

# Exploration of Methods for Characterizing Effects of Chemical Stressors to Aquatic Animals

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## List of Acronyms

ACE	Acute-to-Chronic Estimation
AChE	acetylcholinesterase
ACR	Acute-Chronic Ratio
ALSV	Aquatic Life Screening Value
ALWQC	Aquatic Life Water Quality Criteria
AOP	Adverse Outcome Pathway
CWA	Clean Water Act
EF	Extrapolation Factor
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
HC <sub>5</sub>	The 5 <sup>th</sup> percentile of a SSD, HC stands for “hazard concentration”
ICE	Interspecies Correlation Estimation
LC <sub>50</sub>	Lethal Concentration of 50% of organisms
LOC	Level of Concern
LOEC	Lowest Observable Effect Concentration
MATC	Maximum Allowable Test Concentration (geometric mean of NOEC and LOEC)
NOEC	No Observable Effect Concentration
OECD	Office of Economic Cooperation and Development
OW	Office of Water
OPP	Office of Pesticides
ORD	Office of Research and Development
QSAR	Quantitative Structure-Activity Relationship
SSD	Species Sensitivity Distribution
TCE	Time-Concentration Event
USEPA	U.S. Environmental Protection Agency

## 1. Executive Summary

In order to characterize potential adverse effects of chemicals in the aquatic environment, the United States Environmental Protection Agency (USEPA) uses available toxicity data from studies involving individual test species, which serve as surrogates for untested species. These data are collected for individual organisms exposed to chemicals (*e.g.*, pesticides) and are then frequently extended to represent effects to populations of the same species, populations of similar genera/taxa, or to aquatic ecosystems. The goal of this work is to examine how limited test results can best be used to characterize adverse effects on aquatic animals. To that end, this paper explores two general types of methods that may be used to extrapolate from toxicity test results to taxa-specific and community-based measures of effect relevant to the Office of Pesticide Programs (OPP) and to the Office of Water (OW). These methods include sensitivity distributions and extrapolation factors (EFs) both of which may be used to account for uncertainty, particularly in situations where toxicity data are limited. A portion of this work will address the derivation of an “Aquatic Life Screening Value” that is related to the fifth percentile in a Sensitivity Distribution. ALSVs may be used to screen concentrations of pesticides and effluents in ambient waters and may be used by States and Tribes in the development of water quality standards. Other portions of this work will address other percentiles in sensitivity distributions that can be used to evaluate concentrations of pesticides in ambient water in other ways. This paper describes proposed analyses that will be conducted by USEPA in order to determine the utility of specific methods for development of a common effects characterization methodology for use in ecological assessments of chemicals by USEPA to meet the mandates of the Clean Water Act (CWA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

## 2. Introduction

The mission of the USEPA is to protect human health and to safeguard the natural environment upon which life depends. Consistent with the USEPA’s mission, OPP and OW are both responsible for evaluating the potential effects of chemicals on aquatic life. The process for accomplishing this mission involves three general steps. The first is compiling available toxicity data. Currently, both OPP and OW rely on the same aquatic toxicity test results (*e.g.*, scientific literature, registrant-submitted studies) to characterize the sensitivity of species in the aquatic environment to chemical stressors such as pesticides. The second step, which is the focus of this paper, involves characterization of potential effects of a chemical on the environment. OPP and OW currently use different methods to accomplish this. The third step, which will not be discussed in this paper, involves implementing the results of the effects characterization in order to safeguard the environment. This is accomplished by OPP through risk assessment and OW through criteria development.

In the effects characterizations of OPP and OW, the process used to translate single species toxicity test results to taxa-based and community-based (cross-taxa) adverse effect thresholds has not been consistent. In addition, the tools used in these processes have not been consistently applied across both offices. Toward developing a more transparent and consistent process for

characterizing chemical effects at various levels of organization, *i.e.*, single species, taxa (population) and cross taxa (community), USEPA has conducted six regionally-based public meetings and has drafted three white papers. These papers describe existing approaches and potential tools that may be used by OPP and OW to characterize the distribution of sensitivities in fish, invertebrates and plants in aquatic communities exposed to chemicals.

This particular white paper describes methods that may be used to characterize effects of chemicals on specific taxa (*i.e.*, aquatic vertebrates and invertebrates) and across taxa (*i.e.*, aquatic vertebrates and invertebrates combined). These additional methods may be used to augment the ability of the USEPA, as well as states, local and tribal water management agencies to derive taxa-based and cross-taxa (community-based) toxicity benchmark values for chemicals, such as pesticides, for risk assessment, monitoring and diagnostic purposes. This white paper explores analytical approaches that rely on empirical toxicity test results, particularly in cases where the available data may be limited and there is uncertainty as to the extent that the full distribution of species sensitivities (either at the taxa level or across taxa) is adequately characterized. OW has relied on a process defined in the 1985 Guidelines<sup>1</sup> to characterize community-level effect thresholds (*i.e.*, Aquatic Life Water Quality Criteria) using a defined number of taxa. The available data for chemicals can vary considerably. Therefore, regulatory agencies must have the flexibility to characterize the potential taxa-based and community-based effects of chemicals using the available data even if those data are limited in quantity. Although many of the methods discussed in this paper are currently used to varying extents in both offices, their consistent and integrated use by both offices has yet to be realized. The utility of the specific approaches described in this paper will be evaluated by USEPA and will undergo peer review and additional development prior to implementation by OPP and OW.

In order to characterize potential adverse effects of chemicals in the aquatic environment, it is necessary to use available toxicity test results from individual test species to extrapolate to assessment endpoints that are related to aquatic ecosystems. An assessment endpoint is “an explicit expression of the environmental value to be protected.”<sup>2</sup> Specific assessment endpoints relevant to this effort are based on those currently used by OPP and by OW. For OPP, assessment endpoints are taxa specific and include acute mortality and chronic survival, growth and reproduction of aquatic vertebrates (*i.e.*, fish and aquatic-phase amphibians) and invertebrates. For OW, assessment endpoints also include acute mortality and chronic survival, growth and reproduction but these endpoints are expressed in terms of the aquatic animal community (*i.e.*, a combination of vertebrates and invertebrates). Although the assessment endpoints of OPP and OW differ (*i.e.*, taxa-based vs. community-based, respectively), they both rely upon similar aquatic toxicity test results (See USEPA 1985, USEPA 1994, USEPA 2004). Therefore, it is possible to arrive at both sets of assessment endpoints using similar a common methodology.

Measures of effect, such as acute and chronic toxicity test results, are used to quantitatively represent assessment endpoints (USEPA 1998). For OPP, the measures of effect are the lowest available EC50 (or LC50) values to represent effects from acute exposures of fish and

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<sup>1</sup> “Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses” (USEPA 1985).

<sup>2</sup> <http://www.epa.gov/OCEPATERMS/>

invertebrate species to stressors, as well as the lowest No Observable Effect Concentration (NOEC) values to represent effects to growth and reproduction of fish and invertebrate species resulting from chronic exposures. For OW, the measure of effect is the HC<sub>5</sub>, or the 5<sup>th</sup> percentile of a distribution of acute toxicity test results for genera of aquatic animals (*i.e.*, fish, amphibians and invertebrates) as well as the HC<sub>5</sub> for chronic toxicity test results.

This paper describes two types of methods that may be used to extrapolate from individual species toxicity test results to taxa-based and community-based measures of effect relevant to OPP and OW. These methods are sensitivity distributions and extrapolation factors. Examples of how sensitivity distributions and extrapolation factors are used by regulatory agencies, particularly those in the United States, for characterizing effects of chemicals on aquatic animals are also discussed. For instance, OPP's taxa-specific Aquatic Life Benchmarks (USEPA 2010) for pesticides and OW's community-based Aquatic Life Water Quality Criteria currently use a combination of these methods in characterizing effects to aquatic organisms.

This paper also describes proposed analyses that will be conducted by USEPA in order to determine the utility of specific sensitivity distribution and extrapolation factor approaches for characterizing effects of chemicals on aquatic vertebrates, invertebrates and animal communities. These analyses will use large data sets for chemicals with various adverse outcome pathways<sup>3</sup> that are representative of pesticides with differing modes of action (*e.g.*, acetylcholinesterase inhibition and non-polar narcosis). These data sets will be used to evaluate intra- and inter-species variability by considering test results available for species exposed to the same chemical. The analysis will also consider the uncertainty that results from extrapolating from limited toxicity data to other species within aquatic taxa and across taxa (aquatic communities). Ultimately, the utility of a specific approach will be defined by its ability to predict a measure of effect with a desired level of certainty.

As indicated in the scoping document for this project<sup>4</sup>, “One goal of a common effects characterization methodology is to improve the tools and approaches available to States and stakeholders to derive scientifically defensible water quality criteria that can in turn be used to set water quality standards in a manner consistent with aquatic effects assessments conducted by OPP and OW in compliance with both the CWA and FIFRA.” This could involve use of empirical toxicity test results available for chemicals, predicted or estimated toxicity data using methods discussed in the tools white paper<sup>5</sup> and extrapolation methods described in this paper. To that end, this paper provides a general framework that may be used to conceptualize the development of community level benchmarks that may be considered by stakeholders to set water quality standards. The term “aquatic life screening value” (ALSV) is introduced here to represent community level benchmarks. Since the ALSV may be considered by USEPA, States and Tribes to derive scientifically defensible water quality standards, and because water quality criteria established using the 1985 Guidelines are set to one half of the fifth percentile of a

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<sup>3</sup> An adverse outcome pathway describes the linkage between a molecular-level initiating event for the toxicity of a chemical of interest to an adverse outcome at a biological level of organization of interest for a risk assessment (Ankley *et al.* 2010). More information about this term and its advantages relative to the conceptually similar “mode-of-action” and “mechanism-of-action” can be found in the tools white paper.

<sup>4</sup> Available online at: [http://www.epa.gov/oppefed1/cwa\\_fifra\\_effects\\_methodology/scope.html](http://www.epa.gov/oppefed1/cwa_fifra_effects_methodology/scope.html)

<sup>5</sup> Titled: “Predicting the toxicity of chemicals to aquatic animal species.”

sensitivity distribution, the measure of effect for the acute ALSV is also set to one half of the HC<sub>5</sub> of a sensitivity distribution, and the chronic ALSV is set to the HC<sub>5</sub> of the distribution of chronic toxicity data. It is expected that as USEPA reviews the specific methods described in this paper and in the tools paper, the ALSV framework presented in this paper will evolve to represent the most useful approaches and the conditions under which they should be used.

Following this introduction, this paper has three primary sections. The next section of this white paper (*i.e.*, section 3) serves to characterize available methods for extrapolating from available toxicity test results to taxa-specific and community-based measures of effect relevant to OPP for use in ecological risk assessments and OW for use in development of water quality criteria. The fourth section of this paper describes a proposed analysis that will be conducted by USEPA in order to determine the utility of the available methods for OPP's and OW's effects characterization. And the fifth section of this paper provides a conceptual approach that may be used to integrate chemical-specific toxicity test results, tools and methods for deriving community level benchmarks (*i.e.*, ALSVs) that, 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).

### 3. Methods for Deriving Measures of Effect from Results of Toxicity Tests

This section provides descriptions of methods that can be used to extrapolate from available toxicity test results to measures of effect. These methods include sensitivity distributions and extrapolation factors. Also included are examples of how sensitivity distributions and extrapolation factors are used by regulatory agencies for characterizing effects of chemicals on aquatic animals.

#### 3.1. Sensitivity Distributions

This section describes various distributions used for describing sensitivities of species as well as uncertainty in estimating HC<sub>5</sub> values and applications of sensitivity distributions in limited data situations. This section also includes brief descriptions of regulatory applications of sensitivity distributions.

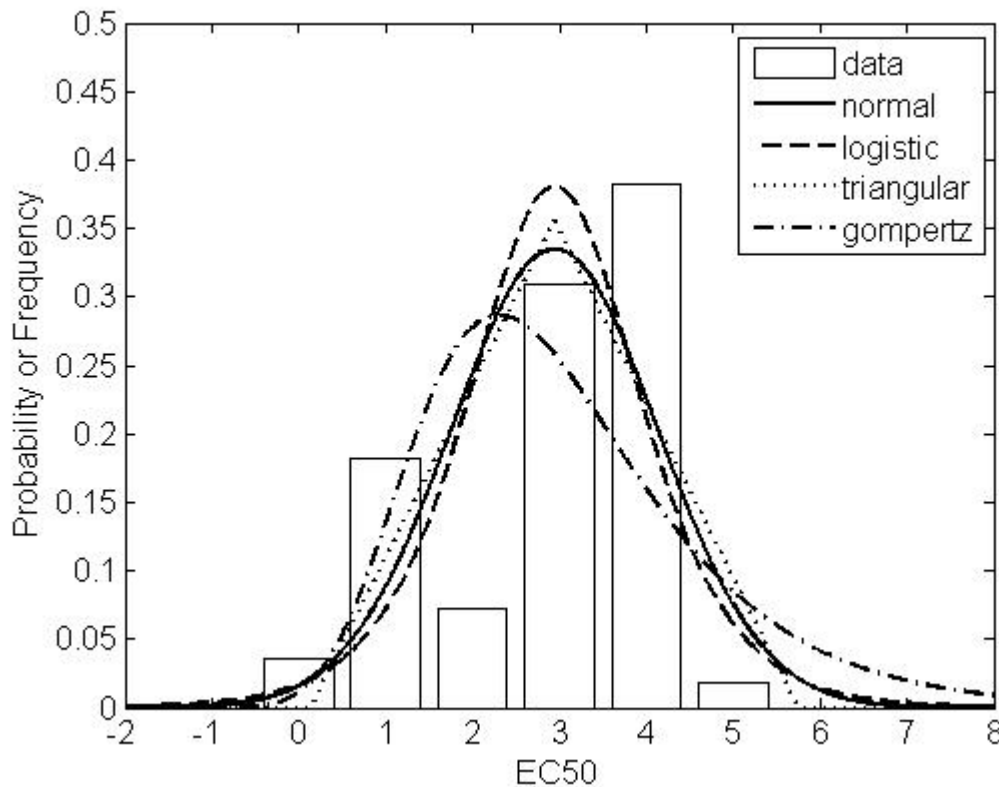
##### 3.1.1. Method Description

“Sensitivity Distribution” is a generic term used to represent a distribution of sensitivities of different biological taxa to the same stressor. Most commonly the taxa are biological species (these distributions are termed: “species sensitivity distributions” and abbreviated SSDs); however, sensitivity distributions may also be developed for genera (*e.g.*, USEPA 1985). For acute exposures, the toxicity test results of interest are EC<sub>50</sub> and LC<sub>50</sub> values of set durations of exposure (*e.g.*, 48-hr for cladocerans, 96-hr for non-cladoceran invertebrates and fish). Separate sensitivity distributions may also be applied to chronic toxicity data by compiling chronic toxicity data for similar test results, such as NOECs, or maximum acceptable toxic concentrations (MATCs; defined as the geometric mean of the NOEC and the lowest observable effect concentration (LOEC)).



