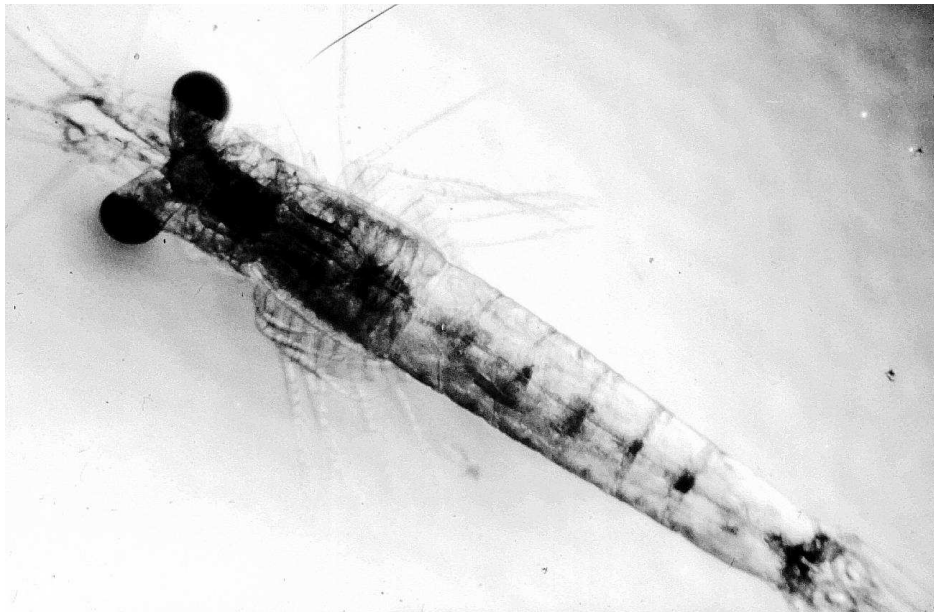


# ***Americamysis bahia* Stochastic Matrix Population Model for Laboratory Populations: Technical Documentation**



By

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## Notice

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## Abstract

The population model described here is a stochastic, density-independent matrix model for integrating the effects of toxicants on survival and reproduction of the marine invertebrate, *Americamysis bahia*. The model was constructed using Microsoft® Excel 2003. The focus of the model is on laboratory populations because neither biological variability of field populations nor variability associated with time-varying toxicant concentrations, as might be expected in field situations, are considered. The model employs several aspects of traditional population viability analysis (PVA), establishing a dose-response relationship between exposure concentrations and estimates of expected minimum population size. This documentation describes the model, and also provides the justification for various default parameters for use when the ideal toxicity data set may not be available.

Keywords: *Americamysis bahia*, PVA, matrix model, risk curves

## Acknowledgments

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## Preface


EPA's Office of Pollution Prevention and Toxic Substances (OPPTS) needs population modeling methods to support assessments of the risks from toxic chemicals to non-target populations of aquatic and terrestrial species ("wildlife"). ORD's Safe Pesticides/Safe Products (SP2) Multi-Year Plan (EPA 2006) and NHEERL's Implementation Plan for SP2 Research (EPA 2005) recommend population modeling approaches to address this need. The modeling effort described herein is specifically targeted towards OPPTS' Office of Pesticide Programs (OPP) in support of their effort to integrate population models into risk assessments associated with the pesticide registration process. The work described in this plan contributes to the accomplishment of SP2 Long-term Goal 2's annual performance goal: provide methods for characterizing population-level risks of toxic chemicals to aquatic life and wildlife.

The model is for the marine invertebrate, *Americamysis bahia*, a common toxicity test organism, and one for which OPPTS has specific test guidelines for acute and chronic tests (OPPTS 850.1035 and OPPTS 850.1050). Currently, acute and chronic toxicity data are treated separately within the risk assessment process for pesticide registration. The model allows the integration of these kinds of data into a single risk assessment endpoint. Because the model uses laboratory derived data for both its demographic parameters and the toxicity dose-response, the target population is essentially a laboratory population.

