.g.*, Lipnick, 1995b; Zeeman, 1995; Zeeman *et al.*, 1995; Karabunarliev *et al*., 1996a; Karabunarliev, 1996b; Russom *et al*., 1997; Walker and Printup, 2008). The proper application and continued acceptance of any structural analog approach requires well-defined and validated methods or models that can be used to systematically identify potential analogs, and subsequently identify appropriate QSAR models. Implicit in the development of these models is the need to define the group of chemicals by some level of similarity. The chemical categories and structural analog methods can be used to help bin chemicals for further use in development of models.

The analog approach is a variation on the grouping process used for chemical categories such that the grouping is only based on a single similar chemical or a limited number of similar chemicals. Traditionally, analog approaches involve predicting an endpoint or property of one chemical based on the available data for the same endpoint or property of a similar chemical. In this case, predictions are largely based on read-across methods. As discussed in Section 4.2.2, "similarity" can be based on common structures/substructures, chemical properties including behavior in physical or biological process, and/or the function/use of the substance (OECD, 2007a).

As mentioned in Section 4.2, (Q)SARs are based on the assumptions that a chemical's structure imparts properties that relate to biological activity, and that a group of chemicals that produce the same activity have something similar about their chemistry / structure. One goal of (Q)SAR approaches is to quantify 'structural similarity' imparting biological activity and identify which other chemicals may be 'similar' with the assumption that an untested chemical may produce the same activity. It is important to clarify that even if chemicals display a high level of structural similarity, they may not be functional analogs. Conversely, chemicals that act similarly are not always structural analogs (Saliner *et al.*, 2005.) Therefore, using a WOE approach, when gathering information to use in (Q)SAR approaches, is critical.

The OECD and USEPA have defined guidelines for identifying similar substances (USEPA, 1999; OECD 2007a) when building chemical categories based on the following commonalities:

- a common functional group or substructure (*e.g.*, phenols, aldehydes);
- a common precursor or break-down product may result in structurally-similar chemicals, which can be used to examine related chemicals such as acids/esters/salts. (*e.g.*, short-chained alkyl-methacrylate esters which are metabolized to methacrylic acid);
- an incremental or constant change (*e.g.*, increased carbon chain length; typically used for physicochemical properties such as boiling point); and

- common constituents or chemical class, similar carbon range numbers - used with substances of Unknown or Variable composition, Complex reaction products or Biological material" (UVCBs) (*e.g.*, series of linear alkyl sulfonates).

Pesticides are commonly grouped based on their similarity in pesticide activity (*e.g.*, insecticides, fungicides, herbicides, *etc.*) and chemical class (*e.g.*, organophosphate, triazine, *etc.*). Pesticides are substances designed to adversely affect organisms, and therefore differ in the chemicals typically addressed under TSCA, which focus on substances used in manufacturing, and specifically exclude those substances covered under Federal Food, Drug, and Cosmetics Act (FFDCA) and FIFRA.

Since pesticide active ingredients are designed to adversely affect the pest or target organism, and information is typically available on the molecular or tissue target site of action, this information can be used to define similarity based on a pesticide activity, common functional group approach and/or pest organism AOP information. As an example, in **Table 2,** a subset of the insecticide MOAs is presented. For each MOA, more than one chemical class is represented; therefore, 'similarity' is based on all three aspects of the substance, its pesticide classification (*e.g.*, insecticide), its pest organism MOA (*e.g.*, acetylcholinesterase inhibition), and structural analog /common functional group (*e.g.*, carbamate, organophosphate.)

Table 2. Target (pest) organism mode of action and associated chemical class for a select group of insecticides (adapted from IARC http://eclassification.irac-online.org/)

Pest Organism Mode of Action	Chemical Class
Acetylcholine esterase inhibitor	Carbamate
	Organophosphate
GABA-gated chloride channel antagonists	Cyclodiene organochlorine
	Phenylpyrazole (Fiprole)
Sodium channel modulators	Pyrethroid
	Organochlorine
Nicotinic Acetylcholine receptor agonists	Neonicotinoid
	Botanical
Juvenile hormone mimics	Juvenile hormone analog
	Carbamate
	Pyridine insect growth regulator

4.2.3.1 Structural Analogs

Structural analogs are chemicals that have a high degree of chemical similarity, but one or more functional groups or substructure(s) has been substituted (Saliner *et al.*, 2007). A structural analog can be defined by a substructure or similarity based on pattern matching or signature analysis (Saliner *et al*., 2007.) In **Figure 6** the structures for two pesticides belonging to the synthetic pyrethroid class of insecticides, permethrin and bifenthrin, are presented. These two chemicals can be defined as structural analogs because all structures share a common substructure (*i.e.*, a cyclopropane carboxylic acid). The difference in potency (**Table 3**) is based

on partitioning and electronic charge characteristics of each chemical within a specific matrix (*i.e.*, biological or other media such as water) and related to the functional groups that are not common among each structure (*e.g.*, fluorine atoms on bifenthrin versus chlorine atoms on permethrin).

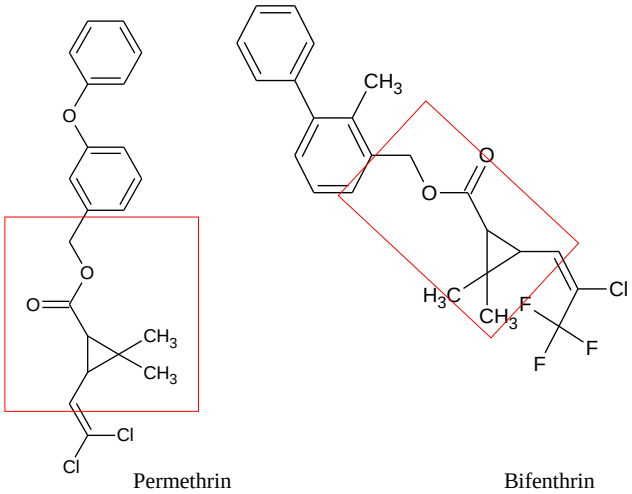

Figure 6. Example of structural analogs involving synthetic pyrethroids.

Table 3. Comparison of LC50 data for structural analogs, permethrin and bifenthrin.

Species	PERMETHRIN 96 hr LC50 based on AI (ug/L)	BIFENTHRIN 96 hr LC50 based on AI (ug/L)	Reference
Bluegill	6.8	0.35	US EPA 2010
Rainbow Trout	2.1	0.15	US EPA 2010

4.2.3.2 Similarity Indices

Another approach is to use algorithms that calculate similarity or distance based on pattern matching. These predictive methods rank chemicals based on characteristics of each structure that are similar (match/overlap) and characteristics that are dissimilar (mismatch/difference) (Monev 2004; Salinar *et al*., 2007). **Figure 7.** Schematic of similarity index measures. provides a schematic of the measures that can be described in similarity indices including attributes that are unique to each chemical (i.e., a and b), attributes common to each (i.e., c) , and, attributes absent from each substance (i.e., d). These techniques can utilize two-dimensional information such as molecular properties or topological indices; *i.e.*, the molecular graph of the structure, or three-dimensional data such as the conformational property of a chemical. Various approaches for calculating similarity indices exist; these approaches include

correlation-type indices (*e.g.*, Tanimoto Index (See Eq. 2; also known as Jaccard coefficient), Hodgkin Ricards Index, cosine-similarity index),

$$T = c/(a + b + c) \qquad \textbf{Eq. 2}$$

dissimilarity measures (*e.g.*, Euclidean distance index (Eq. 3), Hamming distance) which measures mismatches between compounds, and

$$D = \sqrt{a+b} \qquad \textbf{Eq. 3}$$

composite measures which measures both similarity and dissimilarity (*e.g.*, Hamann measure (Eq. 4), Yule measure.)

$$S = (c + d - a - b) / (a + b + c + d) \qquad \textbf{Eq. 4}$$

For an overview of these approaches see Monev (2004), Salinar *et al*. (2007)), and Urbano-Cuadrado *et al*. (2008).

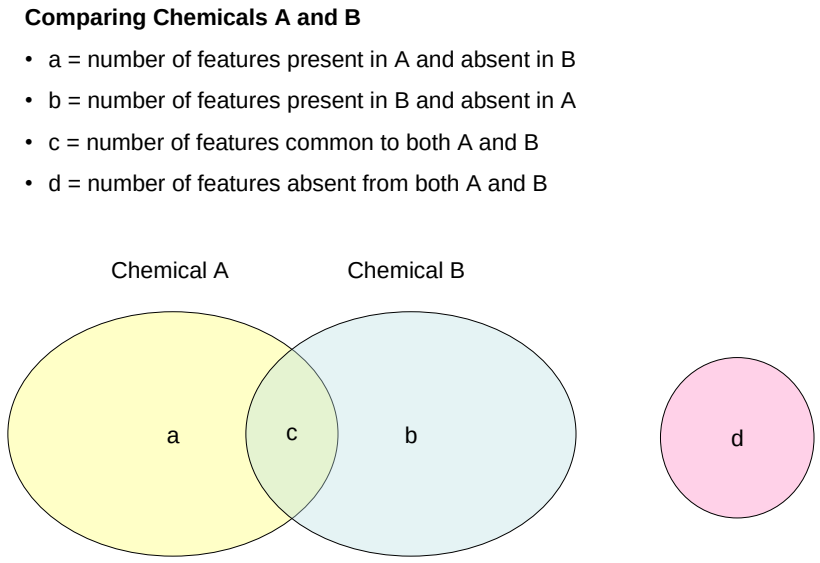

Figure 7. Schematic of similarity index measures.

4.2.4 QSAR Model Development

The development and use of QSAR models has a long and rich history, dating back to the initial work done by Meyer (see overview by Lipnick 1995a) and Overton (1901) in the early 1900s. These works resulted in the development of the Meyer-Overton rule, or general lipid theory of

narcosis, which states that the potency of anesthetics, or narcotics, is directly related to the lipophilicity of the chemical (see review by Lipnick 1995b.) The Meyer-Overton rule, with subsequent refinements by Ferguson (1939), provides the foundation for QSARs used today to predict the narcotic toxicity of industrial organic chemicals (See Könemann, 1981; Veith, *et al.*, 1983).

Another critical step in the development of QSAR modeling was the use of the log of the octanol/water partition coefficient (log P or log K_{ow}) as a modeling parameter to represent the partitioning of chemicals into biological membranes (Hansch and Fujita, 1964; Hansch and Dunn 1972). As represented in **Eq**. 5, the interactions with bio-membranes is explained by the partitioning (P) parameter, with steric properties (S) and/or electronic factors (E_l) used to explain more specific toxicity (*e.g.*, proelectrophilic/electrophilic interactions). Empirically derived constants (a, b, c, x) are obtained by fitting the empirical toxicity data to the equation.

$$\text{Log}(C) = x + a(El) + b(P) + c(S) + ...x(X) \qquad \textbf{(Eq. 5)}$$

As QSAR models have advanced, the approaches have taken on a more mechanistic-approach to model development, linking QSAR model descriptors to knowledge related to mechanism of action of the substance at the molecular target (*e.g.*, Bradbury 1994; Russom *et al*., 1997).

In general, the development of QSAR models follows a logical and step-wise process. First, a set of chemicals with reliable data are collected for a particular biological / chemical activity (see Perkins *et al*., 2003; Tong *et al.*, 2003; Bradbury *et al.*, 2003; Walker *et al*., 2003 for general reviews on approaches related to QSAR development). These test data are used to develop a model to predict values for chemicals which are similar, but lack data. Typically the original data are randomly separated into a test set and a validation set, with the test set used to develop a model and the validation set used to test the assumptions that the model works for chemicals not included in the original model' training set (Leonard and Roy, 2006).

A critical component in this process is reliability and integrity of data (Bradbury *et al.*, 2003; Leonard and Roy 2006). The empirical data used to develop the model must have a well-defined endpoint, and the model should ensure that the data meet quality controls (*i.e.*, were chemical concentrations measured, were standard test protocols followed, *etc*.) To ensure transparency, reliability, and consistency in QSAR models used in risk assessments, the OECD developed a set of guidelines, known as the "Principles for (Q)SAR Validation" (Jaworska *et al*., 2003; http://www.oecd.org/dataoecd/33/37/37849783.pdf) The five OECD principles for (Q)SAR validation are as follows, and will be discussed in more detail in Section 6.1:

- *A defined biological endpoint* - clearly define the test guidelines/methods used, and the specific biological endpoint being predicted (*e.g.*, LC_{50});
- *An unambiguous algorithm* – purpose is to ensure a transparent model, preferably with access to the test set of empirical data and descriptors used in the development of the model, assuring reproducibility of the model predictions;
- *A defined domain of applicability* – define the limitations / bounds of the model including chemical structure, physico-chemical properties, and/or modes of action relevant to the model;

- *Appropriate measures of goodness-of-fit, robustness, and predictivity* – to ensure reliability of predictions and internal performance of the model; and
- *A mechanistic interpretation* – although not always possible, attempts should be made to explain the biological response as it relates to the molecular site of action, and why various descriptors were used in the QSAR model.

Although many people observed that, for narcotic chemicals, the slope of log (Kow) vs. log (acute toxicity) was close to 1 for many aquatic species, Di Toro et al. (2000) were apparently the first to determine a pooled slope using data for many aquatic species. Herein this is called the multi-species QSAR approach to differentiate it from the single-species QSAR approach in which data for individual aquatic species are considered separately. This first multi-species QSAR model, which is referred to herein as the "Kow Target Lipid Model", was applied in several situations (Di Toro and McGrath 2000; McGrath et al. 2004, 2005; Redman et al. 2007; McGrath and Di Toro 2009). Later, Kipta and Di Toro (2009) developed the "polyparameter Target Lipid Model" for narcotic chemicals that uses the Abraham partitioning constants instead of Kow. Acree and coworkers have also used the Abraham partitioning constants (Hoover et al. 2005,2007; Bowen et al. 2006a,b). The polyparameter Target Lipid Model merged polar and non-polar narcotics, as did the use of Kmw instead of Kow by Escher and Hermens (2002). The development of multi-species QSARs for narcotics suggests that it might be possible to develop multi-species QSARs for other modes of action.

The availability of a multi-species QSAR for a mode of action (MOA) would be very useful because it would allow the measure of toxicity to be extrapolated from one species to another over the range of species for which the validity of the multi-species QSAR had been validated. This would provide a rationale for saying, for example, that the rainbow trout is a factor of 1.5 more sensitive than the fathead minnow to all chemicals that have that MOA. In addition, a multi-species QSAR can also be used in the evaluation of the acceptability of results of toxicity tests on all chemicals with that MOA, for species for which the multi-species QSAR has been validated. Because a multi-species QSAR can be used to extrapolate each toxicity value for any chemical with that MOA to all other chemicals that have that MOA, the composite dataset can be used in the derivation of ALSVs for all chemicals that have that MOA.

4.3 Interspecies Correlation Estimation (ICE) Models

4.3.1 Background

Interspecies toxicity extrapolation using regression analysis of acute sensitivity has been explored in ecotoxicology for decades (Kenaga, 1978; Doherty, 1983; LeBlanc, 1984; Thurston *et al*., 1985; Slooff *et al*., 1986; Mayer *et al*., 1987). Although earlier studies were based on limited species, chemicals, and chemical MOAs, they provided evidence that regression models could be used to predict acute toxicity (Kenaga, 1978) and were more robust for closely related species (Slooff *et al*., 1986) and within chemical categories (LeBlanc, 1984). Raimondo *et al*. (2010b) developed interspecies correlation estimation (ICE) models with the most diverse species and chemical database to date and performed quantitative uncertainty analyses in relation

to taxonomic distance, chemical MOA, and model parameters. These analyses provided guidance on model selection and use in ecological risk assessment (Raimondo *et al*., 2010).

In 2003, USEPA first compiled ICE models into a cd-based modeling application that may be used in ecological risk assessment (ICE v1.0; Asfaw *et al*., 2003). High quality databases were expanded and models were updated in the Web-based Interspecies Correlation Estimation application in 2007 (Web-ICE v1.0; Raimondo *et al*., 2007a; http://www.epa.gov/ceampubl/fchain/webice/). Improved standardization criteria were applied to the ICE database along with the addition of expanded data sources and the models were again updated in 2010 (Web-ICE v3.0; Raimondo *et al*., 2010). The ICE model database will continue to be updated in future versions as toxicity data become available and are reviewed for quality assurance. Web-ICE contains modules that develop Species Sensitivity Distributions and predict toxicity to threatened and endangered species from multiple surrogates. Future Web-ICE modules will include models that predict acute toxicity to algal species.

4.3.2 Model Development and Validation

Interspecies Correlation Estimation (ICE) models are log-linear least squares regressions of the acute toxicity of chemicals measured in two species in which the measured toxicity of the surrogate species can be used to predict the toxicity to the target taxon. In the example in depicted in **Figure 8.** Example Interspecies Correlation Estimation Model.

, measured toxicity for the surrogate species, rainbow trout, can be used to predict the toxicity to the target species, razorback sucker. ICE models have been developed and validated for aquatic vertebrates and invertebrates to include freshwater and saltwater species (Raimondo *et al*., 2010b) and wildlife species (Raimondo *et al*., 2007b), and their use in developing SSDs has been demonstrated (Awkerman *et al*., 2008, 2009; Dyer *et al*. ., 2008). ICE models can predict to a species, a genus, or a family. Toxicity data are pooled within genus and family to develop models for these higher taxa. ICE models for both aquatic organisms and wildlife are available on the USEPA Web-based Interspecies Correlation Estimation application (Web-ICE; http://www.epa.gov/ceampubl/fchain/webice/; Raimondo *et al*. 2010). For aquatic organisms, Web-ICE contains 780 species-level models (77 species to 77 species), 289 genus-level models (comparing 62 species to 28 genera), and 374 family-level models (comparing 69 species to 27 families). For wildlife, Web-ICE contains 560 species-level models (comparing 49 species to 49 species) and 292 family-level models (comparing 49 species to 16 families).

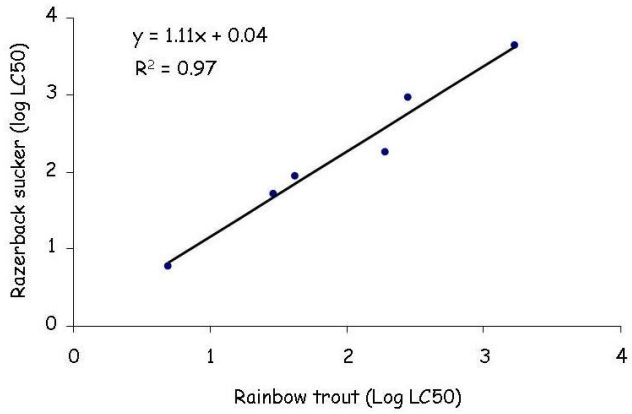

Figure 8. Example Interspecies Correlation Estimation Model.

ICE models are developed from an extensive database of acute toxicity for a diversity of species and a wide range of chemicals. The Web-ICE aquatic toxicity database was compiled from data sources within the USEPA and open literature and is comprised of 5,487 acute EC/LC_{50} values for 180 species and 1,258 chemicals. All data adhered to standard acute toxicity test requirements outlined by the American Society for Testing and Materials (ASTM, 2007 and earlier editions) and the USEPA Office of Prevention, Pesticides, and Toxic Substances (USEPA 1996). Rigorous quality assurance and standardization guidelines were applied to the database and ensured model relationships were reflective of intrinsic species sensitivity and contained minimal extraneous variation (Raimondo et al., 2010b). Toxicity records for metals, pentachlorophenol, and ammonia were normalized according to Ambient Water Quality Criteria methodology (the 1985 Guidelines). Records were excluded if they did not contain the water quality parameters necessary for normalization (*e.g.*, hardness, pH, temperature), an assessment of test quality, or were reported as non-definitive toxicity values (*e.g.*, > 100 µg/L, < 100 µg/L). A detailed description of the Web-ICE database may be found at Raimondo *et al*. (2010b).

Although data standardization can reduce the amount of data available for model development, it is necessary to reduce extraneous sources of variation and ensure that the model reflects species sensitivity relationships with greater certainty. Raimondo *et al*. (2009) explored the variability of acute test type (static, flow-through), concentration reporting (measured, nominal), and organism life stage on ICE models. In general, the results indicated that standardizing test data by acute test type or reported concentration type may not be critical for developing ecotoxicological models using large datasets of log-transformed values.

The ICE models were developed by pairing all species within the database by chemical and fitting log-transformed toxicity to a least squares regression. Each model with a sample size of four or greater was validated using leave-one-out cross-validation. In this approach, each pair of acute values for surrogate and target species is removed from the original model to build a submodel from the remaining data. The submodel is used to predict the toxicity value of the removed target species from the removed surrogate species toxicity value. The differences between the measured and predicted values of all removed datapoints for each model were used

to assess model accuracy. In general, ICE models did not have a tendency for under or over-estimation, based on this assessment of model accuracy

To provide a measure of cross-validation performance of each model, the cross-validation success rate was calculated as the percentage of removed datapoints predicted within 5-fold of the measured value. Cross-validation success rate is provided for each model on Web-ICE. Accuracy of model prediction was strongly related to taxonomic distance of the surrogate and predicted taxa; models built for two species in the same family predicted within 5 and 10-fold of the actual value for 91% and 96% of datapoints, respectively.

Recursive partitioning analysis identified species taxonomic distance, model mean square error, and the distance of the model input value relative to the range of surrogate values from which the model was developed as the most important variables for obtaining robust predictions. Results from these analyses provide user guidelines on model selection (see Section 6.2).

4.3.3 Influence of MOA on ICE Model Predictions

To determine how ICE models are influenced by MOA, each chemical used in models was assigned a broad and specific MOA (**Table 4**). MOA-specific ICE models were built for all possible species pairs and broad and specific MOAs and were developed for 7 broad MOAs (494 models, 46 species) and 15 specific MOAs (424 models, 44 species). MOA-specific models were cross-validated to determine their prediction accuracy and compared to respective models developed from all chemical data.

Table 4. Number of toxicity records and chemicals included in ICE model development

Mode of Action			
Broad	Specific	# records	# chemicals
AChE inhibition		1120	71
	OP AChE inhibition	784	54
	Carbamate AChE inhibition	336	17
Anticoagulation	Anticoagulation (ND)[1]	9	3
Cellular toxicity	Cellular toxicity (ND)	37	6
Corrosive/irritant	Corrosive/irritant (ND)	23	5
Metallic stress		337	14
	Iono-regulatory toxicity	281	7
	Respiratory toxicity	9	2
	Metallic stress (ND)	47	5
Neurotoxicity		668	55
	Pyrethroid neurotoxicity	216	23
	OC neurotoxicity	392	22
	Neurotoxicity (ND)	60	10

Narcosis		1770	376
	Nonpolar narcosis	1326	273
	Polar narcosis	158	40
	Ester narcosis	190	36
	Diester narcosis	32	5
	Narcosis (ND)	64	22
Reactivity	Reactivity (ND)	222	48
Respiratory toxicity		30	8
	Iono-regulatory toxicity	3	1
	Respiratory toxicity (ND)	27	7
Uncoupler/Inhibitor of oxidative phosphorylation		218	19
	Uncoupling oxidative phosphorylation	112	12
	Inhibiting electron transport/ATP synthase	106	7
Uncertain/Undetermined		387	102

[1] ND – specific mode of action not assigned.

MOA-specific models had lower model mean square error (MSE) than respective models developed using multiple and variable MOAs (with the exceptions of the broad MOA metallic stress and two specific MOAs, including ion-regulatory toxicity and organophosphate AChE inhibitors) (**Figure 9.** Models developed from chemicals of diverse modes of action compared to those with MOA-specific data.

). The improvement in prediction accuracy of MOA-based models was dependent on taxonomic relatedness and the type of MOA. Overall, the prediction accuracy of MOA-specific models did not improve for models developed for two species within the same phylum (*e.g.* fish to fish, invertebrate to invertebrate). For models developed for two species within the same kingdom (*e.g.* fish to invertebrate), toxicity predictions from MOA-based models were a significantly improved compared to models developed using all data (**Figure 9.** Models developed from chemicals of diverse modes of action compared to those with MOA-specific data.

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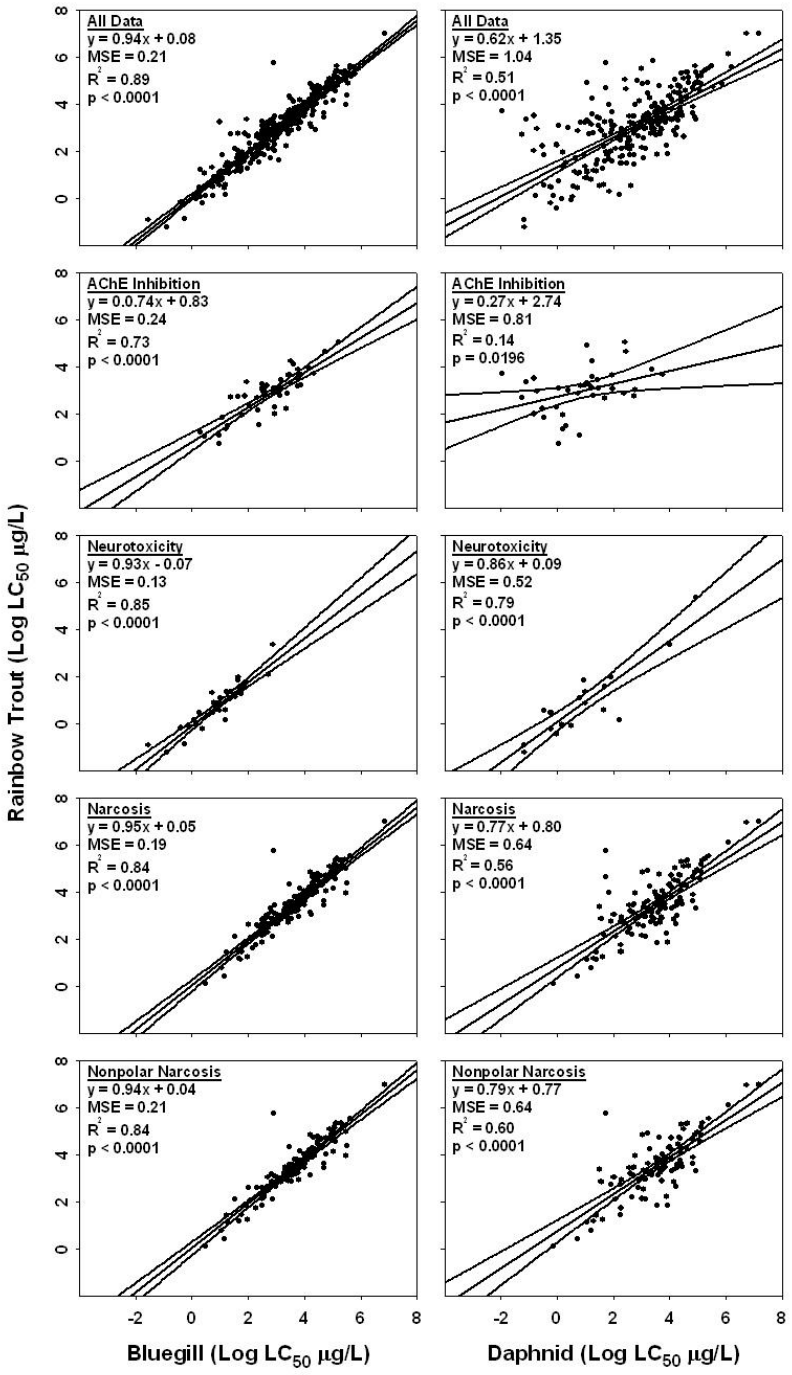


Figure 9. Models developed from chemicals of diverse modes of action compared to those with MOA-specific data.

4.4 Acute-Chronic Ratios (ACRs)

When measured chronic toxicity data are limited or unavailable for aquatic species, it is often necessary to predict chronic data for those species based on their observed acute responses. Acute-Chronic ratios (ACRs) are used to predict chronic toxicity in aquatic organisms when the acute toxicity is known but chronic data are absent or limited/indefinite. ACRs are typically derived for specific species and chemicals in which both acute and chronic data are available and are in turn used to predict chronic toxicity to a different species exposed to the same chemical. As shown in Equation 7, the ACR is the ratio of the acute value to chronic value; where acute value is defined as the lethal concentration (LC_{50}) or effect concentration (EC_{50}) to 50% of the organisms tested under acute durations, and the chronic value is the No Observed Concentration (NOEC) or the Maximum Acceptable Toxicity Concentration (MATC is the geometric mean of the chronic NOEC and the Lowest Observed Effect Concentration [LOEC]):

$$ACR = \text{Acute Value} / \text{Chronic Value} \qquad \textit{Eq. 7}$$

An ACR has a higher level of confidence if the data for the acute and chronic values are from the same study or concurrent studies done by the same investigator, with the same species, using the same batch of chemical, and under similar test conditions. Paired acute and chronic values from the same laboratory are usually not available; therefore ACRs reported in the literature can vary considerably.

Alternatively, ACRs may be standard or fixed values rather than quotients derived from chemical and species specific data. Both OPP and OW currently use ACRs in effects assessments, although slightly differently. A description of the approaches used by OPP and OW to derive ACRs is provided below along with a summary of similarities and differences regarding derivation. In addition, various approaches for estimating ACRs when acute and/or chronic toxicity data are not available for the same species are described in Sections 4.4.4 and 4.4.5. **Error! Reference source not found.**46 4.4.6 contains information and uncertainties associated with the use of standard ACRs.

4.4.1 OPP Approach to Derive ACRs

OPP routinely uses ACRs in ecological risk assessments for pesticides to predict chronic toxicity for aquatic animals when the acute toxicity profile indicates that the most sensitive surrogate aquatic species was not tested in a chronic study or when a data gap exists (*i.e.*, no data were submitted or submitted data were classified as "invalid" (*e.g.*, when the NOEC is less than the lowest concentration tested)). OPP derives ACRs for pesticides based on the ratio of the acute toxicity of a chemical (expressed as an LC_{50} or EC_{50} value from a valid acute study) to its chronic toxicity (expressed as a NOEC value from a valid chronic study). Ideally, the acute and chronic toxicity data used to derive the ACR should be from the same test species. This ACR is then applied to a species within a similar group (e.g., freshwater fish).