Toxicity data are log-transformed and then used to derive a cumulative distribution where sensitivities are ranked from most sensitive to least sensitive. The concentration at the lower 5<sup>th</sup> percentile of the sensitivity distribution is most often the concentration of interest for regulatory purposes (e.g., USEPA, OECD). The 5<sup>th</sup> percentile value is commonly termed the HC<sub>5</sub>, where HC stands for “hazard concentration.” It should be noted that the HC<sub>5</sub> is discussed as a measure of effect throughout this document; however, other percentiles of sensitivity distributions could also be used.

Many different probability distributions exist and are in use for describing the sensitivities of aquatic organisms to chemicals. For instance, Figure 1 depicts normal, logistic, triangular and Gompertz probability distributions of log-transformed, acute toxicity data for the pesticide carbaryl. Carbaryl is a carbamate insecticide and plant growth regulator. For aquatic animals, carbaryl’s mode of action is inhibition of acetylcholinesterase (AChE). Toxicity data for carbaryl were obtained from the database of empirical acute toxicity EC50 and LC50 values underlying Web-ICE<sup>6</sup>. These data are provided in Appendix A. These data are used throughout this white paper to illustrate the applications of the methods that are described. It should be noted that although these data were reviewed to determine suitability for inclusion in Web ICE, these data have not been reviewed for inclusion in OPP ecological risk assessments or OW’s aquatic life water quality criteria development.



**Figure 1. Probability densities of log-normal, log-logistic, log-triangular, and log-gompertz distributions fit to 49 log<sub>10</sub> GMAVs for carbaryl.**

<sup>6</sup> Model and documentation available online at: <http://www.epa.gov/ceampubl/fchain/webice/>

Fitted cumulative distribution functions for the carbaryl data from Figure 1 are depicted in Figure 2. Equations for the probability density function (pdf), cumulative distribution function (cdf), quantile function ( $F^{-1}$ ), mean, and variance of these four distributions are provided in Appendix B. In general, the quantile function provides an estimate of the concentration (in  $\log_{10}$  units) associated with a given percentile of the distribution [*i.e.*,  $HC_5 = F^{-1}(0.05)$ ]. Thus, if the sensitivity data are drawn from a distribution with known parameters (*i.e.*, mean and standard deviation), the  $HC_5$  can be easily obtained.  $HC_5$  values from the fitted cumulative distribution functions for log-normal, log-logistic, log-triangular, and log-gompertz distributions for carbaryl (depicted in Figure 2) are provided in Table 1. These values range 7.9 to 10.5  $\mu\text{g/L}$ .

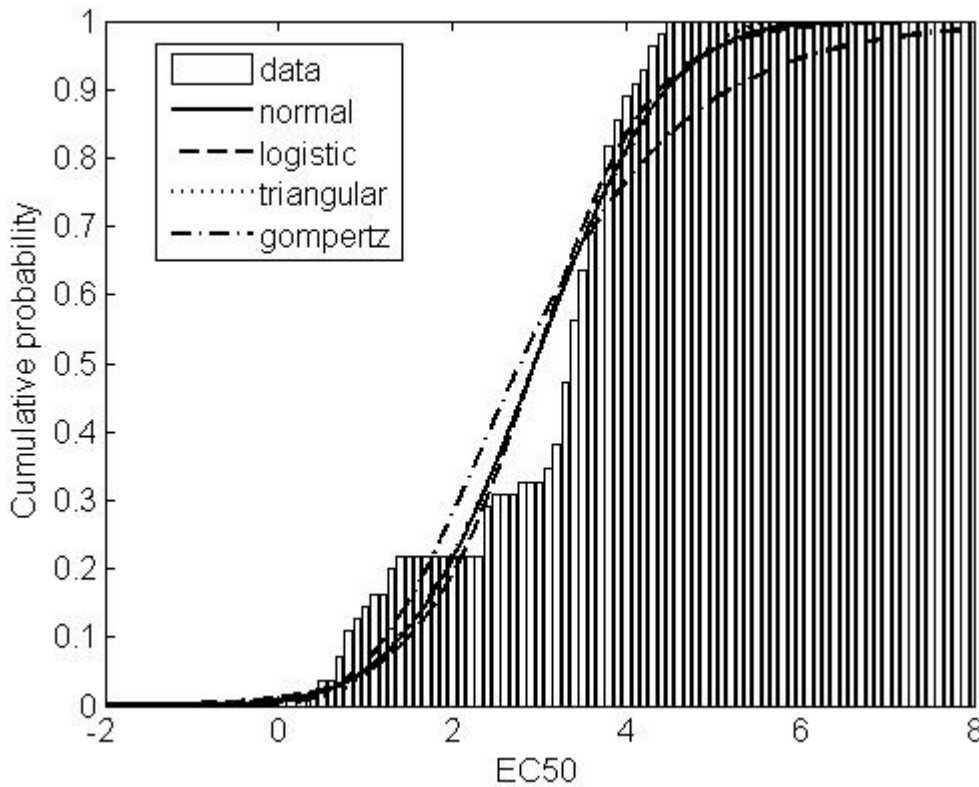


Figure 2. Fitted cumulative distribution functions for log-normal, log-logistic, log-triangular, and log-gompertz distributions plotted against 49  $\log_{10}$ GMAVs for carbaryl.

Table 1.  $HC_5$  values from fitted cumulative distribution functions for log-normal, log-logistic, log-triangular, and log-gompertz distributions plotted against 49  $\log_{10}$ GMAVs for carbaryl.

Distribution	$HC_5$ ( $\mu\text{g/L}$ )	Proportion of data < $HC_5$
Normal	9.6	0.13
Logistic	10.2	0.15
Triangular	10.5	0.15
Gompertz	7.9	0.11

N = 55 GMAVs

Many other distributions have been used for sensitivity distribution approaches, including weibull (Zajdlik & Associates 2005) and burr (Shao 2000). The burr distribution tends to limit to either the reciprocal weibull (Tadikamalla 1980) or the pareto distribution (<http://www.emis.csiro.au/envir/burrliz/>). Sensitivity distributions may also be calculated using non-parametric, distribution-free methods (Newman et al. 2000).

### 3.1.2. Distribution Approaches With Limited Toxicity Test Results

#### 3.1.2.1. Uncertainty in the Estimated HC<sub>5</sub> When Data are Limited

When a distribution is fit to a sample set with limited data, the precision of the estimated parameters of the distribution is uncertain. Thus the precision with which the HC<sub>5</sub> is estimated will vary from chemical to chemical and will often be limited depending on the number of data points and the amount of variability in the data. Several approaches for handling this uncertainty have been developed for sensitivity distributions, typically with the objective of placing confidence limits around the estimated HC<sub>5</sub> (examples are provided in Appendix B). The level of confidence (cl) in the estimated HC<sub>5</sub> can be noted as follows: HC<sub>5</sub><sup>cl</sup>. "cl" denotes the confidence with which the estimated HC<sub>p</sub> is no higher than the true HC<sub>p</sub>. A considerable body of literature on sensitivity distributions employs a confidence level of 95% (*i.e.*, HC<sub>5</sub><sup>95</sup>), though in practice any level of confidence could be specified. In the 1985 Guidelines, a 50% confidence level (corresponding to the median estimate) is used as the best estimate of the HC<sub>5</sub> (*i.e.*, HC<sub>5</sub><sup>50</sup>).

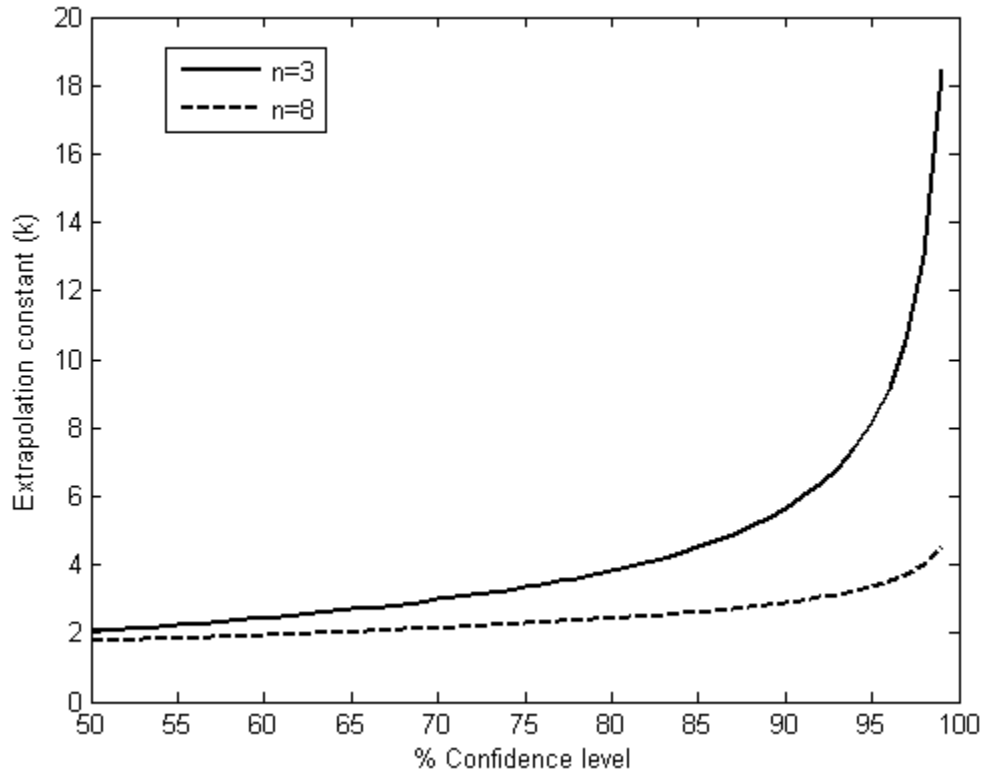
While the methods for developing these confidence limits differ across distributions, most follow a similar procedure, often based on a "standard" form of the distribution. A common method for expressing the location of this concentration is as a function of the sample mean ( $\bar{x}$ , Equation 1), standard deviation ( $s$ , Equation 2) and an extrapolation constant ( $k$ , Equation 3).

$$\text{Equation 1} \quad \bar{x} = \frac{1}{N} \sum_{i=1}^N x_i$$

$$\text{Equation 2} \quad s = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (x_i - \bar{x})^2}$$

$$\text{Equation 3} \quad HC_5^{cl} = \bar{x} - ks$$

Extrapolation constants (not to be confused with extrapolation factors, which are described in section 3.2) have been developed for three distributions commonly used for sensitivity distributions, the log-normal distribution (Aldenberg and Jaworska 2000, Aldenberg et al. 2002), the log-logistic distribution (Aldenberg and Slob 1993) and the triangular distribution (Pennington 2003). These authors provide tables of  $k$  values derived either theoretically or through Monte Carlo simulation. These  $k$  values are provided in Appendix B. For distributions in the location/scale family, values of the extrapolation constants depend only on the parametric distribution, the sample size, and the desired level of confidence. Extrapolation constants increase with the level of confidence required and decrease with sample size (Figure 3).



**Figure 3. Extrapolation constants (k) versus confidence bound on the HC<sub>5</sub> for a standard log-logistic distribution of species sensitivities (k-values generated following methodology of Aldenberg and Slob 1993).**

Extrapolation constants are not the only method for handling uncertainty in an estimated HC<sub>5</sub>. An important limitation to the use of extrapolation constants is that the choice of any given value for an extrapolation constant makes an implicit judgment concerning an acceptable level of uncertainty (*e.g.*, the use of an extrapolation constant corresponding to 95% confidence presumes that the acceptable level of uncertainty is 5%). Another way to quantify the uncertainty surrounding the estimated HC<sub>5</sub> is to estimate its standard error using the delta method (Seber 2002). This requires an estimate of the covariance matrix for the estimated parameters and the quantile function ( $F^{-1}$ ). For example, let  $\theta$  represent a vector of estimated parameters describing a distribution, then for a normal distribution,  $\theta = [\bar{x}, s]$  and:

$$\text{Equation 4. } \hat{\text{COV}}(\theta) = \begin{bmatrix} \hat{\text{var}}(\bar{x}) & \hat{\text{cov}}(\bar{x}, s) \\ \hat{\text{cov}}(\bar{x}, s) & \hat{\text{var}}(s) \end{bmatrix}$$

From the above, the equation for the sampling variance of the HC<sub>5</sub> is:

$$\text{Equation 5. } \text{var}(\text{HC}_5) = \begin{bmatrix} \frac{\partial F^{-1}}{\partial \bar{x}} & \frac{\partial F^{-1}}{\partial s} \end{bmatrix} \text{cov}(\theta) \begin{bmatrix} \frac{\partial F^{-1}}{\partial \bar{x}} & \frac{\partial F^{-1}}{\partial s} \end{bmatrix}^T$$

Where the partial derivatives are evaluated at 0.05. An advantage to the above method is that the resulting variance estimate can be applied to any level of uncertainty deemed acceptable using standard methods for generating confidence intervals.

### 3.1.2.2. Sensitivity Distribution Approaches Using Known Population Variance

The sensitivity distribution approaches described in Section 3.1.2.1 rely upon sample estimates of population parameters (*i.e.*, sample mean and standard deviation). As discussed below, Aldenberg and Luttik (2002) and de Zwart (2002) have explored the applications of known population variance along with sample means in deriving HC<sub>5</sub> values from sensitivity distributions.

Aldenberg and Luttik (2002) proposed the use of known standard deviations in combination with estimated means (from samples) to derive HC<sub>5</sub> values from sensitivity distributions. This approach requires knowledge of the standard deviation from the population of interest, preferably from “similar substances as the one under study.” This approach is limited in its application in that the standard deviation must be obtained from other chemical data sets, which does not allow for a simple application of this method for data limited chemicals.

In order to account for population variability that may be represented by standard deviations of samples with small sample sizes, de Zwart 2002 proposed use of a logistic sensitivity distribution with known variance ( $\beta$ ) that are based on a chemical’s mode of action (Table 2). de Zwart assumed that the intrinsic toxicity of each chemical within a mode of action would change (*i.e.*,  $\bar{x}$  would vary); however, the variability of organism sensitivities to the same chemical will be similar within a mode of action. Using this approach, the HC<sub>5</sub> is calculated using Equation 6.