There are several components to this modeling work. One such component is the mathematical construction of the model which includes the selection of data used to derive the demographic parameters for the base model. These data were taken from the control treatments of historical tests conducted at EPA's Atlantic Ecology Division. An additional component of the modeling effort provides default input values for needed toxicity information that may be missing for a given pesticide. OPPTS has recently updated its toxicity data requirements for the registration of pesticides. However, data that meet these new requirements may not be sufficient for all of the model's needs. Therefore, if population modeling is to be incorporated reasonably soon into the risk assessment process for pesticides, it needs to be done with existing data requirements.

The model is constructed using Microsoft® Excel 2003—filename **MYSID 13x13 MATRIX—LAB EXPOSURE 2009 Version 1.XLS**. A copy of this file is embedded below. Excel's Solver add-in may need to be activated for new users of the model.

## Note

This PDF file has an attached Excel file at the end of this paragraph. You can either double left click or single right click the icon. When you double left click the attached Excel file should open. When you single right click on the icon a pop up menu should appear. From this pop up menu choose either Open File or Save Embedded File to Disk. 

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## Section 1: Introduction

The U.S. Environmental Protection Agency registers pesticides for intended use in the United States under the authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). A pesticide can be licensed for use if it will not cause “unreasonable adverse effects on the environment,” Section 2, Paragraph bb of FIFRA defines this as “any unreasonable risk to man or the environment” or “a human dietary risk” from food residues. The focus of this document is on the environmental portion of the above. Risk assessments for the registration of pesticides have been based historically on the toxicological effects on organism-level vital rates such as survival and reproduction. The intention, however, is to evaluate risks to populations, not to individual organisms. In this context, a population-level endpoint may be more desirable. This documentation shows a different approach for using data from traditional organism-level toxicity tests to estimate population-level risks. The specific application being evaluated is risk to populations of the marine invertebrate *Americamysis bahia* (Molenock, 1969). This is accomplished through a population model which is a mathematical representation using vital rates (e.g., survival and reproduction) to describe population dynamics, such as population growth rate ( $\lambda$ ). Population risks from pesticides (and other chemicals and stressors) are projected using a population model integrating both acute and chronic toxicity data from standard organism-level toxicity tests into predictions of long term viability of a population. This model demonstrates the potential for routine use of population level endpoints in pesticide risk assessments, and paves the way for more detail modeling efforts that will be aimed at more field-like situations. The document not only describes the inner workings of the model, but also provides the necessary documentation of default model input parameters when such parameters are not readily available from the toxicity data.

Population models are a convenient mathematical method to integrate multiple sources of effects (e.g., effects on survival and reproduction) into a single endpoint. There are, however, additional values to using population models instead of, or in addition to, standard toxicity test dose-response data. When examining only organism-level data “it cannot be assumed that large effects on the vital rates translate into large contributions to the effects on  $\lambda$ ” (Caswell 1989). In other words, a compound cannot be assumed to cause the same degree of effect on population growth rate as it does on survival or reproduction independently. Caswell (1996) further states:

The take-home message of every example that has been published so far is that it is not safe to assume that the most obvious effect of a toxicant on the vital rates is the source of that toxicant’s effect on  $\lambda$ . In each case there are large vital rate effects that make only trivial contributions to effects on  $\lambda$  and other, much smaller vital rate effects whose contributions are much larger.

There are many examples of this in the literature. In a study on the effects of cadmium on nematodes, reproduction was more sensitive to cadmium than was juvenile period (duration as juvenile); however, change in juvenile period had a greater effect on population fitness than did effects on reproduction (Kammenga et al. 1996). In addition, Gleason and Nacci (2001) demonstrated that “populations of species that have different life history strategies can respond differently to a stressor producing responses of similar type and magnitude at the individual level.” In summary, not only will population modeling endpoints integrate stress to multiple vital

rates, but they may actually result in a different conclusion relative to which vital rate has the most significant effect on the vitality of the population.