As an example of how OPP regularly makes use of ACRs, acute and chronic toxicity data are routinely submitted for the cladoceran, *Daphnia magna* or *D. pulex*, (daphnids) based on the ecological effect data requirements for pesticides specified in 40 CFR Part 158 Subpart G (CFR,

2010). Acute and chronic data from guideline tests with daphnids are normally used as a surrogate for the freshwater invertebrate taxa; therefore, these data are typically available. In situations where the available toxicity data from registrant-submitted or open literature studies indicate that another tested species from the same taxonomic group, *e.g.*, the amphipod, *Gammarus* sp., is more sensitive than daphnids on an acute exposure basis, the acute value for that species (for the *Gammarus*) are used to predict acute risk to freshwater invertebrates. If chronic toxicity data are not available for the *Gammarus*, an ACR would be calculated based on the ratio of the acute to chronic toxicity data for daphnids and the ACR would be applied to the acute value for *Gammarus* to yield a corresponding chronic toxicity value. In the following example, an ACR of 10 is derived for freshwater invertebrates based on the ratio of the acute and chronic toxicity data for daphnids (48-hr EC_{50} of 20 µg/L ÷ 21-day NOEC of 2 µg/L = 10); the ACR of 10 is then applied to the acute toxicity value for *Gammarus* (96-hr LC_{50} = 1 µg/L) to yield a predicted chronic NOEC of 0.1 µg/L (*Gammarus* LC_{50} of 1 µg/L ÷ ACR of 10 = 0.1 µg/L).

- Daphnid 48-hr EC_{50} = 20 µg/L
- *Gammarus* 96-hr LC_{50} = 1 µg/L
- Daphnid 21-day NOEC = 2 µg/L
- ACR = Daphnid EC_{50} (20 µg/L) ÷ Daphnid NOEC (2 µg/L) = 10
- Predicted chronic NOEC for *Gammarus* = *Gammarus* EC_{50} (1 µg/L) ÷ ACR = 0.1 µg/L

In this example, the predicted chronic toxicity value for *Gammarus* of 0.1 µg/L would be quantitatively used in OPP's ecological risk assessment to derive chronic risk estimates for freshwater invertebrates as it is the most sensitive value for that taxonomic group. Use of the ACR to predict chronic toxicity data for use in risk assessments considers the most acutely sensitive species and provides a means of addressing the uncertainty regarding the potential chronic toxicity of the untested species.

In this example, the predicted chronic toxicity value for *Gammarus* of 0.1 µg/L would be quantitatively used in OPP's ecological risk assessment to derive chronic risk estimates for freshwater invertebrates as it is the most sensitive value for that taxonomic group. Use of the ACR to predict chronic toxicity data for use in risk assessments considers the most acutely sensitive species and provides a means of addressing the uncertainty regarding the potential chronic toxicity of the untested species. Typically, ACRs determined with one species are applied to a different species, as discussed below for freshwater and estuarine/marine fish. . Typically, ACRs determined with one species are applied to a different species, as discussed below for freshwater and estuarine/marine fish.

When considering whether to use an ACR to predict chronic toxicity for aquatic animals, OPP evaluates the available data based on the following factors:

- <u>LC/EC_{50} and chronic NOEC data validity</u>: Acute and chronic data used to derive ACRs must be from scientifically valid studies (*i.e.*, submitted guideline studies that are classified by OPP as either "acceptable" or "supplemental" or studies from the open literature that are classified by OPP as "quantitative").

- Preference for data from the same species: Acute and chronic toxicity values used to calculate the ACR should be from the same species when possible. If data from acute and chronic studies for same species are not available, other prediction methods (e.g., QSAR) may be more appropriate. However, deriving an ACR from acute and chronic data for different species within the same taxonomic group, particularly those that are in the same genus or family (*i.e.*, bluegill sunfish (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*)) may provide a reasonable prediction of the ACR if the acute toxicity database is robust and suggests that the toxicity of the pesticide being assessed is relatively consistent across all species tested within a taxonomic group.
- Studies conducted under similar conditions: When possible, acute and chronic toxicity values used to derive the ACR should be from studies conducted under similar conditions (*e.g.*, flow conditions, dilution water quality, temperature, hardness, pH, *etc*.). When test conditions from the available acute and chronic toxicity studies vary such that the difference is expected to significantly affect the bioavailability and/or toxicity of the pesticide, data from these studies should not be used to derive an ACR. For example, in situations where water hardness or pH is known to affect the toxicity of the pesticide, it would be important that the acute and chronic studies have comparable or similar hardness or pH conditions to allow for comparison of acute and chronic toxicities.
- Use of mollusk shell deposition data: Aquatic invertebrate data submitted to OPP in support of pesticide registration most commonly include studies in two crustaceans (one freshwater and one estuarine/marine) and a marine mollusk. Relating crustacean toxicity data to mollusk shell deposition data is not recommended for ACR derivation based on the variability in responses associated with the measured endpoints (*i.e.*, mortality and growth of crustaceans versus shell growth of mollusks). However, some mollusk studies involve larvae and are not shell deposition studies. In those cases, and depending on the study conditions, these data may be appropriate for deriving ACR values.
- Use of endpoints with ">" values: Three values are necessary for calculation and application of ACRs (two acute and chronic values from the same species to allow for calculation of the ACR and one acute value to which the ACR is applied in order to predict the chronic value). If the available experimental acute toxicity value is expressed as a non-definitive value, such as an LC_{50}>100 mg/L, which is common in limit tests, an ACR should not be calculated using these data. Toxicity values expressed as a non-definitive value often represent the maximum concentration tested and not the inherent toxicity of the chemical. Similarly, indefinite NOEC values where chronic effects were seen at all of the test concentrations should not be used.
- Use of ACRs < 1: ACRs < 1, which occur when the LC/EC_{50} value is lower than the chronic NOEC should not be used; these types of values indicate that a measured 50% effect occurred after short-term exposures at pesticide concentrations that did not produce any effect following chronic exposure. ACRs < 1 may occur if the acute and chronic values are from the following types of studies: (1) those conducted in different species with different sensitivities to the pesticide; (2) those that tested different forms of the pesticide (*i.e.*, technical grade versus formulated product) where one form is more or less toxic than the other; (3) those that used different protocols or test conditions that may have affected the sensitivity of the toxicity study.
- Consideration of mode of action: Mode of action should be considered in deciding whether to apply an ACR from one species in a broad taxonomic group to another

organism within that broad group. For example, it would be inappropriate to apply an ACR for a chitin synthesis inhibitor universally across all aquatic invertebrates. If the mode of toxicity in the surrogate organism being evaluated is not known, it should be discussed as uncertainty in the risk assessment.

4.4.2 OW Approach to Derive ACRs

In deriving National Ambient Water Quality Criteria (NAWQC), OW aims to calculate both an acute value (CMC), and a chronic value (CCC). The Final Chronic Value (FCV) may be considered as one of the values used to approximate the CCC. As noted in the 1985 Guidelines, the CCC is based on aquatic animal chronic exposure data (*i.e.*, the FCV) or calculated based on acute-chronic ratios derived as described herein. OW uses ACRs when insufficient empirical data are available to calculate a FCV containing 8 taxa as defined by the 1985 Guidelines. When chronic data sets do not meet MDRs, a Final Acute-Chronic Ratio (FACR) is derived, when possible, based on the geometric mean of at least three ACRs including (1) at least one fish; (2) at least one invertebrate and (c) at least one acutely sensitive freshwater species (the other two may be saltwater). The chronic value used in the ACR calculation is obtained by calculating the MATC as the geometric mean of the NOEC and LOEC. The FACR then serves as the denominator in deriving the FCV (or CCC) as follows:

$$FAV \div FACR = FCV \qquad \text{(Eq. 8)}$$

where:
FAV = Final Acute Value
FACR = Final Acute Chronic Ratio
FCV = Final Chronic Value
CCC = lowest of FCV_{animal}, $FCV_{residue}$

According to the 1985 Guidelines, OW considers the following four different methods for deriving the FACR, based on trends in variance in the Species Mean Acute Value (SMAV):

1. If the species mean ACR increases or decreases as the SMAV increases, the FACR should be calculated as the geometric mean of the ACRs for only those species who's SMAVs are close to the FAV.
2. If no major trend is apparent and the ACRs for a number of species are within a factor of 10, the FACR should be calculated as the geometric mean of all the species mean ACRs available for both freshwater and saltwater species.
3. If the most appropriate species mean ACR values are less than 2, it is assumed that acclimation has probably occurred during the chronic test. Because chronic toxicity values derived for animals which have acclimated to chronic test conditions are not likely to provide adequate protection to aquatic animals under field conditions, the FCV is equal to the CMC (where the CMC equals the Final Acute Value [FAV] ÷ 2).

The OW criteria development process uses ACRs as in the following example (**Table 5**). Paired acute and chronic test data are available for 3 species: two tests for the Cladoceran (*Daphnia magna*), one for rainbow trout (*Onchorhynchus mykiss*), and a third for a marine mysid (*Americamysis bahia*). The geometric mean of the two available daphnid chronic values is used as the daphnid species specific ACR. Chronic values are the MATC or geometric mean of NOEC and LOEC.

Table 5. Acute and chronic values and ACRs for freshwater contaminant.

Species	Acute Value (µg/L)	Chronic Value (µg/L)	ACR
Cladoceran, (*Daphnia magna*)	84.8	157.9	**2**
Cladoceran, (*Daphnia magna*)	190	30.59	**6.211**
Mysid, (*Americamysis bahia*)	43	5.112	**8.412**
Rainbow trout, (*Oncorhynchus mykiss*)	121	7.861	**15.392**

The first D. magna ACR value is 0.537, therefore it is brought up to 2 for reasons explained above.

The specific calculations involved are as follows:
Final Acute Value = 33.02µg/L[±]
Criterion Maximum Concentration = 33.02/2 = 16.5 µg/L
Final Acute-Chronic Ratio = 6.33
Final Chronic Value = (55.71 µg/L)/6.33 = 8.80 µg/L
[±] *Calculated based on 1985 Guidelines, data not presented.*

When considering if data are available to derive an FACR for use in calculating the FCV or CCC, OW considers the following factors:

- Validity of the chronic data: Chronic test data used in deriving the ACR must be from acceptable studies as specified in the 1985 Guidelines.
- Preference of paired acute and chronic data: OW gives preference to studies where the acute test was conducted as part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water, but in a different study may be used. If no such acute tests are available, results from different laboratories may be used provided that the test conditions are similar.
- Exposure regime of chronic data: Chronic data must be from flow-through tests (or static renewal for daphnids) and the duration of the test must be appropriate to the species.
- Control performance: Control tests must have acceptable survival, growth, and reproduction.
- Dilution water quality: Results of chronic tests conducted in unusual dilution water (*i.e.*, dissolved organic carbon [DOC] > 5 mg/L, *etc*.) should not be used unless data show that DOC, *etc*. do not affect toxicity. In addition, water quality characteristics that have been shown to be related to toxicity (e.g., hardness, pH, *etc*.) should be accounted for.

- Endpoints and exposure duration: Chronic values should be based on endpoints and lengths of exposure appropriate to the species as specified in the 1985 Guidelines.
- Preference for data from same species: Acute and chronic data must be from the same species to generate an ACR. In addition, paired acute and chronic toxicity tests are preferred.

4.4.3 *Similarities and Differences in OPP and OW Approaches to Derive ACRs*

There are a number of similarities and differences regarding the approaches that OPP and OW use to calculate ACRs for use in pesticide ecological risk assessments and NAWQC derivation, respectively. Similarities in approaches and the types of data that are considered in deriving ACRs within OPP and OW include the following:

- Use of all available reliable, scientifically valid, aquatic toxicity data including data from the public literature;
- Preference for acute and chronic data from same species;
- Use of same assessment endpoints (survival, growth, and reproduction);
- Consideration of control performance;
- Consideration of dilution water quality and potential impacts on toxicity; and
- Consideration of chronic data based on similar exposure duration and type of exposure.

A summary of the major differences in OPP and OW ACR derivation is provided in **Table 6.**

Table 6. Differences in OPP and OW ACR derivation methodology.

OPP	OW
ACRs developed when chronic data are not available for most acutely sensitive species or if data gaps exist	ACRs developed when insufficient data are available to calculate a FCV meeting MDRs
ACRs are taxa-specific[1] and based on most acutely sensitive species; one ACR is derived for each taxon, depending on the availability of data	One FACR derived based on the geometric mean of 3 ACRs; 3 separate ACRs are required for FACR derivation
Trends in acute values are not considered; ACR applied to the most acutely sensitive tested	ACRs are compared to SMAVs to review trends among the parameters
No provision for special consideration of chronic data on commercially/recreationally important species	Defaults to commercially/recreationally important species chronic data if less than the calculated FCV
ACR is based on ratio of acute value (LC/EC_{50}) to the chronic NOEC value[2]	ACR is based on ratio of acute LC/EC_{50} to the chronic MATC value or regression-derived EC_{20} value

[1] OPP may derive separate taxa-specific ACRs for freshwater and estuarine/marine fish and invertebrates, depending on the available data.
[2] OPP acute value ≠ OW FAV.

4.4.4 *Deriving ACRs Using Predicted and Empirical Data*

In cases where chronic toxicity data are available for a species, but no acute toxicity data are available, it may be possible to use methods described in this white paper to predict acute

toxicity data for that species (*e.g.*, Web-ICE, read-across). The predicted acute and empirical chronic toxicity data may then be used to derive a chemical-specific ACR.

In other cases, acute LC_{50} data may be available for species for which no chronic toxicity data are available, requiring that a chronic toxicity test result be predicted. Fewer QSAR methods are available for predicting chronic toxicity than exist for acute toxicity and most of what is available is focused on non-polar organic chemicals and narcosis. Predicting chronic values might be obtained using read-across and time-concentration effect (TCE, Section 4.5) models.

Another scenario would be where empirical data for both the acute and chronic values are not available and (Q)SAR models for both acute and chronic toxicity are used to predict the ACR. Once again, with limited QSAR models for chronic toxicity, read-across methods based on chemicals with the same adverse outcome pathway may have the most potential for predicting the chronic toxicity of pesticides.

4.4.5 Default ACRs

As part of analyses underlying the Great Lakes Water Quality Initiative (Host *et al*. 1995), distributions of ACR values from existing AWQC were evaluated to derive a default ACR value of 18. This value, representing the 80th percentile of the available data, was derived independent of the chemical or AOP. TenBrook *et al.* (2010) took a similar approach, but limited the analysis to data for pesticides, and calculated an 80th percentile ACR of 12.4.

This lack of reliable acute and chronic data has made it difficult to calculate chemical class or AOP specific ACRs with a high level of confidence, although some research has been done in this area. Call and coworkers, comparing acute LC50 values to the MATC from an early life stage test (~32d)) using fathead minnows, found the average ACR (range of ACRs in parenthesis) of 4.7 (1.7-8.3) for uncouplers of oxidative phosphorylation, 10.4 (4.5-27.9) for organophosphates, 7.3 (4.6-9.1) for carbamates (Call et al., 1989), and 9.8 (3.3-24.0) for nonpolar narcotic chemicals (Call et al., 1985).

Based on theoretical considerations, one would expect that the magnitude of an ACR for a particular species, or closely related species, would show greater similarity within rather than between adverse outcome pathways. The extent to which this has been demonstrated through analysis of actual data is mixed. Raimondo et al. (2007c) compiled a large database of ACR values and evaluated their distribution relative to AOP (**Table 7**); this analysis did not clearly indicate that classifying chemicals by AOP did much to parse the overall variability reported in the literature. However, other evaluations with more narrowly defined data sets (by species and/or chemical group) have suggested lower degrees of variability in ACRs, at least for some adverse outcome pathways (*e.g.*, Call *et al*. 1989; Di Toro *et al*. 2000; Roex *et al*. 2000).

Table 7. MOA-specific ACRs reported by Raimondo et al. 2007.

MOA/class	Median ACR	90th percentile ACR
AChE inhibitors - carbamates	8.9	28.0
AChE inhibitors - organophosphates	6.2	77.8

Cell function/division	7.4	63.0
Narcosis (non-specific)	10.4	148.9
Ester narcosis	5.6	169.7
Nonpolar narcosis	9.9	148.9
Polar narcosis	14.1	188.5
Neurotoxicity (non-specific)	5.6	109.1
Neurotoxicity – cyclodiene-type	7.8	109.1
Neurotoxicity – DDT-type	3.6	5.1
Neurotoxicity – Pyrethroid	4.7	25.1
Metals	9.1	88.0
Reactivity (non-specific)	7.9	33.7
Reactivity – alkylation/arylation based	17.2	150.8
Reactivity – dinitroaromatic group	14.0	76.1
Respiratory blocker	8.3	68.3
Uncoupler of oxidative phosphorylation	6.2	30.4

4.4.6 Fixed ACRs

Certain regulatory programs, such as the European Union (EU) and the Office of Chemical Safety and Pollution Prevention (OCSPP), use fixed ACRs of 10 for fish and 4 for green algae to provide protection from chronic toxicity to species for which there are no chronic data. However, there are concerns regarding the use of fixed ACRs for the extrapolation of acute to chronic toxicity because of large variation associated with ACR differences in species, chemical class, MOA, and test conditions (Kenaga, 1982; Ahlers *et al*., 2006; Raimondo *et al.*, 2007c). Based on the work of Ahlers *et al*. (2006), ACRs of up to 100 are not protective for all chemicals and trophic levels.

Use of fixed ACRs assumes that the relationship between acute and chronic toxicity is independent of the test species and the test compound including differing mechanisms of biological action from species to species. The ratio of acute and chronic effect levels for different species with different life histories is assumed to be the same. These assumptions may result in under- or over-estimation of toxicity in the field when using fixed ACRs to predict chronic toxicity of pesticides.

4.5 Time-Concentration Effect (TCE) Models

4.5.1 Background

TCE models use time-course to mortality data from acute toxicity tests to extrapolate to a prediction of chronic lethality for a chemical to a particular species. TCEs are derived from generic time-to-event models which employ nonparametric, semi-parametric, and parametric approaches and are not restricted to toxicity-specific mechanisms or applications. Rather, time-to-event models simply assume that an event (e.g. death, birth, disease onset) occurs at some point in time (Newman and Crane 2002), and some models (e.g., accelerated life testing [ALT])

have theory originally routed in industrial reliability studies (Sun *et al.* 1995). Since conventional toxicity metrics such as LC/EC_{50} and NOECs focus on exposure intensity during a constant duration of exposure and time course distinguishes acute and chronic toxicity, TCE models have the potential to provide an understanding of toxic effects over time (Sun *et al.* 1995; Newman and Crane, 2002).

A common assumption of generic TCE models is that a chronic endpoint can be predicted by the time course of that endpoint in acute tests. While the endpoint measured in acute toxicity tests is typically mortality, except where mobility or shell growth are the endpoints of effect concentrations (e.g., EC_{50}), the endpoint of chronic tests, as determined by the LOEC or ECx, may be mortality, reproduction, or growth. Validation of Accelerated Life Testing (ALT) and Linear Regression Analysis (LRA) has shown relatively good accuracy of models to predict chronic mortality (Mayer et al., 1994; Sun et al., 1995; Barron et al., 2008); however, prediction of non-lethal chronic endpoints is less accurate using these models (Barron et al. 2008). Mayer et al. (1986) recommends applying additional safety factors to lethality-based NOEC to include other biologically significant effects.

An additional assumption of TCEs is that time-course to mortality is independent of chemical MOA. Since many TCEs have non-chemical origins (e.g., ALT; Ellersieck et al., 2003), a toxicological mechanism is lacking. A change in MOA between acute toxicity and chronic toxicity could result in poor accuracy of chronic toxicity prediction due to a shift in the dose-response curve (Barron *et al.,* 2008).

TCE models require the input of time-course acute data such as the number of live organisms during each time step of an acute test (*e.g.,* 24-h, 48-h, 72-h and 96-hr). Barron et al., (2008) demonstrated that ALT and LRA had the highest accuracy when the majority of mortality occurred early in the acute test. This same study found that the inclusion of additional time steps (e.g., 12-hr, 36-hr) did not improve the accuracy of chronic toxicity prediction.

The TCE models that have received the most attention and application in predicting chronic toxicity from acute time-course data are the ALT (Sun *et al.*, 1995; Mayer *et al.*, 2002), linear regression analysis (LRA; Mayer et al., 1986; Mayer et al. 1994; Mayer et al. 2002), and the Multi-factor Probit analysis (MPA; Lee *et al.,* 1995; Mayer *et al.,* 2002). The ALT model employs a survival analysis assuming a Weibulll distribution and accelerated life testing theory. This method was originally used to predict "time to failure" for mechanical and electrical devices placed under short-term stress (Ellersieck *et al.*, 2003). The ALT model should be used when there are at least 3 "partial responses" (mortality between 0 and 100% at a given time step) in the acute toxicity test. The LRA model is a two-step linear regression analysis that combines regressions that predict the low lethal concentration at each observation time period and regresses those concentrations against the reciprocal of time. The intercept of this second regression is the chronic NOEC. The LRA model may be used when there are no partial responses during the acute toxicity test. The MPA models are multiple regressions that describe the relationship among exposure concentration, time and probit percent mortality. The model was developed to predict chronic toxicity when acute tests contain varying conditions, such as varying exposure scenarios of effluent tests. The MPA model should be used when there are at least 5 partial responses during the acute test.

5 Examples of Predictive Methods

This paper is not intended to provide an exhaustive overview of computational tools available via government, open access, or commercial sources, but rather an overview of the types of predictive methods that are available to scientists in OW and OPP, focusing on those that are frequently used in environmental risk assessments, and those predictive methods that will directly assist in derivation of aquatic life criteria. **Appendix C** provides information on these methods for predicting toxicity using Expert Systems, Similarity Tools, ICE models, and TCE models. (Q)SAR models also exist for other endpoints such as bioaccumulation (Arnot and Gobas, 2004), and physical chemical properties (European Commission (EC) 1995a,b; Deardon and Worth, 2007). The physical chemical property data will typically be a required input variable for (Q)SAR models. For most of the computational expert systems described below, the data are populated when the structure is identified, but **Appendix D** provides a list of physical chemical property (Q)SAR tools that may be useful when data are not available. (Q)SAR predictive methods used for screening, prioritization and ranking of large chemical inventories will not be covered but are available to risk assessors (See Schmieder *et al*., 2004, Walker *et al*., 2004; Brown and Wania 2008, Jensen *et al*., 2008; Pavan and Worth 2008; Daginnus *et al*., 2009 for examples). Several reviews have been written on the types of predictive methods available (see EC, 1995a,b; Pavan *et al*., 2005a,b; Jensen *et al.*, 2008); however, it should be kept in mind that the inventory of available predictive methods is constantly changing with emerging research in the area

6 Interpretation of (Q)SAR, Read-Across/Bridging, ICE, and TCE Predictions

6.1 (Q)SARs

(Q)SAR approaches are based on assumptions that the biological activity of a chemical is related to the chemical's structure, and therefore have more uncertainty surrounding the predicted value than empirical toxicity tests which follow acceptable test methodologies. Selection of (Q)SAR predictive methods is dependent upon the assessment context (*e.g*., limited documentation for screening/data identification, more complex documentation for effects/risk assessment decisions). Recognizing that these (Q)SAR approaches could assist in filling data gap needs within risk assessments, the OECD developed validation principles for (Q)SARs to help identify scientifically valid models that are reliable and also acceptable for use in the risk assessment process. Five validation principles were identified that addressed both statistical and non-statistical aspects of (Q)SAR models (OECD, 2004).

1. *The (Q)SAR model must be associated with a well defined endpoint*: The purpose of this criterion is to ensure clarity in the predicted endpoint . The model should clearly define the experimental protocols and conditions employed in the development of the model database. For instance, the USEPA's fathead minnow database

(http://www.epa.gov/med/Prods_Pubs/fathead_minnow.htm) was a data source for many (Q)SAR models (Veith *et al*., 1983; 1989; Russom *et al*., 1988; Karabunarliev *et al*., 1996a,b.) The database was developed using an ASTM protocol for acute fish toxicity, endpoints were based on the measured chemical concentrations in water, all fish were from the same culture unit, and the dilution water was from the same source, *etc*. (Russom *et al*., 1997.) Therefore variance associated with changes in experimental procedures / conditions was controlled. It is also important to consider the relevance of the predicted endpoint as it relates to its use in answering a particular hazard assessment question. For instance, the prediction of a no observable effect concentration (NOEC) may be relevant for some risk assessments, but may not be suitable where hypothesis testing does not provide a clear understanding of the uncertainty or precision of the endpoint.

2. *The model must be an unambiguous algorithm*: The purpose of this criterion is to ensure transparency of the model. Under this rule, information on the test set used in development of the model including the effects data points, and all parameters used as input variables must be provided. This information allows for independent assessment of the reproducibility and performance of the model. It should be noted that unambiguous algorithms are not always possible, especially when models are based on CBI data, as in the case of commercial models and the OPPT EcoSAR application.

3. *The model must have a defined domain of applicability*: The purpose of this criterion is to ensure the reliability of the model. All models are developed using a training set of chemicals and an associated list of input variables to predict the endpoint in question. These variables have limits that define the chemical domain that the model describes. For instance, the domain of applicability may be related to certain chemical analogs (*e.g.*, model developed using a test set of organophosphate pesticides), and/or a range of physical/chemical parameters (*e.g.*, chemical in test set had log P values ranging from 0 to 6.) Predictions outside these domains are questionable (not the intent of the model), and are therefore, not reliable for use in a risk assessment.

4. *The model must have appropriate measures of goodness-of-fit, robustness and predictivity*: The purpose of this criterion is to ensure internal performance and predictivity of the model. The internal performance of a model is represented by goodness-of-fit, or how well the model predicted values agrees with observed values, and model robustness, which ensures the model is not unduly affected by outliers or small departures from model assumptions. Measures of goodness of fit for regressions include the coefficient of determination (*i.e.*, R-squared (R^2), and the sum of square. The predictive power of the model is determined by testing data not used in the development of the model, but within the model's domain of applicability, thereby conducting an external validation of the model.

5. *The model should have a mechanistic interpretation*: At times, the mechanism of action may not be fully understood, but if possible, models with a mechanistic understanding should be used. A critical consideration is whether the descriptors used in the models make mechanistic sense. Development of an AOP can assist in this assessment. For

instance, log P is used in most QSAR models to predict toxicity for chemicals acting via a narcosis MOA, which makes mechanistic sense, because log P is used as a surrogate for the biological membrane which is the proposed molecular site of action for narcotic chemicals (Ankley *et al*., 2010).

Under REACH, models that meet the OECD validation principles and are proposed for use in filling data gaps are currently being gathered. A searchable catalog of all models including background information required to validate the models, authors/source of model, related publications, predicted endpoints and related experimental protocols, algorithms with training and validation sets, including all input variables for the models, can be found at the website: http://qsardb.jrc.ec.europa.eu/qmrf/index.jsp.

When using these predictive methods, users should document any interpretations, and /or restrictions related to use of the predicted value. The strengths and weakness of the data prediction method used should be clearly described by the user when such predictions are included in assessments. When any (Q)SAR model has been used to predict toxicity values, the output of the model should be included as an appendix to the assessment to enhance transparency.

6.2 ICE Models

ICE toxicity predictions can be made from a suite of models available for a diversity of surrogate species predicting to the species, genus, and family level. As such, multiple models are frequently available to predict toxicity to a desired species. When comparing and selecting ICE models, the following guidelines should be used (Raimondo et al. 2010b):

1. Relatively low mean square error (MSE) (< approximately 0.22)
2. Large sample sizes (df $\geq$3)
3. Close taxonomic relatedness (Genus > Family >>Class)
4. Use MOA-specific models where available
5. Narrow confidence intervals surrounding prediction
6. Input values occur within the data boundaries of the model.

MSE error is the recommend measure of model robustness. ICE models are based on the acute toxicity available for two species, so available models usually contain different sample sizes. Because MSE is derived using model degrees of freedom, it is an unbiased estimated of model error and variance. In regression models, such as ICE models, R^2 captures the percentage of variability in the data explained by the model and is not adjusted for sample size. Model sample sizes should also be considered in model selection. Models developed with only three data points may have extremely low MSE, which may be an artefact of limited chemical diversity and small domain of applicability.

While these criteria provide the user with guidance, for selecting and evaluating various ICE models, it should be noted that professional judgment is ultimately required to evaluate model predictions. For example, models with MSE error < 0.22 were identified through uncertainty

analyses as having an average accuracy of model predictions within 4 fold of the actual value. Models with MSE > 0.22 will not necessarily yield poor predictions, however the average fold difference between predicted and measured values was 10 (stdev = 37, N = 2542). Additionally, models between closely related species were shown to yield more accurate predictions than models for less related species. However, some exceptions to this trend may apply depending on the availability and variance of the data available between species. In cases where multiple acceptable models exist, documentation of decision making/professional judgement should be provided/maintained.

Prediction confidence intervals should be used in conjunction with MSE and taxonomic relatedness. The size of the confidence interval is inversely relative to the robustness of the prediction. Large confidence intervals may be produced by models with large MSEs as well as other models where the input value (measured surrogate toxicity) is outside the range of surrogate date used to parameterize the model.

ICE estimated confidence intervals represent the range in which the actual toxicity is likely to fall with a certain level of confidence (e.g. 95% confidence). Conservative predictions of toxicity may use the lower confidence limit as the predicted value of toxicity for untested species.

6.3 TCE Models

Chronic toxicity predictions obtained from TCEs are NOECs for survival corresponding to the species, chemical, and test conditions of the entered acute toxicity data. TCE models do not provide a prediction for non-lethal chronic toxicity and may not represent standard chronic test NOECs, which are typically based on the most sensitive endpoint measured (e.g. growth, reproduction). In a dataset comprised of 138 chronic toxicity tests, survival represented the most sensitive endpoint measured in approximately 43% of all tests (Barron et al. 2008). Additional safety factors should be applied to chronic NOECs derived from TCEs to be protective of nonlethal endpoints. A safety factor of 0.2 is recommended based on frequency analysis demonstrating NOECs for survival being 5 times or less than the NOECs for most other endpoints at least 95% of the time (Mayer et al. 1986).

7 Considerations for Using Toxicity Predictions

7.1 WOE and Best Professional Judgment

Predictive methods are not intended to be used as the sole indicator of a chemical's toxicity but rather are intended to be used in concert with other sources of information. As such, they represent only one element in a WOE approach. Output from these predictive methods should be weighted (qualitative/quantitative) according to reliability, availability of specific data types (*e.g.*, *in vivo* study results), and assessment context (*e.g.*, identification of data requirements vs. hazard assessment decision). Under ideal conditions multiple predictions are available from

multiple computational tools, although this may not always be possible. Users should also consider predictive performance when use (Q)SARs in a WOE approach.