Equation 6.  $HC_5 = 10^{\alpha - 2.94\beta}$

**Table 2. Mean  $\beta$  values for various modes of action reported by de Zwart 2002.**

Mode of Action	N	Average $\beta$	Standard error of the mean $\beta$ **
Nonpolar narcosis	34	0.39	0.03
AChE* inhibition (carbamates)	27	0.71	0.03
Photosynthesis inhibition	20	0.60	0.03
Polar narcosis	13	0.31	0.03
AChE* inhibition (organophosphates)	11	0.50	0.05
Uncoupler of oxidative phosphorylation	8	0.38	0.05
Multisite inhibition	6	0.62	0.07
Dithiocarbamates	6	0.57	0.05
Diesters	6	0.42	0.07
Systemic fungicide	5	0.46	0.04
Sporulation inhibition	5	0.37	0.05
Neurotoxicity (pyrethroids)	4	0.65	0.03
Neurotoxicity (cyclodiene-type)	4	0.61	0.01
Plant growth inhibition	4	0.52	0.06
Membrane damage by superoxide formation	3	0.69	0.01
Cell division inhibition	3	0.63	0.21
Systemic herbicide	3	0.52	0.12
Plant growth regulator	3	0.44	0.10
Neurotoxicant (DDT-type)	2	0.50	0.13
Amino acid synthesis inhibition	2	0.47	0.03
Germination inhibition	2	0.40	0.02
Quinolines	2	0.28	0.02
Reactions with carbonyl compounds	2	0.28	0.07

\*AChE = acetylcholinesterase

$$** SEM = \frac{(stdev\ of\ \beta)}{\sqrt{N}}$$

### 3.1.2.3. Methods for Deriving Chronic Distributions Using Acute Toxicity Test Results

Two approaches are described in the literature (de Zwart 2002, Douboudin et al. 2004) that use available acute toxicity data to create chronic sensitivity distributions. In both approaches it is possible to derive chronic HC<sub>5</sub> values using no chronic toxicity data.

de Zwart developed a regression for the acute and chronic mean values (termed  $\alpha_{acute}$  and  $\alpha_{chronic}$ , respectively; Equation 7). Using this equation, it can be concluded that the average acute toxicity for a chemical is a factor of 13-24x higher than the average chronic toxicity. de Zwart concluded that this analysis could be applied to assign surrogate chronic species distribution parameters in cases where acute toxicity data are available but chronic data are not available. Using this approach, a chronic HC<sub>5</sub> could be derived using Equation 6 and the appropriate  $\beta$  from Table 2.

Equation 7.  $\alpha_{chronic} = 1.053 * \alpha_{acute} - 1.430$  (de Zwart 2002)

In Duboudin et al. (2004)'s approach, acute mean ( $\mu_A$ ) and standard deviations ( $\sigma_A$ ) based on available invertebrate data are used to calculate mean values ( $\mu_c$ ) representative of chronic effects using equations 8 and 9 for vertebrates and invertebrates, respectively. These equations were derived from regressions of empirical acute and chronic data available for vertebrates (22 chemicals;  $r^2 = 0.854$ ) and invertebrates (15 chemicals,  $r^2 = 0.917$ ). The test chemicals included metals, inorganic chemicals and organic chemicals, some of which were pesticides (e.g., lindane, azinphos-methyl, diazinon, atrazine).

Equation 8.  $\mu_{Cv} = 0.82 * \mu_{Av} - 0.62 * \sigma_{Av} - 0.49$  (vertebrates)

Equation 9.  $\mu_{Ci} = 0.58 * \mu_{Ai} - 1.96 * \sigma_{Ai} - 1.60$  (invertebrates)

### 3.1.2.4. Illustration of Distribution Approaches Involving Limited Data

Toxicity test results for carbaryl were used to illustrate the different distributional approaches described in Section 3.1.1. Relative to many other pesticides, there are a great deal of aquatic toxicity test results available for carbaryl in the scientific literature, as well as from studies submitted by carbaryl registrants to fulfill FIFRA data requirements. In order to illustrate the uses of the methods described above in determining the HC<sub>5</sub> of for a chemical using a small data set, a subset of aquatic toxicity test results for carbaryl from Appendix A is used here to simulate a chemical with limited toxicity data (Table 3). Since this effort is focused on applications of extrapolation factors that are relevant to OPP and OW, the subset of data is based on the FIFRA data requirements for pesticides. Under FIFRA, acute toxicity data are required for two freshwater fish (typically the rainbow trout, *Oncorhynchus mykiss* and the bluegill sunfish, *Lepomis macrochirus*) and one freshwater invertebrate (typically the waterflea, *Daphnia magna*).

**Table 3. Subset of acute toxicity data for carbaryl used to illustrate use of sensitivity distribution approaches with limited data.**

Taxa	Species	Test Result (µg/L)	Geometric mean of test result for species (µg/L)
Aquatic invertebrate	<i>Daphnia magna</i>	5.6*	7.5
		10.1*	
Fish (warm water)	Bluegill sunfish	5230**	5230
Fish (cold water)	Rainbow trout	1090**	1841
		3110**	

\*48-h EC50

\*\*96-h LC50

For this small data set, the average ( $\bar{x}$ ) and standard deviation ( $s$ ) of the log transformed geometric means of the toxicity test results for the species are 2.62 and 1.53, respectively. These values are used to generate median and 95<sup>th</sup> percentile HC<sub>5</sub> values (Table 4) using Equation 3 and the k values from the log-normal distribution (Aldenberg and Jaworska 2000, Aldenberg et al. 2002), the log-logistic distribution (Aldenberg and Slob 1993) and the triangular distribution (Pennington 2003). The k values used for N=3 are provided in Appendix B. These values are

also used in the approaches where the variance is known (*i.e.*, de Zwart 2002). For the de Zwart method, equation 6 was used with a  $\beta$  value of 0.71, which is based on the mode of action representative of carbaryl (*i.e.*, AChE inhibition from a carbamate). Median HC<sub>5</sub> values calculated with these methods range 0.31-3.4  $\mu\text{g/L}$ . It is notable that the 95<sup>th</sup> percentile HC<sub>5</sub> values are nine orders of magnitude lower than the median HC<sub>5</sub> values. It is also notable that the median HC<sub>5</sub> values are all below the HC<sub>5</sub> values generated for the fitted distributions from the full carbaryl data set, which were 9.6, 10.2 and 10.5  $\mu\text{g/L}$  for the log-normal, log-logistic and log-triangular distributions, respectively (Table 1).

**Table 4. Median and 95<sup>th</sup> percentile HC<sub>5</sub> values ( $\mu\text{g/L}$ ) derived for subset of carbaryl data.**

Distribution (method source)	Median HC <sub>5</sub>	HC <sub>5</sub> <sup>95</sup>
Log-normal (Aldenberg and Jaworska 2000)	0.46	$7.3 \times 10^{-10}$
Log-logistic (Aldenberg and Slob 1993)	0.31	$1.5 \times 10^{-10}$
Log-logistic (de Zwart 2002)	3.4	Not available
Log-triangular (Pennington 2003)	0.52	$7.2 \times 10^{-10}$

If no chronic toxicity data were available for carbaryl, de Zwart's work could also be used to derive a chronic HC<sub>5</sub>. In this approach, equation 7 could be used to estimate a mean of the chronic toxicity data ( $\alpha_{\text{chronic}} = 1.33$ ). Using this with the  $\beta$  value of 0.71 and equation 6, the resulting HC<sub>5</sub> would be 0.17  $\mu\text{g/L}$ .

### 3.1.3. Examples of Regulatory Applications of Sensitivity Distributions

#### 3.1.3.1. OPP

OPP has used species sensitivity distributions to characterize risks of pesticides to specific taxa, (*e.g.*, fish) of concern. This approach involved generating separate distributions of acute toxicity data for fish and aquatic invertebrates. Estimated and measured concentrations of pesticides in surface water were compared to the two joint probability distributions to consider the proportions of fish and invertebrates that may be impacted by the assessed pesticide (USEPA 2007a, USEPA 2007b).

Sensitivity distributions are also incorporated into OPP's aquatic level II risk assessment model (v.2.0, 3/16/2004). Although this model is still in development, it is intended for use in OPP to estimate the likelihood and magnitude of effect on aquatic species that are vulnerable to pesticide exposure. This model uses available acute toxicity data to develop separate distributions for fish and aquatic invertebrates. The model calculates the 5<sup>th</sup>, 50<sup>th</sup> and 95<sup>th</sup> percentiles of the distributions to generate probit-concentration response curves. These are used to determine the magnitude of effect to specific taxa resulting from an exposure concentration (USEPA 2004b).

#### 3.1.3.2. OW

OW currently derives Aquatic Life Water Quality Criteria (ALWQC) for communities of aquatic animals using a triangular distribution of toxicity test results for genera. This approach combines toxicity data for vertebrates (*i.e.*, fish and amphibians) and invertebrates (*e.g.*, cladocerans, insects). The acute criterion is set to half of the 5<sup>th</sup> percentile of the distribution, based on the



1985 Guidelines. The 5<sup>th</sup> percentile is calculated using the four most sensitive acute toxicity genus mean values. The 5<sup>th</sup> percentile is divided by 2 to derive a final acute criterion (*i.e.*, the criterion maximum concentration) in order to adjust the 5<sup>th</sup> percentile EC50 value to an EC “low” value which is intended to estimate a level of toxicity between 0 and 10% such that the final value is statistically indistinguishable from mortality allowable in control treatment and considered background. The overall intention of the acute and chronic criteria is to establish concentrations of chemicals that, if not exceeded, aquatic animals should not be unacceptably affected (directly).

#### 3.1.3.3. Europe

The preferred approach of the EU for deriving environmental quality standards for substance concentrations in inland surface waters as well as in transitional, coastal and territorial waters is statistical extrapolation using a SSD (European Commission 2003). In this approach, it is also preferred that the dataset used in the SSD contain more than 15, but at least 10 NOECs from “long-term” toxicity tests, for different species covering at least 8 taxonomic groups.

The Netherlands requires at least four chronic NOECs for different taxonomic groups for a refined effects assessment. The Netherlands (RIVM 2001) “estimated risk levels” are derived using the SSD method of Aldenberg and Jaworska (2000). That is, HC<sub>5</sub> values are calculated based on a log-normal SSD.

#### 3.1.3.4. OECD

The sensitivity distribution methodology for the Organization of Economic Cooperation and Development (OECD) method is similar to the European Union (EU) SSD methodology; however, no data distribution type is assumed *a priori*. The SSD can follow any data distribution, although log-normal and log-logistic (*e.g.*, Burr Type III) distributions are most likely. The best model is assessed using the Kolmogorov-Smirnov goodness-of-fit test. The “maximum tolerable concentration” is represented as either the median of the 5<sup>th</sup> percentile value or the lower 95% confidence limit of the 5<sup>th</sup> percentile depending on the goal of the end user. If sufficient chronic data exist to meet all of the taxonomic requirements, then a final chronic value can be calculated. If species level data are used, then the final chronic value is equal to the maximum tolerable concentration. Unlike the USEPA method, however, the OECD method requires NOECs from eight taxonomic groups, and does not allow for use of Actual Chronic Ratios (ACRs); however, appropriate QSAR values can be used as surrogates if laboratory data are unavailable.

#### 3.1.3.5. Australia and New Zealand

Australia and New Zealand derive guideline trigger values from toxicity data. The Australia/New Zealand guidelines use the same SSD method as the Netherlands, but with a curve-fitting procedure that overcomes the problem of data that do not fit an assumed distribution. Using the program BurrliOZ v. 1.0.13 (CSIRO 2001; Campbell et al. 2000), data are first fitted to one of a family of Burr distributions (Burr 1942; the log-logistic distribution is in the Burr family). After an appropriate distribution is chosen, then the calculation to estimate the 95<sup>th</sup> protection level is

the same as the Netherlands' methodology but utilizes extrapolation constants ( $k$ ) derived for each of the distributions.

#### 3.1.3.6. University of California, Davis Methodology

This methodology was developed by researchers at the University of California, Davis (TenBrook et al. 2010) for the derivation of pesticide water quality criteria for the Sacramento and San Joaquin Basins, although the method is applicable to pesticide water quality criteria derivation for all freshwater aquatic systems. This methodology was first finalized as a 2009 report to the Central Valley Regional Water Quality Control Board and has been used to derive aquatic water quality criteria for bifenthrin, chlorpyrifos, cyfluthrin, lambda-cyhalothrin, diazinon, diuron and malathion<sup>7</sup>.

The authors of this methodology evaluated many of the current water quality criteria derivation methodologies and incorporated elements from several of them, in addition to providing their own specific modifications of these elements, throughout the derivation process. The SSD procedure described in this approach is the result of a synthesis of elements from the SSD procedures of the Dutch (RIVM), Australian and New Zealand (ANZEC&ARMCANZ), and 1985 *Guidelines* procedures, with additional modifications. Based on this analysis, the Burr III distribution should be used for the SSD method (acute and chronic). Similar to the 1985 *Guidelines* approach, this SSD method uses the median value of the 5<sup>th</sup> percentile of the SSD, but species level data are used, rather than aggregating to the genus level. The acute value is determined by dividing the median of the 5<sup>th</sup> percentile by 2, and the chronic value is the 5<sup>th</sup> percentile value without additional adjustment. The authors also recommend calculating 95% confidence intervals surrounding the 1<sup>st</sup> and the 5<sup>th</sup> percentiles using a bootstrapping procedure that is described in detail within the methods manual and is also included with the BurrliOZ freeware described above. The use of the SSD method is based on the availability of toxicity test results for species in 5 different families.

### 3.2. Extrapolation factors

#### 3.2.1. Method Description

Extrapolation factors (EFs) are set values that are applied to available toxicity test results to account for various sources of uncertainty in extrapolating from individual species toxicity data to measures of effect. Several different names have been used to describe these factors, including assessment factors, safety factors, application factors and uncertainty factors. These factors are used as follows: available toxicity data are identified for a chemical and the lowest toxicity test result is divided by the EF. The advantage of this approach is that it is simple, easy to use and requires little expenditure of time or resources in terms of reviewing toxicity data or calculating the final measures of effect.

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<sup>7</sup> available at:

[http://www.swrcb.ca.gov/rwqcb5/water\\_issues/tmdl/central\\_valley\\_projects/central\\_valley\\_pesticides/criteria\\_method/index.shtml](http://www.swrcb.ca.gov/rwqcb5/water_issues/tmdl/central_valley_projects/central_valley_pesticides/criteria_method/index.shtml)

### 3.2.2. Uncertainty Associated with use of Extrapolation Factors

There is uncertainty associated with this approach in terms of accurately characterizing effects of stressors on measures of effect given limited availability of data. Since the EF is applied to the most conservative toxicity data available for a chemical, the magnitude of the EF is typically dependant on the extent of available data. Important considerations for this include: species that are tested and the adverse outcome pathway of the chemical.

Additional sources of uncertainty can be attributed to differences in: sensitivity among individuals within a species due to different test conditions (intra-species variability) and sensitivity of test species and species of concern (inter-species variability). EFs may be designed to account for some of these sources of uncertainty and variability.

### 3.2.3. Examples of Regulatory Applications of Extrapolation factors

In regulatory applications, EFs generally include default values applied to acute toxicity test results to derive water quality criteria. EFs are used by various agencies in the United States (*e.g.*, USEPA 1995), Canada (*e.g.*, MENVIQ 1990, Rev. 1992) and Europe (European Commission 2003) to establish levels of concern for aquatic species, taxa, communities and ecosystems. Described below are two approaches involving EFs that are used by United States regulatory agencies. Specifically, these include the OPP Aquatic Benchmarks and the Great Lakes Water Quality Initiative for deriving criteria with limited toxicity data. The OPP Aquatic Benchmarks and Great Lakes Water Quality Initiative methods differ in their methods and intended measures of effect (*i.e.*, the lowest available toxicity data for a taxa and the HC<sub>5</sub> for a community, respectively). Also described below is an approach presented in the scientific literature by Pennington 2003, which is based on various sensitivity distribution assumptions and considers chemical mode of action. It should be noted that the approaches described below are not necessarily equivalent because they vary in the uncertainty, measures of effect and assessment endpoints they are intended to represent.