The particular mathematical model chosen for this product is a stochastic, density independent matrix population model created with Microsoft® Excel 2003. The model evaluates exposure to a constant concentration of a toxicant on laboratory populations. The normal operation of the model includes a series of such concentrations, resulting in a dose-response for the probability of decline in a population. The endpoint for the model derives from population viability analysis (PVA) that is commonly used in conservation biology, and requires estimates of variability in population growth rate. The PVA is based on the concept of quasi-extinction probabilities introduced by Ginzburg et al. (1982). As explained by Morris and Doak (2002):

The basic goal of PVA is to predict the future with some reasonable degree of assurance. We can do this much better if we don't try to predict when the very last desert tortoise in Las Vegas County may die, but, rather, when the population will reach a small enough number that many additional genetic and ecological problems will further threaten it, making it perilously at risk.

The population threshold at which a group of organisms is at risk is referred to as the quasi-extinction threshold. In this context, risk is described as the probability of a population declining below this threshold.

PVA analysis requires incorporating estimates of vital rates and their variability into projections of quasi-extinction. The source of the variability for the mysid model comes from the variance in mysid vital rates under unstressed conditions (i.e., laboratory survival and reproduction) and the uncertainty associated with responses to toxicants. This version of the model does not incorporate biological variability within field populations or variability associated with time-varying concentrations of a toxicant. It is expected that the output from this model will augment the current use of mysid chronic test data in the FIFRA risk assessment process. Although the model was developed primarily for use within EPA's Environmental Fate and Effects Division, Office of Pesticides Programs' (OPP) risk assessments for registration of pesticides, its use is not restricted to that program.

The model described herein provides a mechanism for expressing the toxicity of single compounds as a series of risk curves. The risk curves are built using PVA. These also are referred to as "risk of decline curves" (Akçakaya 2000). The concept was originally described by Ginzburg et al. (1982), but was not specifically referred to as risk curves. Simply put, a risk curve is a plot of the probability of decline relative to the magnitude of the decline. The probability is greatest for a small threshold of decline and least for a very large threshold. Risk curves can be expressed either as a threshold population size—estimating the probability of declining below that threshold (e.g., McCarthy 1996, McCarthy and Broome 2000, and Taylor et al. 2003)—or as percentage decline (e.g., Inchausti and Weimerskirch 2004, and Schtickzelle et al. 2005). The latter is the way the risk curves are expressed in this mysid model. With either method, however, a judgment has to be made to determine the threshold or percentage decline of concern. This is not always an easy judgment to make. As Morris and Doak (2002) point out, "the size of the quasi-extinction threshold is often the subject of argument." This issue is eliminated if risk is evaluated by comparing the area between different risk curves, each representing a different environmental scenario (Burgman et al. 1993, see Figure 3.17). In the case of the mysid model presented here these different scenarios represent different toxicant

concentrations. McCarthy and Thompson (2001) took this one step further showing that the area to the left<sup>1</sup> of a single quasi-extinction curve was equivalent to the expected minimum population size. This expected minimum is expressed as either an absolute number of organisms or as a percentage of the current population size—depending on how the threshold is expressed. Thus, using a series of toxicant concentrations in a model will allow the construction of a dose-response curve with expected minimum population size as the response.