Keeping in mind the OECD Validation Principles for use of QSARs, and the Bradford Hill criteria for identification of AOPs, users should recognize that these are prediction methods and they have associated limitations. With every model used, the rules, restrictions, and limitations associated with the model should be considered, and the following questions should be asked: (1) are there particular groups of chemicals identified as potential issues as they relate to the specific models, and (2) what are the bounds of the model and is the prediction exceeding the limits of the model?

When using read-across approaches, it is important that the data sets being used to interpolate/extrapolate the unknown value are structurally similar. It is also important to remember that extrapolating out from a data set where the chemical to be estimated is very 'distant' from other chemicals identified as similar may have unknown errors and should be avoided.
For any (Q)SAR predictive methods, the user should determine whether model outputs for selected parameters (*i.e.*, fate, effects) are consistent with what is known for the chemical under evaluation. In some cases, some toxicity data on either fish and/or invertebrates may exist and the reviewer should evaluate whether the model outputs are consistent with what has been measured. The ability of the (Q)SARs to reliably predict the toxicity of other chemicals depends on the extent to which structurally similar chemicals with similar MOAs are represented in the training set of data used to populate the model. As such, (Q)SAR model results must be put into the context of any existing data and what is known about the chemical. Additionally, for each of the (Q)SAR models, the structural moieties and/or MOA on which the model predicts toxicity are provided by the model. The reviewer should be aware of this output and make an effort to determine whether these moieties and/or MOA are consistent with their understanding of the chemical.

With any model, as with measured toxicity data, there are uncertainties regarding the variability and relevancy of predicted values. (Q)SAR estimates should only be considered when actual measured chemical-specific data are not available. The decision to use such estimates should be weighed against other sources of data that may be available. When used, SAR model output should be properly identified and the uncertainties associated with the values must be discussed in the assessment. Ultimately, the use of SARs is dependent on the best professional judgment of the user.

The use of (Q)SARs is dependent on multiple lines of evidence and must be viewed in the context of the model's domain of applicability; these multiple lines of evidence must be integrated. Some QSAR models like ASTER warn users that certain chemical structures are not in their domain. Users are cautioned to examine model outputs carefully to determine whether predictions are consistent with what may be known about the chemical however limited.

7.2 Selection of Descriptors in (Q)SAR models

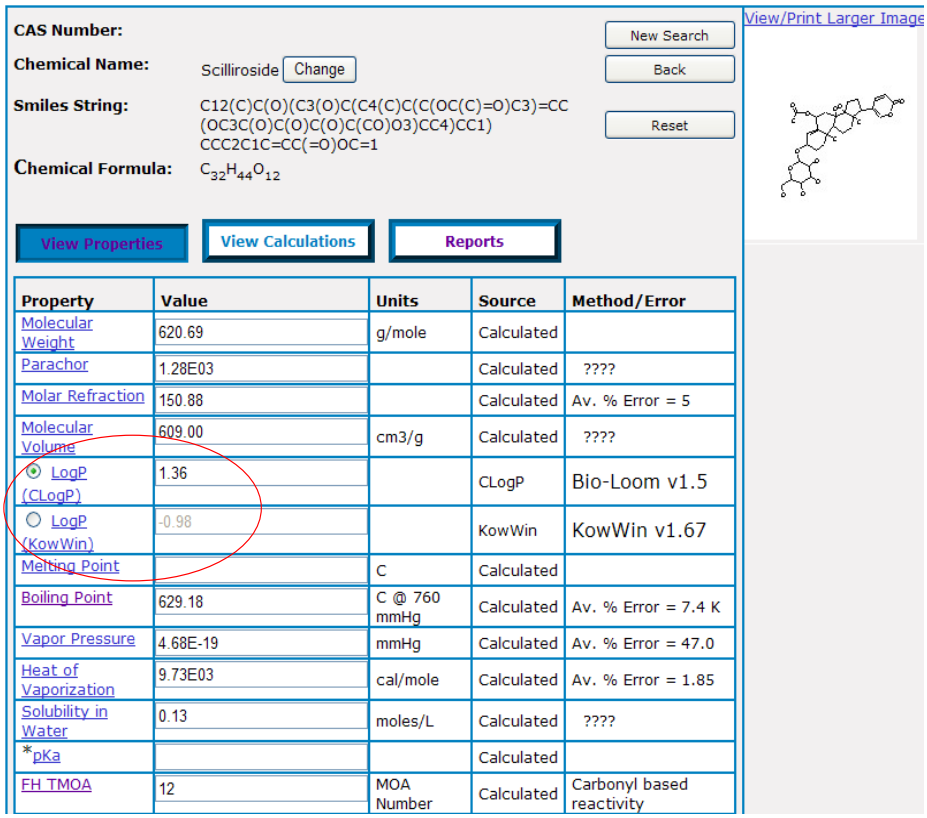
There are a large number of descriptor variables that can be used in the development of a QSAR to predict the toxicity of a substance. Many of these descriptors are calculated using first order principles, *i.e.*, calculated based on theory as it relates to chemical structure and not experimentation. In theory, these have less error because they would not have error related to experimentation; however, selection of these parameters should be based on how they relate and help explain the mechanistic interpretation of the AOP. For instance, log P is a logical parameter to explain partitioning into biological membranes since it is by definition a measure of lipophilicity and membranes are by nature lipophilic. But it is less clear what parameters should be used in defining inhibition of an acetylcholinesterase inhibitor. Due to limited data, many QSAR models combine data from various mechanisms and use a global QSAR approach (Netzeva *et al*., 2007) which incorporates parameters associated with hydrophobicity, electronic, and possibly steric information regarding each compound in the test set (*i.e.*, Hansch approach.) Ensuring an understanding of what each parameter in an equation explains as it relates to toxicity becomes more complicated. In addition, some global QSARs include a disproportionately large number of descriptor variables in comparison to the number of chemicals used in developing the model, and users should be wary of overfitting the data For instance, even though small standard errors are related to greater predictivity of a model, an overfitted model could result where the standard error is actually smaller than the experimental error associated with the biological test data, which is not recommended (Wold et al., 1984). To this end, techniques such as genetic algorithms, Principle Component Analysis (PCA) or Factor Analysis (FA) can be used to identify appropriate descriptor variables (OECD, 2007d).

Many (Q)SAR models do not address the three-dimensional nature of the chemical which may be critical in its interactions with biological matrices. Log P has been used historically to describe the partitioning of chemicals into biological membranes, and works well in QSARs developed for baseline narcosis (see **Table 8**). However, regressions using log P for mechanisms such as polar narcosis, uncouplers of oxidative phosphorylation, etc. show lower goodness-of-fit measures, presumably because steric and electronic effects are not addressed by log P. **Table 8** provides QSAR models developed for non-polar and polar narcotic MOAs for three different species. A slope approaching unity (1) would mean that the descriptor variable, in this case log P, adequately describes the toxicity that is being modeled. The models for guppy, fathead minnow, and the ciliate, *Tetrahymena*, all have slopes approaching unity ranging from 0.871-0.929 for the non-polar narcotic MOA implying that log P is an adequate descriptor of toxicity. It is a different case for the chemicals acting via a polar narcosis MOA, where the slopes range from 0.46-0.65, implying that log P is NOT describing all aspects of the toxicity. This makes sense when you consider that log P captures only the partitioning aspect and not the hydrophilic or polar nature of the chemical in question.

Table 8. Examples of baseline narcosis and polar narcosis models using only log P as a means of predicting toxicity.

Organism		Endpoint	Slope	Intercept	R^2	N	Source
Guppy	Baseline narcosis	LC_{50}	0.871	1.13	0.976	50	Könemann 1981
Guppy	Polar narcosis	LC_{50}	0.46	3.04	0.824	11	Könemann and Musch 1981
Fathead minnow	Baseline narcosis	LC_{50}	0.94	1.25	0.94	60	Russom *et al*., 1997
Fathead minnow	Polar narcosis	LC_{50}	0.65	2.29	0.90	39	Veith and Broderius, 1987
Ciliate	Baseline narcosis	IGC_{50}	0.929	2.639	0.986	20	Schultz 1996
Ciliate	Polar narcosis	IGC_{50}	0.574	0.8652	0.756	30	Schultz 1987

It is important to remember that, as with any QSAR model, the prediction of log P has error associated with it, and the reliability, predictivity, and appropriateness of the model is related to the knowledgebase behind the predictive method. For instance, Benfenati and coworkers, predicted the log P for a series of pesticides using four log P models, and found that performance varied depending on the chemical class (Benfanati *et al*., 2003.) In **Figure 10,** the property screen from ASTER (http://cfint.rtpnc.epa.gov/aster/) for the rodenticide Scilliroside is provided. Note that the two predictions of the octanol/water partition coefficient; the USEPA Episuite's KowWin model (-0.98) and BioByte's CLOG P (1.36) are more than 2 log units different, which could greatly impact toxicity predictions using QSARs.



CAS Number:

Chemical Name: Scilliroside Change

Smiles String: C12(C)C(O)(C3(O)C(C4(C)C(C(OC(C)=O)C3)=CC(OC3C(O)C(O)C(O)C(CO)O3)CC4)CC1)CCC2C1C=CC(=O)OC=1

Chemical Formula: $C_{32}H_{44}O_{12}$

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Property	Value	Units	Source	Method/Error
Molecular Weight	620.69	g/mole	Calculated	
Parachor	1.28E03		Calculated	????
Molar Refraction	150.88		Calculated	Av. % Error = 5
Molecular Volume	609.00	cm3/g	Calculated	????
LogP (CLogP)	1.36		CLogP	Bio-Loom v1.5
LogP (KowWin)	-0.98		KowWin	KowWin v1.67
Melting Point		C	Calculated	
Boiling Point	629.18	C @ 760 mmHg	Calculated	Av. % Error = 7.4 K
Vapor Pressure	4.68E-19	mmHg	Calculated	Av. % Error = 47.0
Heat of Vaporization	9.73E03	cal/mole	Calculated	Av. % Error = 1.85
Solubility in Water	0.13	moles/L	Calculated	????
*pKa			Calculated	
FH TMOA	12	MOA Number	Calculated	Carbonyl based reactivity

Figure 10. Log P predictions for Scilliroside using KowWin and Clog P predictive methods.

Another reason that log P calculation methods may be a poor representation of the actual physical/chemistry properties of a substance is that the calculation is based on fragments of the chemical, typically added up with correction factors applied, thereby losing any information about intra-molecular interactions (Benfenatti *et al*., 2003.) For these reasons, researchers have explored other models for predicting how a chemical partitions into tissues. One such method is the use of empirical measures in liposomes vs. measurements of octanol/water partitioning. Liposomes, which are an artificial phospholipid bilayer, are better mimics of a biological membrane, having regions with hydrophobic, hydrophilic, charged or neutral properties (Smejtek and Wang, 1993; Escher and Schwarzenbach, 1996).

Although the partitioning coefficients developed with liposomes are better at describing activity in biological membranes, there is not a large database of measured values for use in model development, such as the database that exists for octanol/water partition coefficient measurements. Additionally, models are not as readily available for predicting the membrane / water partition coefficient; however, this is changing with the advancement in QSPR (quantitative structure-property relationship) prediction methods using linear solvation free energy relationships (LSERs). LSER approaches to predicting lipophilicity have been around since the early 1980s (Abraham *et al*., 1983; Abraham *et al*., 1985; Abraham 1993), and differ from fragment contribution methods such as log P in that they use quantum chemical calculations to predict solute/solvent interactions by accounting for the dipolarity / polarizability

of the chemical, electrostatic interactions, hydrogen bonding interactions, and steric measures such as molar volume to predict lipophilicity. Previously, these models were not readily available due to computational requirements, but recent advances have allowed for availability of these calculations via personal computers. Once again, these models must be taken in the context of the associated knowledgebase. Tetko and coworkers found issues related to predictivity when comparing 96,000 substances from Pfizer and Nycomed company in-house data sets of measured lipophilicity, with 30 prediction methods including those utilizing LSER approaches (Mannhold *et al*., 2009; Tetko *et al.*, 2010). These models performed well on publicly available measures of lipophilicity, but not as well on structures not previously made available (i.e., not part of the models domain of applicability).

7.3 Empirical Test Data

In keeping with the first and fourth OECD validation principles, some QSAR models use training sets of test data generated by single research laboratories specifically developed for use in model development. These single laboratory data sets may reduce experimental variability due to genetic variability of test species, animal husbandry, variation in testing protocols, etc. Examples of species having these types of data sets in ecotoxicology, include the guppy (Könemann, 1981), the fathead minnow (Russom et al., 1997), the ciliate, *Tetrahymena (Schultz, 1996),* and the bacterium, *Vibrio fischeri* (Kaiser and Palabrica 1991). Some QSAR models may be based on training sets gleaned from the open literature. These models may have more variability due to inconsistent test methodologies and data analysis. Therefore, users of QSAR models should be knowledgeable of the training set used to develop the model. Another limitation in the development of QSAR models for a specific test species, are logistical constraints related to aquaculture, husbandry and test methodologies.

Pesticides are complicated by the fact that they exist as formulated products which may include 'inert' ingredients that may not be completely identified, but may affect the ultimate toxicity of the substance. Therefore, when selecting test data for use in (Q)SAR approaches, such as read-across, ICE, *etc.*, it is important to consider only test results based on the active ingredient.

It is presumed that for the OW/OPP common assessment efforts, most data gathering will be on the parent compound, but some active ingredients become more toxic upon degradation. Having a clear understanding of degradate exposure and toxicity is necessary, and predictive methods such as the Metabolism Simulator under development by USEPA (see Jones *et al*., 2009) may be useful in identifying degradates of interest. It should be kept in mind though that a degradate may not have the same MOA or even fall within the same chemical category as the parent compound. As such, toxicity predictions for the degradate may be based on a different working QSAR model than the parent compound. It is also recommended that when deciding which (Q)SAR model to use, consideration be given to the extent to which various models yield predictions that best approximate measured values.

7.4 Selecting Chemical Category and/or AOP

One of the largest sources of uncertainty regarding the use of QSARs is that the models are typically developed for relatively generic structures and AOPs; however, conventional pesticides have been engineered to be toxic, oftentimes to specific taxa, through relatively specific MOAs. Thus, a chemical may have a number of substructures (moieties) that have associated toxicities based on what may be dissimilar MOAs, while the entire chemical structure may impart a specific AOP and toxicity. For example, the insecticide carbaryl, is classified in EcoSAR as a neutral organic and it predicts the toxicity of carbaryl based on an ester moiety contained within the compound. However, the overall MOA of this N-methyl carbamate is to inhibit acetylcholine esterase. The extent to which the component toxicities contribute to the overall activity of the compound is an important consideration and can be a challenge to decipher. As compounds are transformed/degraded to smaller components, the importance/relevance of substructure-activity relationships increases as the overall activity of the parent compound may no longer dominate. Consideration should be given to the extent to which the compound retains structural similarities to the parent and/or chemicals with known toxicities and are accurately predicted by the QSAR model.

As outlined in the IPCS framework document (Boobis *et al*., 2008), all aspects of the experimental design should be considered when determining the AOP, since differences in the exposure duration (acute vs. chronic), life-stage, and concentrations could result in very different adverse outcomes in the whole organism. Most of the methods for determining MOA are based on acute toxicity data, and may not be reflective of chronic exposure.

7.5 ICE Models

While the taxonomic relatedness of surrogate and predicted species within ICE models is an important consideration for model selection, evaluation of ICE models should be a holistic evaluation of all available models and their attributes. Models are available to predict to a species, genus, and family, and models predicting to all three taxonomic levels may be available for a species of interest. Additionally, available measured toxicity values for multiple surrogate species may also increase the number of models from which to choose. Model uncertainty analyses have identified guidelines that may be used in model selection. However, the recommended rules of thumb are intended as guidance and should not be interpreted as requirements. For example, while selecting a model with the most taxonomically related species is recommended, model mean square error and predicted toxicity confidence intervals may indicate otherwise. Additionally, MOA-specific models may provide more accurate predictions for two species with greater taxonomic distances. Additionally, it is important that the input value of the surrogate species is within or close to the range of toxicity values used to develop the model.

8 Example Applications and Proposed Analyses of Predictive Methods

In order to begin assessing the feasibility of using the predictive methods described in this white paper, an effort was undertaken to work through the mechanics of some of the tools and the logic displayed in **Figure 11** (from "*Exploration of Methods for Characterizing Effects of Chemical Stressors to Aquatic Animals*"), and to perform a preliminary comparison of acute and chronic ALSVs with actual FAVs from published criteria documents. These brief comparisons do not constitute a critical evaluation of any one method, but are used to illustrate the proposed analyses that will be done to evaluate the various tools.

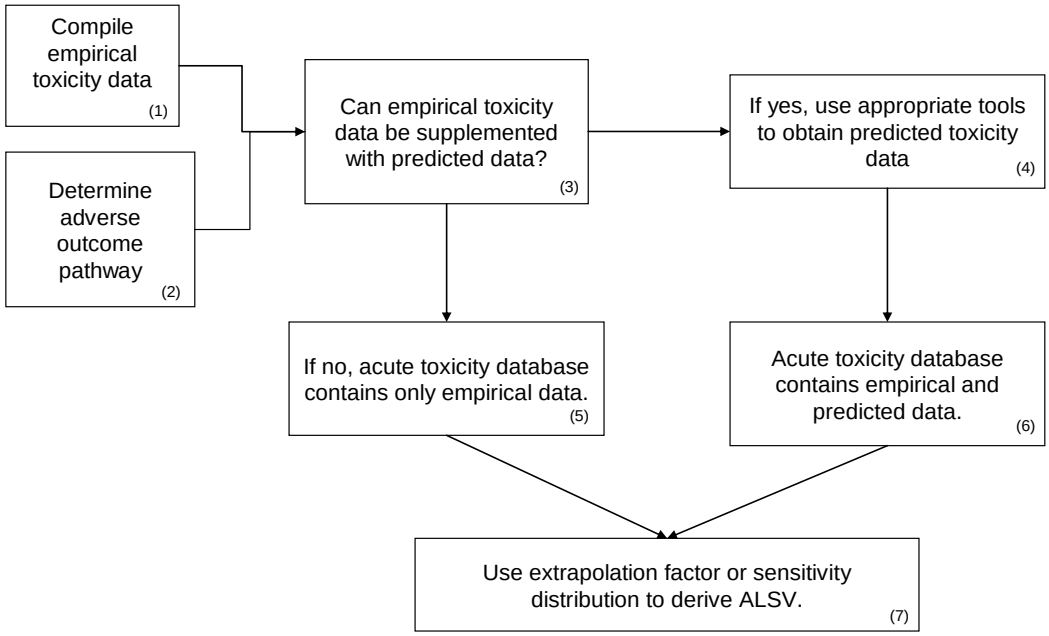


Figure 11. A Conceptual Framework for Deriving the Acute ALSV.

8.1 Empirical Data and Important Values from Existing ALWQC Documents (Box 1 and 2 of Figure 11)

For these examples, pesticides were sought that met the following criteria: 1) have an established ALWQC, 2) the ALWQC had enough toxicity data to meet minimum data requirements as described in the 1985 Guidelines, 3) important values such as the FAV and the FCV were calculated by formulae consistent with the 1985 Guidelines, and 4) represent different toxicological MOAs. Four pesticides were identified that met these criteria: Acrolein, Diazinon, Chlorpyrifos and Tributyltin (TBT). The acute MOAs for these chemicals were listed in Web-ICE as reactive (Acrolein), anticholinesterase –OP (Diazinon and Chlorpyrifos) and uncoupler/inhibitor of oxidative phosphorylation (TBT). Important values (**Table 8**) for these chemicals for the example applications were obtained from the criteria documents.

Table 8. Important values found in ALWQC documents for Acrolein, Diazinon, Chlorpyrifos and TBT

Chemical	FAV	FCV	CMC	CCC	FACR
	(ug/L)	(ug/L)	(ug/L)	(ug/L)	
Acrolein (2009)	5.92	3	3	3	1.906 = 2.0 [1]
Diazinon (2005)	0.3397	0.1699	0.1699	0.1699	1.328 = 2.0 [1]
Chlorpyrifos (1986)	0.0485 [3]	0.012 [4]	0.024 [4]	0.012 [4]	4.046
TBT (2003)	0.9177	0.0723	0.4589	0.0074 [2]	12.69

1 - Per 1985 guidelines, if geomean of ACRs are< 2,FACR = 2

2 - CCC is less than FCV to account for concerns regarding imposex in female snails.

3 - FAV recalculated to reflect the addition of sensitive cladoceran, Critera FAV =0.1669 ug/L

4 - Values are not from criteria document, see text for further description.

When a new pesticide is registered, a minimum of 3 acute values (a cold water fish, a warm water fish and an invertebrate) and 2 chronic values (either a cold water or warm water fish and an invertebrate) are used to develop benchmarks for assessing potential aquatic risks (US EPA 2007). The three acute values (48/96 hr EC/LC50) are typically from toxicity tests using rainbow trout (*O. mykiss*), bluegill (*L. macrochiris*) and a cladoceron species *(e.g., D. magna*). The two chronic values are typically represented by toxicity tests using fathead minnow (*P. promelas*, early life stage or full life-cycle) and a cladoceron species (eg. 21 day life-cycle test, *D. magna*). Therefore, criteria documents were used as a source of toxicity data that could populate this minimum data set for each of the four pesticides (**Table 9**). There is one important exception. The 1986 criteria document for chlorpyrifos did not have acute or chronic values for a cladoceran species. Therefore, a data search was completed in ECOTOX which yielded two acceptable acute tests (Harmon et al. 2003 & El-Merhibi et al. 2003) and two acceptable chronic tests (Rose et al. 2001 and Rose et al. 2002). Mean acute and chronic values (the geometric mean (geomean) of the NOEC and LOECs) were calculated from the studies for each species. *C.dubia* was the most sensitive species tested for chlorpyrifos and would have been ranked first in species sensitivity within the criteria document. Therefore, for the purposes of this analysis only, a new chlorpyrifos FAV was calculated, along with the commensurate CMC and CCC (**Table 8**).

These data (**Table 9**) were subsequently used as a baseline of minimum data available to develop ALSVs using the various predictive methods, assuming no other toxicity data were available. Resultant ALSVs were then compared to criteria FAVs (**Table** 8. Important values found in ALWQC documents for Acrolein, Diazinon, Chlorpyrifos and TBT
12-14).

Table 9. Species mean acute and chronic values found in criteria documents.

Chemical	Acute Values (ug/L)			Chronic Values (ug/L)	
	Oncorhynchus mykiss	Lepomis macrochiris	Cladoceran spp.	Pimephales promelas	Cladoceran spp.
Acrolein (2009)	16	27.19	<39.76 [1]	11.4 [2]	23.83 [1,2]
Diazinon (2005)	425.8	459.6	0.3773 [3, 4]	40.73 [5]	0.3382 [2,3]

Chlorpyrifos (1986)	8.485	10	0.053 [3,8]	2.263 [6]	0.05 [8]
TBT (2003)	4.571	8.3	4.3 [1]	0.2598 [6]	0.1896 [2,7]

1 - Daphnia magna

2 - Life-cycle test

3 - Ceriodaphnia dubia

4 – Diazinon did have a D.magna A.V., but no corresponding C.V., therefore C. dubia was used.

5 - Geomean of two ELS tests (Norberg King 1989, Jarvinen and Tanner 1982)

6 – ELS test

7 - Geomean of two life-cycle tests (Brook et al. 1986, ABC Labs, Inc. 1990.)

8 - SMAVs not available in 1986 criteria document. Values are from studies found in literature that would be acceptable for inclusion in criteria development consistent with the 1985 Guidelines. AVs from Harmon et al. 2003 & El-Merhibi et al. 2003 CVs are from Rose et al. 2001 and Rose et al. 2002.

8.2 Methods for Deriving Acute ALSVs

Following the conceptual framework from **Figure 11**, **Table 9** represents the actions described in the Box 1 of **Figure 11**; "Compile empirical toxicity data". Assuming no additional toxicity data are available and that additional data can be predicted using tools described in this paper, the decision from Box 3 would be, "Yes". Moving to Box 4 , the next step is to use the appropriate tools to then predict toxicity data. The rest of Section 8.2 is divided into providing examples where 1) empirical data are supplemented with predicted values from Web-ICE and the OECD Toolbox to populate a species sensitivity distribution of 8 taxa when possible (box 6); and 2) development of ALSVs (box 7). ALSVs were developed in two ways. First, predictive methods were used to populate MDRs as described in the currently accepted paradigm of the 1985 Guidelines and an ALSV was calculated in an identical manner as an FAV. Second, the ALSV was calculated as the HC5 concentration using examples of SSD approaches independent of the 1985 Guidelines.

8.2.1 Supplementing Empirical Data with Predicted Values for Missing MDRs (Box 4 of Figure 11).

The FIFRA registration process for pesticides results in a minimum of toxicity data for three of the eight acute FW MDRs required by the 1985 Guidelines. The following sections provide examples of two methods used to populate the remaining acute freshwater MDRs with predicted acute values and the resultant ALSVs are then compared to FAVs derived from experimentally determined acute values. The MDRs, as outlined in the 1985 Guidelines, require that data be available for at least eight genera with a specified taxonomic diversity, in order to address a wide variety of the taxa constituting an aquatic animal community. For freshwater criteria, the MDRs are:

MDR #1. a salmonid fish
MDR #2. a nonsalmonid fish

MDR #3. a species from a third chordate family
MDR #4. a planktonic crustacean
MDR #5. a benthic crustacean
MDR #6. an insect
MDR #7. a species from a family in a phylum other than Chordata or Arthropoda
MDR #8. a species from a family in another order of insect or in a fourth phylum

8.2.1.1 Web-ICE

Web-ICE models were strategically used to predict acute values for additional species that would meet MDRs (**Table 10**). Acute values were predicted for fathead minnow (MDR #3), an amphipod (*Gammarus fasciatus*, MDR #5), a midge (*Chironomus plumosus*, MDR #6), a stonefly (*Claassenia sabulosa*), MDR #8), and the eastern oyster (*Crassostrea virginica*, MDR #7. It is recognized that the eastern oyster is a saltwater species, and use herein does not imply that acute values for saltwater species can be used to satisfy freshwater species requirements in Agency risk assessments or water quality criteria, However, given the assumption in this scenario that there are no acute toxicity data beyond rainbow trout (MDR #1), bluegill (MDR #2) and cladoceron species (MDR #4), there are no predicted species available that meet the 7th MDR and correlate with any of those 3 surrogate species. In choosing species that met MDRs, care was taken to choose the most appropriate surrogate species for each of these predictions. Although it may be intuitive to use an invertebrate surrogate for a prediction of an invertebrate acute, mathematical constraints in the model may reduce the robustness of the relationship. In this example, rainbow trout and bluegills were used as surrogates. Model predictions are most robust when there is a high number of data points (*i.e.*, toxicity tests) correlating the two species. When a low number of tests (*e.g.*, <5) are used in the ICE model, the range of exposure becomes limited and, therefore, predictions may fall outside of the domain of the model. Also, with a low number of tests, the confidence intervals for each predicted acute value often become larger. Therefore, this exercise sought taxa combinations that met minimum data requirements and had the highest number of toxicity test results correlating the two species. Model statistics varied among the species combinations (**Table 11**). The number of tests correlating the taxa ranged from 7 (Bluegill → Stonefly) to 95 (Bluegill → Eastern oyster) while R^2 ranged from 0.311 (Rainbow trout → the midge) to 0.825 (Rainbow trout → fathead minnow). After predicted values were obtained, acute ALSVs were calculated for each chemical using formulae from the 1985 Guidelines (n=8, the four most sensitive species are bolded in **Table 10**).

Table 10. Meeting minimum data requirements of the 1985 Guidelines using Web-ICE (acute values, 95% confidence limits are in parentheses).

Chemical	Species Mean Acute Values (ug/L)			Predicted Acute Values (LC/EC50 ug/L)					Acute ALSV
	Oncorhynchus mykiss[1]	Lepomis macrochiris[2]	Ceriodaphnia sp.[4]	Pimephales promelas[3]	Gammarus fasciatus[5]	Chironomus plumosus[6]	Crassostrea virginica[7,11]	Claassenia sabulosa[8]	(ug/L)
				RBT[9]	RBT[9]	RBT[9]	Bluegill[10]	Bluegill[10]	
Acrolein (2009)	**16**	27.19	<39.76	54.9	**16.7**	**14.9**	36.8	**1.6**	0.8886
				(33.7 - 89.3)	(3.6 - 76.7)	(2.6 - 84.5)	(20 - 67.6)	(0.6 - 4.5)	
Diazinon (2005)	425.8	459.6	**0.3773**	1011.6	**153.1**	**185.3**	299.9	**4.54**	0.0368
				(736.7 - 1389.2)	(53.3 - 439.6)	(61.2 - 561)	(203.5 - 442.1)	(1.0 - 21.2)	
Chlorpyrifos (1986)	**8.485**	10	**0.053**	31.2	10.9	**9.2**	17.5	**1.12**	0.0113
				(18.3 - 53.2)	(2.0 - 58.7)	(1.4 - 62.2)	(8.5 - 36.0)	(0.4 - 3.1)	
TBT (2003)	**4.571**	8.3	**4.3**	18	7.6	**5.7**	15.3	**1**	0.6566
				(10.1 - 32.2)	(1.1 - 45.7)	(0.7 - 46.6)	(7.3 - 32.0)	(0.4 - 3.0)	

Note: Bolded values were used to calculate ALSV with an n=8

1 - The family Salmonidae in the class Osteichthyes;
2 - One other family (preferably a commercially or recreationally important, warmwater species) in the class Osteichthyes (*e.g.*, bluegill, channel catfish);
3 - A third family in the phylum Chordata (*e.g.*, fish, amphibian);
4 - A planktonic crustacean (*e.g.*, cladoceran, copepod);
5 - A benthic crustacean (*e.g.*, ostracod, isopod, amphipod, crayfish);
6 - An insect (*e.g.*, mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge);
7 - A family in a phylum other than Arthropoda or Chordata (*e.g.*, Rotifera, Annelida,Mollusca);
8 - A family in any order of insect or any phylum not already represented.
9 - Rainbow Trout (O. mykiss) was used as a surrogate for these species
10 - Bluegill (L. macrochiris) was used as a surrogate for these species
11-No predicted freshwater species were available that met MDR #7 using SMAVs from available freshwater surrogates (*i.e.*. RBT, blugill and cladoceran)

Table 11. Summary of model statistics used to predict acute values.