#### 3.2.3.1. OPP Aquatic Benchmarks

The OPP benchmarks were developed in response to recommendations and input from stakeholders, who were concerned about potential effects of pesticides with no existing Aquatic Life Water Quality Criteria. OPP developed a webpage of non-regulatory taxa-specific endpoints referred to as “OPP Aquatic Benchmarks”<sup>8</sup>. These Benchmarks are based on the most sensitive acute and chronic toxicity test results for fish and invertebrates (considering registrant-submitted studies and the scientific literature) from OPP’s ecological risk assessments of specific pesticides. OPP’s acute toxicity test results are lethal concentrations to 50% of the animals tested (LC<sub>50</sub>) and adverse effect concentrations for 50% of the animals tested (EC<sub>50</sub>) values for freshwater vertebrates and invertebrates. Chronic toxicity test results are no observed adverse effect concentrations (NOECs) for the same taxa. Benchmarks are calculated by multiplying each lowest toxicity result for a taxon by its respective Level of Concern (acute risk LOC = 0.5; chronic risk LOC = 1.0), which is based on OPP’s ecological risk assessment process. LOCs are

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<sup>8</sup> OPP Aquatic Benchmark Table. Available online at:  
[http://www.epa.gov/oppefed1/ecorisk\\_ders/aquatic\\_life\\_benchmark.htm](http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm)

the Agency's interpretative policy and are used to analyze potential risk to non-target organisms and the need to consider regulatory action. LOCs are used to indicate when a pesticide use (as directed on the label) has the potential to cause adverse effects on non-target organisms (USEPA 2004). In the context of OPP's risk assessment, any water concentrations exceeding a benchmark for a specific taxon have potential to cause adverse effects to that taxon. OPP's benchmarks may be useful for interpreting the potential effects of pesticides in surface water in cases where there is no existing Aquatic Life Water Quality Criteria. If the FIFRA data requirements are fulfilled, then benchmarks should be available for freshwater fish and invertebrates.

To put the OPP benchmark approach in terms of EFs, this is equivalent to dividing the lowest acute toxicity test result for a taxon by 2 and the lowest chronic toxicity test result by 1. For the acute benchmark, this is comparable to OW's approach for calculating acute criteria value by dividing the 5<sup>th</sup> percentile LC50 value 2 to establish an LC "low" (see section 3.1.3.2).

To illustrate the methods employed in deriving OPP Aquatic Benchmarks the same subset of aquatic toxicity data for carbaryl used to illustrate SSD methods is used here to simulate a chemical with limited toxicity data (Table 3). For the OPP Aquatic Benchmarks, two acute benchmarks are selected: one for aquatic invertebrates and one for fish. In this example, toxicity data are available for one species of invertebrate (*i.e.*, *D. magna*). The lowest single toxicity test result available for *D. magna* is used to derive the freshwater invertebrate acute toxicity benchmark by dividing by 2. Therefore, the freshwater invertebrate acute benchmark is 2.8 µg/L. Although toxicity data are available for two species of fish, the lowest single result of all the tests is selected and then divided by 2 to calculate the fish benchmark. The resulting freshwater vertebrate benchmark value for this example is 545 µg/L.

### 3.2.3.2. Great Lakes Water Quality Guidance (and similar approaches)

The Great Lakes Water Quality Guidance (USEPA 1995) was developed by USEPA with participation from the eight Great Lakes states (*i.e.*, Illinois, Indiana, Michigan, Minnesota, New York, Ohio, Pennsylvania and Wisconsin) as part of the Great Lakes Water Quality Initiative. The purpose of this guidance is to help establish consistent, enforceable, long-term protection with respect to all types of pollutants. The guidance includes a two-tiered approach to derive criteria, including: 1) Tier I values for which data meet the minimum data requirement as given in the 1985 Guidelines<sup>9</sup>, and 2) Tier II values where there is an absence of the full set of data needed to meet Tier I data requirements. Due to limited data used to derive Tier II criteria, these values are likely to be more uncertain compared to Tier I criteria. Tier II criteria are intended to be more protective, on average, than what Tier I criteria would be for the same chemicals if

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<sup>9</sup> For acute criteria, the data requirements include toxicity data for:

- a salmonid fish
- a nonsalmonid fish
- a species from a third chordate family
- a planktonic crustacean
- a benthic crustacean
- an insect
- a species from a family in a phylum other than Chordata or Arthropoda
- a species from a family in another order of insect or in a fourth phylum

sufficient data were available. Of interest here are the Tier II criteria values, which are derived using EFs.

For the Tier II method, all acute toxicity data are collected for a chemical. The geometric mean of the toxicity data for each genus is calculated. The number of data requirements that are met is determined. In order to calculate an acute Tier II criterion, the dataset must contain an acute toxicity result for at least one daphnid species (*i.e.*, *Ceriodaphnia sp.*, *Daphnia sp.*, or *Simocephalus sp.*) The number of data requirements that are met is used to determine the appropriate EF (Table 5). When the EFs are applied to the lowest genus mean acute value, the result is intended to approximate the 5<sup>th</sup> percentile of a species sensitivity distribution (triangular continuous probability distribution) as used in the 1985 Guidelines. With this method, the acute Tier II criterion is calculated by dividing the lowest genus mean acute value by the appropriate EF (this result is termed the “secondary acute value” abbreviated SAV) and then dividing that value by 2. The EFs were calculated using 29 toxicity datasets analyzed to determine the effects of removing taxa on calculation of the 5<sup>th</sup> percentile (Host et al. 1995). These factors are intended to address variability in interspecies sensitivity to chemical stressors.

**Table 5. Extrapolation factors used by Great Lakes Water Quality Guidance, Michigan DEQ, Ohio EPA, USDOE, and UC Davis in deriving criteria when data requirements defined in 1985 Guidelines are not met.**

Number of data requirements that are met	Great Lakes Water Quality Guidance*	Michigan DEQ*	Ohio EPA*	USDOE*	USDOE (no daphnid)	UC-Davis*
1	21.9	Not applicable	21.9	20.5	242	570
2	13.0	13.0	13.0 or 7.9 **	13.2	64.8	36
3	8.0	8.0	8.0	8.6	36.2	7.8
4	7.0	7.0	7.0	6.5	20.1	5.1
5	6.1	6.1	6.1	5.0	12.9	3.8
6	5.2	5.2	5.2	4.0	9.2	NA
7	4.3	4.3	4.3	3.6	7.2	NA

\*Data from at least one daphnid species is available.

\*\* If the family salmoindae is not represented, this value is 13.0. If the family salmoindae is represented, this value is 7.9.

NA = not applicable

A number of similar approaches are in use by state agencies (*i.e.*, Michigan Department of Environmental Quality (MDEQ; MNDEQ 2006) and Ohio EPA (Ohio EPA 2008)) and the U.S. Department of Energy (USDOE; Suter and Tsao 1996). These approaches involve EFs that are modifications of the Great Lakes Water Quality Guidance (Table 5). The MDEQ and Ohio EPA EFs are identical to the Great Lakes Water Quality Guidance, except that the Michigan approach does not provide an EF when only one data requirement is met and the Ohio EPA has two different EFs available when two data requirements are met: one for when a salmonid test result is available, and one for when a salmonid test result is not available. The USDOE differs from the Great Lakes Water Quality Guidance in that it provides a separate set of EFs for use when no daphnid test results are available.

For the Great Lakes Water Quality Guidance and other similar methods described above (*i.e.*, MI DEQ, Ohio EPA, USEOE), the example data set for carbaryl (Table 3) fulfills 3 data requirements (*i.e.*, a salmonid fish, a non-salmonid fish and a planktonic crustacean). Therefore,

the EF of 8.0 is used. For this guidance, the lowest mean value of toxicity test results available for a genus is selected. In this example, the most sensitive genus is *Daphnia sp.*, with a mean toxicity test results of 7.5 µg/L. The SAV of 0.94 µg/L is calculated by dividing the daphnid toxicity value by 8.0. As indicated above, this value is intended to be representative of the 5<sup>th</sup> percentile of a triangular distribution, as calculated according to the 1985 Guidelines.

With the UC-Davis Methodology (TenBrook et al. 2010), acute criteria are derived using EFs when toxicity data are not available for the 5 required species defined in this method. The EFs for this method (Table 5) were empirically derived by applying the procedure of Host et al. (1995), using a database of ten of the pesticides also used to determine the most appropriate SSD data distribution. EFs based on having 1 through 5 of the required taxa were generated for each of the ten pesticides, and then a final EF was calculated for taxonomic samples sizes of 1-5. This approach requires that one of the data values be from a daphnid species. The EFs were cross validated by comparing them to criterion values derived using the SSD method. For 2-5 taxonomic groups, the EF approach (lowest acute value in the dataset divided by the appropriate EF) yielded lower criteria than the SSD approach. The EF approach for one taxon yielded higher criteria estimates for two pesticides in the dataset. For these pesticides, daphnids were not the most sensitive species and the EF approach overestimated the HC<sub>5</sub> by a factor of 8-10. Thus, an additional safety factor of ten was included for cases where data for only one taxon is available.

To illustrate the use of the UC-Davis methodology using the carbaryl dataset used previously (Table 3), 3 of the 5 data requirements are met by the example data set for carbaryl, resulting in the use of 7.8 as an EF. The lowest species mean acute value (*i.e.*, 7.5 µg/L) is divided by this EF to yield a value of 0.96 µg/L. This result is similar to the result produced by the Great Lakes Water Quality Guidance (*i.e.*, 0.94 µg/L).

#### 3.2.4. Extrapolation factors Described in the Scientific Literature

Pennington 2003 expanded upon the work of de Zwart (2002) who explored the use of a logistic sensitivity distribution with known variability ( $\beta$ ) based on chemical mode of action (see section 3.1.2.2). Pennington's work is essentially an application of de Zwart's work on SSDs but is expressed in the form of EFs. Pennington considered the application of known  $\beta$  values in combination with uncertainty associated with sample mean values based on varying N, where  $N \leq 8$ . Pennington determined EFs (note that the author termed them "application factors") relating the lowest sample toxicity test result to HC<sub>5</sub> values from log-normal, log-logistic and log-triangular distributions in combination with the range of  $\beta$  values recommended by de Zwart (0.24-1.4). Using this approach, HC<sub>5</sub> values could be estimated with 50% and 97.5% confidence by dividing the lowest available toxicity test result by the EF representing the appropriate N for the sample of interest. Table 6 provides the EFs for estimating HC<sub>5</sub> values with 50% confidence. Pennington also determined EFs using  $\beta$  values for Quinolines (*i.e.*,  $\beta = 0.28$ ) and AChE inhibition by organophosphates (*i.e.*,  $\beta = 0.71$ ). Table 7 provides the EFs for estimating HC<sub>5</sub> values with 50% confidence and assuming a log-logistic distribution.

**Table 6. Extrapolation factors reported by Pennington (2003) for estimating HC<sub>5</sub> values with 50% confidence assuming different distributions. Values derived using range of  $\beta$  values recommended by de Zwart (2002).**

N	Log-normal	Log-logistic	Log-triangular
2	24	27	23
3	10	12	9.4
4	6.0	7.1	5.5
5	4.2	4.9	3.9
6	3.1	3.5	2.8
7	2.5	2.8	2.3
8	2.0	2.3	1.9

**Table 7. Extrapolation factors reported by Pennington (2003) for estimating HC<sub>5</sub> values with 50% confidence for quinolines and organophosphates.**

N	quinolines	organophosphates
2	3.8	25
3	2.8	12
4	2.3	7.2
5	2.0	4.9
6	1.7	3.7
7	1.5	2.9
8	1.4	2.2

The carbaryl dataset in Table 3 is used to illustrate the application of Pennington’s EFs. When considering Pennington’s EFs for the log-normal, log-logistic and log-triangular distributions when considering the availability of 3 toxicity test results, the HC<sub>5</sub> values range 0.63-0.80  $\mu\text{g/L}$  (Table 8). These are comparable to the results of the Great lakes water quality guidance (0.94  $\mu\text{g/L}$ ) and TenBrook et al. 2010 (0.96  $\mu\text{g/L}$ ), which are intended to approximate the HC<sub>5</sub> values of the triangular and burr distributions, respectively.

**Table 8. Example OPP benchmarks and Tier II criterion for sub-set of carbaryl data.**

Description	Test result ( $\mu\text{g/L}$ )	Extrapolation factor	Value ( $\mu\text{g/L}$ )
Log-normal (Pennington 2003)	7.5	10	0.75
Log-logistic (Pennington 2003)	7.5	12	0.63
Log-triangular (Pennington 2003)	7.5	9.4	0.80

#### 4. Proposed Analyses of Methods for Deriving Measures of Effect from Results of Toxicity Tests

The goal of this work is to examine how limited test results can best be used to characterize adverse effects on aquatic animals. To that end, Section 3 explores two general types of methods (*i.e.*, sensitivity distributions and extrapolation factors) that may be used to extrapolate from toxicity test results to taxa-specific and community-based measures of effect relevant to OPP and to OW. A portion of this work will address the derivation of an “Aquatic Life Screening Value” that is related to the fifth percentile in a Sensitivity Distribution (*i.e.*, the HC<sub>5</sub>). ALSVs may be

used to screen concentrations of pesticides and effluents in ambient waters and may be used by States and Tribes in the development of water quality standards. ALSVs are further discussed below in Section 5. Other portions of this work will address other percentiles in sensitivity distributions that can be used to evaluate concentrations of pesticides in ambient water in other ways (*e.g.*, for ecological risk assessment). This section describes the analyses that USEPA proposes to conduct to determine the utility of these methods to estimate  $HC_p$  based on "data-limited" sets. These proposed analyses will not simply compare published methods, but rather will use these methods as sources of ideas for development of improved methods for estimating the  $HC_p$  to a specified level of confidence.

#### 4.1. Analytical Strategy

##### 4.1.1 Definitions

The measurement of effect of interest to this effort is the  $HC_p$ , where "p" denotes the proportion of species adversely affected by a pesticide under laboratory test conditions, which can be confidently estimated for "data-rich" chemicals (*i.e.*, those with large amounts of toxicity test data available). Because there will be uncertainty about the "true" value of the  $HC_p$ , especially for "data-limited" chemicals, the proposed work will develop methods for the statistic  $HC_p^{cl}$  (See section 3.1.2.1). These methods will be developed to accommodate a range of values for p and cl, specific values for which can be established as appropriate for specific applications.

Because the term extrapolation factor has been used by different authors to mean different things (see Section 3), it is important to define its meaning for the analyses proposed here. Simply put, an EF is a factor applied to estimate the  $HC_p^{cl}$  based on a value derived from the available data. In more specific terms, it is the expected ratio between a location statistic (*e.g.*, mean) within the data set of interest and the  $HC_p^{cl}$ . An EF might be applied to the lowest value among the available data (such as in the Host et al. (1995) approach described in Section 3.2.3.2), or to some other characteristic of the data, such as the mean value. Accordingly, the magnitude of an EF will depend upon several factors, including the statistic used to describe the available data (minimum, mean), the confidence level to which the  $HC_p$  is estimated (higher confidence will result in larger EFs), as well as the amount and type of available data (fewer data will generally result in larger EFs). The focus of the work outlined below will be to evaluate the ability of different EF derivation approaches to estimate  $HC_p^{cl}$ , recognizing that this may vary depending on the nature of available data.