*Americamysis bahia* has been a standard toxicity test species for several decades, and the utility of population level toxicity endpoints for this species was demonstrated almost from the beginning (Gentile et al. 1982, 1983). In spite of this, the application of population models in the interpretation of the effects of toxicants on this species has not been routinely used. Recently there have been some attempts at developing matrix population models for *A. bahia*. Kuhn et al. (2000, 2001) developed a deterministic age class matrix model with a daily time step using demographic data from standard 28-day chronic early life-cycle toxicity tests. The matrix model covered approximately one-third of the mysid's expected 90 d life history—using 28 separate daily age classes. The authors demonstrated that the traditional chronic test results were likely to be protective of mysid population growth. A different daily time step matrix model for *A. bahia* was created by Raimondo et al. (2005a,b). These authors also used demographic data from traditional chronic toxicity tests. However, they developed a stage class model with seven different stages based on age. The oldest stage in the model was represented by late breeders (25 to 29 d). The authors of this stage class model also differed from Kuhn et al. (2000, 2001) by incorporating stochastic simulations so that quasi-extinction estimations could be made, but complete risk curves were not created. Both of the above matrix models were successfully used to demonstrate the utility of population models for mysids, but neither fit the exact needs of the current model.

The desired matrix model covers the entire life-cycle of *A. bahia*—approximately 90 days. The models by Kuhn et al. (2000, 2001) and Raimondo et al. (2005a,b) could have been modified to cover this time frame. The former was not used for this modification because daily age classes would have made the model size too large for ease of use. The latter could have been modified by adding one or a few additional stages to cover the remainder of the life cycle. This was not done because it would still result in a model with a daily time step. In the next phase in the development, the mysid model presented here will become part of a periodic series covering an entire year. This means that a separate matrix model will be needed for each time step within the year. Covering an entire year with a series of matrices will allow OPP to evaluate annual pesticide concentration time series for their risks to populations. Currently OPP only uses a few single concentration values (e.g., peak values and 21 d averages) from their annual estimated exposure scenarios in the derivation of their risk quotients. Using a series of matrices will allow the entire concentration time series to be used, and will allow the incorporation of time-varying pesticide concentrations into the modeled effects. This in turn will allow exposure scenarios that are more similar to what is expected in nature. To avoid using 365 separate matrices associated with a daily time step, a model using a longer time step will be needed. A monthly time step is convenient, because a year is easily divided into thirteen 4-week “months”. This time step,

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<sup>1</sup> To the right of the risk curve applies in situations where the degree of population change is expressed as percentage decline—as with this mysid model. To the left applies for those curves expressed as population thresholds.

however, will be too long because the month would represent an exceedingly large portion of the mysid life cycle. A weekly time step lets the life history be divided into 13 separate weekly age classes. Although this will require 52 separate matrices when the annual version of the mysid model is developed, it is a reasonable compromise, and certainly more manageable than 365 daily matrices.

The rest of this document is divided into five sections. The next section describes the model structure, and includes a summary of the baseline data used to develop the biological parameters for the model. The third section details the minimum toxicity data requirements needed for the model. Data submitted to OPP must meet certain minimum acceptability requirements. These data, however, may not provide all of the information needed for a successful use of the population model. Therefore, this third section also provides the technical documentation for the selection of default values when desired data are absent. A summary of the data used to determine the variability in model parameters (these are the source of stochasticity needed for PVA analysis) is provided in the fourth section. The fifth section is an example showing a comparison among model runs using data from toxicity tests conducted with the insecticide endosulfan. This data set is sufficient to provide all of the necessary parameters for running the model. The model was also run assuming that the data were not sufficient and that default values for survival kinetics and reproduction dose-response curves were needed. This fifth section provides an example of the difference in the endpoint relative to the concentration-response curve when using a complete data set versus using default values for survival kinetics or reproduction dose-responses. The sixth, and final, section contains a detailed description of each Excel spreadsheet used in this version of the model.

## Section 2: Model Structure—Summary of Demographic Parameters for *Americamysis bahia* for the Matrix Population Model

The model is an age class, density independent, stochastic matrix population model. The life history of *Americamysis bahia* is divided into 13 one-week age classes, covering the expected duration of the life cycle. The model time step (projection interval) is also one week. This means that at each step of a model simulation an individual either dies or survives and grows into the next age class. Density independent means that the survival and reproduction rates are not influenced by the size of the population. Although density dependence has been incorporated into mysid models for various reasons (e.g., Raimondo et al. 2005a), the relationship between density and vital rates has not been quantified. The reference given for the existence of density dependence is Lussier et al. (1988). That reference, however, only has two minor statements about density dependence. The first:

Population density within a culture affects reproduction. A culture that is too densely populated will cease reproduction.