Surrogate species	Predicted species	N	DF	R^2
Rainbow trout	*Pimephales promelas*	81	79	0.825
Rainbow trout	*Gammarus fasciatus*	36	34	0.311
Rainbow trout	*Chironomus plumosus*	20	18	0.531
Bluegill	*Crassostrea virginica*	95	93	0.549
Bluegill	*Claassenia sabulosa*	7	5	0.585

The performance of Web-ICE in accurately predicting acute values for use in ALSV derivation varied by chemical (**Table 12**). For TBT, the models performed reasonably well in that the Web-ICE derived ALSV was 1.4 fold less than the criteria FAV, thus achieving the goal of having the ALSV being a somewhat conservative estimate of the FAV. For acrolein, diazinon and chlorpyrifos, the Web-ICE ALSVs were 6.6, 9.2 and 4.3 fold respectively lower than the criteria FAV (**Table 12**). The Web-ICE ALSV for diazinon is particularly troublesome in that it is 9 fold lower the most sensitive species in the restricted data set and even if one considers all the data in the criteria document, this observation remains the same.

Table 12. Web-ICE ALSV estimates compared to FAV in criteria documents.

	Criteria	Web-ICE
Chemical	FAV	ALSV
	(ug/L)	(ug/L)
Acrolein (2009)	5.92	0.8886
X Fold Difference		**-6.6**
Diazinon (2005)	0.3397	0.0368
X Fold Difference		**-9.2**
Chlorpyrifos (1986)	0.0485	0.0113
X Fold Difference		**-4.3**
TBT (2003)	0.9177	0.6566
X Fold Difference		**-1.4**

Proposed analyses will determine when Web-ICE is a useful tool and when it is not. Types of analyses proposed will : 1) comparing individual species predictions to experimentally determined values; 2) comparing the shapes of predicted species sensitivity distributions to experimentally determined distributions; 3) explore species sensitivity distributions developed using different predicted taxa meeting the 1985 Guidelines MDRs; 4) consideration of biases in surrogate species that may, in-turn, bias the predicted values; 5) compare the accuracy of ICE predictions among types of MOA; 6) determine if there are characteristics of surrogate data sets that limit, or enhance, the predictability of regression models used in ICE.

8.2.1.2 Read-Across

As another example of a means to populate MDRs with predicted acute values, the OECD (Q)SAR application toolbox (V. 1.1.02, Nov. 2009) was used to fill data gaps using read-across techniques for single chemicals. The toolbox software, as well as guidance documents, can be downloaded free of charge from the following URL: http://www.oecd.org/document/54/0,3343,en_2649_34379_42923638_1_1_1_1,00.html#Download_qsar_application_toolbox.

Step by step instruction is available in the guidance document, but some description here is necessary to document various steps that influence the predicted acute values in **Table 13**. From the start up screen (**Figure 12**) of the QSAR application toolbox, the "Flexible Track" was used to identify the target chemical, gather analogue data, and fill data gaps.

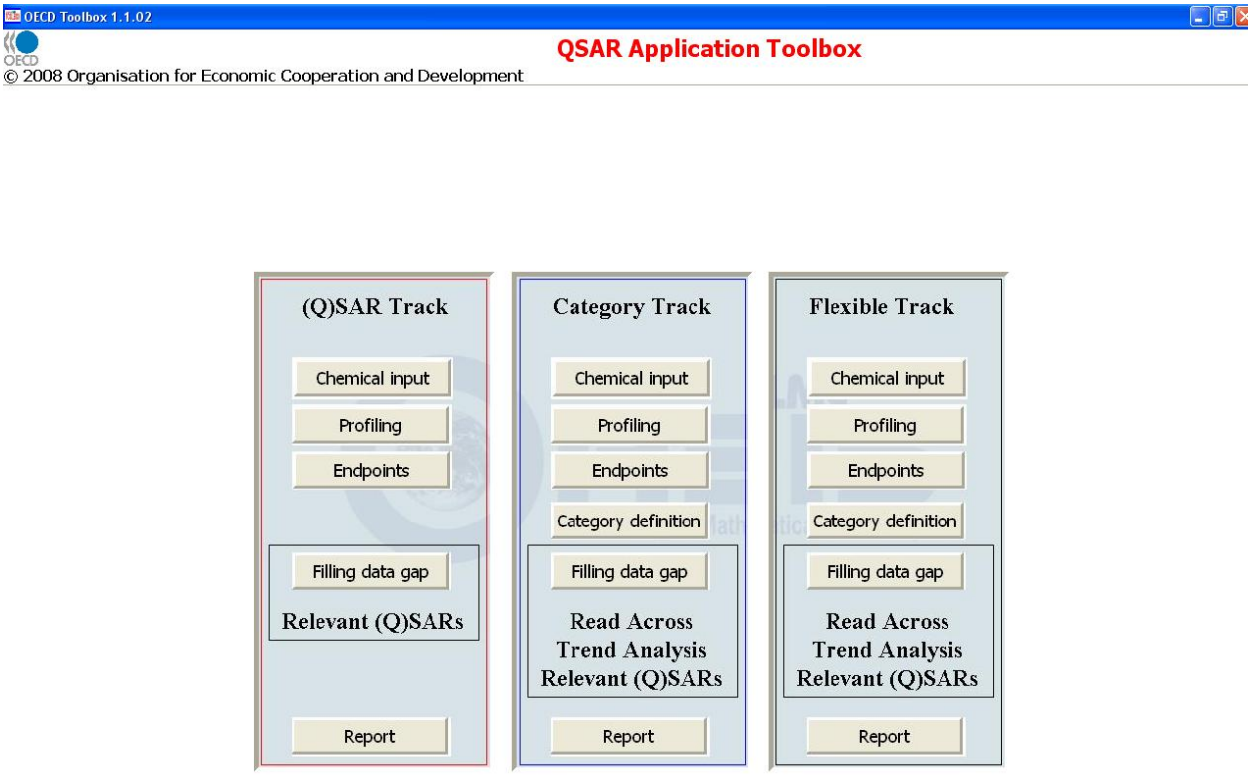


Figure 12. Start-up screen of the OECD toolbox.

After identifying the target chemical, users are prompted to "Profile" the chemical. In profiling, the software can affiliate the target chemical with previously defined categories of chemical structures, or mode of action. For acute values for aquatic species, the toolbox guidance recommends using the OASIS Acute toxicity MOA, Verhaar and ECOSAR classifications (**Figure 13**). **Figure 13** demonstrates an example using diazinon. Note that ECOSAR classifies diazinon as an ester, phosphate ester, and as a pesticide. OASIS classifies it simply as a "Reactive Unspecified", while the Verhaar scheme classifies diazinon as Class 5 (Not possible to classify this chemical).

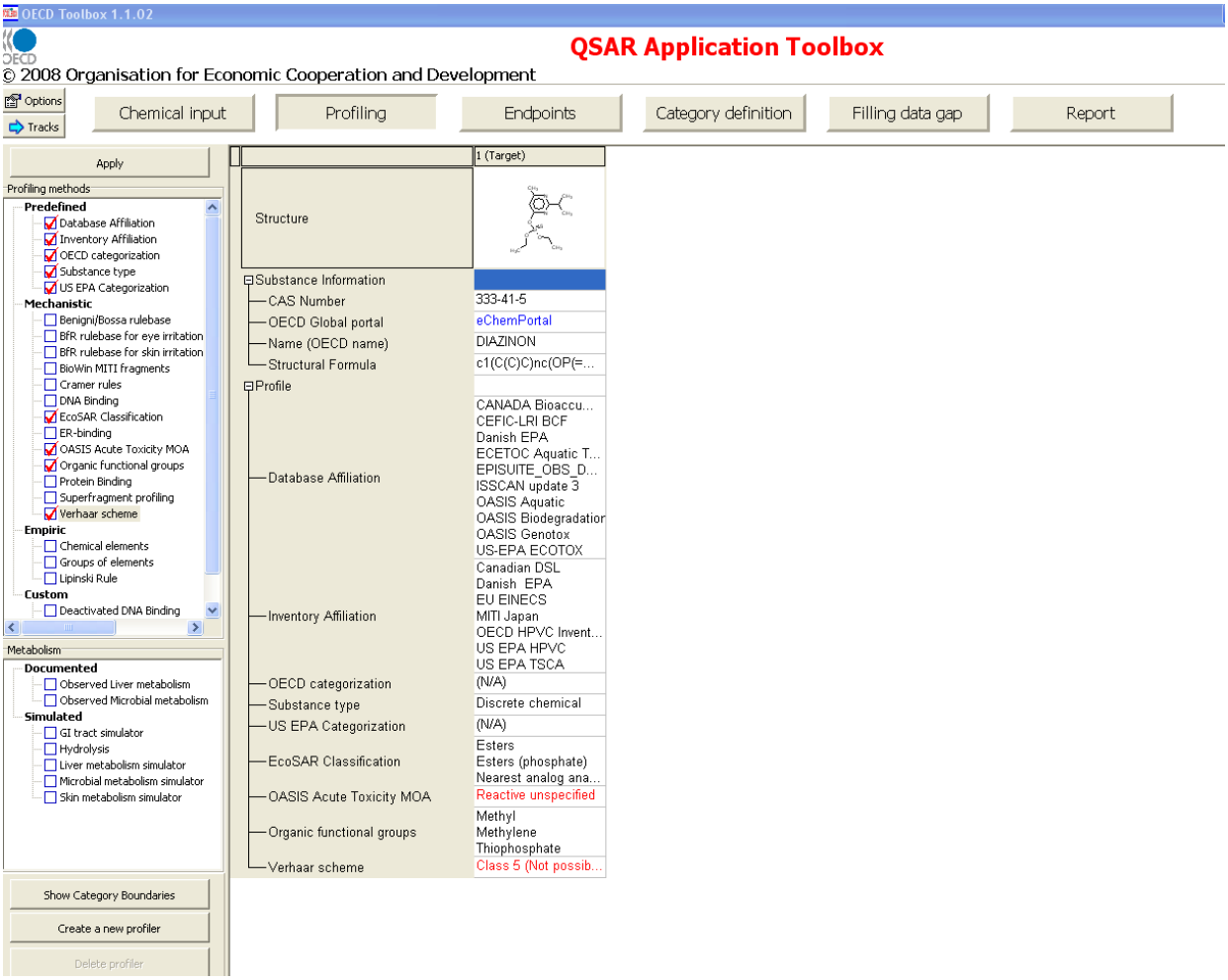


Figure 13. "Profiling" Diazinon in the OECD Toolbox.

Next, users are asked to identify endpoints of interest. The first step is to identify what databases will be queried. Depending on the user's purpose, all or few of the databases can be selected. For the purposes of this example, 5 databases were chosen to focus on acute values for aquatic species: The ECETOC Aquatic toxicity, EPISUITE_OBS_data, ISSCAN UPDATE 3, Japan Aquatic, OASIS Aquatic and USEPA ECOTOX. After identifying the databases, the software gathers the requested data (**Figure 14**).

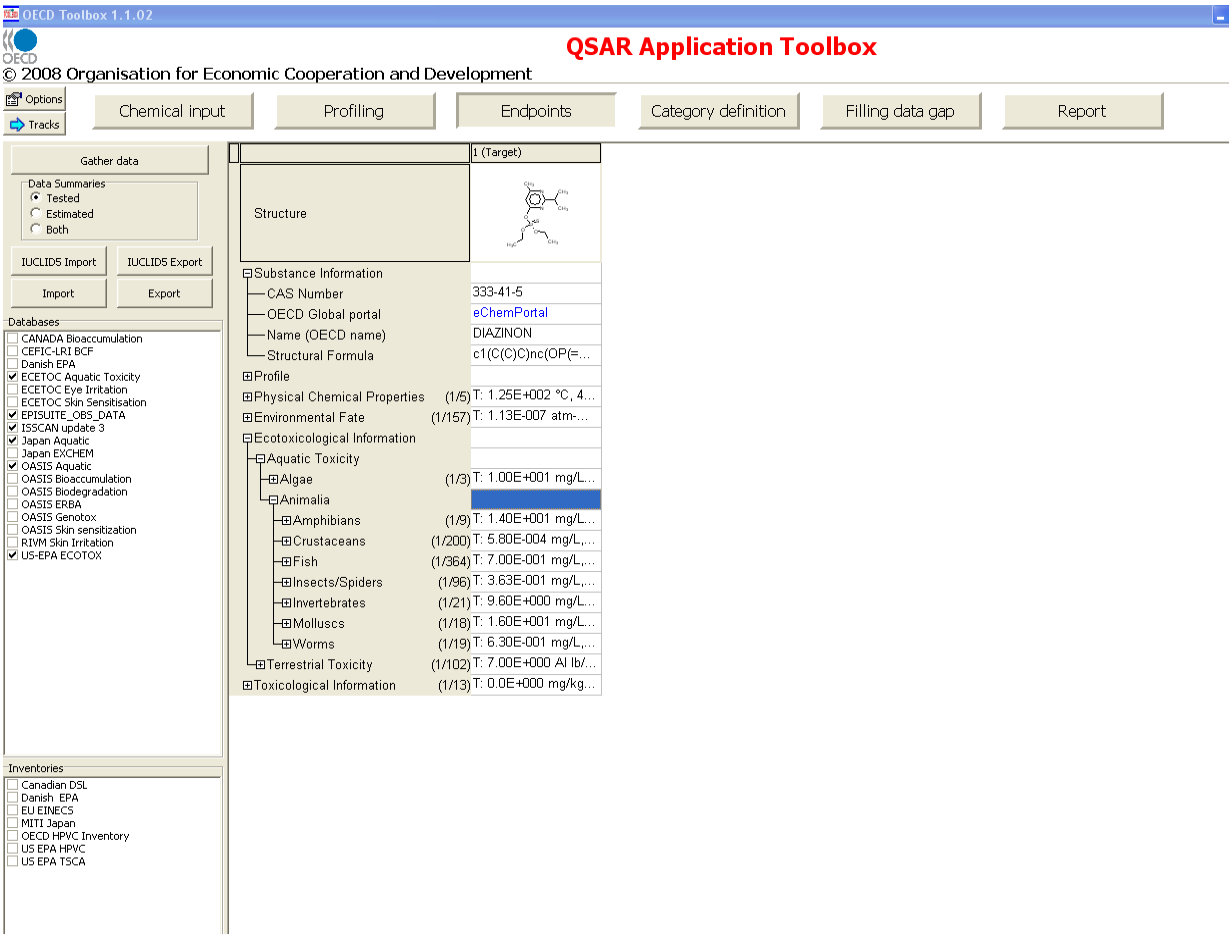


Figure 14. Identified databases and available data for the target chemical diazinon in the OECD toolbox.

After gathering the toxicity data for the target chemical, the next step is to define the category from which analogue data will be gathered. In this example, the ECOSAR classification was used which yielded 170 analogue structures. The other two categories (Verhaar scheme and OASIS Acute toxicity MOA) were not helpful in this example. Since the Verhaar scheme classified diazinon as "unclassifiable", there were no other analogues for comparison. Since the OASIS classification labeled diazinon as "reactive unspecified", it was lumped with 14,156 other analogues. After choosing the ECOSAR classification, the user is prompted to identify what data should be gathered. Through a series of check boxes on pop-up windows, only acute values for Amphibians, Crustaceans, Echinoderms, Fish, Insects, Invertebrates, Molluscs and Worms were selected for either 48 or 96 hr exposures (**Figure 15**).

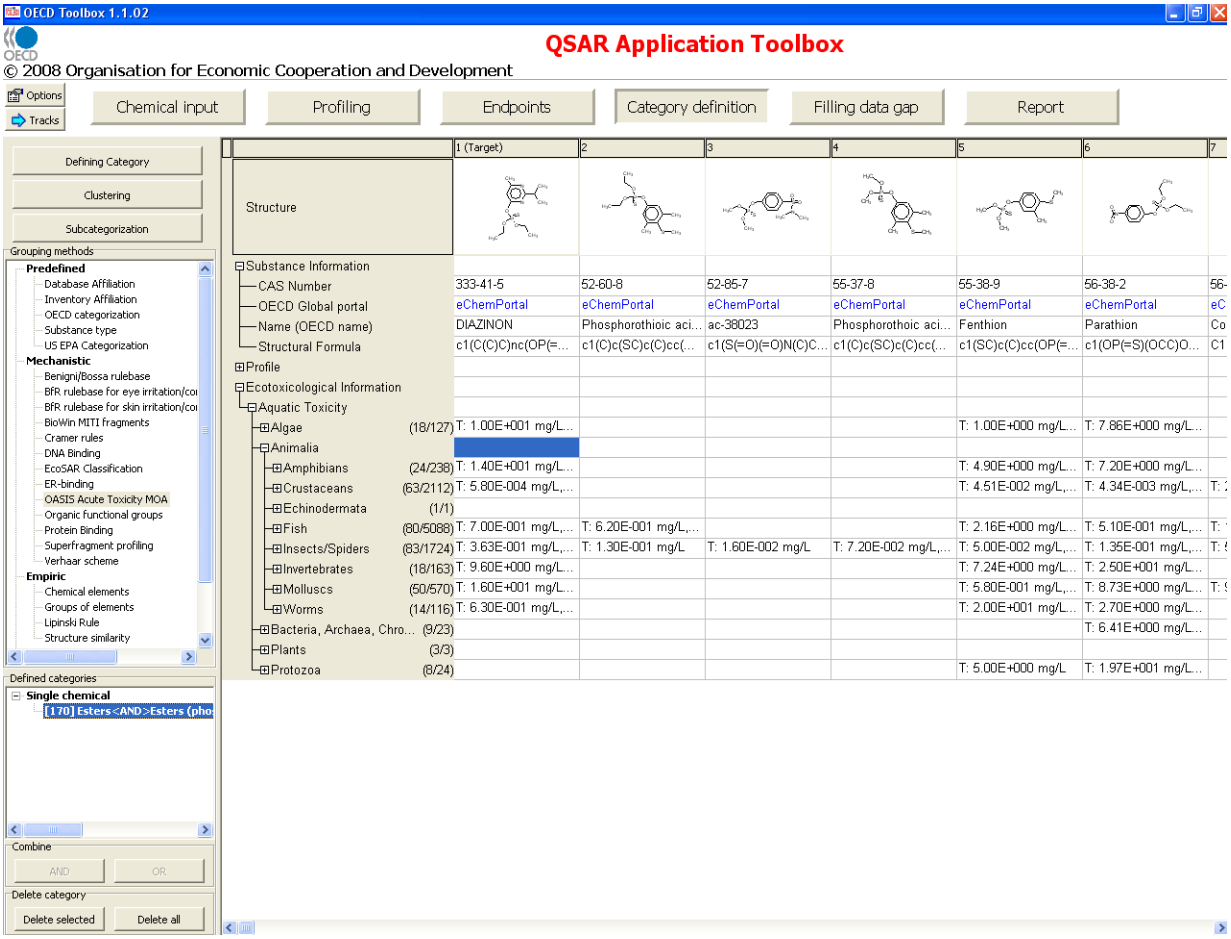


Figure 15. Data identified from the Category definition screen for Diazinon in the OECD toolbox.

The final step is to move onto the "Filling data gap" module of the toolbox. The first step is to identify the species of interest (e.g. *P. promelas*) for which the toolbox will gather analogue data. The data are gathered and subsequently graphed according to a "descriptor". The default descriptor is Log_Kow_EPISUITE. In the diazinon example, there were 18 analogues with 48 hr LC50 data for fathead minnow (**Figure 16**). The toolbox then identified 5 (may be more or less) of the closest analogues (as defined by the descriptor) and calculated an average LC50 value for those 5 points. In **Figure 16**, the 5 analogues used to calculate the average LC50 are identified as the maroon colored points, the target value is in red and all others are in blue. At this point, users can further refine the points by subcategorization. For this example, a refinement of structure similarity using the Dice index (**Figure 17**) was used and all data points with structures having similarity index of 70% or less were removed. Applying that filter to diazinon fathead minnow acute values, resulted in having data from 7 analogues from which an average LC50 was calculated.

These steps were repeated for all four of the pesticides and for multiple species to meet MDRs. Unfortunately, it was not possible for acrolein and TBT because too few analogues are available in the database. Predicted acute values and calculated ASLVs are illustrated in **Table 13**.

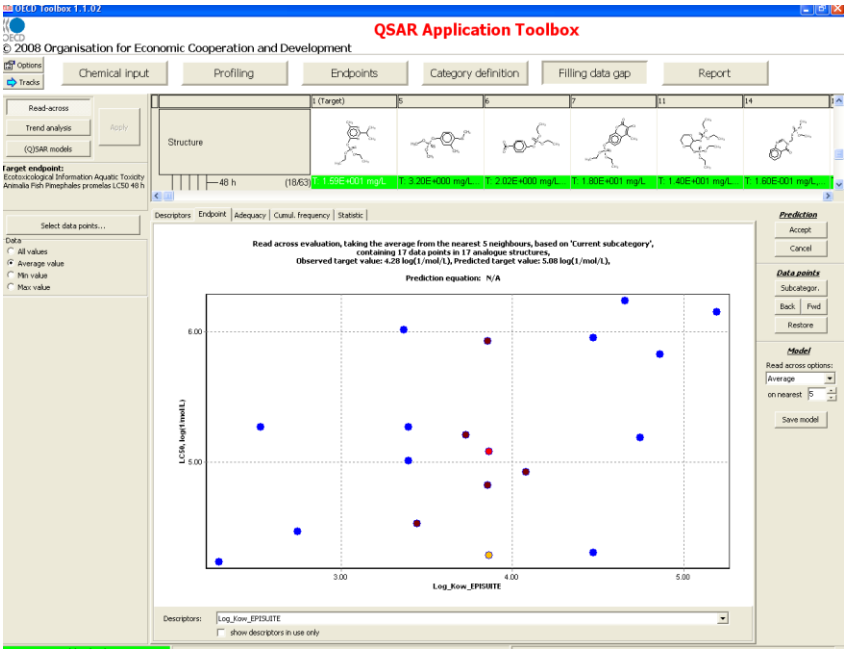


Figure 16. Read-across evaluation screen for Diazinon in the OECD toolbox.

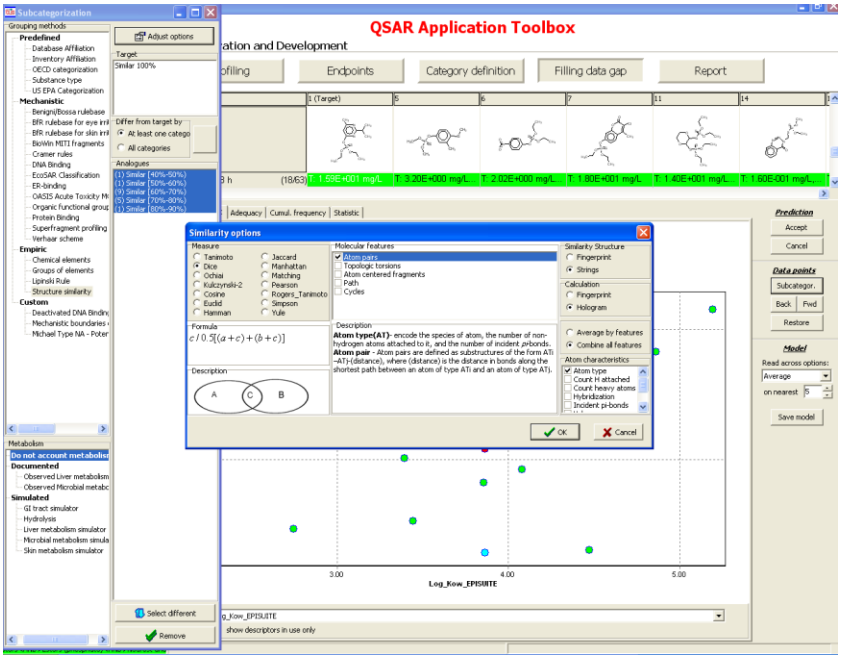


Figure 17. Analogue structure similarity indexs in the OECD toolbox.

Table 13. Meeting minimum data requirements of the 1985 Guidelines using Read-across from OECD toolbox.

(LC/EC50s, 95% confidence limits are in parentheses)

Chemical	Species Mean Acute Values (ug/L)			Predicted Acute Values (ug/L)					ALSV	Criteria FAV
	Oncorhynchus mykiss[1]	Lepomis macrochiris [2]	Cladoceran sp.[4]	Pimephales promelas[3]	Gammarus fasciatus[5]	Pteronarcys californicus [6]	Crassostrea virginica [7, 9]	Dugesia tigrina [8]	(ug/L)	(ug/L)
Diazinon (2005)	**425.8**	459.6	**0.3773**	1350	**3.18**	**6.81**	437	3060	0.0206	0.3397
				(31 - 58.2)	(0.014 - 700)	(0.67-690)	(96.5 - 1970)	(0.979 - 9.55)	**X Fold Difference**	-16.5
Chlorpyrifos (1986)	**8.485**	**10**	**0.053**	1310	41.7	**9.99**	140	3420	0.0141	0.0485
				(56.4 - 30300)	(0.29 -5820)	(0.35 -286)	(14.3 - 1360)	(1090 - 10700)	**X Fold Difference**	-3.5

Note: Bolded values were used to calculate ALSV with n = 8

1 - The family Salmonidae in the class Osteichthyes;
2 - One other family (preferably a commercially or recreationally important, warmwater species) in the class Osteichthyes (*e.g.*, bluegill, channel catfish);
3 - A third family in the phylum Chordata (*e.g.*, fish, amphibian);
4 - A planktonic crustacean (*e.g.*, cladoceran, copepod);
5 - A benthic crustacean (*e.g.*, ostracod, isopod, amphipod, crayfish);
6 - An insect (*e.g.*, mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge);
7 - A family in a phylum other than Arthropoda or Chordata (*e.g.*, Rotifera, Annelida,Mollusca);
8 - A family in any order of insect or any phylum not already represented.
9 - Saltwater species retained for read-across comparison to Web-ICE

Like WEB-ICE, the OECD toolbox using the read-across application met the overall goal of ALSV derivation in that it provided predicted acute values that led to a conservative estimate of the FAV for both diazinon (-16.5 fold) and chlorpyrifos (-3.5 fold). And, like Web-ICE, diazinon had the largest discrepancy between the ALSV and the criteria-derived FAV.

Proposed analyses will further explore the use of the OECD toolbox with its read-across, trend analysis and (Q)SAR model applications. In this example, some observations and points of investigation are apparent. Obviously, the more data that are available from similarly structured chemicals, the more useful this tool will be. In the case of acrolein and TBT, sufficient data are not available to begin using any of these predictive methods as employed currently. In the case of the two organophosphates, sufficient data were available for a number of different analogues with similar structures to consider their use. Additionally, more work needs to be completed to identify profiles more closely linked to adverse outcome pathways. For example, using the default recommended aquatic acute toxicity classifications (ECOSAR classification, OASIS Acute Toxicity and Verhaar Classification) all four pesticides fell into the same classification schemes closely linked to the 3 discreet MOAs they represent. Other classification schemes such as Protein Binding may yield a more refined inference to MOA as compared to the OASIS Acute Toxicity MOA (e.g. reactive unspecific). In our example, the default descriptor that identifies the 5 closest analogues was log K_{ow}. The toolbox has dozens of other physical and chemical descriptors that would likely be more appropriate for anticholinesterase pesticides such as diazinon or chlorpyrifos. Proposed analyses will attempt to identify the most appropriate descriptors for various pesticide MOAs. Also in our examples, the Dice similarity index for chemical structure was used with a 70% similarity cut off. It is not known at this point, what similarity index is most appropriate for each type of chemical structure. Proposed analyses will be completed to identify the most appropriate similarity indexes with MOA or structure type. Finally, this was a very quantitative example of read-across. Proposed analysis will also describe how the technique can be used in a qualitative manner to rank relative potencies of chemical structures among taxa types.

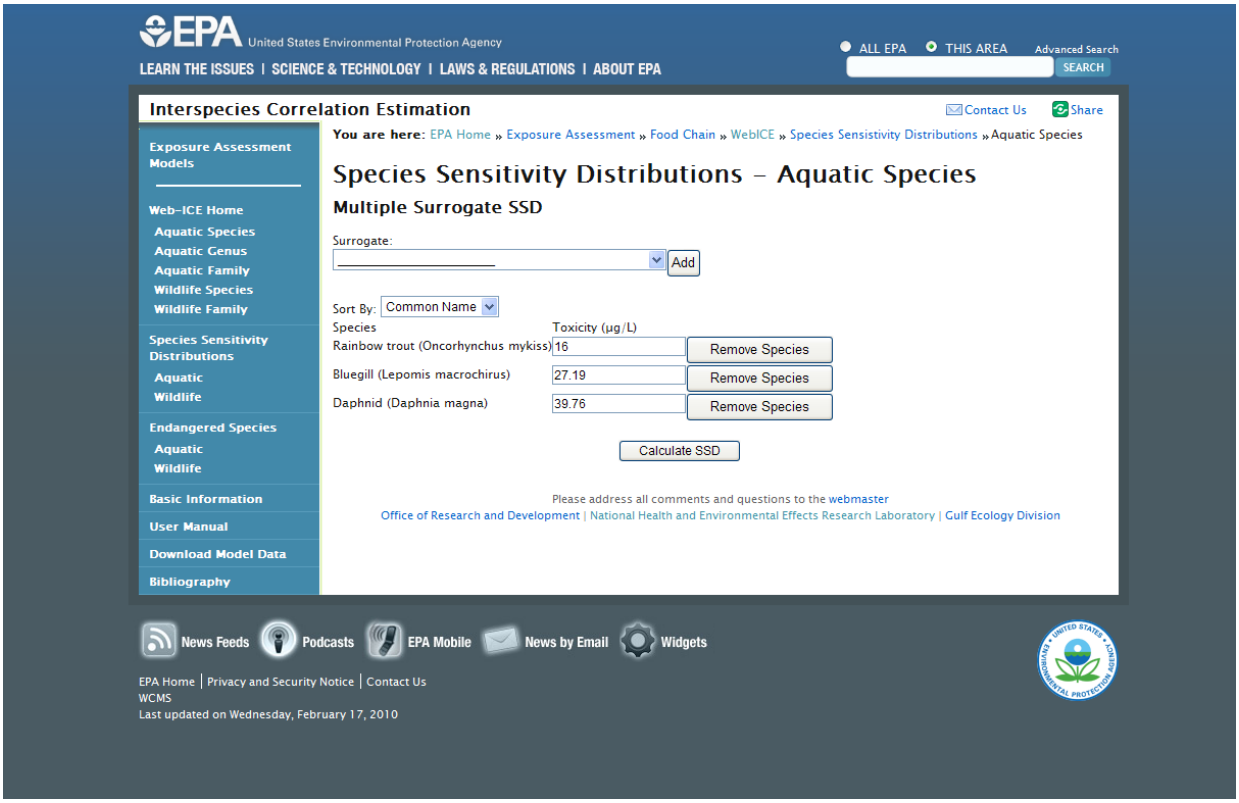
8.2.2 Only Use Results of Toxicity Tests for Chemical and Taxa of Interest

The previous two predictive methods were examples of how one may be able to supplement data sets to meet MDRs and subsequently calculate an ALSV using an SSD approach. The following section provides examples of how one may generate an ASLV by calculating an ALSV using the available toxicity data (Box 7 in **Figure 11**). These calculations can be accomplished by: 1) Calculating the ALSV using extrapolation factors, or 2) using an SSD methodology. The companion paper ("*Exploration of Methods for Characterizing Effects of Chemical Stressors to Aquatic Animals*") illustrates an example of using GLI extrapolation factors and therefore will not be duplicated here. Therefore, this section focuses on two examples of SSD approaches.