Analysis of the continuum of available aquatic animal data for pesticides will allow for inference on data sufficiency that may be dependent on the taxonomic sensitivity to a given pesticide AOP, and provide a scientifically defensible basis for the use of available benchmark methods discussed in this white paper. Because USEPA will investigate several techniques for specifying  $HC_p^{cl}$ , it will be necessary to determine which techniques produce the most accurate estimates. This will be particularly challenging because, even with "data rich" pesticides, the "true"  $HC_p$  is unknown. The work proposed here will use two strategies for addressing this



unknown. First, data rich sets will be used to specify a "reference"  $HC_{p,ref}$  to provide a surrogate for the true  $HC_p$  for use in the evaluations. Second, the statistical characteristics of data rich sets will be used to develop distributions for which the true  $HC_p$  is known and from which samples can be drawn to test methodologies for data-limited sets. All of the proposed analyses described below will entail various evaluations of accuracy and precision. However, in the interests of readability and brevity, this is not separately noted for each task.

#### 4.1.2. Anticipated Sources of Variation in the Estimated $HC_p$

Many factors affect the value of an  $HC_p$  estimate derived from test data, including (but not limited to): 1) total number of test results available for analysis; 2) taxonomic diversity of available test results; 3) degree of within species (or genus) test replication and associated variability; and 4) knowledge of the AOP of the pesticide. The proposed analyses will explore each of these potential influences.

#### 4.1.3. Analytical Steps

The general analytical strategy will involve two steps. In the first step,  $HC_p$  estimation will be evaluated under "data rich" conditions. This work will support decisions regarding the best distributional assumptions and estimation techniques for sensitivity distribution analysis. It will also allow specification of an  $HC_{p,ref}$  to use in assessing method performance under "data limited" conditions. In the second step, data-limited samples of the data-rich sets will be constructed and their theoretical distributions will be used to test the performance of various options for sensitivity distribution analysis relative to the  $HC_{p,ref}$  values. Extrapolation factors for data-limited conditions will also be developed by various approaches described in Section 3 based on both distributional analyses and sampling of data-rich sets and theoretical distributions, and their uncertainty described relative to the  $HC_{p,ref}$  values. Unless specifically noted below, the same methods will be applied regardless of whether the toxicity values pertain to acute versus chronic endpoints or pertain to aquatic invertebrates, fish, or both.

#### 4.1.4. Data

Proposed analyses will be conducted using acute and chronic toxicity data for chemicals with modes of action representative of pesticides (*e.g.*, AChE inhibition). Acute toxicity values will be obtained from the Web-ICE database of empirical EC50 and LC50 values that are used to generate the ICE models. Chronic toxicity values will be obtained from a database called AquaChronTox, which is currently under development by USEPA. AquaChronTox contains chronic toxicity test results for aquatic animals from studies identified in the primary literature, OPP Data Evaluation Records, and the fathead minnow database managed by scientists at USEPA.

## 4.2. Specific Analyses

### 4.2.1. Analyses of Data-Rich Sets

Regardless of what method is employed, the estimation of an  $HC_p$  requires the estimation of a quantile from a sample of data. Further, it is desirable that the estimate be an unbiased estimate of the “population” quantile of interest (*i.e.*, the quantile of the distribution of sensitivities of *all* aquatic invertebrates, *all* fish, or both in a given aquatic community). As noted in Section 3, this requires choice of a distribution to fit to the sample and choice of a method for fitting the distribution. Specifically, the proposed analyses are:

- 1) Compare the fit of commonly used (log) distributions (Triangular, Normal, Logistic, Gompertz, Weibull, and Burr) to full sets of data for “data rich” chemicals and to composite distributions based on these data-rich sets.
- 2) Explore multiple methods for fitting the distributions to the data. In particular, the following will be compared:
  - a) linear regression techniques on transformed data (*e.g.*, after Erickson and Stephan 1988)
  - b) maximum likelihood (Edwards 1992), and
  - c) moment estimators (equating the sample mean and standard deviation with the parametric equations for the distribution of interest and solving for the distributional parameters). This is a commonly used method in the literature on Species Sensitivity Distributions (*e.g.*, Aldenberg and Slob 1993, Aldenberg and Jaworska 2000)
- 3) In performing the above comparisons, the proposed analyses will include investigation of methods for using information contained in:
  - a) replicate tests on a given species (genus) beyond simply taking the geometric mean
  - b) adverse outcome pathway of the pesticide (for example using AOP-specific variance estimates, de Zwart 2002).
  - c) taxonomic diversity of the “data rich” sample.
  - d) right-handed censoring of data sets.
- 4) Overall results will be used to assign an  $HC_{p,ref}$  for each data-rich chemical

USEPA will also consider distribution free methods (*e.g.*, Newman et al 2000) and compare the results of these methods to commonly used distributions and methods for fitting distributions to available data.

### 4.2.2 Analyses of Data-Limited Sets

#### 4.2.2.1 Direct Application of Distributional Analysis

Given an estimate of the  $HC_{p,ref}$  (*i.e.*, from 4.2.1 above) USEPA will explore the accuracy of  $HC_p$  estimates from distributional analysis of limited subsets of the same data. The same steps and

issues as in section 4.2.1 will be followed for this analysis of smaller sets. It will not necessarily be assumed that the best choices under “data rich” conditions will prove to be best under “data limited” conditions.

#### 4.2.2.2 Extrapolation Factors from Distributional Analysis

- 1) Toxicity values will be subject to analysis of variance to determine the variance of toxicity values for a chemical across all species (genera). For chemicals with the same AOP, an AOP-specific variance (including standard error of the variance estimate) will be estimated. The result of this task will be distributions of variances applicable to different AOPs, as well as a distribution of variances across all chemicals.
- 2) Based on the procedures of de Zwart (2002) and Pennington (2003), analyses of “data limited” subsets will be conducted using the AOP-specific variances determined in Task 1 to derive extrapolation factors for different “data limited” sample sizes.
- 3) The proposed analyses in Tasks 1 and 2 will be extended to consider the influence of taxonomic diversity of the subsamples on the accuracy of  $HC_p^{cl}$  estimates. This analysis will include consideration of species for which OPP typically receives toxicity test results.

#### 4.2.2.3. Extrapolation Factors from Statistical Re-sampling

- 1) For each “data rich” pesticide, extrapolation factors will be generated using the approach of Host et al. (1995), but expanding this approach to:
  - a) consider the impact of using individual toxicity test results rather than geometric mean values as the basis of the toxicity data subsets, and
  - b) describe how estimated extrapolation factors vary with taxonomic diversity of the underlying subsamples.
- 2) The relationship between the extrapolation factors for individual chemicals determined under Task 1 will be analyzed regarding their similarity within and across different AOPs.

#### 4.2.3. Use of Predicted Values

The availability of toxicity values that comprise the data for these analyses (LC50, EC50, NOEC, etc.) differs widely among pesticides and may be very limited in some cases, especially for recently developed pesticides. To address this, the analyses proposed above (sections 4.2.1-4.2.2) will be repeated in a second phase including the use of predictive methods (*i.e.*, QSAR, Read-Across/Bridging, ICE, and TCE) to augment empirical data. The use of acute-chronic ratios (below) will also be evaluated for augmenting scarce data on chronic toxicity.

#### 4.2.4 Considerations for Proposed Analyses of Chronic Toxicity

Two important issues arise in the estimation of an  $HC_p$  for chronic toxicity. First, the definition of a “chronic effect” is not always consistent, with some researchers classifying any toxicity test with a duration longer than an acute exposure period as a chronic test, whereas others apply more stringent standards (*e.g.*, only results of full or partial life-cycle tests, as defined in the 1985

Guidelines). Second, chronic test results (NOEC, LOEC and MATC) indicate an uncertain (or at least variable) degree of effect, though use of exposure-effect analysis and point estimates is increasing. To the extent possible, USEPA will re-analyze chronic toxicity data (currently being compiled in AquaChronTox) to obtain point estimates of effect (*e.g.*, EC20s) that will be subsequently used for HC<sub>p</sub> estimation. Whatever the chronic test result (ECx, NOEC, etc.), distributional analysis of empirical data will proceed largely as described in sections above (4.2.1 – 4.2.2). Proposed analyses that will be unique to chronic data include estimation of acute-chronic ratios.

In general, fewer chronic test results are available compared to acute tests. This naturally leads to investigation of methods to supplement chronic data through analysis of the relationship between acute and chronic toxicity. With paired acute and chronic data this is a straightforward exercise and generates a distribution of ACRs. Historically, researchers have used quantiles (often the 80<sup>th</sup> percentile) of the ACR distribution to specify a default ACR (Host et al. 1995, TenBrook et al. 2010). When paired data do not exist, several methods may be applied, making use of predictive methods (see Tools paper). The proposed analyses will include the following evaluations of the potential for using ACRs to strengthen estimates of chronic HC<sub>p</sub> and HC<sub>p</sub><sup>cl</sup>:

- 1) Use “data rich” chemicals to compile a distribution of ratios of HC<sub>p,acute</sub> to HC<sub>p,chronic</sub> and examine both the variance and quantiles of the resulting distribution.
- 2) Evaluate alternatives for estimating an ACR from chronic toxicity values for which paired acute values are not available, including ICE, QSARs, read-across, and using acute values from closely-related species (see Tools paper for a description of these methods).
- 3) Evaluate and develop guidance for applying read-across methods to estimate chronic values and ACRs for species having acute data but no chronic data.
- 4) Evaluate time-concentration effect (TCE) models (Mayer et al. 1994) for predicting results of chronic toxicity tests, including relationships between mortality and sublethal endpoints under chronic exposure.
- 5) Use screened chronic EC20s from AquaChronTox to determine the degree to which species-specific ACRs are predictable within AOPs, with a particular focus on well-developed methods for the narcosis AOP (Di Toro et al. 2000; Undine and DiToro 2009).
- 6) Development of AOP-specific default ACRs for all chemicals within a specific AOP by using a percentile of the distribution of available empirical ACRs across all taxa for the most sensitive taxa for each chemical where valid ACRs are available.

## 5. Potential Approach for Deriving Aquatic Life Screening Values for Chemicals with Limited Datasets

One of the objectives of this effort is to provide a conceptual approach that may be used to integrate chemical-specific data, tools and methods for deriving community level benchmarks (*i.e.*, ALSVs) that may be used by States and Tribes to set scientifically defensible water quality

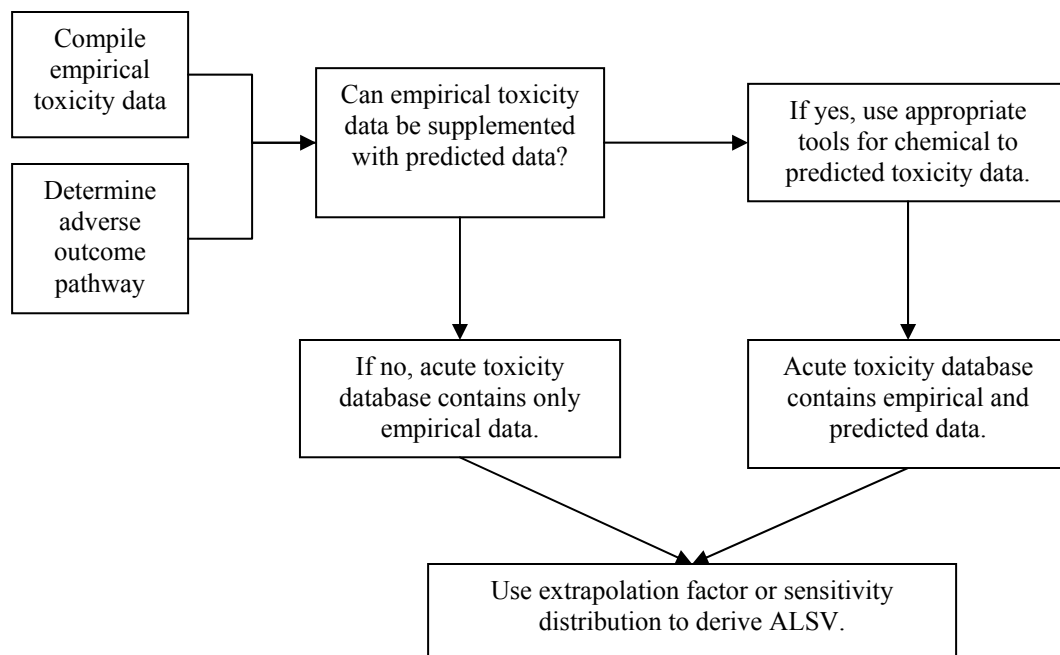
standards for pollutants for which USEPA Aquatic Life Water Quality Criteria are not yet available, and to interpret pesticide monitoring data. This is of particular interest for chemicals with limited toxicity data. Based on the information described above and in the tools paper, there are multiple approaches that may be used to accomplish this objective. Although the USEPA has not yet evaluated the specific sensitivity distribution and extrapolation factor methods described above, these methods may be considered to characterize the effects of chemicals to aquatic organisms. The appropriateness of specific methods may depend upon the amount of available data, the type of available data (*e.g.*, specific species for which toxicity data are available) and the mode of action of the chemical.

This section presents a potential framework for determining when to use different methods for chemicals with limited data. This framework is intended as an illustration of how various methods described in this paper and tools described in the tools white paper may be integrated to characterize the effects of a chemical on aquatic communities (not specific taxa). It is expected that once the methods described above are reviewed that this framework will be revised to incorporate the methods determined to have the greatest utility.

The term used to represent the community level benchmark that is derived from this framework is the aquatic life screening value (ALSV). Since different approaches are available for characterizing acute and chronic toxicity data, two separate ALSV frameworks are described below. Since the ALSV may be considered by USEPA, States and Tribes to derive scientifically defensible water quality criteria, and because water quality criteria established using the 1985 Guidelines are set to one half of the fifth percentile of a sensitivity distribution, the measure of effect for the acute ALSV is also set to one half of the HC<sub>5</sub> of a sensitivity distribution, and the chronic ALSV is set to the HC<sub>5</sub> of the distribution of chronic toxicity data. Due to uncertainty associated with limited data used to derive HC<sub>5</sub> values, ALSV calculations should incorporate conservative estimate of the HC<sub>5</sub>.

### 5.1. Derivation of Acute ALSV

A conceptual framework for deriving the acute ALSV is provided in Figure 4. The first step in deriving an acute ALSV is to compile all available acute toxicity data for aquatic animals. Data acceptability is based on standards established by OW (USEPA 1985) and by OPP (USEPA 1994, USEPA 2004). As noted previously, acute toxicity test results for aquatic invertebrates are defined as 48-h or 96-h EC<sub>50</sub> or LC<sub>50</sub> values. For vertebrates, acute toxicity test results are defined as 96-h LC<sub>50</sub>s.