Followed latter by:

A steady decline of a culture is more difficult to ascertain and may result from a combination of factors such as inadequate nutrition, over-harvesting, chronic disease, contaminant organisms, high mysid density, high male-to-female sex ratio, or sublethal toxicity from algae, *Artemia* cysts or dilution water.

There is insufficient information to accurately portray density dependence; therefore, it was not incorporated into this model. Finally, the need for stochasticity is because the model output relies on an application population viability analysis (PVA) within risk curves. This means that the output is based on the probability or the likelihood that a population will decline. The underpinning of this type of analysis requires an estimate of variability in population growth rate. This variability is described in Section 4: *Sources of Stochasticity*.

The matrix population model developed for *A. bahia* is shown in Figure 1. A  $P_i$  value is the probability the  $i^{th}$  age class survives during a time step (one week), therefore moving into age class  $i+1$ . The  $F_i$  values represent the fecundity for the  $i^{th}$  age classes, which is the number of young produced and surviving the time interval. The calculations for these parameters are shown below in Equations 1 and 2. The grey area in the upper left of the matrix represents the portion of the life cycle for which measured values are available. The matrix age classes, however, cover the entire life history of *A. bahia*. This is needed because the sensitivity to a toxicant is in part a function of the duration of exposure. Representing the entire life history in the matrix allows tracking the potential effect of a toxicant on individuals of different ages no matter how old they are.

Age	1	2	3	4	5	6	7	8	9	10	11	12	13
1	0	$F_2$	$F_3$	$F_4$	$F_5$	$F_6$	$F_7$	$F_8$	$F_9$	$F_{10}$	$F_{11}$	$F_{12}$	$F_{13}$
2	$P_1$	0	0	0	0	0	0	0	0	0	0	0	0
3	0	$P_2$	0	0	0	0	0	0	0	0	0	0	0
4	0	0	$P_3$	0	0	0	0	0	0	0	0	0	0
5	0	0	0	$P_4$	0	0	0	0	0	0	0	0	0
6	0	0	0	0	$P_5$	0	0	0	0	0	0	0	0
7	0	0	0	0	0	$P_6$	0	0	0	0	0	0	0
8	0	0	0	0	0	0	$P_7$	0	0	0	0	0	0
9	0	0	0	0	0	0	0	$P_8$	0	0	0	0	0
10	0	0	0	0	0	0	0	0	$P_9$	0	0	0	0
11	0	0	0	0	0	0	0	0	0	$P_{10}$	0	0	0
12	0	0	0	0	0	0	0	0	0	0	$P_{11}$	0	0
13	0	0	0	0	0	0	0	0	0	0	0	$P_{12}$	0

**Figure 1.** The 13x13 matrix population model used for *Americamysis bahia*.  $P_i$  and  $F_i$  are defined with Equations 1 and 2. The grey area represents the extent of the standard 28d chronic test.

The survival and reproduction (maternity) data used to calculate baseline (or control) survival probabilities and fecundities are shown in Table 1. These data were summarized from ten, 28-day chronic tests conducted at the Atlantic Ecology Division (AED) between the years of 1987 and 1993. To be included in the dataset for estimating the demographic parameters, tests had to meet reproductive and temperature requirements of the current OPPTS test guidelines<sup>2</sup>. This meant that tests from the late 1970s and early 1980s were not included because they were conducted at a lower temperature, which delays reproduction.

<sup>2</sup> Ecological Effects Test Guidelines. OPPTS 850.1350. Mysid Chronic Toxicity Test. Office of Prevention, Pesticides and Toxic Substances.