8.2.2.1 Calculating an ALSV using a modified SSD Methodology (Box 7, Figure 11)

Two different methods are readily available for estimating an ALSV using a SSD methodology. The first example described here is also within the Web-ICE tool. Within Web-ICE there is an option for calculating an SSD with as few as 1 species. In this example, the 3 acute values (**Figure 18**) from **Table** 9. Species mean acute and chronic values found in criteria documents. **9** were used to calculate a HC5 for each of the 4 pesticides (**Table 14**).

Figure 18. Estimating an ASLV using the SSD function of Web-ICE Data here are for Acrolein.



The HC5 estimates using the Web-ICE log-logistic assumed distribution were relatively accurate and conservative for 2 of the chemicals: acrolein (-4.3 fold) and TBT (-2.4 fold). However, diazinon (62.3 fold) and chlorpyrifos (22.1 fold) were both severely overestimated. As described earlier when Web-ICE was being used to extrapolate single species, the proposed analyses will describe the reasons behind these apparent biases to assess characteristics of datasets that are most amenable to the use of this tool.

A second example of how minimum toxicity data sets can be used to estimate HC5s from assumed distribution was completed. Rainbow trout, bluegill and cladoceran acute values from **Table** 9. Species mean acute and chronic values found in criteria documents. **9** were used with an assumed Log-logistic SSD according to methods proposed by de Zwart (2002). The log-logistic equation from the Species Sensitivities Distribution section (Appendix

B) of the companion white paper "*Exploration of Methods for Characterizing Effects of Chemical Stressors to Aquatic Animals*" was used to calculate HC5s (**Table 14**). Two parameters (α and β) were determined for each of the four pesticides (**Table 14**). The α parameter was the average $\log_{10}$ of the 3 acute values for each chemical, while β was taken from de Zwart (2002) specific to the chemicals MOA as listed in Web ICE

In this example, HC5s were very similar to the FAV. In the case of diazinon, the two values were identical. The maximum difference between the FAVs and the HC5s was -3.62 fold and all had a negative bias, meaning that they were conservative estimates.

Table 14. Predicted HC5 concentrations assuming Log-logistic shape of SSD specific to MOA

Chemical	Experimentally derived Acute Values (ug/L)					ASLV	Criteria FAV
	Oncorhynchus mykiss	Lepomis macrochiris	Cladoceran sp.	Avg Log	β-deZwart[1]	HC5	
				A	β	Mg/L	µg/L
Acrolein (2009)[2]	16	27.19	39.76	1.41	0.28	3.876	5.92
Log	1.20	1.43	1.60	**x-fold difference**			**-1.53**
Diazinon (2005)[3]	425.8	459.6	0.3773	1.62	0.71	0.341	0.3397
Log	2.63	2.66	-0.42	**x-fold difference**			**1.00**
Chlorpyrifos (1986)[3]	8.485	10	0.053	0.22	0.71	0.013	0.0485
Log	0.93	1.00	-1.28	**x-fold difference**			**-3.62**
TBT (2003)[4]	4.571	8.3	4.3	0.74	0.38	0.416	0.9177
Log	0.66	0.92	0.63	**x-fold difference**			**-2.21**

1 - β values are those from de Zwart, 2002 and are dependent of MOA designation

2 – The β value for Acrolein was that of Reaction with carbonyl compounds

3 - The β value for Diazinon and Chlorpyrifos was that of Acetylcholinesterase inhibitors: organophosphates

4 - The β value for TBT was that of uncouplers of oxidative phosphorylation

One obvious and perhaps important difference in these two SSD examples is de Zwart's incorporation of MOA in the HC5 estimate as parameters that help shape the Log-logistic curve.

8.3 Additional Applications of Predictive Tools

8.3.1 EFED QSAR Guidance

Guidance and use of (Q)SAR models to predict environmental fate and toxicity values when chemical-specific data are not available for EFED risk assessment is provided in **Appendix A**. (Q)SAR may be used in effects assessments for all types of risk assessments conducted by OPP.

8.3.2 Comparison of endosulfan and its degradate, endosulfan sulfate, toxicities using Web-ICE

Section 8.2.1.1 discussed the application of Web-ICE models for predicting acute toxicity values to meet MDRs. This section demonstrates another potential application of Web-ICE models that compares toxicities of a chemical and it's degradate, which may have limited test data. The organochlorine insecticide, endosulfan, and it's degradate endosulfan sulfate appear to be of relatively equal toxicity, with endosulfan sulfate generally being slightly less toxic than the parent compound. Limited toxicity data for endosulfan sulfate allow direct comparison of toxicities for bluegill (*Lepomis macrochirus*), carp (*Cyprinus carpo*), sheepshead minnow (*Cyrpinodon variegates*), daphnid (*Daphnia magna*), and the mysid shrimp (*Americamysis bahia*), and show measured toxicity within an order of magnitude of each other (Error! Reference source not found.). Since direct comparisons of endosulfan and endosulfan sulfate were limited to only five aquatic species, the Web-ICE (Web-based Interspecies Correlation Estimation v 3.0; http://www.epa.gov/ceampubl/fchain/webice/) application was used to predict toxicity to endosulfan sulfate for additional species.

Endosulfan sulfate toxicity was predicted for species for which a measured endosulfan toxicity value existed, but the degradate toxicity was lacking. Predicted toxicity values could only be obtained where models exist for both the taxa of interest (the predicted taxa) and the surrogate for which measured toxicity is available. Therefore, the data presented in **Table 14** are for species for which predictions could be made from the measured data and where models were available.

For aquatic species, surrogate selection and assessment of model predictions for aquatic species followed Web-ICE user guidance (Raimondo et al. 2010). To predict toxicity of endosulfan sulfate to the untested species listed in **Table 14** (*e.g.*, rainbow trout, fathead minnow), all species, genus, and family models were extracted for each potential surrogate (bluegill, carp, sheepshead minnow, *Daphnia magna*, and the mysid shrimp) and predicted species. Where multiple models existed for a predicted species (multiple surrogates, multiple taxonomic levels) models were sorted by MSE in ascending order. For all predicted species except the scud, *Gammarus lacustris*, low MSE correlated with high R^2, close taxonomic distance, and high cross-validation success rate. For predicted species with multiple models, all degrees of freedom (df) were greater than 10 and p-values less than 0.01. For all species with multiple models except for the scud, toxicity predictions and confidence limits were calculated using the top 2 or 3 models based on the criteria above (low MSE). Confidence intervals were used to confirm that the models with the lowest MSE, highest R^2, highest cross-validation success rate, and closes taxonomic distances yielded the most robust predictions.

All models for the scud contained relatively equal and large MSE (>1.48), low R^2 (<0.53) and low cross-validation success rates (< 51%), which indicate high model uncertainty. Since these model criteria were generally the same for all scud models and confidence intervals of all model predictions were generally equal, taxonomic relatedness was used to select the best surrogate (Mysid shrimp). Results of the extrapolations support the measured data in demonstrating similar toxicity of endosulfan and endosulfan sulfate, with the degradate being slightly less toxic than the parent compound.

Table 14. Comparison of the acute toxicity predictions for endosulfan and endosulfan sulfate using Web-ICE and measured data for aquatic species. The upper table includes species with toxicity values for both endosulfan and endosulfan sulfate. The lower table contains species for which endosulfan sulfate toxicity was predicted from Web-ICE.

Species	Endosulfan		Endosulfan Sulfate	
	Measured Acute Toxicity (ug/L)	MRID or Ecotox reference	Measured Acute Toxicity (ug/L)	MRID or Ecotox reference
Bluegill	1.7		3.8	
Carp	0.1	Sunderam et al. 1992; (ECOTOX 5850)[a]	2.2	45421402[a]
Daphnia magna	166	5008271	300	45421403[a]
Sheepshead minnow	1.3[b]	Shimmel 1981; (Ecotox 3740)	3.1	46382603
Mysid shrimp	0.83[b]	Shimmel 1981; (Ecotox 3740)	7.9	46406401

Species	Endosulfan		Endosulfan Sulfate	
	Measured Acute Toxicity (ug/L)	MRID or Ecotox reference	Web-ICE Predicted Toxicity and 95% c.i.[c](ug/L)	Surrogate (input value) - Model level
Rainbow trout (*Oncorhynchus mykiss*; Salmonidae)	0.83	136999	4.23 (3.32-5.39)	Bluegill (3.8) - Species
Channel catfish (*Ictalurus punctatus*; Ictaluridae)	1.5	40094602	1.95 (0.77 – 4.92)	Common carp (2.2) - Family
Fathead minnow (*Pimephales promelas*; Cyprinidae)	1.5	40094602	2.89 (1.35 – 6.21)	Common carp (2.2) - Species
Scud (*Gammarus lacustris*; Gammaridae)	5.8	40094602	30.15 (8.27 – 109.93)	Mysid (7.9) - Genus
Stonefly (*Pteronarcys californica*; Pteronarcyidae)	2.3	40094602	2.23 (0.49 – 10.08)	Bluegill (3.8) - Family
Striped mullet (*Mugil cephalus*; Mugilidae)	0.38	40098001	3.06 (0.66-14.25)	Bluegill (3.8) - Family
Pink shrimp *(Penaeus dourarum, Farfantepenaeus duorarum*; Penaeidae)	0.04	5005824	1.03 (0.28 – 3.82)	Bluegill (3.8) - Family
Grass shrimp (*Palaemonetes pugio*; Palaemonidae)	1.31	5005824	156.2 (90.76 – 268.71)	Daphnid (300) Genus
Eastern oyster (*Crassostrea virginica*; Ostreidae)	0.45	128688	8.55 (3.71 – 19.71)	Bluegill (3.8) - Species

[a] study classified as supplemental

[b] geometric means
[c] confidence interval

9 References

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Appendix A: EFED Guidance on Use of Structure-Activity Relationships

Executive Summary

This document is intended as an example of how quantitative structure-activity relationships ([Q]SARs can be used for estimating chemical/physical and toxicity characteristics of pesticides. Although QSARS have been used in other areas of EPA to estimate the potential toxicity of chemicals where measured data are not available, the use of these models in the Environmental Fate and Effects Division (EFED) has been sporadic. Even where QSARs have served as a source of information on a compound, their utility to risk assessment is typically based on a weight-of-evidence approach where the predictive capacity of the models among other lines of evidence is a critical factor for determining whether the information can be used qualitatively versus quantitatively. This weight-of-evidence approach considers the predictive capacity of the model for chemicals with known physical/biological characteristics compared to similarly structured compounds where such characteristics are unknown.

Available QSAR Tools

Two SAR tools most readily available to ecological risk assessors in OPP include **Eco**logical **S**tructure **A**ctivity **R**elationships (ECOSAR)[1] and the **As**sessment **T**ools for the **E**valuation of **R**isk[2] (ASTER). Both tools rely on the chemical name, CAS number, and the chemical's structure as represented by a **S**implified **M**olecular **I**nput **L**ine **E**ntry **S**ystem (SMILES) string. Both have undergone Agency peer review and are posted to the Agency's intranet. Information on chemical-specific SMILES strings can be found using some freely accessible tools on the internet. ChemiSpider[3] maintained by the Royal Society of Chemistry and the Toxicology Data Network (ToxNet)[4] maintained by the National Library of Medicine both provide SMILES string information along with chemical/physical properties of a chemical.

The user manual for ECOSAR[5] can be found at http://www.epa.gov/oppt/newchems/tools/ecosarusersguide.pdf. An example of the ECOSAR data entry window is depicted in **Figure A1**; **Figure A2** depicts the ECOSAR results window with chemical class assignments and toxicity estimates across various organisms.

The ASTER model allows the user to modify chemical/physical properties of the pesticide to more accurately define these parameters if measured values are available. ASTER requires the

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[1] USEPA 2009*a*. Ecological Structure Activity Relationships (ECOSAR). http://www.epa.gov/oppt/newchems/tools/21ecosar.htm

[2] USEPA 2009*b*. Assessment Tools for the Evaluation of Risk (ASTER). U.S. EPA, National Health and Environmental Effects Research Laboratory (NHEERL), Mid-Continent Ecology Division (MED), http://www.epa.gov/med/Prods_Pubs/aster.htm

[3] Royal Society of Chemistry. 2009. ChemSpider. http://www.chemspider.com/

[4] National Library of Medicine. 2009. Toxicology Data Network (TOXNET). http://toxnet.nlm.nih.gov/

[5] USEPA. 2009*d*. User's Guide for the ECOSAR Class Program, MS-Windows Version 0.99d November 1998. Prepared by W. Meylan and P. H. Howard, Syracuse Research Corporation Environmental Sciences Center, 6225 Running ridge Road, North Syracuse, NY 13210. http://www.epa.gov/oppt/newchems/tools/ecosarusersguide.pdf

chemical CAS Number, chemical name and SMILES string as inputs. The model allows the use to modify the mode of action leading to the most toxic response to ensure protection. The user should review the model's output to determine whether its estimates of chemical/physical properties are consistent with available measured values and adjust the input where necessary. The user manual for ASTER can be found at http://www.epa.gov/med/Prods_Pubs/ASTER_Quick_User_Guide.pdf . An example of the ASTER data entry window is depicted in **Figure A3**; **Figures A4** and **A5** depict the ASTER output windows for estimated chemical properties and ecotoxicological hazard, respectively. The toxicity values depicted in **Figure A5** represent the geometric means of each of the taxa represented for all of the data passing the ASTER filter. For carbaryl, the mean 96-hr LC_{50} value for fish is 3.253 mg/L.

SAR programs typically used by ecological risk assessors in OPP estimate toxicity to aquatic organisms and do not currently estimate the toxicity of chemicals to nonmammalian terrestrial organisms. The SARs in ECOSAR express correlations between a compound's physicochemical properties and its aquatic toxicity within specific chemical classes; whereas, the SARs in ASTER express correlations between a compound's physiochemical properties (K_{ow}) and its aquatic toxicity within specific chemical modes of action. QSARs within ASTER use the K_{ow} as the chemical attribute (descriptor) for estimating toxicity and ASTER will not provide estimates for chemicals with K_{ow} values outside the range of its training set.

Other useful tools include the Distributed Structure-Searchable Toxicity (DSSTox)[6] public database network. DSSTox is a project of EPA's Office of Research and Development National Center for Computational Toxicology that is helping to build a public data foundation for improved structure-activity and predictive toxicology capabilities. The DSSTox website (http://www.epa.gov/ncct/dsstox/) provides a public forum for publishing downloadable, structure-searchable, standardized chemical structure files associated with toxicity data (USEPA 2009). An example of the DSSTox data entry window is depicted in **Figure A6** using carbaryl as an example; **Figure A7** depicts the DSSTox search results summary and the number of files containing data which exactly match and partially match for carbaryl. **Figure A8** depicts where there are exact matches on data for carbaryl; in the far lower right column of the output, acronyms are listed for files with data on carbaryl. If the acronym EPAFHM (EPA Fathead Minnow) dataset is selected, DSSTox displays the acute toxicity data and mode of action of carbaryl (**Figure A9**). The output indicates that carbaryl has a mean LC_{50} of 8.75 mg/L representing the geometric mean of 4 experiments and that carbaryl's mode of action is through acetylchlolinesterase inhibition.

An important use of QSARs is in the identification of potential degradates of concern An additional tool for determining potential degradates of concern is MetaPath[7]. MetaPath is a

[6] USEPA 2009f. Distributed Structure-Searchable Toxicity (DSSTox) Database Network. http://www.epa.gov/ncct/dsstox/index.html

[7] Jones, W. J., P. K. Schmieder, R. C. Kolanczyk, and O. Mekenyan. Development of a Searchable Metabolite Database and Simulator of Xenobiotic Metabolism. Presented at BOSC Computational Toxicology Research Program Review, Research Triangle Park, NC, September 29 - 30, 2009.

computational tool developed for the storage and analysis of metabolic pathways and associated metadata. The tool is capable of text and chemical structure/substructure searching as well as comparing metabolites formed across chemicals, species, and/or experimental conditions. The database has been constructed primarily from in vivo rat metabolism studies of pesticides. This system also serves as a foundation of an expert system to predict metabolite formation. **Figure A10** depicts the MetaPath window for selecting chemicals of interest and the associate degradation pathway reported in rat studies.

For ECOSAR and ASTER, the user should determine whether model outputs for both fate and effects parameters are consistent with what is known for the chemical under evaluation. In some cases, some toxicity data on either fish and/or invertebrates may exist and the reviewer should evaluate whether the model outputs are consistent with what has been measured. The ability of the SARs to reliably estimate the toxicity of other chemicals depends on the extent to which similarly structure chemicals with similar modes of action are represented in the training set of data used to populate the model and estimate relationships. As such, SAR model results must be put into the context of any existing data and what the reviewer may already know about their chemical. Additionally, for each of the SAR models, the structural moieties and/or mode of action on which the model estimates toxicity are provided by the model. The reviewer should be aware of this output and make an effort to determine whether these moieties and/or mode of action are consistent with their understanding of the chemical.

The use of SARs is dependent on multiple lines of evidence and must be viewed in the context of the model's domain of applicability; these multiple lines of evidence must be integrated. Some SAR models like ASTER warn users that certain chemical structures are not in their domain. Users are cautioned to examine model outputs carefully to determine whether estimates are consistent with what may be known about the chemical however limited.

With any model as with measured toxicity data, there are uncertainties regarding the variability and relevancy of estimates. The decision to use such estimates should be weighed against other sources of data that may be available. When used, SAR model output should be properly identified and the uncertainties associated with the values must be discussed in the assessment. Ultimately, the use of SARs is dependent on the best professional judgment of the user and are part of a weight-of-evidence approach toward characterizing potential chemical hazards.

One of the largest sources of uncertainty regarding the use of SARs is that the models are typically developed for relatively generic structures and modes of action; however, conventional pesticides have been engineered to be toxic, oftentimes to specific taxa, through relatively specific modes of action. Thus, a chemical may have a number of substructures (moieties) that have associated toxicities based on what may be dissimilar modes of action while the entire chemical structure may impart a specific mode of action and toxicity. For example, the insecticide carbaryl, is classified in ECOSAR as a neutral organic and the tool estimates the toxicity of carbaryl based on an ester moiety contained within the compound. However, the overall mode of action of this N-methyl carbamate is to inhibit acetylcholine esterase. The

extent to which the component toxicities contribute to the overall activity of the compound is an important consideration and challenge to decipher. As compounds are transformed/degraded to smaller components, the importance/relevance of substructure activity relationships increases as the overall activity of the parent compound may no longer dominate. Consideration should be given to the extent to which the compound retains structural similarities to the parent and/or chemicals with known toxicities and are accurately estimated by the QSAR model.

As mentioned previously, some SARs are based on the structure and mode of action of chemicals. Again though, the mode of action may be relatively generic even though the specific mode of action for a pesticide may not be reported and/or evaluated. ECOSAR may group chemicals as acting through nonpolar narcosis; however, this relatively broad mode of action may include chemicals with widely divergent modes of action. Chemical mode of action may also differ between target and non-target species; however, by the same token, for most of the SAR estimates, while the species may be different the mode of action across those species is the same.

Criteria for Use of [Q]SARs

Using OECD validation principles as template/guidance for the use of QSAR models,[8] the following attributes should be examined for completeness. The model must estimate a defined endpoint, *e.g.*, a 96-hr lethal concentration to 50% of the test organisms (LC_{50}), a no-observed adverse effect concentration (NOAEC), or a maximum acceptable toxic concentration (MATC). As such, the model must be transparent as to the types of studies included in model database. The user must in turn determine whether these defined endpoints are relevant to the effects assessment.

The model must be based on an unambiguous algorithm. That is to say that the mathematical relationship on which the model is based, must be clearly identified. If the model is regression-based and uses K_{ow}, then the exact mathematic expression (function) should be identified and its derivation transparent. Known limitations of the model should be identified. For example, in the output for the ASTER model (**Figure A4**), estimates for environmental fate properties like vapor pressure are based on averages. The fifth column of the output table indicates the method used to calculate the vapor pressure and the percent error (47%) associated with the estimate.

As with any mathematical relationship it is constructed over a specified domain that is dictated by the range of chemical data contained within its training set. Attempting to estimate the toxicity of chemicals that fall outside of the estimation capabilities of the model could lead to erroneous values. As such, the user should be cognizant of the domain of applicability for the model and should not extend analyses beyond that range. For example, in **Figure A2** depicting output from ECOSAR, the user is warned that chemical solubility may limit the model's ability to estimate certain effects. It also notes that for fish and daphnids the model does estimate

[8] OECD. 1007. Report on the Regulatory Uses and Applications in OECD Member Countries of (Quantitative Structure-Activity Relationship [(Q)SAR] Models in the Assessment of New and Existing Chemicals. ENV/JM/MONO(2006)25.
http://www.olis.oecd.org/olis/2006doc.nsf/LinkTo/NT00003B0A/$FILE/JT03221927.PDF

toxicity for compounds with log octanol-water partition coefficient (log K_{ow}) values of 5 and that for green algae, the cutoff is a log K_{ow} of 6.4. The molecular weight cutoff for the model is also provided (*i.e.*, 1000 g/mole).

Again, since QSARs are typically based on mathematical relationships and are regression-based, these models can be evaluated for their ability to estimate the training values used to derive them. Models should contain an analysis of their goodness of fit, robustness and predictivity. Typically, the mean square error term and/or regression coefficient (r^2) of the model serve as measures of the ability of the model to represent the data in its training set. Robustness of the model can be evaluated by examining how toxicity estimates vary with relatively minor changes in structure. Such evaluations may be accomplished through examining toxicity estimates across several degradates. For example, **Figure A4** depicts ASTER-estimated fate properties for the carbamate insecticide carbaryl. ASTER reports a log octanol-water partition coefficient (log P) of 2.38, vapor pressure of 1.9×10^{-4}, and solubility of 7.68×10^{-4} moles/L (154 mg/L) for carbaryl. Measured values reported in the environmental fate and ecological risk assessment written in support of the reregistration eligibility decision (RED) on carbaryl are near identical for log P; however, the ASTER estimate for vapor pressure differs from the measured value by 3 orders of magnitude and the ASTER-estimated solubility is roughly 5X greater than what is reported in the RED. In cases where the model allows the user to modify environmental fate characteristics to reflect measured values, the user should consider doing so to determine the extent to which the toxicity values are affected.

Ideally, the toxicity estimated for a chemical should be mechanistically plausible and be consistent with what has been demonstrated for similarly structured chemicals, *e.g.*, analogs. Additional guidance on the use of QSAR models may also be available from the tool developers as well and should ideally contain defined criteria for determining whether the model is likely to be biased either positively or negatively. For example, in **Figure A2** depicting output from the ECOSAR model for carbaryl, the toxicity estimates are based on a neutral organic SAR for chemicals containing esters. It is clear from the structure of carbaryl depicted in **Figure 3** that compound has a well defined ester linkage. However, as stated previously, it is also known that carbaryl's primary mode of action is to inhibit acetyl cholinesterase. As such, the toxicity estimates based on the ester moiety by itself may or may not be reflective of the toxicity associated with the entire chemical.

Finally, the strengths and weakness of the data estimation method used should be clearly described by the user when such estimates are included in assessments. When any SAR model has been used to estimate toxicity values, the output of the model should be included as an appendix to the assessment to enhance transparency.

As an example of how these tools can be used, consider carbaryl again. Registrant-submitted and open literature studies indicate that carbaryl can readily degrade. One of its primary degradates is 1-naphthol. **Figure A10** depicting the MetaPath output also indicates that 1-naphthol is a primary degradate and the window in the left of the output provides the SMILES string for the compound. **Figure A11** depicts chemical information on 1-naphthol entered into

ECOSAR and **Figure A12** depicts the ECOSAR output for the chemical with a fish 96-hr LC_{50} of 7.959 mg/L. The ASTER outputs for 1-naphthol are depicted in **Figures A13** and **A14** and indicate the mean acute LC_{50} value for freshwater fish is 1.78 mg/L. When naphthol is entered into DSSTox (**Figure A15**), the tool indicates that there are no exact matches but 9 partial matches for the compound. If 1-naphtol is selected from the chemicals depicted among the partial matches and the EPA fathead minnow database is then selected, DSSTox provides the measured LC_{50} values (4.63 mg/L) and the indicates that the mode of action is through polar narcosis (**Figure A16**). **Table 1** summarizes the measured and estimated toxicity values for carbaryl and its primary degradate 1-naphthol; the most sensitive measured values used in the carbaryl risk assessment are also provided for comparison. Although QSAR model estimates for carbaryl appear to be considerably higher than the most sensitive toxicity value (Atlantic salmon 96-hr LC_{50}=0.22 mg/L) used in the ecological risk assessment of carbaryl, the mean measured 96-hr LC_{50} value across all freshwater fish species is roughly 2.6 mg/L and is relatively consistent with QSAR estimates. Similarly for 1-napthol, the most sensitive measured toxicity value is 0.75 mg/L; however, values ranged as high at 1.6 mg/L. Therefore, QSAR-estimated values for both carbaryl and its 1-naphthol degradate are not substantially different than measured toxicity values. .

Table 1. Summary of acute toxicity estimates for carbaryl and its primary degradate 1 naphthol from three predictive tools for freshwater fish.

Method	Carbaryl Toxicity Estimate for Fish (mg/L)	Naphthol Toxicity Estimate for Fish (mg/L)
ECOSAR (estimated)	19.79	7.95
ASTER (estimated)	3.25*	1.78*
DSSTox (estimated)	8.75	4.63
Measured (most sensitive)	0.22**	0.75

*Geometric mean of all freshwater fish acute toxicity data passing the ASTER filter.

** Most sensitive freshwater fish 96-hr LC_{50}

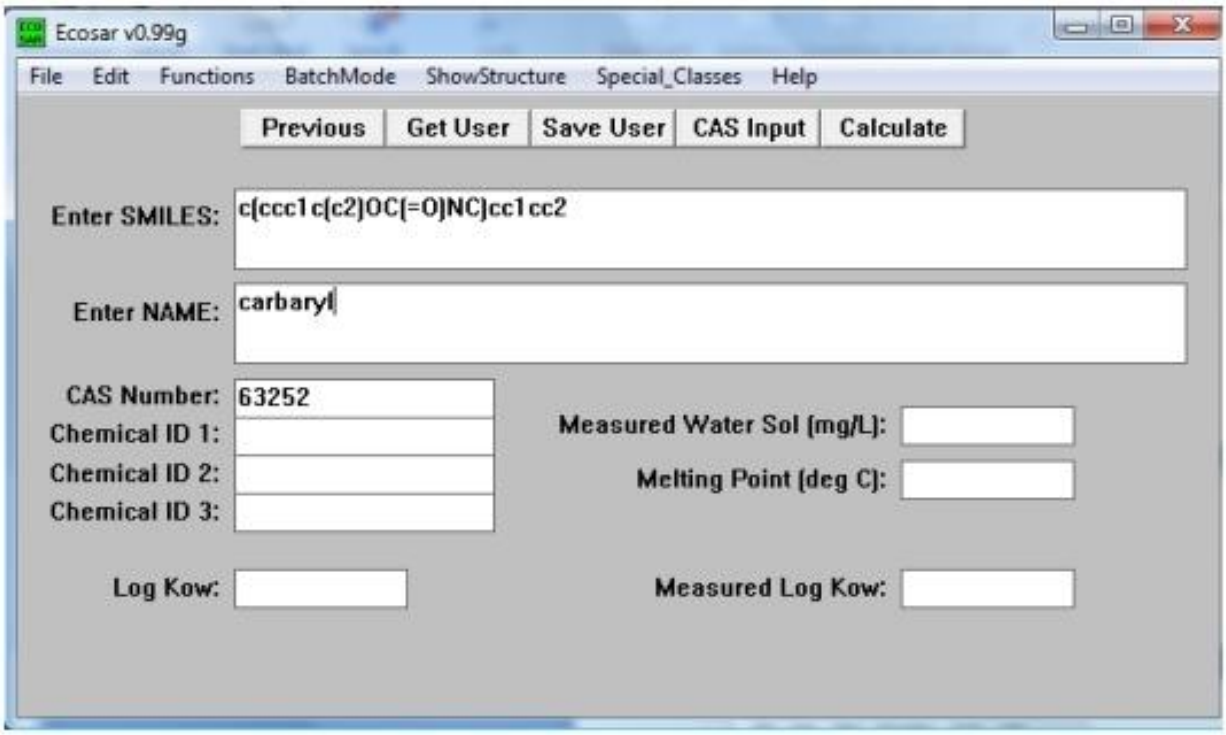


Figure A1. ECOSAR chemical input screen. Information depicted is for the carbamate insecticide carbaryl.