**Figure 4. Conceptual framework for deriving acute ALSV.**

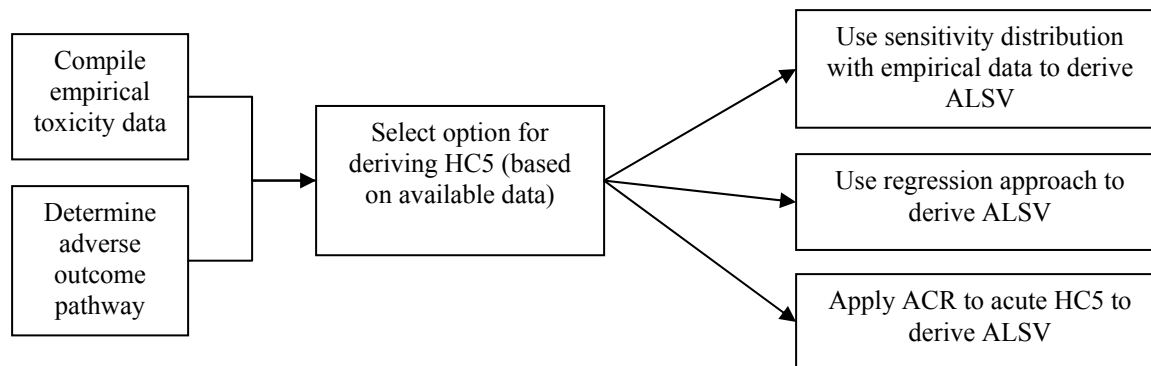
Once the acute toxicity database has been developed for a chemical, it is necessary to determine whether the empirical data can be supplemented with predicted or estimated toxicity data. This can be determined by considering the chemical's mode of action in the context of the utility of available tools. More information on specific tools and their applicability to chemicals with specific adverse outcome pathways is provided in the tools white paper. If toxicity data can be predicted or estimated for a chemical, then these data can be combined with available empirical data to represent the acute toxicity database for that chemical. If tools are not available to reliably predict or estimate the acute toxicity of the chemical, then the empirical data alone will represent the acute toxicity database for the chemical.

Once the acute toxicity database is available for a chemical, an ALSV can be derived. As noted above, there are two major approaches that may be used to derive an HC<sub>5</sub>. Extrapolation factors based on extrapolation from the most sensitive toxicity data to the HC<sub>5</sub> of a sensitivity distribution may be used. Also, an HC<sub>5</sub> may be derived from sensitivity distribution based on the mean and variance of the acute toxicity database. The specific extrapolation factors and distribution methods that may be most appropriate for various situations (considering number of available toxicity data and adverse outcome pathway of a chemical) will be determined after USEPA has evaluated the specific methods described above.

## 5.2. Derivation of Chronic ALSV

A conceptual framework for deriving the acute ALSV is provided in Figure 5. Like the acute ALSV, derivation of the chronic ALSV starts with compiling acceptable chronic toxicity test

results for aquatic animals. Chronic toxicity test results, which are based on various sublethal effects and exposure durations, differ much more from each other compared to acute toxicity test results, which are set durations of exposure most often based on mortality. In order to compare the relative sensitivities of species chronically exposed to a chemical, effort should be invested to make the test results as consistent as possible in terms of duration of exposure and the measure of effects (*e.g.*, NOECs, MATCs). Although limited tools are available for estimating chronic toxicity data, it may be possible to supplement the empirical toxicity data using predicted or estimated chronic values (*e.g.*, using ACRs or ACE models applied to acute toxicity data).



**Figure 5. Conceptual framework for deriving chronic ALSV.**

Once the chronic toxicity database is established, several options are available for deriving the HC<sub>5</sub>. One option is to use a sensitivity distribution to derive the HC<sub>5</sub>. A second option would be to apply a regression approach to derive the HC<sub>5</sub> (*e.g.*, de Zwart 2002, Duboudin et al. 2004). Another option, which is similar to the one used in the 1985 Guidelines, would be to apply an ACR to the acute HC<sub>5</sub>, which is derived for the acute ALSV. The ACR may be based on empirical toxicity data, a combination of empirical and predicted or estimated data for specific species or a default values. Default values may be generically applied to all chemicals (*e.g.*, Great Lakes Water Quality Guidance), or it may be possible to use a default ACR based on the mode of action of the chemical (*e.g.*, Raimondo et al. 2007).

### 5.3. Illustration of one approach for deriving ALSVs

Although there are many different tools and methods available for estimating an ALSV, one approach is used here to illustrate a potential way to derive acute and chronic ALSVs when data are limited. The approach for deriving the acute ALSV involves the use of the Web-ICE tool. Within Web-ICE there is an option for calculating a SSD with toxicity test results from as few as 1 species. This SSD is a log-logistic distribution (de Zwart 2002) of estimated toxicity data. In this example, the example data set used previously in this paper (Table 3) were used to derive a HC<sub>5</sub> of 12 µg/L (95%CI = 1.1-89) for carbaryl. This value is similar to the HC<sub>5</sub> value generated for the fitted log-logistic distribution from the full carbaryl data set, which was 10.2 µg/L (Table 1). Therefore, the acute ALSV for this example would be calculated by dividing the HC<sub>5</sub> by 2, resulting in a value of 6 µg/L. Note that this HC<sub>5</sub> estimate is based on estimated toxicity data for 42 species of freshwater and saltwater fish and invertebrates. The HC<sub>5</sub> estimate does not include

estimated toxicity test results from species where the input values were not within model maximum and minimum values.

The chronic ALSV could be calculated by applying an ACR to the HC<sub>5</sub> from the acute data. Raimondo et al. (2007) published ACRs based on mode of action. The median and 90<sup>th</sup> percentile ACRs for AChE inhibition by carbamates are 8.9 and 28, respectively. If the median ACR of 8.9 were applied to the acute HC<sub>5</sub>, the resulting chronic HC<sub>5</sub> would be 1.3 µg/L. If the 90<sup>th</sup> percentile ACR were applied to the acute HC<sub>5</sub>, the chronic HC<sub>5</sub> would be 0.43 µg/L. One of these chronic HC<sub>5</sub> values could be used to represent the chronic ALSV.

## 6. Conclusions

This paper identifies several different methods for extrapolating from available toxicity data to measures of effect relevant to the survival, growth and reproduction of aquatic vertebrates, invertebrates and animal communities. These methods include: sensitivity distributions and extrapolation factors. This paper describes the analyses that USEPA proposes to conduct to determine the utility of the specific methods in characterizing effects to aquatic organisms both in data-rich and data-limited instances. This paper also provides a conceptual approach that may be used to integrate chemical-specific data, tools and methods for deriving community level benchmarks (*i.e.*, ALSVs) that may be used by USEPA, States and Tribes to derive scientifically defensible water quality criteria or in ecological risk assessments. After the USEPA finishes its analysis of available tools and methods, this conceptual approach will be refined to incorporate specific methods and considerations for deriving ALSVs for chemicals.

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**Appendix A. Acute toxicity data available for Carbaryl in Web-ICE master database.**

Common Name	Species	Taxa	Test Duration	Dose Type	Test Type	Acute Toxicity Value (ug/L)	Data Source Category
African clawed frog	<i>Xenopus laevis</i>	Amphibia	96h	LC50	S	1730	ECOTOX
Amphipod	<i>Echinogammarus tibaldii</i>	Crustacea	96h	LC50	NR	6.5	ECOTOX
Amphipod	<i>Gammarus fasciatus</i>	Crustacea	96h	LC50	S	26	Mayer and Elliersieck
Amphipod	<i>Gammarus italicus</i>	Crustacea	96h	LC50	NR	28	ECOTOX
Amphipod	<i>Gammarus lacustris</i>	Crustacea	96h	LC50	S	22	Mayer and Elliersieck
Amphipod	<i>Gammarus pseudolimnaeus</i>	Crustacea	96h	LC50	S	7.2	ECOTOX
Amphipod	<i>Gammarus pseudolimnaeus</i>	Crustacea	96h	LC50	S	7	ECOTOX
Amphipod	<i>Gammarus pseudolimnaeus</i>	Crustacea	96h	LC50	S	13	ECOTOX
Amphipod	<i>Gammarus pseudolimnaeus</i>	Crustacea	96h	LC50	S	16	ECOTOX
Amphipod	<i>Gammarus pseudolimnaeus</i>	Crustacea	96h	LC50	S	16	ECOTOX
Amphipod	<i>Gammarus pseudolimnaeus</i>	Crustacea	96h	LC50	S	7.2	Mayer and Elliersieck
Amphipod	<i>Gammarus pseudolimnaeus</i>	Crustacea	96h	LC50	S	7	Mayer and Elliersieck
Amphipod	<i>Pontoporeia hoyi</i>	Crustacea	96h	LC50	R	250	ECOTOX
Apache trout	<i>Oncorhynchus gilae</i>	Fishes	96h	LC50	S	1435	Mayer 2008
Apache trout	<i>Oncorhynchus gilae</i>	Fishes	96h	LC50	S	1500	ECOTOX
Apache trout	<i>Oncorhynchus gilae</i>	Fishes	96h	LC50	S	1540	ECOTOX
Apache trout	<i>Oncorhynchus gilae</i>	Fishes	96h	LC50	S	1650	ECOTOX
Apache trout	<i>Oncorhynchus gilae</i>	Fishes	96h	LC50	S	1430	ECOTOX
Appalachian crayfish	<i>Cambarus bartonii</i>	Crustacea	96h	LC50	R	2240	ECOTOX
Appalachian crayfish	<i>Cambarus bartonii</i>	Crustacea	96h	LC50	R	2900	ECOTOX
Appalachian crayfish	<i>Cambarus bartonii</i>	Crustacea	96h	LC50	R	2410	ECOTOX
Appalachian crayfish	<i>Cambarus bartonii</i>	Crustacea	96h	LC50	R	2230	ECOTOX
Appalachian crayfish	<i>Cambarus bartonii</i>	Crustacea	96h	LC50	R	940	ECOTOX
Argentine common toad	<i>Bufo arenarum</i>	Amphibia	96h	LC50	S	24640	ECOTOX

Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	1150	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	905	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	1430	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	1350	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	1180	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	4500	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	1000	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	900	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	1270	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	500	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	2010	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	1100	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	1000	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	2070	Mayer and Elliersieck
Atlantic salmon	<i>Salmo salar</i>	Fishes	96h	LC50	S	250	Mayer and Elliersieck
Backswimmer	<i>Notonecta undulata</i>	Insecta	96h	LC50	S	200	ECOTOX
Backswimmer	<i>Notonecta undulata</i>	Insecta	48h	LC50	S	360	ECOTOX
Bayou darter	<i>Etheostoma rubrum</i>	Fishes	96h	LC50	S	2020	ECOTOX
Black bullhead	<i>Ameiurus melas</i>	Fishes	96h	LC50	S	20000	Mayer and Elliersieck
Black crappie	<i>Pomoxis nigromaculatus</i>	Fishes	96h	LC50	S	2600	OPP
Black crappie	<i>Pomoxis nigromaculatus</i>	Fishes	96h	LC50	S	2600	Mayer and Elliersieck
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	14000	OPP
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	5230	Mayer and Elliersieck
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	5200	Mayer and Elliersieck
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	8200	Mayer and Elliersieck
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	6760	Mayer and Elliersieck
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	5600	ECOTOX
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	1000	ECOTOX
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	2200	ECOTOX
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	F	5047	Mayer and Elliersieck
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	6200	Mayer and Elliersieck
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	7400	Mayer and Elliersieck
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	2600	Mayer and Elliersieck
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	1800	Mayer and Elliersieck
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	5200	Mayer and Elliersieck

Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	7000	Mayer and Elliersieck
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	16000	Mayer and Elliersieck
Bluegill	<i>Lepomis macrochirus</i>	Fishes	96h	LC50	S	5400	Mayer and Elliersieck
Bonytail	<i>Gila elegans</i>	Fishes	96h	LC50	S	3490	Literature
Bonytail	<i>Gila elegans</i>	Fishes	96h	LC50	R	2020	ECOTOX
Bonytail	<i>Gila elegans</i>	Fishes	96h	LC50	R	650	ECOTOX
Bonytail	<i>Gila elegans</i>	Fishes	96h	LC50	S	3500	ECOTOX
Bonytail	<i>Gila elegans</i>	Fishes	96h	LC50	S	3490	ECOTOX
Bonytail	<i>Gila elegans</i>	Fishes	96h	LC50	S	3580	ECOTOX
Bonytail	<i>Gila elegans</i>	Fishes	96h	LC50	S	3400	ECOTOX
Bonytail	<i>Gila elegans</i>	Fishes	96h	LC50	S	3044	Mayer 2008
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	1130	Mayer and Elliersieck
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	2130	Mayer and Elliersieck
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	2100	Mayer and Elliersieck
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	1200	Mayer and Elliersieck
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	4560	Mayer and Elliersieck
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	1290	Mayer and Elliersieck
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	1450	ECOTOX
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	1070	ECOTOX
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	1100	ECOTOX
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	5400	ECOTOX
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	2500	ECOTOX
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	900	ECOTOX
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	3700	ECOTOX
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	680	Mayer and Elliersieck
Brook trout	<i>Salvelinus fontinalis</i>	Fishes	96h	LC50	S	3000	Mayer and Elliersieck
Brown trout	<i>Salmo trutta</i>	Fishes	96h	LC50	S	700	ECOTOX
Brown trout	<i>Salmo trutta</i>	Fishes	96h	LC50	F	2000	Mayer and Elliersieck
Brown trout	<i>Salmo trutta</i>	Fishes	96h	LC50	S	6300	Mayer and Elliersieck
Brown trout	<i>Salmo trutta</i>	Fishes	96h	LC50	S	1950	ECOTOX
Cape Fear shiner	<i>Notropis mekistocholas</i>	Fishes	96h	LC50	S	4510	Literature
Cape Fear shiner	<i>Notropis mekistocholas</i>	Fishes	96h	LC50	S	4264	Mayer 2008
Cape Fear shiner	<i>Notropis mekistocholas</i>	Fishes	96h	LC50	S	4510	ECOTOX
Catla	<i>Gibelion catla</i>	Fishes	96h	LC50	R	6400	ECOTOX
Channel catfish	<i>Ictalurus punctatus</i>	Fishes	96h	LC50	S	7790	Mayer and Elliersieck