**Table 1.** List of chemicals used in the evaluation of control demographic parameters for the mysid *Americamysis bahia*. The six digit number following the chemical name refers to the AED experiment number which takes the form of YYMM##. Note, there is no experiment number for triethylene glycol/acetone.

Compound	$l(1)^a$	$l(2)$	$l(3)$	$l(4)$	$m_3^b$	$m_4$
Acenaphthene 890703	0.967	0.933	0.700	0.567	6.086	5.417
Acenaphthene 890805	0.933	0.833	0.633	0.450	4.127	7.825
Aniline 871001	0.950	0.933	0.783	0.683	3.195	6.500
Carbofuran 921001	1.000	1.000	0.900	0.767	3.100	4.091
Dichlorvos 900101	0.917	0.900	0.867	0.750	3.875	6.479
Flouranthene 910702	0.883	0.867	0.833	0.700	0.625	2.545
Propoxur 900601	0.867	0.800	0.783	0.683	2.938	5.813
Pyrene 920701	1.000	1.000	0.850	0.767	1.769	3.423
Triethylene glycol/acetone	1.000	0.933	0.833	0.733	1.533	2.114
Thallium 880210	0.950	0.883	0.717	0.617	0.600	3.643
Mean	0.947	0.908	0.790	0.672	2.785	4.785
SD	0.0023	0.0043	0.0070	0.0103	2.913	3.589

<sup>a</sup>  $l(x)$  = the probability of survival from birth to age  $x$ .

<sup>b</sup>  $m_x$  = maternity rate. Average total number of young per female at age  $x$ .

*Americamysis bahia* is a *birth-flow* (Caswell 2001) population, meaning births occur continuously over the projection interval. Because of this, the age of individuals within a given age class is not precisely known. For example, those individuals within age class 1 are between 0 and 1 week old; those within age class 2 are between 1 and 2 weeks old; and so on. The probability of an individual in a given age class surviving to the next age class ( $P_i$ ) depends on the age of that individual; therefore, there needs to be some sort of average for each age class<sup>3</sup>. This probability is estimated using Equation 2.24 from Caswell (2001):

$$P_i = \frac{l(i) + l(i+1)}{l(i-1) + l(i)} \quad \text{Equation 1}$$

Likewise the number of births in a projection interval has to be averaged in some way because individuals born near the beginning of the interval have to survive the entire interval before being counted and those born near the end of the interval have a higher probability of surviving until the end of that interval. Caswell's equations 2.34 and 2.35 are used to estimate the average fecundity for a given age class ( $F_i$ )<sup>4</sup>:

$$F_i = l(0.5) \cdot \left( \frac{m_i + P_i \cdot m_{i+1}}{4} \right) \quad \text{Equation 2}$$

<sup>3</sup> See Section 2.4.1 of Caswell (2001) for a more complete explanation.

<sup>4</sup> Note: Equation 2.34 from Caswell has 2 in the denominator instead of 4. This is because Caswell's maternity rate represents the number of females per female. Since here the maternity rate is the total number of young, this change converts the results to number of females per female.

Where  $l(0.5)$  is approximately equal to  $(l(0)+l(1))/2$ . Note: when expressed in terms of probability  $l(0)$  is 1.0.

The survival rates and fecundity values for the first 4 age classes can be calculated directly from the available data. These parameters for the remainder of the age classes, however, have to be estimated. One option for these latter survivals is to assume that the survival rate for weeks 4 through 12 are the same and use a survival probability of 0.0 for week 13 so that life ends. Survival, however, is a function of time and most, if not all species fit the model of either a Type I, Type II or Type III survivorship curve. Knowing this a survivorship curve can be fit to the averages from the data in Table 1 and estimates made of the survival probabilities for weeks 4 through 13. Survivorship curves can typically be fit with a Weibull function. There are several versions of the Weibull equation; the one used herein is:

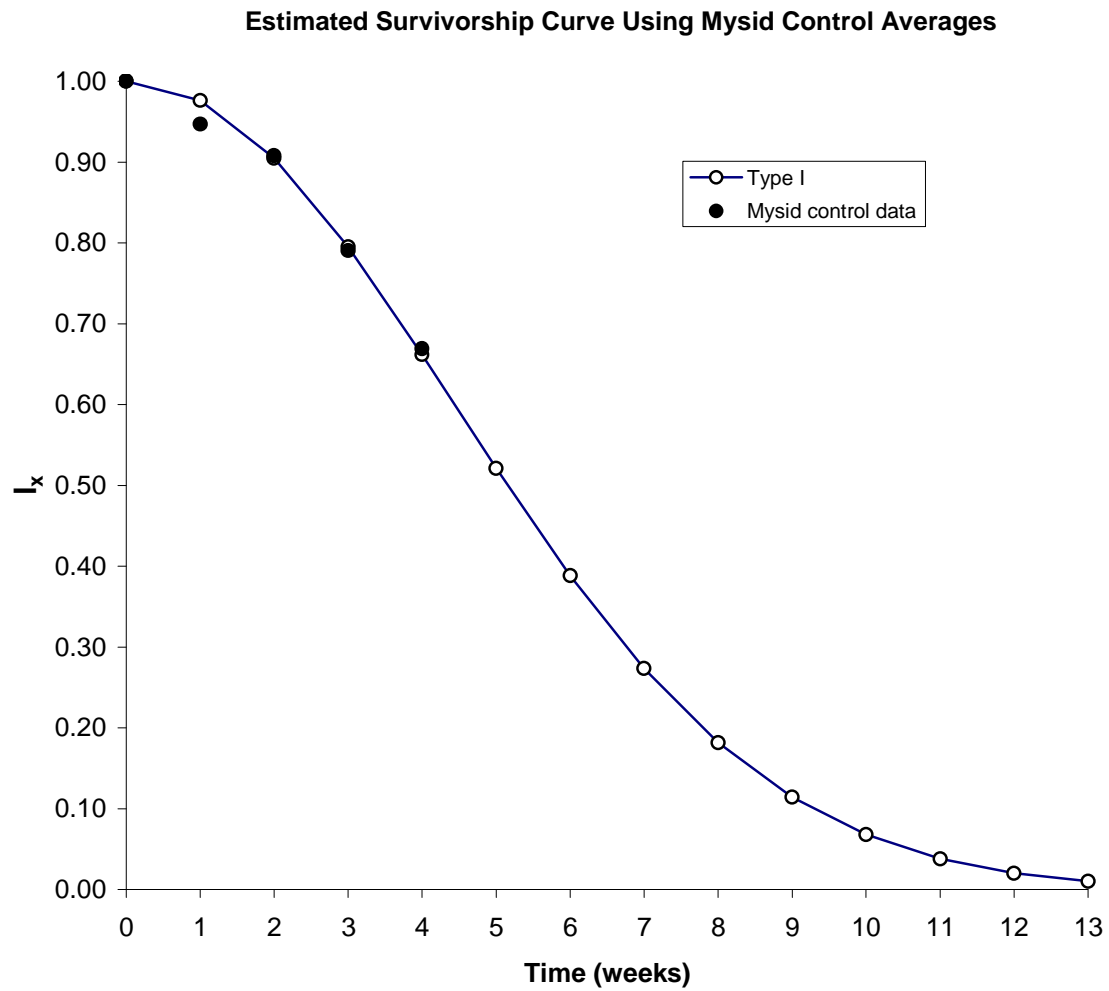
$$S(t) = e^{-(k_2 t)^{k_1}} \quad \text{Equation 3}$$

This is Equation 6.2.3 from Lee and Wang (2003), where  $t$  is time and  $S(t)$  is the probability of survival at time  $t$ . The constants  $k_1$  and  $k_2$  are shape parameters. If  $k_1$  is greater than 1.0, then the survivorship curve is Type I, if equal to 1.0, the curve is a Type II, and if less than 1.0, a Type III. The longer the life span of the organism, the smaller the value of  $k_2$ . Figure 2 shows a Weibull fit to the average survival values from all 10 tests (Table 1). The shape parameters are  $k_1 = 2.045$  and  $k_2 = 0.162$ . Appendix E gives a more complete explanation of how these parameters were derived, as well as the general relationship between the two shape parameters.