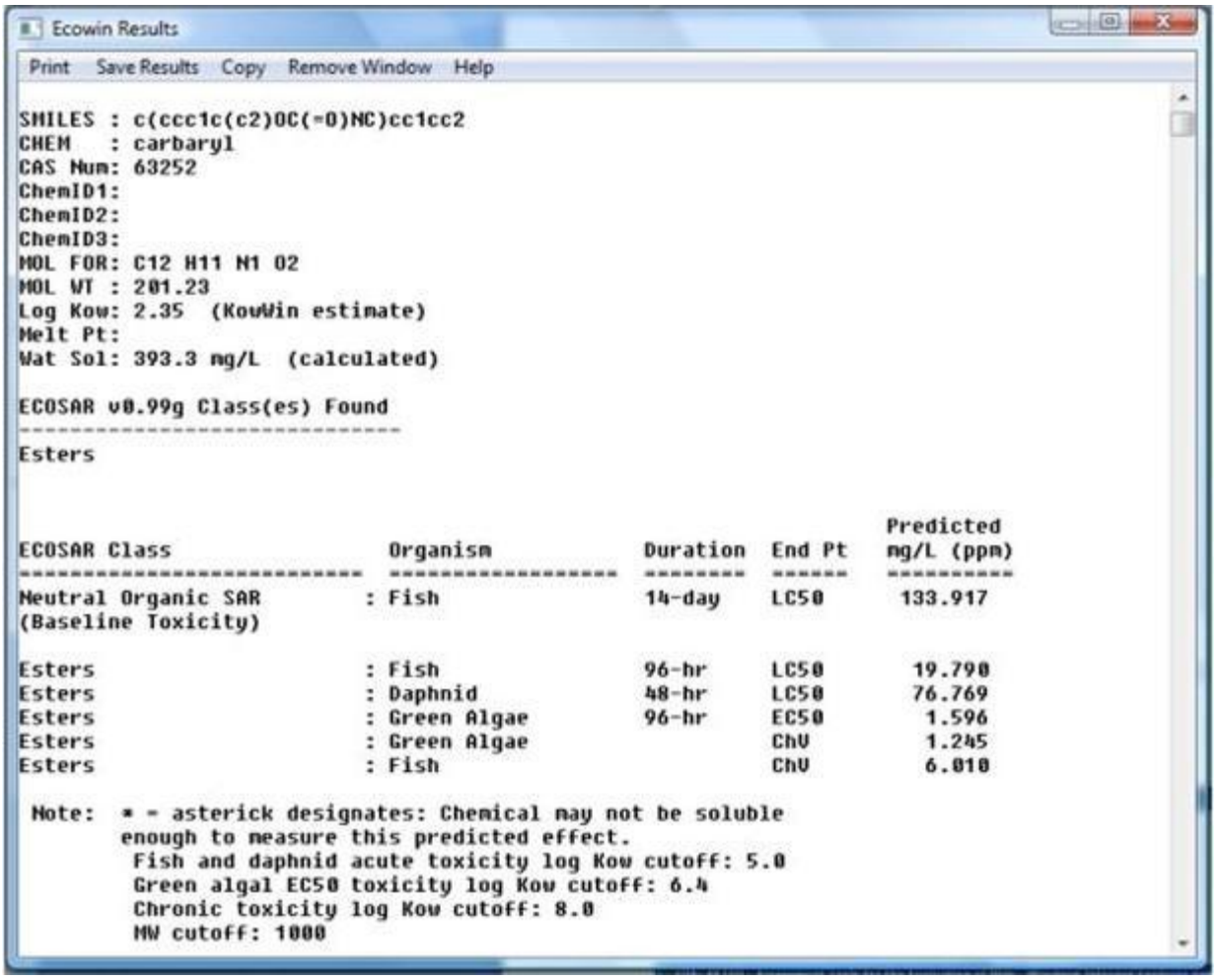


Figure A2. ECOSAR output window screen shot. Screen is depicting estimates for the carbamate insecticide carbaryl.

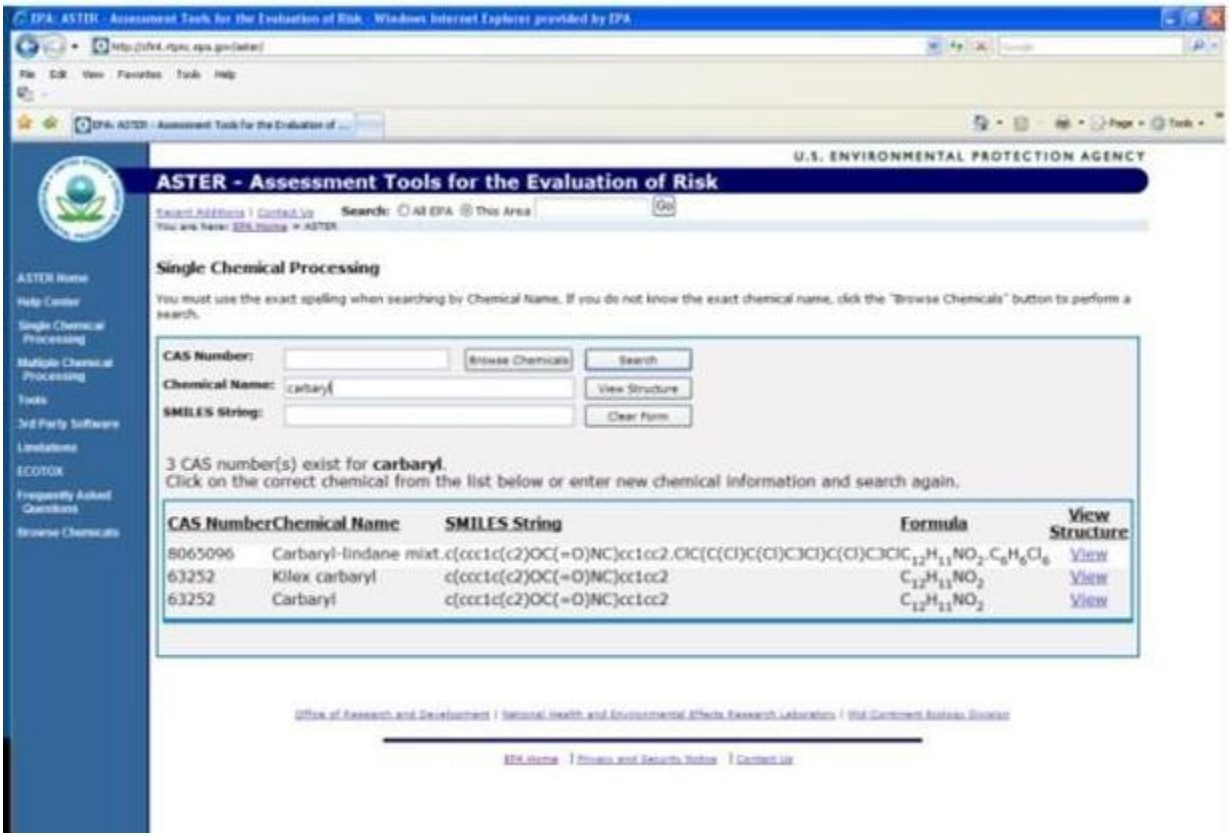


Figure A3. ASTER chemical data entry screen.

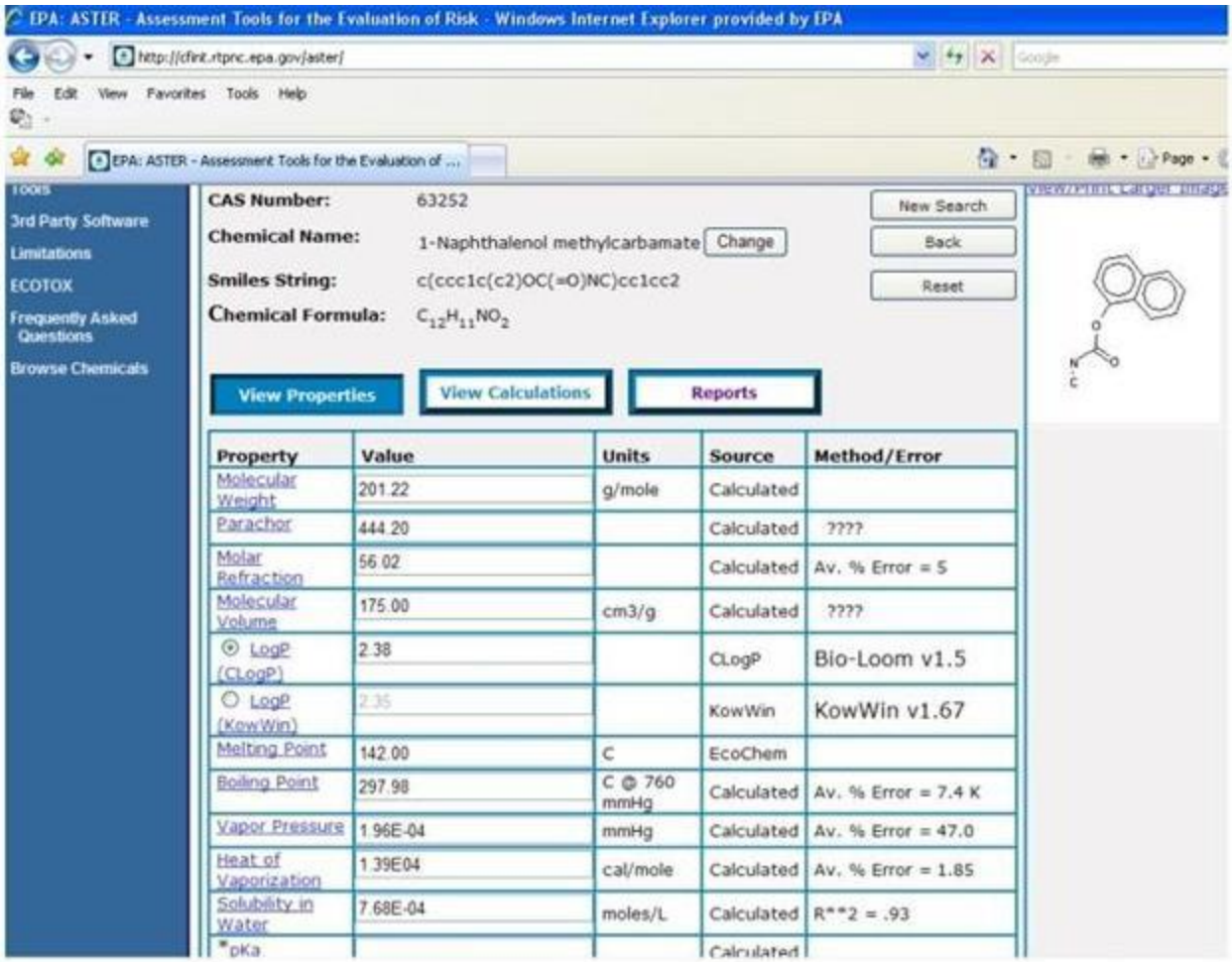


Figure A4. ASTER chemical property screen. Screen is depicting estimates for the carbamate insecticide carbaryl (1-naphthalenol methylcarbamate; CAS 63252).

III. ECOTOXICOLOGICAL HAZARD ASSESSMENT

Table 1. Geometric Means of all data passing the ASTER filter by Species Group

Habitat	Species	Effect	Count	Geo Mean	Min	Max
A	Algae - Acute	POP	5	3788 ug/L	2797 ug/L	6101.4 ug/L
A	Algae - Chronic	POP	2	3162 ug/L	2000 ug/L	5000 ug/L
A	Amphibian - Acute	MOR	4	7049 ug/L	2100 ug/L	24640 ug/L
A	Amphibian - Chronic	BCM	3	2475 ug/L	1000 ug/L	7580 ug/L
A	Arthropod - Acute	MOR	56	82.15 ug/L	1.7 ug/L	9750 ug/L
A	Arthropod - Acute	PHY	5	13.43 ug/L	5.6 ug/L	115 ug/L
A	Arthropod - Chronic	MOR	1	3500 ug/L	3500 ug/L	3500 ug/L
A	Fish - Acute	MOR	136	3253 ug/L	250 ug/L	58000 ug/L
A	Fish - Chronic	GRO	5	1590 ug/L	720 ug/L	4050 ug/L
A	Fish - Chronic	MOR	5	1978 ug/L	720 ug/L	7000 ug/L
A	Mollusc - Acute	BEH	1	10300 ug/L	10300 ug/L	10300 ug/L
A	Mollusc - Acute	GRO	3	1965 ug/L	1500 ug/L	2300 ug/L
A	Mollusc - Acute	MOR	11	13766 ug/L	3850 ug/L	48500 ug/L
T	Bee - Acute	MOR	4	19.52 mg/kg bdwt	2.721 mg/kg bdwt	268.8 mg/kg bdwt
T	Bird - Chronic	REP	3	215.44 mg/L	40 mg/L	500 mg/L
T	Mammal - Chronic	BCM	5	240.22 mg/L	10 mg/L	2000 mg/L
T	Mammal - Chronic	BCM	9	7.72 mg/kg	1 mg/kg	50 mg/kg
T	Mammal - Chronic	BCM	4	33.44 mg/kg bdwt	10 mg/kg bdwt	50 mg/kg bdwt
T	Mammal - Chronic	BEH	2	70.71 mg/kg	50 mg/kg	100 mg/kg
T	Mammal - Chronic	BEH	1	50 mg/kg bdwt	50 mg/kg bdwt	50 mg/kg bdwt
T	Mammal - Chronic	GRO	13	1814 mg/L	10 mg/L	10000 mg/L
T	Mammal - Chronic	GRO	15	99.57 mg/kg	7.2 mg/kg	20000 mg/kg
T	Mammal - Chronic	GRO	9	175.29 mg/kg bdwt	50 mg/kg bdwt	500 mg/kg bdwt
T	Mammal - Chronic	MOR	10	263.01 mg/kg	19.8 mg/kg	20000 mg/kg
T	Mammal - Chronic	MOR	6	382.36 mg/kg bdwt	100 mg/kg bdwt	500 mg/kg bdwt
T	Mammal - Chronic	PHY	1	10 mg/L	10 mg/L	10 mg/L
T	Mammal - Chronic	PHY	1	50 mg/kg	50 mg/kg	50 mg/kg
T	Mammal - Chronic	PHY	1	50 mg/kg bdwt	50 mg/kg bdwt	50 mg/kg bdwt
T	Mammal - Chronic	REP	15	136.63 mg/kg	25 mg/kg	300 mg/kg
T	Mammal - Chronic	REP	9	119.27 mg/kg bdwt	25 mg/kg bdwt	500 mg/kg bdwt
T	Terrestrial Worm - Acute	MOR	1	20 mg/kg	20 mg/kg	20 mg/kg

Figure A5. ASTER ecptoxicological hazard assessment. Screen is depicting estimates for the carbamate insecticide carbaryl (1-naphthalenol methylcarbamate; CAS 63252).

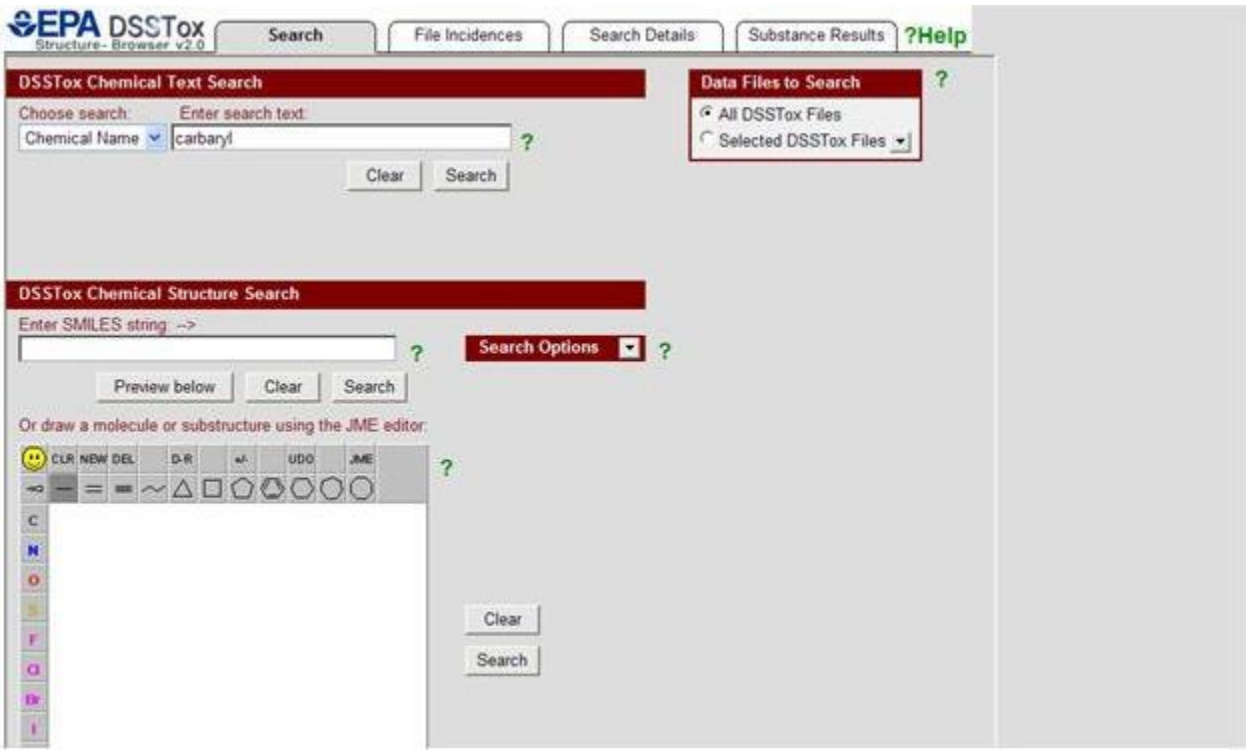


Figure A6. DSSTox chemical data entry screen where carbaryl has been entered. The drop down menu in the upper box has been set to Chemical Name and carbaryl has been entered as the search text. Options in the Choose search window also include CAS number.

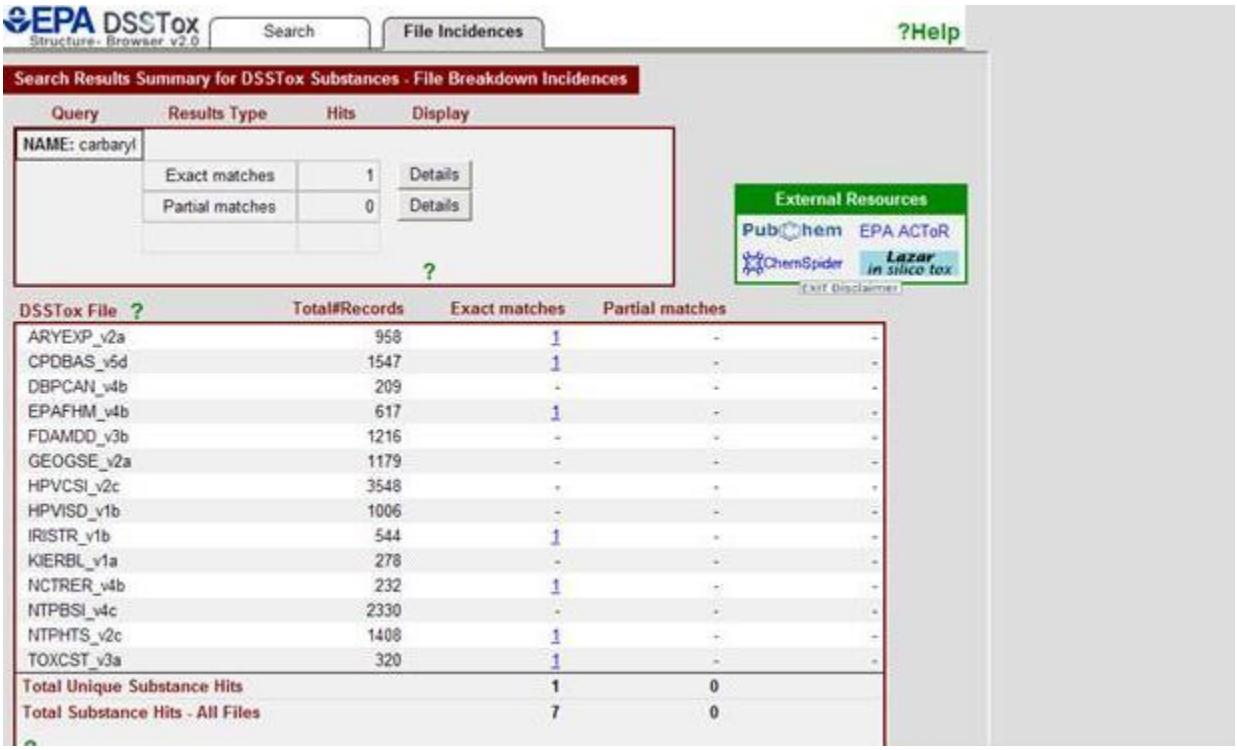


Figure A7. DSSTox search results using carbaryl as an example. In this example the upper box of DSSTox search window indicates that for carbaryl, there is one exact match and no partial matches. The lower box indicates there are records (data) for carbaryl in 7 of the databases in which it searched. Double clicking on the box labeled "Exact matches" will provide more detail on those matches.

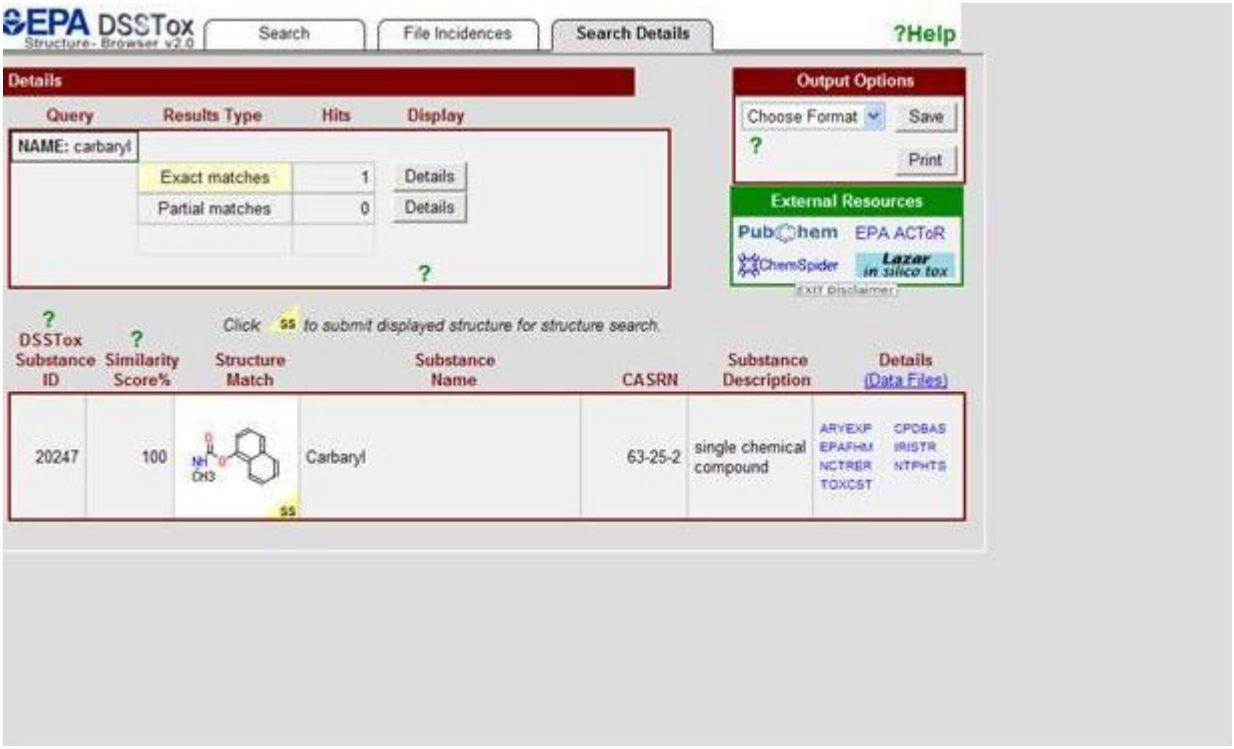


Figure A8. DSSTox search details screen using carbaryl as an example. In this example, the uppermost box entitled "Exact matches" has been double clicked and the lowermost box now displays the similarity score, structure, CAS number, and hot links to each of the databases containing chemical-specific information on carbaryl. Double clicking on the hotlinks will display the chemical-specific data.

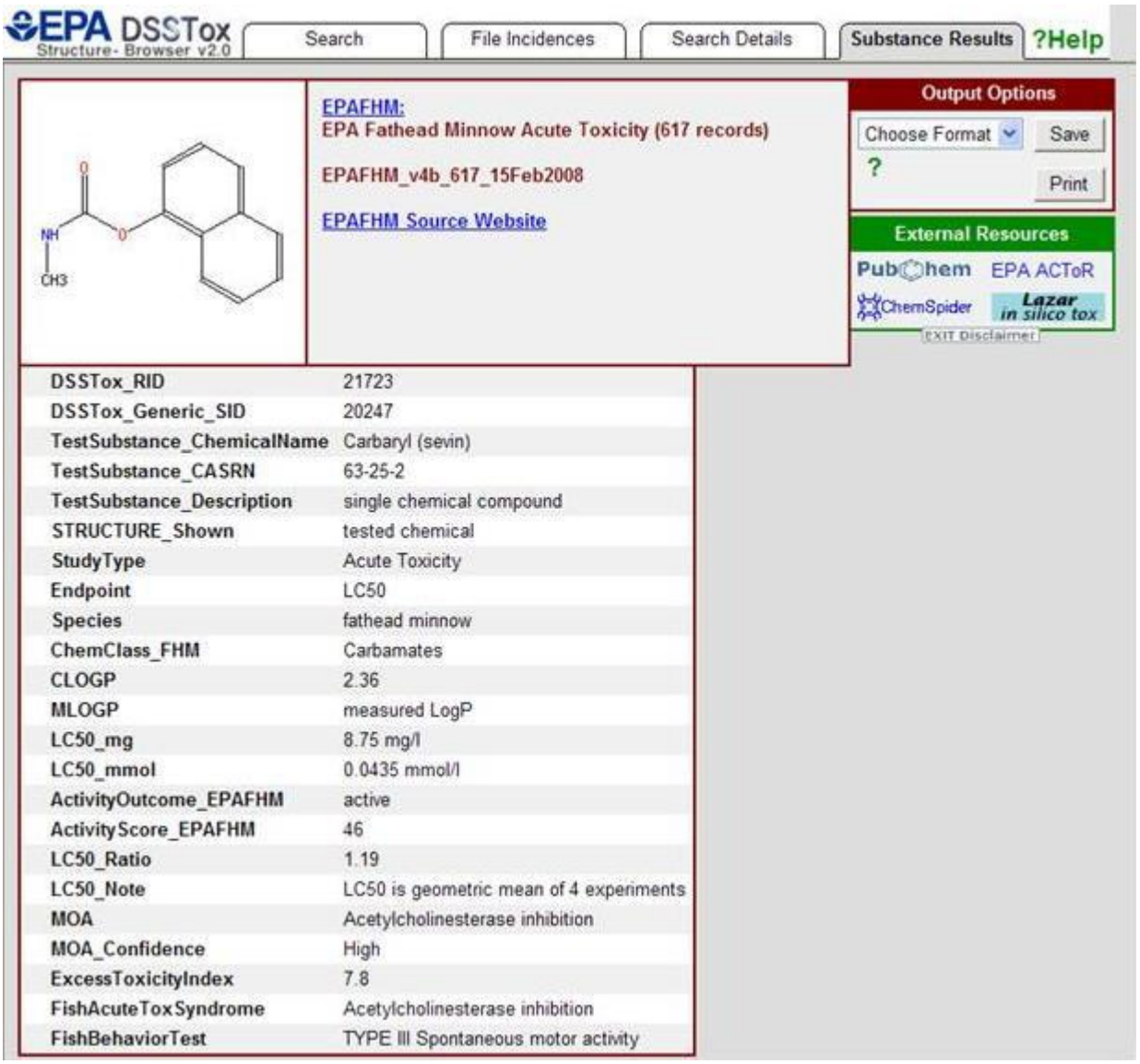


Figure A9. DSSTox substance results for EPA fathead minnow (EPAFHM) acute toxicity data using carbaryl as an example. In this example, the EPAFHM chemical/physcial and biological data are displayed for carbaryl.

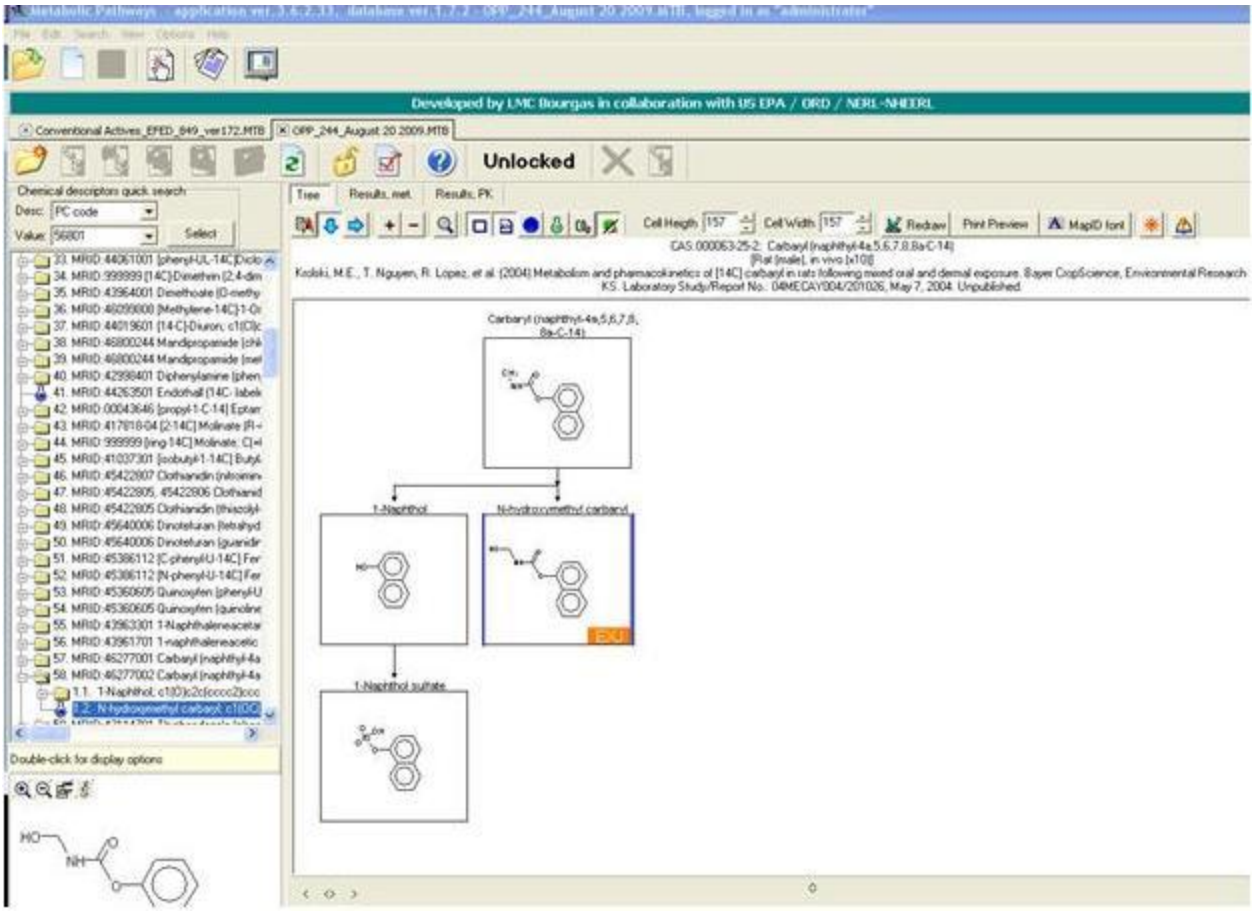


Figure A10. Metabolism Pathway (MetaPath) chemical selection window depicting carbaryl and it primary degradates. Header over the metabolism pathway (map) in lower right is the open literature citation from which the metabolism data were derived. In this example, the upper left pull down menu (Desc) under chemical description quick search has been set to "PCCode" and the PC Code of carbaryl (56801) has been entered as the Value. The metabolic pathway for carbaryl is displayed in the window to the right showing the parent compound along with three of its degradates. Above the metabolic pathway is the full reference for the study used to depict the metabolites.