Channel catfish	<i>Ictalurus punctatus</i>	Fishes	96h	LC50	S	15800	Mayer and Elliersieck
Channel catfish	<i>Ictalurus punctatus</i>	Fishes	96h	LC50	NR	140	ECOTOX
Channel catfish	<i>Ictalurus punctatus</i>	Fishes	96h	LC50	S	1300	ECOTOX
Channel catfish	<i>Ictalurus punctatus</i>	Fishes	96h	LC50	S	1560	ECOTOX
Channel catfish	<i>Ictalurus punctatus</i>	Fishes	96h	LC50	F	17300	Mayer and Elliersieck
Chinook salmon	<i>Oncorhynchus tshawytscha</i>	Fishes	96h	LC50	F	2400	Mayer and Elliersieck
Climbing perch	<i>Anabas testudineus</i>	Fishes	96h	LC50	R	5500	ECOTOX
Coho salmon	<i>Oncorhynchus kisutch</i>	Fishes	96h	LC50	S	4340	Mayer and Elliersieck
Coho salmon	<i>Oncorhynchus kisutch</i>	Fishes	96h	LC50	S	2700	Mayer and Elliersieck
Coho salmon	<i>Oncorhynchus kisutch</i>	Fishes	96h	LC50	S	2400	Mayer and Elliersieck
Coho salmon	<i>Oncorhynchus kisutch</i>	Fishes	96h	LC50	S	1750	Mayer and Elliersieck
Coho salmon	<i>Oncorhynchus kisutch</i>	Fishes	96h	LC50	S	1150	Mayer and Elliersieck
Coho salmon	<i>Oncorhynchus kisutch</i>	Fishes	96h	LC50	S	997	ECOTOX
Coho salmon	<i>Oncorhynchus kisutch</i>	Fishes	96h	LC50	S	1300	ECOTOX
Coho salmon	<i>Oncorhynchus kisutch</i>	Fishes	96h	LC50	S	764	ECOTOX
Colorado squawfish	<i>Ptychocheilus lucius</i>	Fishes	96h	LC50	SR	1310	Literature
Colorado squawfish	<i>Ptychocheilus lucius</i>	Fishes	96h	LC50	S	3070	Literature
Colorado squawfish	<i>Ptychocheilus lucius</i>	Fishes	96h	LC50	R	1310	ECOTOX
Colorado squawfish	<i>Ptychocheilus lucius</i>	Fishes	96h	LC50	S	3100	ECOTOX
Colorado squawfish	<i>Ptychocheilus lucius</i>	Fishes	96h	LC50	S	3070	ECOTOX
Colorado squawfish	<i>Ptychocheilus lucius</i>	Fishes	96h	LC50	S	4060	ECOTOX
Colorado squawfish	<i>Ptychocheilus lucius</i>	Fishes	96h	LC50	S	2320	ECOTOX
Colorado squawfish	<i>Ptychocheilus lucius</i>	Fishes	96h	LC50	S	3038	Mayer 2008
Common carp	<i>Cyprinus carpio</i>	Fishes	96h	LC50	S	4220	ECOTOX
Common carp	<i>Cyprinus carpio</i>	Fishes	96h	LC50	S	5280	Mayer and Elliersieck
Common carp	<i>Cyprinus carpio</i>	Fishes	96h	LC50	S	1700	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	5000	Mayer and Elliersieck
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	2221	Mayer 2008
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	1553	Mayer 2008
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	2250	Literature
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	3950	Mayer and Elliersieck
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	6700	Mayer and Elliersieck
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	970	Mayer and Elliersieck
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	7100	Mayer and Elliersieck



Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	2169	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	1500	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	NR	3950	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	NR	970	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	NR	3950	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	NR	6000	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	NR	5000	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	2300	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	2250	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	2530	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	2000	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	6000	Mayer and Elliersieck
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	1600	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	1550	ECOTOX
Cutthroat trout	<i>Oncorhynchus clarkii</i>	Fishes	96h	LC50	S	1550	ECOTOX
Daphnid	<i>Ceriodaphnia dubia</i>	Crustacea	48h	EC50	R	3.06	ECOTOX
Daphnid	<i>Ceriodaphnia dubia</i>	Crustacea	48h	LC50	S	11.6	ECOTOX
Daphnid	<i>Daphnia magna</i>	Crustacea	48h	EC50	R	10.1	ECOTOX
Daphnid	<i>Daphnia magna</i>	Crustacea	48h	LC50	S	9.5	ECOTOX
Daphnid	<i>Daphnia magna</i>	Crustacea	96h	LC50	S	3280	ECOTOX
Daphnid	<i>Daphnia magna</i>	Crustacea	48h	LC50	S	16760	ECOTOX
Daphnid	<i>Daphnia magna</i>	Crustacea	48h	LC50	S	7.2	ECOTOX
Daphnid	<i>Daphnia magna</i>	Crustacea	48h	LC50	R	1900	ECOTOX
Daphnid	<i>Daphnia magna</i>	Crustacea	48h	LC50	R	5400	ECOTOX
Daphnid	<i>Daphnia magna</i>	Crustacea	48h	LC50	NR	230	ECOTOX
Daphnid	<i>Daphnia magna</i>	Crustacea	48h	EC50	S	5.6	Mayer and Elliersieck
Daphnid	<i>Daphnia obtusa</i>	Crustacea	48h	EC50	S	0.0115	ECOTOX
Daphnid	<i>Daphnia pulex</i>	Crustacea	48h	LC50	S	6.4	Mayer and Elliersieck
Daphnid	<i>Simocephalus serrulatus</i>	Crustacea	48h	EC50	S	8.1	Mayer and Elliersieck
Daphnid	<i>Simocephalus serrulatus</i>	Crustacea	48h	EC50	S	7.6	Mayer and Elliersieck
Daphnid	<i>Simocephalus serrulatus</i>	Crustacea	48h	EC50	S	11	Mayer and Elliersieck
Desert pupfish	<i>Cyprinodon macularius</i>	Fishes	96h	LC50	S	7710	ECOTOX
Dragonfly	<i>Austrolestes colenisonis</i>	Insecta	48h	LC50	S	3130.9	ECOTOX
Dragonfly	<i>Xanthocnemis zealandica</i>	Insecta	48h	LC50	S	600	ECOTOX

Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	7000	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	13000	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	F	8930	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	F	6670	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	F	10400	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	F	9470	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	5210	Literature
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	5171	Mayer 2008
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	5200	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	5210	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	7430	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	5860	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	5720	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	5240	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	4380	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	3940	ECOTOX
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	7700	Mayer and Elliersieck
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	14000	Mayer and Elliersieck
Fathead minnow	<i>Pimephales promelas</i>	Fishes	96h	LC50	S	14600	Mayer and Elliersieck
Flying barb	<i>Esomus danricus</i>	Fishes	96h	LC50	NR	34670	ECOTOX
Fountain darter	<i>Etheostoma fonticola</i>	Fishes	96h	LC50	S	2020	Literature
Fountain darter	<i>Etheostoma fonticola</i>	Fishes	96h	LC50	S	2020	ECOTOX
Fountain darter	<i>Etheostoma fonticola</i>	Fishes	96h	LC50	S	1615	Mayer 2008
Gangetic mystus	<i>Mystus cavasius</i>	Fishes	96h	LC50	R	4600	ECOTOX
Goldfish	<i>Carassius auratus</i>	Fishes	96h	LC50	S	12800	Mayer and Elliersieck
Goldfish	<i>Carassius auratus</i>	Fishes	96h	LC50	S	13200	Mayer and Elliersieck
Gray treefrog	<i>Hyla versicolor</i>	Amphibia	96h	LC50	S	2470	ECOTOX
Green frog	<i>Rana clamitans</i>	Amphibia	96h	LC50	S	11320	ECOTOX
Green frog	<i>Rana clamitans</i>	Amphibia	96h	LC50	S	17360	ECOTOX
Green frog	<i>Rana clamitans</i>	Amphibia	96h	LC50	S	22020	ECOTOX
Green sunfish	<i>Lepomis cyanellus</i>	Fishes	96h	LC50	S	9460	Mayer and Elliersieck
Green sunfish	<i>Lepomis cyanellus</i>	Fishes	96h	LC50	S	11200	Mayer and Elliersieck
Greenthroat darter	<i>Etheostoma lepidum</i>	Fishes	96h	LC50	S	2140	Literature
Greenthroat darter	<i>Etheostoma lepidum</i>	Fishes	96h	LC50	S	2143	Mayer 2008
Greenthroat darter	<i>Etheostoma lepidum</i>	Fishes	96h	LC50	S	2140	ECOTOX

Greenthroat darter	<i>Etheostoma lepidum</i>	Fishes	96h	LC50	S	2140	ECOTOX
Guppy	<i>Poecilia reticulata</i>	Fishes	96h	LC50	S	6500	ECOTOX
Guppy	<i>Poecilia reticulata</i>	Fishes	96h	LC50	S	9740	ECOTOX
Guppy	<i>Poecilia reticulata</i>	Fishes	96h	LC50	S	3840	ECOTOX
Isopod	<i>Caecidotea brevicauda</i>	Crustacea	96h	LC50	S	280	Mayer and Elliersieck
Kuncho river prawn	<i>Macrobrachium lamarrei</i>	Crustacea	96h	LC50	R	19	ECOTOX
Lake trout	<i>Salvelinus namaycush</i>	Fishes	96h	LC50	F	2300	Mayer and Elliersieck
Lake trout	<i>Salvelinus namaycush</i>	Fishes	96h	LC50	S	872	Mayer and Elliersieck
Lake trout	<i>Salvelinus namaycush</i>	Fishes	96h	LC50	S	920	Mayer and Elliersieck
Lake trout	<i>Salvelinus namaycush</i>	Fishes	96h	LC50	S	690	Mayer and Elliersieck
Lake trout	<i>Salvelinus namaycush</i>	Fishes	96h	LC50	S	740	Mayer and Elliersieck
Largemouth bass	<i>Micropterus salmoides</i>	Fishes	96h	LC50	S	6400	OPP
Largemouth bass	<i>Micropterus salmoides</i>	Fishes	96h	LC50	S	6400	Mayer and Elliersieck
Leon springs pupfish	<i>Cyprinodon bovinus</i>	Fishes	96h	LC50	S	2017	Mayer 2008
Leon springs pupfish	<i>Cyprinodon bovinus</i>	Fishes	96h	LC50	S	4540	ECOTOX
Midge	<i>Chironomus plumosus</i>	Insecta	48h	EC50	S	10	ECOTOX
Mississippi grass shrimp	<i>Palaemonetes kadiakensis</i>	Crustacea	96h	LC50	S	5.6	Mayer and Elliersieck
Mysid	<i>Mysis relicta</i>	Crustacea	96h	LC50	R	230	ECOTOX
Oligochaete	<i>Lumbriculus variegatus</i>	Annelida	96h	LC50	S	8200	ECOTOX
Oligochaete	<i>Lumbriculus variegatus</i>	Annelida	48h	LC50	S	13000	ECOTOX
Paper pondshell	<i>Anodonta imbecillis</i>	Mollusca	48h	LC50	R	25600	ECOTOX
Paper pondshell	<i>Anodonta imbecillis</i>	Mollusca	48h	LC50	R	23700	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1880	Literature
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	F	1330	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	3000	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1950	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1450	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	780	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	3500	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1600	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	2300	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	2080	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1900	Mayer and Elliersieck

Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1360	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1460	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1090	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1200	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1866	Mayer 2008
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1350	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1470	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	R	5400	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	R	4330	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	5400	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	2830	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	4340	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	800	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1500	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	800	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	2200	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	900	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	2200	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	2800	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1900	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1880	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	3110	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	2260	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	2070	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	2000	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1240	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1220	ECOTOX
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1200	Mayer and Elliersieck
Rainbow trout	<i>Oncorhynchus mykiss</i>	Fishes	96h	LC50	S	1100	Mayer and Elliersieck
Razorback sucker	<i>Xyrauchen texanus</i>	Fishes	96h	LC50	S	4350	Literature
Razorback sucker	<i>Xyrauchen texanus</i>	Fishes	96h	LC50	S	4325	Mayer 2008
Razorback sucker	<i>Xyrauchen texanus</i>	Fishes	96h	LC50	S	4400	ECOTOX
Razorback sucker	<i>Xyrauchen texanus</i>	Fishes	96h	LC50	S	4350	ECOTOX
Razorback sucker	<i>Xyrauchen texanus</i>	Fishes	96h	LC50	S	4420	ECOTOX
Razorback sucker	<i>Xyrauchen texanus</i>	Fishes	96h	LC50	S	4290	ECOTOX

Redear sunfish	<i>Lepomis microlophus</i>	Fishes	96h	LC50	S	11200	ECOTOX
Shortnose sturgeon	<i>Acipenser brevirostrum</i>	Fishes	96h	LC50	S	1810	Literature
Shortnose sturgeon	<i>Acipenser brevirostrum</i>	Fishes	96h	LC50	S	1949	Mayer 2008
Shortnose sturgeon	<i>Acipenser brevirostrum</i>	Fishes	96h	LC50	S	1810	ECOTOX
Snail	<i>Bellamya bengalensis</i>	Mollusca	96h	LC50	S	9020	ECOTOX
Snail	<i>Bellamya bengalensis</i>	Mollusca	96h	LC50	S	4320	ECOTOX
Snail	<i>Bellamya bengalensis</i>	Mollusca	96h	LC50	S	13500	ECOTOX
Snail	<i>Bellamya bengalensis</i>	Mollusca	96h	LC50	S	9600	ECOTOX
Southern leopard frog	<i>Rana sphenoccephala</i>	Amphibia	96h	LC50	S	7964	Mayer 2008
Southern leopard frog	<i>Rana sphenoccephala</i>	Amphibia	96h	LC50	S	8400	ECOTOX
Spotfin Chub	<i>Erimonax monachus</i>	Fishes	96h	LC50	S	3410	Literature
Spotfin Chub	<i>Erimonax monachus</i>	Fishes	96h	LC50	S	3416	Mayer 2008
Spotfin Chub	<i>Erimonax monachus</i>	Fishes	96h	LC50	S	3410	ECOTOX
Stonefly	<i>Claassenia sabulosa</i>	Insecta	96h	LC50	S	5.6	Mayer and Elliersieck
Stonefly	<i>Pteronarcella badia</i>	Insecta	96h	LC50	S	29	Mayer and Elliersieck
Stonefly	<i>Pteronarcella badia</i>	Insecta	96h	LC50	S	13	Mayer and Elliersieck
Stonefly	<i>Pteronarcella badia</i>	Insecta	96h	LC50	S	11	Mayer and Elliersieck
Stonefly	<i>Pteronarcella badia</i>	Insecta	96h	LC50	S	29	ECOTOX
Stonefly	<i>Pteronarcella badia</i>	Insecta	96h	LC50	S	13	ECOTOX
Stonefly	<i>Pteronarcella badia</i>	Insecta	96h	LC50	S	11	ECOTOX
Stonefly	<i>Pteronarcella badia</i>	Insecta	96h	LC50	S	1.7	Mayer and Elliersieck
Stonefly	<i>Pteronarcys californica</i>	Insecta	96h	LC50	S	2	ECOTOX
Stonefly	<i>Pteronarcys californica</i>	Insecta	96h	LC50	S	4.8	Mayer and Elliersieck
Striped black fly	<i>Simulium vittatum</i>	Insecta	48h	LC50	S	23.72	ECOTOX
Striped catfish	<i>Mystus vittatus</i>	Fishes	96h	LC50	R	2400	ECOTOX
Striped catfish	<i>Mystus vittatus</i>	Fishes	96h	LC50	S	11500	ECOTOX
Swamp lymnaea	<i>Lymnaea stagnalis</i>	Mollusca	48h	LC50	S	21000	ECOTOX
Tiger frog	<i>Rana tigrina</i>	Amphibia	96h	LC50	R	5680	ECOTOX
Two-spot barb	<i>Puntius ticto</i>	Fishes	96h	LC50	S	3700	ECOTOX
Virile crayfish	<i>Orconectes virilis</i>	Crustacea	96h	LC50	R	1890	ECOTOX
Western toad	<i>Bufo boreas</i>	Amphibia	96h	LC50	S	12300	Literature
Western toad	<i>Bufo boreas</i>	Amphibia	96h	LC50	S	12303	Mayer 2008
Western toad	<i>Bufo boreas</i>	Amphibia	96h	LC50	S	12300	ECOTOX
Western toad	<i>Bufo boreas</i>	Amphibia	96h	LC50	S	12310	ECOTOX
Yellow fever mosquito	<i>Aedes aegypti</i>	Insecta	96h	LC50	S	336	ECOTOX

Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	F	1420	Mayer and Elliersieck
Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	S	3750	Mayer and Elliersieck
Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	S	5000	Mayer and Elliersieck
Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	S	350	Mayer and Elliersieck
Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	S	480	Mayer and Elliersieck
Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	S	3800	Mayer and Elliersieck
Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	S	3400	Mayer and Elliersieck
Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	S	5400	Mayer and Elliersieck
Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	S	5100	Mayer and Elliersieck
Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	S	4200	Mayer and Elliersieck
Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	S	4000	Mayer and Elliersieck
Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	S	745	Mayer and Elliersieck
Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	S	1200	Mayer and Elliersieck
Yellow perch	<i>Perca flavescens</i>	Fishes	96h	LC50	S	13900	Mayer and Elliersieck

## Appendix B. Equations for the Parametric sensitivity distribution Distributions

In the equations below, we use standard statistical notation to describe common functions associated with probability distributions. The probability density function (pdf) will be referred to as  $f$ . The cumulative distribution function (cdf) will be referred to as  $F$ . The quantile function, which is the inverse of the cdf, will be referred to as  $F^{-1}$ . We provide formulae for the mean and variance of each distribution as functions of the parameters of the distribution. While these equate to the parameters  $\mu$  and  $\sigma$  for the normal distribution, this will not generally be the case, so we avoid using those symbols for the mean and variance, respectively.