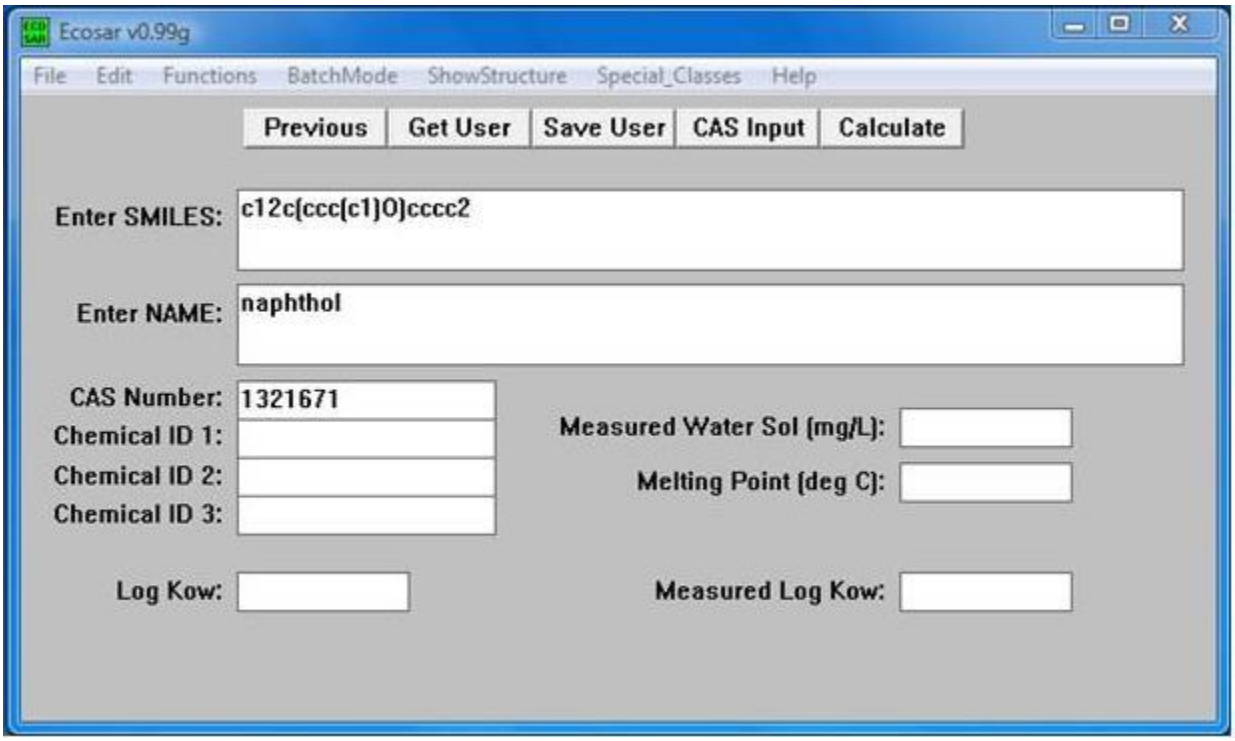


Figure A11. ECOSAR chemical input screen. Information depicted is for the carbaryl degradate naphthol. In this example, the SMILES string, Name and CAS Number have been entered. Alternatively, the tab "CAS Input" could be selected and by entering the CAS Number (1321671), ECOSAR will automatically populate the window. Afterward, the user would double-click the tab "Calculate".

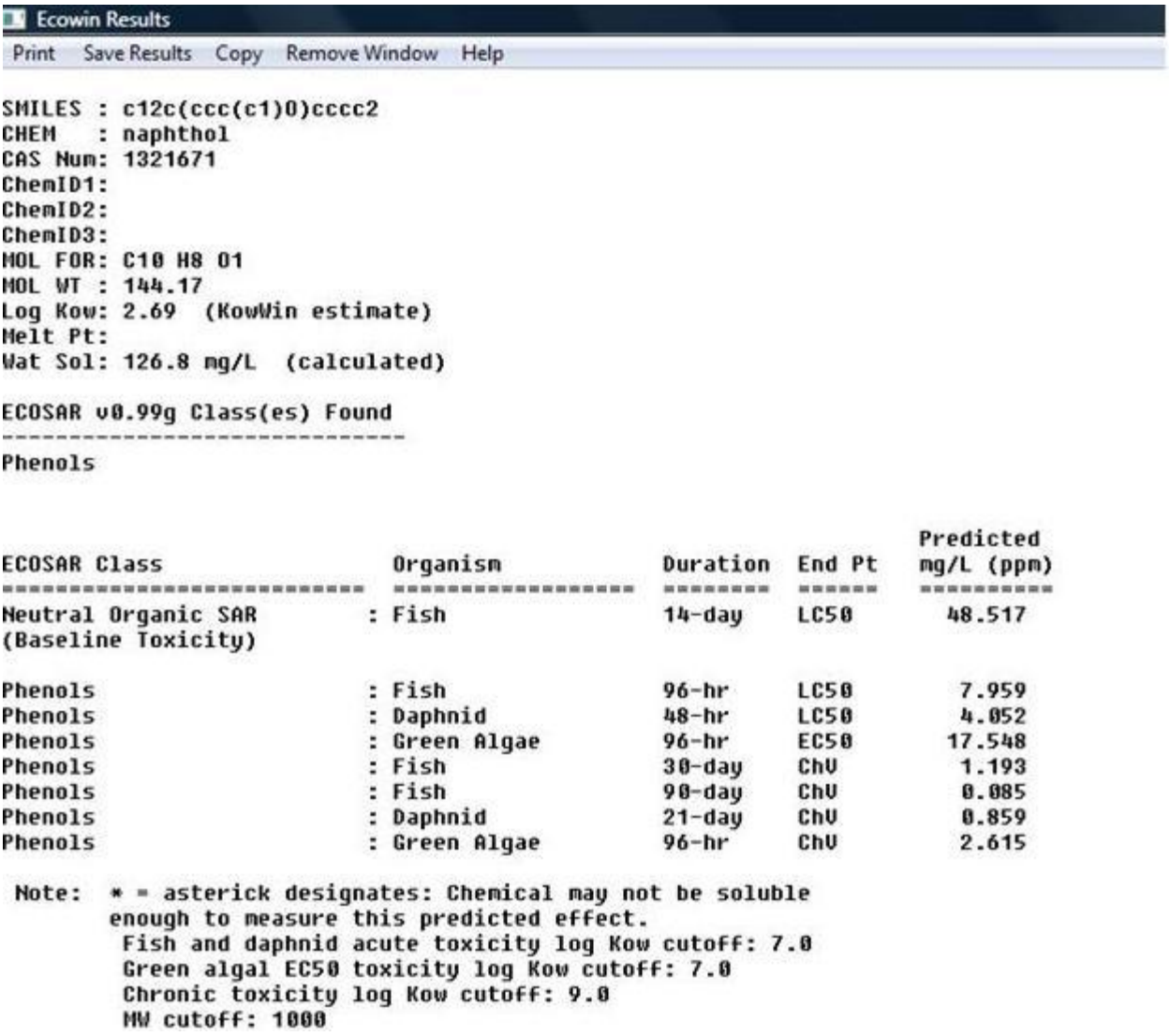


Figure A12. ECOSAR output window screen shot. Screen is depicting estimates for the carbaryl degradatenaphthol. Toxicity estimates are calculated for both neutral organics and phenols. Both acute (LC50 and EC50) and chronic (ChV) toxicity values for aquatic animals and nonvascular plants are provided.

I. CHEMICAL IDENTIFICATION

Parameter	Value
Name	1-Naphthalenol
CAS Number	90153
SMILES	c(ccc1c(c2)O)cc1cc2
Formula	$C_{10}H_8O$

II. ENVIRONMENTAL EXPOSURE ASSESSMENT

Parameter	Value	Source	Reference
Molecular Weight (g/mole)	144.17	Calculated	
Melting Point (C)	95.00	EcoChem	
Boiling Point (C)	278.00	EcoChem	
Vapor Pressure (mm of Hg)	7.55E-04	Calculated	
Ht Vaporization (cal/mole)	1.32E04	Calculated	
Solubility in Water (mg/L)	Not available for this chemical		
CLogP	2.65	CLogP	83337
KowWin	2.69	KowWin	83336
pKa	Not available for this chemical		
Adsorption Coef (log Koc)	LogP value required		
Henry's Constant (atm-m**3/mole)	Not available for this chemical		
Log10 (Henry's Constant)(atm-m**3/mole)	Not available for this chemical		
Hydrolysis Half-life (days)	Hydrolysis unlikely		
BioDegradation Data	Linear Model Prediction : Biodegrades Fast Non-Linear Model Prediction: Biodegrades Fast Ultimate Biodegradation Timeframe: Weeks Primary Biodegradation Timeframe: Days-Weeks MITI Linear Model Prediction : Not Readily Degradable MITI Non-Linear Model Prediction: Not Readily Degradable	BioWin	83338
Mackay Level 1 Environmental Partitioning @ 25C	No value was available for aqueous solubility No value was available for log P (log Kow) There is not enough information for the fugacity model		

** Denotes the LogP value used in calculations

Figure A13. ASTER chemical output screen for carbaryl degradate 1-naphthol (aka 1-naphthalenol).

III. ECOTOXICOLOGICAL HAZARD ASSESSMENT

Table 1. Geometric Means of all data passing the ASTER filter by Species Group

Habitat	Species	Effect	Count	Geo Mean	Min	Max
A	Arthropod - Acute	BEH	1	730 ug/L	730 ug/L	730 ug/L
A	Arthropod - Acute	MOR	8	5260 ug/L	200 ug/L	32170 ug/L
A	Arthropod - Acute	PHY	1	730 ug/L	730 ug/L	730 ug/L
A	Fish - Acute	MOR	10	1784 ug/L	330 ug/L	4630 ug/L
A	Mollusc - Acute	GRO	4	1463 ug/L	800 ug/L	2100 ug/L
A	Mollusc - Acute	MOR	2	3427 ug/L	2700 ug/L	4350 ug/L
T	Mammal - Chronic	GRO	1	6500 mg/L	6500 mg/L	6500 mg/L
T	Mammal - Chronic	GRO	1	0.25 mg/kg bdwt	0.25 mg/kg bdwt	0.25 mg/kg bdwt

Table 2. Median Acute Data values

Habitat	Taxon Group	Name	Endpoint	Effect	Measure	Media	Dur (d)	Exposure	Median Conc	Source	Ref No
A	Arthropod	Americamysis bahia	LC50	MOR	MORT	SW	4	R	200.00 ug/L	ECOTOX - EDS	88960
A	Arthropod	Cambarus bartoni	LC50	MOR	MORT	FW	4	R	12860.00 ug/L	ECOTOX - EDS	19508
A	Arthropod	Daphnia magna	EC50	BEH	MOTL	FW	2	S	730.00 ug/L	ECOTOX - EDS	88959
A	Arthropod	Daphnia magna	EC50	ITX	IMBL	SW	2	S	730.00 ug/L	ECOTOX - EDS	88960
A	Arthropod	Neotrypaea californiensis	EC50	MOR	MORT	SW	2	S	3300.00 ug/L	ECOTOX - EDS	4825
A	Arthropod	Neotrypaea californiensis	EC50	MOR	MORT	SW	2	S	3500.00 ug/L	ECOTOX - EDS	4825
A	Arthropod	Orconectes virilis	LC50	MOR	MORT	FW	4	R	32170.00 ug/L	ECOTOX - EDS	19508
A	Arthropod	Orconectes virilis	LC50	MOR	MORT	FW	4	R	30980.00 ug/L	ECOTOX - EDS	19508
A	Arthropod	Upogebia pugettensis	EC50	MOR	MORT	SW	2	S	4400.00 ug/L	ECOTOX - EDS	4825
A	Arthropod	Upogebia pugettensis	EC50	MOR	MORT	SW	2	S	4500.00 ug/L	ECOTOX - EDS	4825
A	Fish	Anabas testudineus	LC50	MOR	MORT	FW	4	R	3000.00 ug/L	ECOTOX - EDS	4969
A	Fish	Catla catla	LC50	MOR	MORT	FW	4	R	4300.00 ug/L	ECOTOX - EDS	4969
A	Fish	Cyprinodon variegatus	LC50	MOR	MORT	SW	4	R	1800.00 ug/L	ECOTOX - EDS	88960
A	Fish	Lepomis macrochirus	LC50	MOR	MORT	SW	4	S	750.00 ug/L	ECOTOX - EDS	88960
A	Fish	Lepomis macrochirus	LC50	MOR	MORT	FW	4	R	760.00 ug/L	ECOTOX - EDS	88959
A	Fish	Mystus cavasius	LC50	MOR	MORT	FW	4	R	330.00 ug/L	ECOTOX - EDS	4969
A	Fish	Mystus vittatus	LC50	MOR	MORT	FW	4	R	1100.00 ug/L	ECOTOX - EDS	4969
A	Fish	Pimephales promelas	LC50	MOR	MORT	FW	4	F	4120.00 ug/L	ECOTOX	15031
A	Fish	Pimephales promelas	LC50	MOR	MORT	FW	4	F	4630.00 ug/L	MED	12447
A	Mollusc	Clinocardium nuttallii	LC50	MOR	MORT	SW	2	R	4350.00 ug/L	ECOTOX - EDS	17741
A	Mollusc	Clinocardium nuttallii	LC50	MOR	MORT	SW	4	R	2700.00 ug/L	ECOTOX - EDS	17741
A	Mollusc	Crassostrea gigas	EC50	DVP	DFRM	SW	2	S	800.00 ug/L	ECOTOX - EDS	4825
A	Mollusc	Crassostrea virginica	EC50	DVP	DVLP	SW	2	S	2100.00 ug/L	ECOTOX - EDS	88960
A	Mollusc	Crassostrea virginica	EC50	DVP	NORM	SW	2	R	2100.00 ug/L	ECOTOX - EDS	88959

Figure A14. ASTER ecotoxicological hazard output screen for carbaryl degradate 1-naphthol. The uppermost window contains acute and chronic toxicity estimates based on behavior (BEH), growth (GRO), mortality (MOR) and physiology (PHY) across both aquatic and terrestrial animals; these estimates represent the geometric mean (plus minimum and maximum values) of all the data passing the ASTER filter by species group. The lowermost window depicts median acute toxicity values for specific species.

EPA DSSTox Structure- Browser v2.0

Search | File Incidences | Search Details | ?Help

Details

Query	Results Type	Hits	Display
NAME: naphthol	Exact matches	0	Details
	Partial matches	9	Details

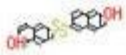
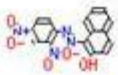
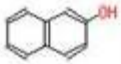
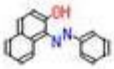
?

Output Options

Choose Format | Save | Print

?

Click SS to submit displayed structure for structure search.

DSSTox Substance ID	Similarity Score%	Structure Match	Substance Name	CASRN	Substance Description	Details (Data Files)
25429	N/A	 SS	6,6'-dithiodi(2-naphthol)	6088-51-3	single chemical compound	KIERBL NTPBSI NTPHTS
29258	N/A	 SS	1-[(E)-(2,4-dinitrophenyl)diazenyl]-2-naphthol	3468-63-1	single chemical compound	HPVCSI
27061	N/A	 SS	2-naphthol	135-19-3	single chemical compound	HPVCSI KIERBL
21135	N/A	 SS	1-Phenylazo-2-naphthol	842-07-9	single chemical compound	CPDBAS KIERBL NTPBSI

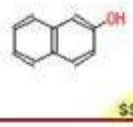
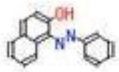
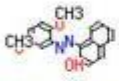
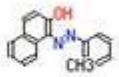
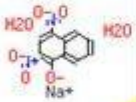
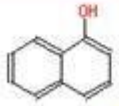
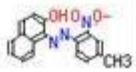
27061	N/A		2-naphthol	135-19-3	single chemical compound	HPVCSI	KIERBL
21135	N/A		1-Phenylazo-2-naphthol	842-07-9	single chemical compound	CPDBAS NTPBSI	KIERBL
24838	N/A		1-[(E)-(2,5-dimethoxyphenyl) diazenyl]-2-naphthol	6358-53-8	single chemical compound	NTPBSI	
25808	N/A		1-((2-Methylphenyl)azo)-2-naphthol	2646-17-5	single chemical compound	NTPBSI	
22304	N/A		2,4-Dinitro-1-naphthol sodium salt dihydrate (Martius yellow)	101836-92-4	single chemical compound	EPAFHM	
21793	N/A		1-Naphthol	90-15-3	single chemical compound	EPAFHM HPVISD	HPVCSI
21226	N/A		1-[(E)-(4-methyl-2-nitrophenyl) diazenyl]-2-naphthol	2425-85-6	single chemical compound	CPDBAS	NTPBSI

Figure A15. DSSTox search details for naphthol.

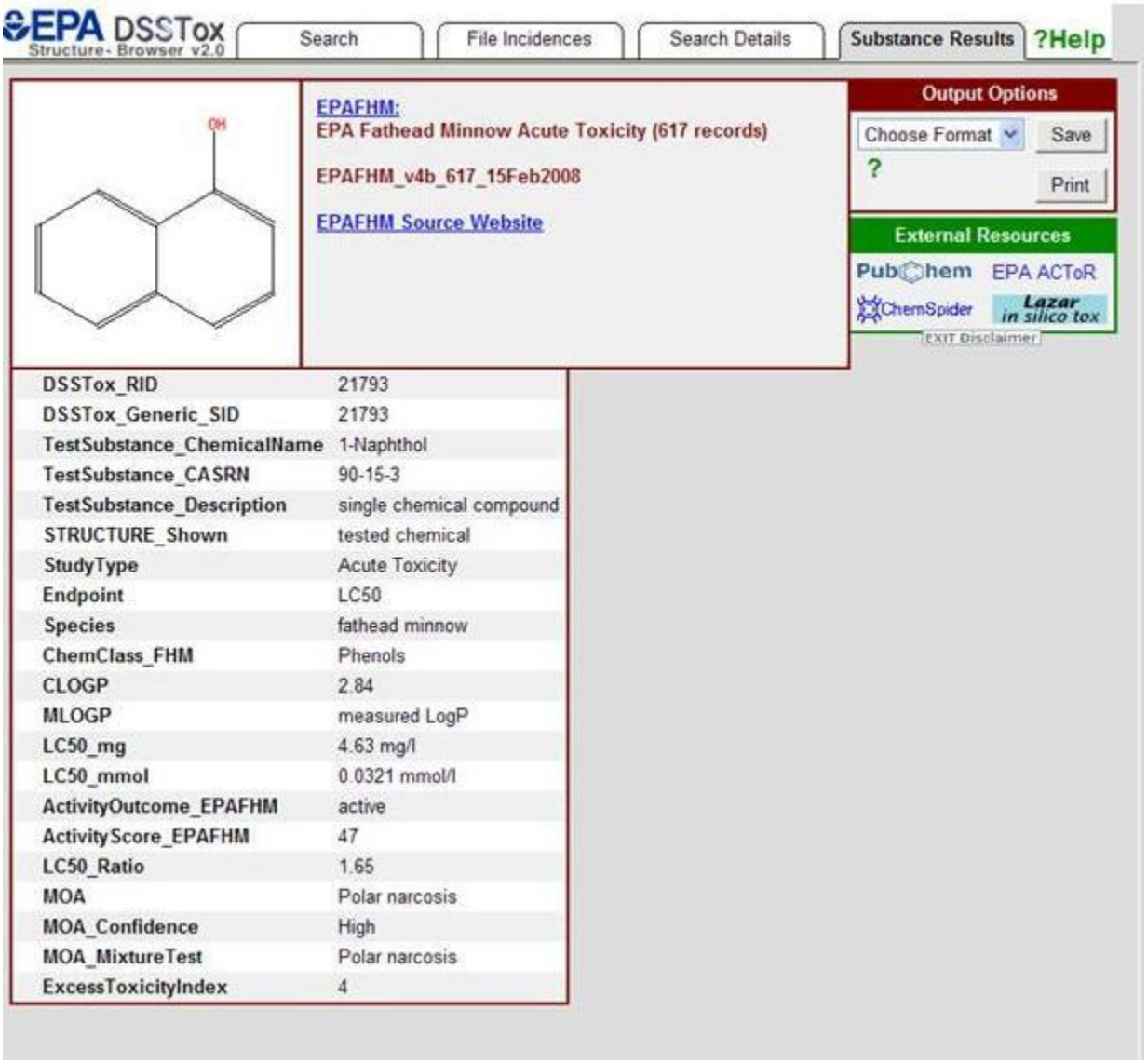


Figure A16. DSSTox substance results for 1-naphthol using the EPA Fathead Minnow (EPAFHM) database.

Appendix B. Overview of modes of action identified, associated chemical classes, and pest organisms.

Modes of Action	Chemical Class	Example chemicals	Pest Organism	Reference
Acetylcholinesterase Inhibition - Carbamate	Carbamates	Carbaryl	Insect	IRAC 2010
Acetylcholinesterase Inhibition - Organophosphates	Organophosphates	Chlorpyrifos	Insect	IRAC 2010
Acylation based reactivity	Ketenes, Acid Halides, Dialkyl carbonyl chlorides, Carboxylic acid anhydrides	Ethenone	Fish	Russom *et al*, 1997
Alkylation and Arylation based reactivity	Epoxides, Aziridines, Sulphonic/Sulphuric/Phosphoric acid esters, Halogenated acids/amides/ethers/sulphides/amines; Diazo compounds, Mustard compounds, Some alkyl/aryl halides, Propargylic alcohols, unsaturated aldehydes, acrylates, quinones, allylic compounds	Ethylene oxide	Fish	Russom *et al*, 1997
Anticoagulant: Vitamin-K antagonist	Coumarins, 4-Hydroxycoumarins, 1,3-Indandione	Warfarin	Rodent	Pelz 2005
Base-line / Non-polar narcosis	Various	1-Octanol	Fish	Russom *et al*, 1997
B-Halogenated Alcohol Based Reactivity	Beta-halogenated alcohols	1,2-Dichloropropanol	Fish	Russom **et al**, 1997
Bleaching: Inhibition of 4-hydroxyphenyl-pyruvate-dioxygenase (4-HPPD)	Triketone, Isoxazole, Pyrazoles, Unclassified	Mesotrione	Plant	HRAC 2010
Bleaching: Inhibition of carotenoid biosynthesis at the phytoene desaturase step (PDS)	Pyridazinones, Pyridinecarboxamides, Unclassified	Norflurazon	Plant	HRAC 2010
Block lipid synthesis by inhibiting enoyl-acy carrier protein reductase	Chlorinated phenoxyphenol	Irgasan (Triclosan)	Fungi, Bacteria	Russell 2004
Blocks potassium ion channels in nerve fibers	Aminopyridine	4-Aminopyridine	Bird	Choquet 1992
Carbonyl based reactivity	Lactones (alpha, beta, and unsaturated), Aldehydes	Acetaldehyde	Fish	Russom *et al*, 1997
Chloride channel activators	Glycosides	Abemectin	Insect	IRAC 2010
Ecdysone agonists / moulting disruptors	Diacylhydrazines	Chromafenozide	Insect	IRAC 2010
Ester narcosis	Esters	Methyl acetate	Fish	Russom *et al*, 1997
GABA-gated chloride channel antagonists - Cyclodiene organochlorines	Cyclodiene organochlorines	Endosulfan	Insect	IRAC 2010
GABA-gated chloride channel antagonists - Phenylpyrazoles	Phenylpyrazoles (Fiproles)	Fipronil	Insect	IRAC 2010
Host defense inducer: salicylic pathway	Benzothiadiazole	Probenazole	Fungi	FRAC 2010
Hydrazine Based Reactivity	Hydrazines	1,2-Diethylhydrazine	Fish	Russom *et al*, 1997
Hypercalcemia resulting in calcification of soft tissue	Vitamin D analogs	Cholecalciferol	Rodent	Marshall 1984
Inhibition of AA and Protein Synthesis: methionin biosynthesis	Anilinopyrimidines	Cyprodinil	Fungi	FRAC 2010
Inhibition of AA and Protein Synthesis: Protein synthesis	Hexopyranosyl antibiotic, Glucopyranosyl antibiotic, Tetracycline antibiotic	Blasticidin-S	Fungi	FRAC 2010
Inhibition of acetyl CoA carboxylase (ACCase)	Aryloxyphenoxyproprionates; Cyclohexanediones	Diclofop-methyl	Plant	HRAC 2010
Inhibition of ALS/AHAS (acetolactate/actohydroxy acid synthase)	Sulfonylureas; Imidazolinones; Triazolopyrimidines; Pyrimidinyl(thio)benzoates; Sulfonylaminocarbonyltriazolinones	Imazapyr	Plant	HRAC 2010

Inhibition of ATP production	Thiophenecarboximides	Silthiofam	Fungi	FRAC 2010
Inhibition of auxin transport	Phthalamates; Semicarbazones	Naptalam	Plant	HRAC 2010
Inhibition of cell division; Inhibition of very long chain fatty acids (VLCFA)	Chloroacetamides; Acetamides; Oxyacetamides; Tetrazolinones, Unclassified	Metolachlor	Plant	HRAC 2010
Inhibition of cell membrane permiability, fatty acids	Carbamates	Propamocarb	Fungi	FRAC 2010
Inhibition of cell wall (cellulose) synthesis	Nitriles; Benzamides; Triazolocarboxamides	Dichlobenil	Plant	HRAC 2010
Inhibition of Complex I - NADH oxidoreductase	Pyrimidinamines	Diflumetorim	Fungi	FRAC 2010
Inhibition of Complex II: succinate-dehydrogenase	Phenylbenzamides, Pyridinyl-ethyl-benzamides, furan-carboxamides, Oxathin-carboximides, Thiazole-carboximides, Pyrazole-Carboximides, Pyridine-carboximides	Carboxin	Fungi	FRAC 2010
Inhibition of Complex III: cycochrome (ubiquinone oxidase at Q0 site	Methoxyacrylates, Methoxycarbamates, Oximinoacetates, Oximinoacetamides, Oxazolidinediones, Dihydrodioxazines, Imidazolinones, benzylcarbamates	Fluoxastrobin	Fungi	FRAC 2010
Inhibition of Complex III: cycochrome bc1 (ubiquinone reductase) at Q1 site	Cyanoimidazole, Sulfamoyltriazole	Amisulbrom	Fungi	FRAC 2010
Inhibition of DHP (dihydropteroate) synthase	Carbamates	Asulam	Plant	HRAC 2010
Inhibition of EPSP (5-enolpyruvylshikimate-3-phosphate) synthase	Glycines	Glyphosate	Plant	HRAC 2010
Inhibition of Glutamine synthetase	Phosphinic acid	Glufosinate-ammonium	Plant	HRAC 2010
Inhibition of lipid peroxidation	Aromatic hydrocarbons, 1,2,4-Thhiadiazoles	Dicloran (DCNA)	Fungi	FRAC 2010
Inhibition of lipid synthesis - not ACCase inhibition	Thiocarbamates; Phosphorodithioates; Benzofuranes; Chloro-carbonic acids	EPTC	Plant	HRAC 2010
Inhibition of Lycopene cyclase	Triazole	Amitrol	Plant	HRAC 2010
Inhibition of mitosis / microtubule polymerization inhibitor	Carbamates	Chlorpropham	Plant	HRAC 2010
Inhibition of Mitosis/Cell Division: Beta-tubulin	Benzimidazoles, Thiophanates, N-phenyl carbamates, Toluamides	Benomyl	Fungi	FRAC 2010
Inhibition of Mitosis/Cell Division: Cell division	Phenylureas	Pencycuron	Fungi	FRAC 2010
Inhibition of Mitosis/Cell Division: delocalisation of spectrin-like proteins	Pyridinylmethylbenzamides	Fluopicolide	Fungi	FRAC 2010
Inhibition of nucleic acid synthesis: DNA topoisomerase Type II	Carboxylic acids	Oxolinic acid	Fungi	FRAC 2010
Inhibition of nucleic acid synthesis: DNA/RNA synthesis	Isoxazoles, Isothiazolones	Octhilinone	Fungi	FRAC 2010
Inhibition of nucleic acid synthesis: Purine metabolism (adenosin-demainase)	Hydroxy-(2-amino)pyrimidines	Ethirimol	Fungi	FRAC 2010
Inhibition of nucleic acid synthesis: RNA polymerase I	Acylalanines, Oxazolidinones, Butyrolactones	Metalaxyl	Fungi	FRAC 2010
Inhibition of phospholid biosynthesis: methyltransferase	Phosphorothiolates; Dithiolanes	Iprobenfos	Fungi	FRAC 2010
Inhibition of phospholipid biosynthesis and cell wall deposition	Cinnamic acid amides, Valinamide carbamates, Mandelic acid amides	Dimethomorph	Fungi	FRAC 2010
Inhibition of photosynthesis at photosystem II, Site A	Triazine, Triazinones, Triazolinone, Uracils, Pyridazinones, Phenyl-carbamates	Atrazine	Plant	HRAC 2010
Inhibition of photosynthesis at photosystem II, Site A but different binding behavior	Ureas; Amides	Linuron	Plant	HRAC 2010
Inhibition of photosynthesis at photosystem II, Site B	Nitriles; Benzothiadiazinone, Phenylpyridazines	Benzothiadiazole	Plant	HRAC 2010

Inhibition of protoporphyrinogen oxidase (PPO)	Diphenyl ethers; Phenylpyrazoles; N-phenylphthalimides; Thiadiazoles; Oxadiazoles; Triazolinones; Oxazolidinediones; Pyrimidindiones; Unclassified	Fomesafen	Plant	HRAC 2010
Inhibition of signal transduction: G-proteins	Quinolines, Quinazolinones	Quinoxyfen	Fungi	FRAC 2010
Inhibition of signal transduction: Osmotic (MAP/histidine kinase)	Phenylpyrroles; Dicarboximides	Iprodione	Fungi	FRAC 2010
Inhibition of sterol biosynthesis class I (demethylation): c14-demethylase	Piperazines, Pyridines, Imidazoles, Triazoles	Fenarimol	Fungi	FRAC 2010
Inhibition of sterol biosynthesis class II (amines): ergosterol	Morpholines, Piperidines, Spiroketal-amines	Piperalin	Fungi	FRAC 2010
Inhibition of sterol biosynthesis class III: 3-keto reductase	Hydroxyanilides	Fenhexamid	Fungi	FRAC 2010
Inhibition of sterol biosynthesis class IV: Squalene epoxidase	Thiocarbamates, Allyamines	Terbinafine	Fungi	FRAC 2010
Inhibitors of acetyl CoA carboxylase - Lipid synthesis, growth regulation	Tetronic acid	Spiromesifen	Insect	IRAC 2010
Inhibitors of cell wall synthesis: chitin synthase	Peptidyl pyrimidine nucleoside	Polyoxin B	Fungi	FRAC 2010
Inhibitors of cell wall synthesis: trehalase and inositol biosynthesis	Glucopyranosyl antibiotic	Validamycin	Fungi	FRAC 2010
Inhibitors of chitin biosynthesis, type 0, Lepidopteran	Benzoylureas	Diflubenzuron	Insect	IRAC 2010
Inhibitors of chitin biosynthesis, type 1 Homopteran	Unclassified	Buprofezin	Insect	IRAC 2010
Inhibitors of melanin synthesis in cell wall: dehydratase	Cyclopropanecarboxamide, Carboxamide, Propionamide	Fenoxanil	Fungi	FRAC 2010
Inhibitors of melanin synthesis in cell wall: reductase	Isobenzofuranone, Pyrroloquinolinone, Triazolobenzothiazole	Tricyclazole	Fungi	FRAC 2010
Inhibitors of mitochondrial ATP synthase	Thioureas, Organotins, Sulfite esters, Bridged diphenyl	Fenbutatin oxide	Insect	IRAC 2010
Inhibitors of oxidative phosphorylation: ATP synthase	Triphenyl tin compounds	Fentin acetate	Fungi	FRAC 2010
Isocyanate based reactivity	Isocyanates, Isothiocyanates	Butyl isocyanate	Fish	Russom *et al*, 1997
Juvenile hormone mimics	Juvenile hormone analog, Carbamates, Pyridine insect growth regulator	Methoprene	Insect	IRAC 2010
Microbial disruptors of insect midgut membranes (includes transgenic crops expressing B.t. toxins)	Biopesticides	Bacillus thuringiensis	Insect	IRAC 2010
Microbial disruptors of pathogen cell membranes	Bacillus subtilius and fungicidal lipopeptides they produce	Bacillus sp.	Fungi	FRAC 2010
Microtubule assembly inhibition	Dinitroanilines, Phosphoroamidates, Pyridines, Benzamides, Benzenedicarboxylic acids	Trifluralin	Plant	HRAC 2010
Miscellaneous nonspecific (multi-site) inhibitors	Alkyl halides, Unclassified, Inorganic compounds	Chloropicrin	Insect	IRAC 2010
Mitochondrial complex I electron transport inhibitors	Unclassified, Phenoxypyrazole, Pyridazinones, Pyrazole, Biopesticides	Rotenone	Insect	IRAC 2010
Mitochondrial complex II electron transport inhibitors	Pyrazole	Cyenopyrafen	Insect	IRAC 2010
Mitochondrial complex III electron transport inhibitors (Coupling site II)	Unclassified, Naphthoquinone derivative, Strobilurin	Hydramethylnon, Antimycin	Insect, Fish	IRAC 2010
Mitochondrial complex IV electron transport inhibitors (cytochrome oxidase)	Inorganic compounds, Unclassified	Zinc phosphide, Sodium azide	Insect, Rodent, Bacteria	IRAC 2010
Mitochondrial complex V electron transport inhibitor - ATP synthase	Sulfite ester	Propargite	Insect	IRAC 2010
Moulting disruptor, Dipteran	Triazine	Cyromazine	Insect	IRAC 2010
Multi-site contact inhibitor activity	Inorganic compounds, Dithiocarbamates and relatives, Phthalimides, Chloronitriles, Sulfamides, Guanidines, Triazines, Quinones	Captafol	Fungi	FRAC 2010

N-Halogenated Acetophenone Based Reactivity	Beta-halogenated acetophenones	alpha-Bromo-p-nitroacetophenone	Fish	Russom *et al*, 1997
Nicotinic Acetylcholine receptor agonists	Neonicotinoids, Botanical	Dinotefuran	Insect	IRAC 2010
Nicotinic Acetylcholine receptor allosteric activators	Spinosyns	Spinosad	Insect	IRAC 2010
Nicotinic acetylcholine receptor channel blockers	Nereistoxin analogues	Thiosultap-sodium	Insect	IRAC 2010
Nitroso Based Reactivity	Nitroso compounds	N-Nitrosomethylamine	Fish	Russom *et al*, 1997
Octopaminergic receptor agonists	Amidine	Amitraz	Insect	IRAC 2010
Oxime Based Reactivity	Oximes	Nifuroxime	Fish	Russom *et al*, 1997
Pheromone: Attract thru odor	Pheromone analogs	Calcium lactate	Insect	IRAC 2010
Photosystem I electron diversion	Bipyridilium	Paraquat	Plant	HRAC 2010
Polar narcosis	Anilines, Phenols, Pyridines	Phenol	Fish	Russom *et al*, 1997
Reactive nitriles	Allylic/Propargylic nitriles; alpha halogenated nitriles	Malononitrile	Fish	Russom *et al*, 1997
Ryanodine receptor modulators	Diamide	Chlorantraniliprole	Insect	IRAC 2010
Selective homopteran feeding blockers	Pyridine azomethines, Pyridine carboxamides	Flonicamid	Insect	IRAC 2010
Sodium channel modulators - organochlorines	Organochlorine	Organochlorines	Insect	IRAC 2010
Sodium channel modulators - pyrethoids	Pyrethroid	Pyrethroid	Insect	IRAC 2010
Sulfhydryl Based Reactivity	Disulfides, Sulfenyl halides, Peroxides, Thiocyanates	Dimethyl disulfide	Fish	Russom *et al*, 1997
Synthetic Auxins	Phenoxycarboxylic acids; benzoic acids; pyridine carboxylic acids; Quinoline carboxylic acids; Unclassified	Dicamba	Plant	HRAC 2010
Uncertain mode of action	Various	Azadirachtin, Dazomet, Fosetyl-Al	All groups	IRAC 2010, HRAC 2010
Uncoupler of oxidative phosphorylation	Arylpyrroles, Dinitrophenol For Plants: Dinitrophenylcrotonates, 2,6-Dinitroanilines, Pyrimidinone-hydrazones	Meptyl dinocap, Dinoseb, Bromethalin	Fungi, Plant, Rodent	FRAC 2010, HRAC 2010, van Lier 1988
Voltage-dependent sodium channel blockers	Oxadiazine, Semicarbazone	Metaflumizone	Insect	IRAC 2010

Appendix C. Computational tools available via government, open access, or commercial sources that are available to risk assessors in OW and OPP

Expert Systems for Predicting Toxicity

ASTER (ASsessment Tools for the Evaluation of Risk)

URL: http://cfint.rtpnc.epa.gov/aster/
Owner: USEPA ORD
Overview: Freely-available within USEPA firewall, or via Aventail AAA access. System is windows-based. ASTER is designed to provide high quality data for discrete chemicals, when available in the associated databases (i.e., ECOTOX and EcoChem), and QSAR-based predictions when data are lacking. Toxicity QSAR models are MOA-based. System can be run in either a batch mode or interactive / single chemical mode. Outputs to Excel format or HTML.
Coverage: Version 2.00 links to the most recent version of ECOTOX and EcoChem databases for empirical data. Includes 9 acute QSARs models for 7 MOAs (nonpolar narcosis, polar narcosis, ester narcosis, uncouplers of oxidative phosphorylation, reactive diesters, reactive carbonyls (3 equations), and reactive acrylates), and 3 chronic QSAR models for three MOA (nonpolar narcosis, polar narcosis, uncouplers of oxidative phosphorylation) for fathead minnow. Predicts toxicity for rainbow trout, bluegill, daphnid, and catfish for 2 MOA (nonpolar narcosis and polar narcosis) based on species extrapolation from fathead minnow LC50. When QSAR models do not exist, the system provides an estimated acute MOA. Models are primarily based on the fathead minnow database (Russom et al., 1997).
Input requirements: Log P and chemical structure via SMILES string, but if structure is in the supporting database, you only need enter the CAS number and both values will be populated.
Known limitations: Models perform well within certain log P ranges, with a domain of applicability of log P ranging from 0 to 6.0. Within the domain of applicability, MOA assignments default to nonpolar narcosis, if substructure fragments associated with other MOA are not identified. Models not available for metals or organometallics. QSAR models do not function outside of the domain of applicability. MOA assessments will function for organic structures within the domain of applicability. Models were built for use under TSCA and therefore do not include QSAR models for MOA related to many pesticide activities. MOA SAR includes substructures related to insecticides, but do not include substructures associated with fungicides, rodenticides, and herbicides.

DEMETRA (Development of Environmental Modules for Evaluation of Toxicity of pesticide Residues in Agriculture)

URL: http://www.demetra-tox.net/index.php?option=com_frontpage&Itemid=1
Owner: European Union Funded
Overview: Freely-available; online and PC-based application. Models are a hybrid of regression types (e.g., partial least squares (PLS), multi-linear regression (MLR), and classification algorithms (e.g., adaptive fuzzy partition). The main goal is to derive models that integrate the best algorithms obtained, and this forms the basis for a hybrid system software to be used for predictive purposes.

Coverage: Includes QSAR models to predict the acute toxicity for rainbow trout (96 hr LC50), and daphnid (48 hr LC50.) QSAR models were specifically developed using a training set including 20 pesticide classes including: organotins, organochlorines, organophosphates, carbamates, formamidines, terpenes, pyrethroids, phenols, spinosyns, pyrroles, pyridazinones, benzoylureas, *etc*.
Input requirements: Chemical structure and electronic descriptors which must be calculated using external computational tools (not included in DEMETRA)
Known limitations: Models have been published, but the level of independent validation of models is unknown. Main limitation is the need to input molecular descriptors that must be calculated using third-party applications, based on recommendations by DEMETRA site. Recommended computational tools are commercially available for cost. It is unknown if these parameters can be predicted using the OECD Toolbox.

EcoSAR: (Ecological Structure-Activity Relationship)

URL: http://www.epa.gov/oppt/newchems/tools/21ecosar.htm
Owner: USEPA OPPT
Overview: Freely-available, PC-based system. Models based on data submitted by manufacturers following OPPT guidelines, much of which is confidential business information (CBI), and data collections developed for use in QSAR modeling such as the USEPA fathead minnow database (Russom et al., 1997). System can be run in either a batch mode or interactive / single chemical mode. Outputs are delimited file formats and in 'summary' or 'full' reports.
Coverage: Version 1.00 includes over 600 QSAR models; models are based on 120 chemical classes. EcoSAR includes acute predictions of LC/EC50 for fish (saltwater and freshwater), mysid shrimp (96 hr) and algae (72 and 96 hr), and daphnid (48 hr); chronic toxicity predictions of chronic value (MATC) for fish (saltwater and freshwater), mysid shrimp, daphnid, and algae. Most acute models are based on a QSAR model, but some estimations are based on ACRs.
Input requirements: Log P and chemical structure via SMILES string, but if structure is in the supporting database, you only need enter the CAS number and both values will be populated.
Known limitations: Models perform well within certain log P ranges, with a domain of applicability of log P ranging from -3 to 8.0. Models were built for use under TSCA and therefore do not include models for MOA related to many pesticide activities. QSARs for some species are limited depending on the chemical class. Most models exist for neutral organics (narcotic-like compounds).

MCASE™

URL: http://www.multicase.com
Owner: MultiCASE, Inc.
Overview: Commercially available; MCASE is a knowledge-based system using fragment methodology to develop QSAR models for non-congeneric databases. MCASE (and MC4PC) evaluate the structural features of a set of non-congeneric molecules and identify the sub-structural fragments, referred to as *biophores* within MCASE™, that may be responsible for the observed activity (i.e. chemical functionalities). The chemicals containing the same *biophore* are grouped into subsets for which independent QSAR models are developed. The descriptors of

these models are called modulators and consist of fragments found within the individual sets as well as calculated transport and partition properties and quantum mechanical indices. The result of this operation is a set of QSAR models built for the congeneric sets of molecules containing the same *biophore* (identified as the "chemical functionality" responsible for the observed property. The domain of validity of the methodology is linked (and assessed) as a function of the probability that the corresponding *biophore* is related to activity and the determination that every three bonded non-hydrogen atom groups has been seen and therefore evaluated by the model builder or not seen and therefore of questionable effect on the prediction results. Batch mode is available.
Coverage: Includes QSAR models for acute toxicity (LC50) for bluegill (96 hr), fathead minnow (96 hr), guppy (14 d), rainbow trout (48 hr), and red killifish (48 hr). Also includes an NR50 (50% inhibition of netural red uptake) for predicting cytotoxicity in goldfish.
Input requirements: Chemical structure; via SMILES, drawing, *etc*., but if structure is in the supporting database, you only need enter the CAS number and chemical information will be populated.
Known limitations: The data used in development of the aquatic toxicity models is unknown at this time, therefore it is unknown how the models would perform with pesticides. Fathead minnow has the largest number of compounds with 683 (most likely USEPA fathead minnow database).

OECD (Q)SAR Application Toolbox

URL: http://www.oecd.org/document/54/0,3343,en_2649_34379_42923638_1_1_1_1,00.html
Owner: OECD
Overview:. Freely –available; PC-Based application.
Coverage: Includes ECOTOX database, and other large collections for empirical data; includes EcoCHEM, EcoSAR, and many other chemical information databases, includes models used in EcoSAR, ASTER, and other large computational tools. OECD is currently in version 1.1.02; with version 2.0 to be released at the end of 2010. The OECD Toolbox is designed to be a decision support system, where users identify their chemical of concern, pull in empirical data from associated databases, predict data if models available, provide predictive methods to build chemical categories and read-across tables, includes structural similarity methods, and QSAR model builders.
Input requirements: Chemical structure; via SMILES, drawing, *etc*., but if structure is in the supporting database, you only need enter the CAS number and chemical information will be populated.
Known limitations: System is currently in beta-testing, and limitations have not been fully documented, but limitations associated with models mentioned in this document that have been included in the OECD Toolbox, would apply.

TerraQSAR™

URL: http://www.terrabase-inc.com/
Owner: TerraBase, Inc.

Overview: Commercially available; Stand-alone Windows-Based application. QSARs are based on a proprietary probabalistic neural network model.
Coverage: Includes a module to predict acute LC50 to *Daphnia magna*, and a model for 96 hr LC50 to the fathead minnow.
Input requirements: Chemical structure; via SMILES, drawing, *etc*., but if structure is in the supporting database, you only need enter the CAS number and chemical information will be populated.
Known limitations: The data used in development of the daphnid and fathead models is unknown at this time; therefore, it is unknown how the models would perform with pesticides.

TOPKAT

URL: http://accelrys.com/products/discovery-studio/predictive-toxicology.html
Owner: Accelrys, Inc.
Overview: Commercially available; TOPKAT runs on PCs under Windows 95/98/NT. TOPKAT is a toxicity prediction program, which uses electrotopological states (Kier and Hall, 1999) as well as shape, symmetry, MW, and logP as descriptors to build statistically robust Quantitative Structure Toxicity Relationship (QSTR) models for over 18 endpoints. TOPKAT will validate its assessments via a univariate analysis of the descriptors, a multivariate analysis of the fit of the query structure in Optimum Prediction Space (OPS), and by similarity searching in descriptor space. QSAR models are preselected by the software based on the chemical class. The program can be executed in batch mode and the result is available in a format that can be imported into Microsoft Excel for Windows. TOPKAT makes visible experimental test data if available for the chemicals of interested
Coverage: DS TOPKAT includes models for fathead minnow LC50 and daphnid EC50.
Input requirements: Chemical structure; via SMILES, drawing, *etc*., but if structure is in the supporting database, you only need enter the CAS number and chemical information will be populated.
Known limitations: TOPKAT produces information for the (Q)SAR applicability domain at several levels: 1) the prediction is within the OPS of the model; 2) the model is within the limits of OPS; 3) all fragments identified in a molecule are known to the model. Users need to be aware that predictions may be outside the domain of applicability; OPS is the preferred mode. The data used in development of the daphnid and fathead models is unknown at this time; therefore it is unknown how the models would perform with pesticides.

Similarity Tools

AIM: (Analog Identification Methodology)

URL: https://aim.epa.gov
Owner: USEPA OPPT
Overview: AIM was designed to identify structurally analogous compounds based on a three pass atom-fragment matching algorithm. The substructure library contains 645 atom-fragment definitions along with super fragments for identifying important ring systems. The library focuses mostly on molecular features of neutral organic chemicals, but the predictive method will also provide results for salts and organometallic compounds. However, AIM does not

consider oxidation state which is often an important consideration when performing a toxicity assessment on metal containing compound and compounds with complex organic cations and anions may give unexpected result. Analog identification is based on structures within the currently linked databases. Current data sets cover 31,031 compounds, and therefore are limited to these structure sets. The empirical data are weighted more towards human health endpoints and do not include as much ecotoxicology data, although ECOTOX data set is to be included by 2011. The current AIM methodology requires exact matching with respect to rings in the candidate compound. No substitutions are allowed (e.g. phenyl ring for a pyridine ring). The same number of rings is also required (e.g. dichlorodiphenylsilane will not be identified as an analog for trichlorophenylsilane). Methodology to remove this limitation is under investigation. The number of analogs included in the analog list - If Pass 1 locates seven or more analogs, Pass 2 and Pass 3 are not currently implemented; therefore, some additional good analogs may not appear in the results..

Coverage: AIM accesses 10 data sources: TSCATS (USEPA TSCA test submission), HSDB (National Library of Medicine's Toxicology Data Network, Hazardous Substances Data Bank), IRIS (USEPA Integrated Risk Information System), National Institute of Health's NTP (National Toxicology Program), Centers for Disease Control and Prevention's ATSDR (Agency for Toxic Substances and Disease Registry), US EPA's HPV (High Production Volume) Challenge Program, US EPA's DSSTox website (see below), NIOSH's RTECS (Registry of Toxic Effects of Chemical Substances), OECD's IUCLID (International Uniform Chemical Information Database) of HPV chemical data reported by European industry, and the National Advisory Committee for the Development of Acute Exposure Guideline Levels (AEGL) for Hazardous Substances, AEGL rank.

Known limitations: AIM was designed to identify analogs only for neutral organic compounds. Other chemical classes should not be run through AIM. Analog identification is based on structures within the currently linked databases. Current data sets cover 31,031 compounds, and therefore are limited to these structure sets. The empirical data are weighted more towards human health endpoints and do not include as much ecotoxicology data, although ECOTOX data set is to be included by 2011. The current AIM methodology requires exact matching with respect to rings in the candidate compound. No substitutions are allowed (e.g. phenyl ring for a pyridine ring). The same number of rings is also required (e.g. dichlorodiphenylsilane will not be identified as an analog for trichlorophenylsilane). Methodology to remove this limitation is under investigation. The number of analogs included in the analog list - If Pass 1 locates seven or more analogs, Pass 2 and Pass 3 are not currently implemented; therefore, some additional good analogs may not appear in the results.

DSSTox (Distributed Structure-Searchable Toxicity) Structure-Browser

URL: http://www.epa.gov/ncct/dsstox/index.html

Owner: USEPA National Computational Toxicology Center (NCCT)

Overview: The USEPA DSSTox Structure-Browser, developed from available structure-viewing freeware and open-source programming methods, delivers a simple, easy-to-use structure-searching capability through the chemical inventory of published DSSTox Data Files. Search is initiated by entering either a chemical name, SMILES string, CAS Registry number, InChI™ (International Chemical Identifier) code, drawing the chemical, or structural formula (e.g.,

C4H5Cl). Users can also select how they want to search for similarity; i.e., exact match, substructure search, or similarity with defined threshold percentage.
Coverage: System searches all the DSSTox databases including the European Bioinformatics Institute (EBI) ArrayExpress Repository for Gene Expression Experiments, NTP bioassay data, NTP high-throughput screening data, National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) Series Experiments, USEPA Estrogen Receptor Ki binding study, the University of California, Berkeley Carcinogenic Potency data set, USEPA fathead minnow acute toxicity data, USEPA water disinfection by-products carcinogenicity predictions, FDA's Maximum Daily Dose data set, USEPA HPV data, IRIS, FDA estrogen receptor binding data, USEPA's ToxCast™, and the Istituto Superiore di Sanita chemical carcinogen data set. Users can select to search on all data sets or limit searches to specific data sets.
Known limitations: Current data sets cover 7,410 compounds, and therefore are limited to these structure sets. The empirical data are weighted more towards human health endpoints and do not include as much ecotoxicology data.

OECD (Q)SAR Application Toolbox

URL: http://www.oecd.org/document/54/0,3343,en_2649_34379_42923638_1_1_1_1,00.html
Owner: OECD
Overview:. Freely –available; PC-Based application.
Coverage: Version 1.1.02 includes 17 databases and 8 chemical inventories with a total of 323,403 chemicals included in similarity searches. System includes predictive methods to build chemical categories and read-across tables based on structural similarity or mechanistic information. System provides estimates of similarity based on Tanimoto coefficient, Dice, Ochial, and Kulcynski-2 methods. Users can set similarity threshold limits (% similarity) and molecular features (e.g., atom pairs, functional groups).
Input requirements: Chemical structure; via SMILES, drawing, *etc*., but if structure is in the supporting database, you only need enter the CAS number and chemical information will be populated.
Known limitations: System is currently in beta-testing, and limitations have not been fully documented.

ICE Models

Web-ICE

URL: http://www.epa.gov/ceampubl/fchain/webice/
Owner: USEPA ORD
Overview: Freely-available; web-based application
Coverage: Web-ICE version 3.1 includes 1443 models predicting toxicity to aquatic species, genera, and families and 852 models predicting to wildlife species and families. Aquatic models are based on 5501 EC/LC50 values of 180 species and 1266 chemicals. Wildlife models are developed from 4329 acute LD50 value for 156 species and 951 chemicals. Models described in Section 4.3 are available on Web-ICE . All models within Web-ICE are statistically significant

and those with at least four datapoints have been cross-validated, the results of which are provided to the user to assist with model selection.

Web-ICE also contains modules that develop Species Sensitivity Distributions from ICE-predicted toxicity and entered measured data. The endangered species module of this application predicts toxicity to selected listed species using all available models and multiple surrogates. The MOA-specific models, as well as full documentation of the data used to develop the models will be available in an update of the application to be posted in late 2010. ICE models for algal species will be posted to Web-ICE in 2011. User guidance provides assistance with model selection and results interpretation.
Input requirements: Acute EC/LC50 value in ug/L for aquatic species, acute LD50 in mg/kg body weight for wildlife.
Known limitations: Models are restricted to species-pairs available from the database. Variability in underlying databases influences model uncertainty.

Risk Assessment Tools (RA)

URL: http://www.setac.org/node/97
Owner: SETAC press (Mayer *et al.* 2010)
Overview: CD-based application available for purchase ($75)
Coverage: ICE models contained in RA are developed from 6 datasets: 1) acute aquatic organism (4890 EC/LC50 tests), 2) aquatic plant (1439 EC/LC50 tests), 3) wildlife acute (997 LD50 tests), 4) wildlife subacute (490 LC50 tests), 5) aquatic chronic (214 tests), and 6) wildlife chronic (98 tests). The RA documentation does not provide the number of significant models developed for each dataset.
Input requirements: Acute values corresponding to each dataset.
Known limitations: Models are restricted to species-pairs available from the database. Variability in underlying databases influences model uncertainty. Chronic tests were not standardized for test duration or most sensitive endpoint measured. Model cross-validation, uncertainty analyses, and influence of MOA have not been conducted for this application.

TCE Models

Acute-to-Chronic Estimation (ACE) v 2.0

URL: http://www.epa.gov/ceampubl/fchain/ace/index.htm
Owner: USEPA ORD
Overview: freely available internet executable application (Ellersieck *et al.*, 2003)
Coverage: ACE v 2.0 contains three TCE models: ALT, LRA, and MPA. ACE software documentation indicates that the models predict to the chronic NOEC for lethality.
Input requirements: time-course data for acute toxicity studies to include the number of individuals alive at each time step and each concentration.

Known limitations: Model output is for chronic lethality. Model uncertainty analyses have not been thoroughly conducted.

Risk Assessment Tools (RA)

URL: http://www.setac.org/node/97
Owner: SETAC press (Mayer *et al.* 2010)
Overview: Cd-based application available for purchase ($75)
Coverage: The ACE application within RA contains two TCE models: ALT and LRA. RA documentation indicates these models predict to the chronic MATC for lethality. RA and ACE v 2.0 were developed by the same authors, however potential differences in algorithms that result in predictions to MATC and NOECs, respectively, are unclear.
Input requirements: time-course data for acute toxicity studies to include the number of individuals alive at each time step and each concentration.
Known limitations: Model output is for chronic lethality. Model uncertainty analyses have not been thoroughly conducted.

Appendix D: Predictive methods for use in determining mode of action and physical chemical properties			
TITLE	**Brief description**	**Web address**	**Owner**
Tools and Websites for use in Determining MOA of chemical of interest			
ASTER	Predicts acute mode of action based on fish model	http://www.epa.gov/med/Prods_Pubs/aster.htm http://cfint.rtpnc.epa.gov/aster/	EPA
Classification of Herbicides	Table of herbicidal mode of action, chemical family and associated active ingredients	http://www.hracglobal.com/Publications/ClassificationofHerbicideModeofAction/tabid/222/Default.aspx	HRAC
Compendium of Pesticides	Provides CAS number, chemical names, structures, INCHI notation, pesticidal activity, molecular formula, etc. for more than 1100 substances. Site is indexed by chemical name, CAS number and pesticide classification	http://www.alanwood.net/pesticides/index.html	Alan Wood
Fungicide Resistance Action Committee	Includes link to fungide mode of action maps as well as FRAC code list of modes of action, with chemical groups and associated active ingredients	http://www.frac.info/frac/index.htm	FRAC
Herbicide MOA classification	Mode of action, chemical family, active ingredient and formulated products for herbicides	http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/prm6487	Alberta Canada
Herbicide MOA classification	Mode of action, chemical family, active ingredient, and formulated products for herbicides along with info on half-life	http://www.omafra.gov.on.ca/english/crops/facts/00-061.htm	Ontario Canada
Herbicide MOA classification	Provides search tool for herbicide with output of HRAC Group, mode of action, example trade names, and company producing AI	http://www.weedscience.org/summary/ChemFamilySum.asp	Weed Science
Insecticide Resistance Action Committee	MOA classification poster, as well as species specific information on mode of action	http://irac-online.org/	IRAC
IRAC (Insecticide Resistance Action Committee)	Lists mechanism of action of insecticide; drop down boxes provide information on associated chemical classes and specific active ingredients	http://www.irac-online.org/eClassification/	IRAC
OECD Toolbox	The Toolbox is a software application intended to be used by governments, chemical industry and other stakeholders in filling gaps in (eco)toxicity data needed for assessing the hazards of chemicals. The Toolbox incorporates information and predictive methods from various sources into a logical workflow. Crucial to this workflow is grouping chemicals into chemical categories. Has several mechanistically-based categories	http://www.oecd.org/document/54/0,3343,en_2649_34379_42923638_1_1_1_1,00.html	OECD
Plant Hormone information	Provides detailed information on plant hormones including function	http://www.plant-hormones.info/	BBSRC
Ware and Whitacre	Overview of Insecticide classes, mode of action, and structures	http://ipmworld.umn.edu/chapters/ware.htm	Univ MN

Wildlife Active Ingredients	Provides overview of active ingredients, including mode of action	http://icwdm.org/handbook/pestchem/active.asp	Internet Center for Wildlife Damage Management
World of Herbicides Map	Graphic of herbicide mode of action. Chemical structures are lumped based on activity	http://www.hracglobal.com/Publications/WorldofHerbicidesMap/tabid/354/Default.aspx	HRAC
Sources of Physical Chemical Property Data			
ASTER	Predict p-chem and fate properties to assist in environmental assessment	http://cfint.rtpnc.epa.gov/aster/	USEPA
EPISuite	Predict p-chem and fate properties to assist in environmental assessment. Following models included: KOWWIN™, AOPWIN™, HENRYWIN™, MPBPWIN™, BIOWIN™, BioHCwin, KOCWIN™, WSKOWWIN™, WATERNT™, BCFBAF™, HYDROWIN™, KOAWIN and AEROWIN™, and the fate models WVOLWIN™, STPWIN™ and LEV3EPI™	http://www.epa.gov/oppt/exposure/pubs/episuite.htm	USEPA
Sparc Performs Automated Reasoning in Chemistry (SPARC)	Calculates a large number of physical/ chemical parameters from pollutant molecular structure and basic information about the environment (media, temperature, pressure, pH, etc.).	http://ibmlc2.chem.uga.edu/sparc/	USEPA
OECD Toolbox	The Toolbox is a software application intended to be used by governments, chemical industry and other stakeholders in filling gaps in (eco)toxicity data needed for assessing the hazards of chemicals. The Toolbox incorporates information and predictive methods from various sources into a logical workflow. Crucial to this workflow is grouping chemicals into chemical categories. The seminal features of the Toolbox are: 1. Identification of relevant structural characteristics and potential mechanism or mode of action of a target chemical. 2. Identification of other chemicals that have the same structural characteristics and/or mechanism or mode of action. 3. Use of existing experimental data to fill the data gap(s).	http://www.oecd.org/document/54/0,3343,en_2649_34379_42923638_1_1_1_1,00.html	OECD
NoMiracle Toolbox	NoMiracle, an integrated European research project, will develop novel methods and computational tools to better evaluate chemical risks	http://nomiracle.jrc.ec.europa.eu/Lists/Toolbox/Exposure.aspx	EU