### Location/scale family of distributions

The following four distributions (normal, logistic, triangular and gompertz) are from the family of distributions often referred to as the location/scale family. They are characterized by a location parameter and a scale parameter. The standard form is a reference form of the distribution with known parameter values, often chosen so that  $E(\bar{x}) = 0$  and  $E(s) = 1$ . For example, the standard normal distribution has  $\mu = 0$  and  $\sigma = 1$ . For a location/scale distribution, any parameterization can be expressed as a linear transformation of the standard form. Such transformations have played a large role in the development of the theory of assessment factors for sensitivity distributions, which are typically derived for the standard form and shown to apply to all other parameterizations.

### Assessment Factors

For standard forms in the location scale family, the distance between the mean and the HC<sub>5</sub> is proportional to the standard deviation of the distribution. This fact has allowed the development of distribution-specific assessment factors that depend only on sample size and the desired level of confidence with which the HC<sub>5</sub> is to be bounded. For example, in a normal distribution, the fifth quantile is approximately 1.64 standard deviation units to the left of the mean. In other words, in a standard normal distribution:

$$F^{-1}(0.05) \approx \mu - 1.64\sigma.$$

More generally, letting  $K_{dist}$  represent the scaled distance between the mean and the fifth percentile in the standard form of a specific distribution, then for *any* parameterization of that distribution,  $F^{-1}(0.05) \approx \mu - K_{dist}\sigma$ . Because  $\mu$  and  $\sigma$  are unknown, the sample mean  $\bar{x}$  (Equation 1) and standard deviation  $s$  (Equation 2) are substituted and it is important to characterize, the expected sampling distribution of the statistic  $\bar{x} - K_{dist}s$  around a “true” fifth percentile ( $\mu - K_{dist}\sigma$ ). Assessment factors ( $k$ ), at a given level of confidence (say 95%), are chosen such that 95% of the expected distribution of  $\bar{x} - K_{dist}s$  would lie to the right of  $\bar{x} - ks$ . In the case of the standard normal, the sampling distribution of  $\bar{x} - ks$  follows a t distribution with  $N-1$  degrees of freedom (Aldenberg et al. 2002). However, in practice, the sampling distribution of the fifth percentile is most often estimated through computer simulation (Aldenberg and Slob 1993, Pennington 2003). With known assessment factors, the

concentration that represents the 5<sup>th</sup> percentile EC<sub>50</sub> value (untransformed) of the distribution (HC<sub>5</sub>) can be estimated with a given level of confidence (corresponding to the value of k) using the following equation:

$$HC_5 = 10^{\bar{x} - ks}$$

### Goodness of Fit

Relatively little work has been done on assessing goodness of fit for fitted sensitivity distributions. Newman et al. (2000) calculated goodness-of-fit tests for 30 sets of effects data (NOEC, EC<sub>50</sub> and LC<sub>50</sub> values) fitted with a normal distribution and found that the assumption of (log) normality was often violated.

### Normal distribution

A normal probability distribution describes data that are dispersed symmetrically around a mean value, forming a characteristic “bell shaped” probability density. The normal distribution has two parameters,  $\mu$  and  $\sigma$ . Assuming the effects data have been log<sub>10</sub> transformed, the equations for the pdf, cdf, and quantile function are:

$$f(x | \mu, \sigma) = \frac{1}{\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

$$F(x | \mu, \sigma) = \int_{-\infty}^x f(z) dz$$

$$F^{-1}(p) = \Phi(p)$$

Neither the cdf ( $F$ ) nor quantile function ( $F^{-1}$ ) have explicit forms. However, both can be readily approximated to arbitrary precision in most mathematical software.

Mean:

$$\mu$$

Variance:

$$\sigma^2$$

The standard normal distribution has mean 0 and unit variance when:

$$\mu = 0$$

$$\sigma = 1$$

Parameters for the normal distribution ( $\mu$  and  $\sigma$ ) can be estimated using the typical moment estimators ( $\bar{x}$  and  $s$ , as given in equations B1 and B2). Maximum likelihood estimates are:



$$\hat{\mu} = \bar{x} = \frac{1}{N} \sum_{i=1}^N x_i$$

$$\hat{\sigma} = s \frac{N-1}{N} = \frac{1}{N} \sum_{i=1}^N (x_i - \bar{x})$$

When sample size is large,  $\bar{x}$  and  $s$  approach  $\mu$  and  $\sigma$ , respectively and  $\bar{x} - 1.64s$  is a precise estimator for  $\mu - 1.64\sigma$ . When the sample size is small (*i.e.*,  $N < 30$ ), a student's t-distribution can be used to describe the expected variation in  $\bar{x} - 1.64s$  around  $\mu - 1.64\sigma$ . Thus, for the log normal distribution, assessment factors ( $k_s$ , Aldenberg and Jaworska 2000), dependent on sample size and desired confidence level, can be derived from a t distribution with  $N - 1$  degrees of freedom. Table B1 provides assessment factors from Aldenberg and Jaworska (2000) for generating 95% and 50% confidence limits on the 5<sup>th</sup> percentile of the sensitivity distribution. Aldenberg et al. (2002) provide a more extensive table with many more sample sizes represented.

**Table B1. t-value (one-tailed probability) and lower k values (representing 95% confidence in resulting HC<sub>5</sub>) and median k values (representing 50% confidence) for deriving HC<sub>5</sub> values from log-normal distributions. k values from Aldenberg and Jaworska (2000).**

N	t-value	Aldenberg and Jaworska (2000)	
		median	lower
2	6.3138	2.3387	26.2597
3	2.9200	1.9384	7.6956
4	2.3534	1.8295	5.1439
5	2.1318	1.7793	4.2027
6	2.0150	1.7505	3.7077
7	1.9432	1.7318	3.3995
8	1.8946	1.7187	3.1873
9	1.8595	1.7091	3.0312
10	1.8331	1.7016	2.9110
11	1.8125	1.6957	2.8150
12	1.7959	1.6910	2.7363
13	1.7823	1.6870	2.6705
14	1.7709	1.6837	2.6144
15	1.7613	1.6808	2.5660
20	1.7291	1.6712	2.3960
30	1.6991	1.6620	2.2198
50	1.6766	1.6549	2.0650
100	1.6604	1.6498	1.9265
200	1.6525	1.6473	1.8372
500	1.6479	1.6458	1.7630
$\infty$	1.6449	1.6449	1.6449

Wagner and Løkke (1991) used a logarithmic normal distribution in estimating percentiles on species sensitivity distributions. This approach involves using the mean and standard deviation of natural log transformed effects concentrations for available species exposed to the same stressor. Later, Aldenberg and Joworska (2000) used the same method, but used a  $\log_{10}$  transformation. The two methods reached identical conclusions, as expected.

### Logistic distribution

The logistic distribution shares the “bell shape” probability density with the normal distribution, but differs from the normal distribution in the tails. This difference is particularly relevant for this analysis because it is concerned with the 5<sup>th</sup> percentile of the distribution, which lies in the tails. Like the normal distribution, the log-logistic distribution is described by two parameters,  $\alpha$  and  $\beta$ . Various log-logistic sensitivity distribution approaches have been suggested in the sensitivity distribution literature (Kooijman 1987; Van Straalen and Denneman 1989; Aldenberg and Slob 1993; de Zwart 2002).

$$f(x: \alpha, \beta) = \frac{\exp(-(x - \alpha) / \beta)}{\beta(1 + \exp(-(x - \alpha) / \beta))^2}$$

$$F(x: \alpha, \beta) = \frac{1}{1 + \exp(-(x - \alpha) / \beta)}$$

$$F^{-1}(p) = \alpha + \beta \ln\left(\frac{p}{1 - p}\right)$$

Mean:

$$\alpha$$

Variance:

$$\frac{\pi^2}{3} \beta^2$$

The standard logistic distribution has mean 0 and variance  $\frac{\pi^2}{3}$  when:

$$\alpha = 0$$

$$\beta = 1$$

The logistic distribution is typically fit using the following moment estimators:

$$\hat{\alpha} = \bar{x} \text{ and}$$

$$\hat{\beta} = \frac{s}{\pi} \sqrt{3}$$

Closed-form maximum likelihood estimates do not exist, but are easily obtained numerically. However, Kooijman (1987:273) found that maximum likelihood estimates of  $\alpha$  and  $\beta$  tended to “seriously underestimate”  $\beta$  for small sample sizes. Aldenberg and Slob (1993) used Monte Carlo simulation to derive k values (Figure 3, Table B2) that can be used along with the sample mean and standard deviation to account for uncertainty in the HC<sub>5</sub>.

**Table B2. Lower k values (representing 95% confidence in resulting HC<sub>5</sub>) and median k values (representing 50% confidence) for deriving HC<sub>5</sub> values from log-logistic distribution (from Aldenberg and Slob 1993).**

N	median	lower
2	2.49	27.7
3	2.05	8.14
4	1.92	5.49
5	1.85	4.47
6	1.81	3.93
7	1.78	3.59
8	1.76	3.37
9	1.75	3.19
10	1.73	3.06
11	1.72	2.96
12	1.72	2.87
13	1.71	2.80
14	1.70	2.74
15	1.07	2.68
20	1.68	2.49
30	1.66	2.28
50	1.65	2.10
100	1.64	1.95
200	1.63	1.85
500	1.63	1.76
∞	1.62	1.62

### Triangular distribution (general)

The triangular distribution is so named for the triangular shape of its probability density function. The general triangular distribution is described by three parameters,  $a$ ,  $b$ , and  $c$  (minimum, maximum and mode, respectively). Most applications of the triangular distribution to species sensitivity data use the two-parameter symmetric form (Fig. B1). The triangular distribution also underlies the calculations of final acute values under the 1985 Guidelines.

If:  $a \leq x \leq c$

$$f(x) = \frac{2(x-a)}{(b-a)(c-a)}$$

$$F(x) = \frac{(x-a)^2}{(b-a)(c-a)}$$

If:  $c < x \leq b$

$$f(x) = \frac{2(b-x)}{(b-a)(b-c)}$$

$$F(x) = 1 - \frac{(b-x)^2}{(b-a)(b-c)}$$

Mean:

$$\frac{a+b+c}{3}$$

Variance:

$$\frac{a^2 + b^2 + c^2 - ab - ac - bc}{18}$$

A symmetric triangular distribution occurs when  $c = \frac{a+b}{2}$ . Substituting these into the above equations yields the equations for the symmetric triangular distribution.

### Triangular distribution (symmetric)

If:  $a \leq x \leq \frac{a+b}{2}$

$$f(x|a,b) = \frac{4(x-a)}{(b-a)^2}$$

$$F(x|a,b) = \frac{2(x-a)^2}{(b-a)^2}$$

If:  $\frac{a+b}{2} < x \leq b$

$$f(x|a,b) = \frac{4(b-x)}{(b-a)^2} \text{ if}$$

$$F(x|a,b) = 1 - \frac{2(x-b)^2}{(b-a)^2}$$

If:  $p \leq 0.5$

$$F^{-1}(p) = a + \sqrt{\frac{p(b-a)^2}{2}}$$

If:  $p > 0.5$

$$F^{-1}(p) = b + \sqrt{\frac{(1-p)(b-a)^2}{2}}$$

Mean:

$$\frac{a+b}{2}$$

Variance

$$\frac{(b-a)^2}{24}$$

The symmetric triangular distribution has mean zero and variance 1 when:

$$a = -\sqrt{6}$$

$$b = \sqrt{6}$$

This is the form reported and used by van Straalen (2002). Erickson and Stephan used percentile methods to fit the triangular distribution, which were used to generate Figure 1. Note that the following moment estimators could also be used:

$$\hat{a} = 2\bar{x} - \hat{b} \text{ and}$$

$$\hat{b} = (s + \bar{x})\sqrt{6}$$

Van Straalen (2002) examined the quantiles of a symmetric triangular distribution for estimating percentiles of species at risk. Pennington (2003) used Monte Carlo simulations to develop assessment factors (k, described above) applicable to all symmetric triangular distributions. Pennington (2003) recommended 50<sup>th</sup> and 95<sup>th</sup> percentile k values that varied based on N (Table B3).

**Table B3. k values for calculating 50th and 95th percentile HC<sub>5</sub> values from a triangular distribution (Pennington 2003).**

N	50 <sup>th</sup> percentile	95 <sup>th</sup> percentile
2	2.3	25.5
3	1.9	7.7
4	1.8	5.0
5	1.7	4.1
6	1.7	3.6
7	1.7	3.4
8	1.7	3.1

### Gompertz distribution

The gompertz distribution is the only asymmetric distribution among the four considered here (Fig. B1), tending to be slightly right-skewed. Like the other three distributions it has two parameters ( $\mu, \beta$ ). Newman et al. (2000) compared the performance of the gompertz distribution to normal and logistic distributions for sensitivity distributions and generally found the gompertz

to be the best of the three parametric distributions (based on  $\chi^2$  analysis of fit). However, Newman et al. (2000) further argued for the adoption of non-parametric bootstrapping approaches over all parametric methods.

$$F(x) = e^{-\frac{e^{-(x-\mu)}}{\beta}}$$

$$f(x) = \frac{1}{\beta} e^{-\frac{(x-\mu)}{\beta}} e^{-e^{-\frac{(x-\mu)}{\beta}}}$$

$$F^{-1}(p) = \mu - \beta \ln(-\ln(p))$$

Mean:

$$\mu + \beta\gamma, \text{ where } \gamma = \text{Euler-Mascheroni constant.}$$

Variance

$$\frac{\beta^2 \pi^2}{6}$$

The standard gompertz distribution has:

$$\mu = 0$$

$$\beta = 1$$

Under these conditions, the gompertz distribution has mean  $\gamma$  and variance  $\frac{\pi^2}{6}$ . The gompertz distribution is typically fit using maximum likelihood. Note the following moment estimators could also be used:

$$\hat{\beta} = \frac{s}{\pi} \sqrt{6}$$

$$\hat{\mu} = \bar{x} - \hat{\beta}\gamma$$