We have no maternity rate data beyond that for week 4. Maternity rates for weeks 5 through 13, therefore, are assumed to be the same as week 4. Note, however, that because the equation for fecundity includes a survival rate the fecundity rates decline as the mysids age. Table 2 shows the numbers used to set the baseline parameters for the 13x13 matrix. These parameters are those used to calculate the stable age distribution<sup>5</sup> that is in turn used as the default *t-zero* population in the model simulations. The end user, however, can substitute any values in the *t-zero* vector.

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<sup>5</sup> Calculated using PopTools version 2.7.5 available on the internet. URL <http://www.cse.cisiro.au/poptools>.



**Figure 2.** Weibull function fit to the first 4 weeks of survival data for *Americamysis bahia*.

**Table 2.** Summary of life table parameters for the matrix population model used for *Americamysis bahia*. The probability of surviving from birth to a given age is  $l(i)$ . The probability of surviving from a given age to the next age is  $P_i$ . The maternity rate for a given age is  $m_i$ . All ages from week 4 on have the same maternity rate. Finally,  $F_i$  is the fertility of age class  $i$ . Note that a week 14 is added in order to correctly calculate  $P_i$  for week 13 (we assumed at the maximum age of 13 weeks that 1% of the initial population would still be alive—see Appendix E for a more complete explanation).

Age $_i$ (weeks)	$l(i)$	$P_i$	$m_i$	$F_i$
0	1.000	-	-	-
1	0.976	0.952	0	0
2	0.905	0.904	0	0.622
3	0.795	0.856	2.785	1.700
4	0.661	0.812	4.785	2.141
5	0.521	0.769	4.785	2.091
6	0.388	0.727	4.785	2.041
7	0.273	0.688	4.785	1.995
8	0.182	0.651	4.785	1.951
9	0.114	0.615	4.785	1.909
10	0.068	0.582	4.785	1.870
11	0.038	0.547	4.785	1.829
12	0.020	0.517	4.785	1.793
13	0.010	0.500	4.785	1.182
14	0.005			

### Section 3: Minimum Toxicity Data Requirements for *Americamysis bahia* Matrix Population Model—Justification for Default Parameters

The use of the *Americamysis bahia* population model for assessing population level effects from toxicants assumes that the minimum data available from the acute test will be a 96 h acute *LC50* (along with its 95% confidence interval) and an *LOEC* (Lowest Observable Effect Concentration) for reproduction from a 28 d chronic<sup>6</sup>. If these data are not available, then the model cannot be used. No other data besides those provided in the OPPTS test guidelines are required. Besides these data, the following additional information is desirable, but defaults are available and explained below: survival dose-response curve; *LC50* vs time data; and reproduction dose-response curve.

#### *Survival Dose-Response Curve*

Equation 4 is used to model survival dose-response curves. Information about this equation and other forms of it are shown in Appendix A. Ideally, there would be enough data to fit this curve and get an estimate of the slope. If enough data are not available, then the exposure response uses a default slope of 3.3. This corresponds to the default used by the Office of Pesticide Programs (OPP) for the probit, 4.5 (Urban and Cook 1986). Both Equation 4 and the probit method (Equation 5) yield essentially the same dose-response curve. Figure 3 shows the relationship between the probit slope and the slope of the logistic equation for a range of dose-response curves. To achieve the data set eighteen probit curves were created using slopes ranging from 1.5 to 10 with an *LC50* of 2.5 for each data set. A best fit for these data to the logistic equation using least squares regression (adjusting only the slope) was then made.

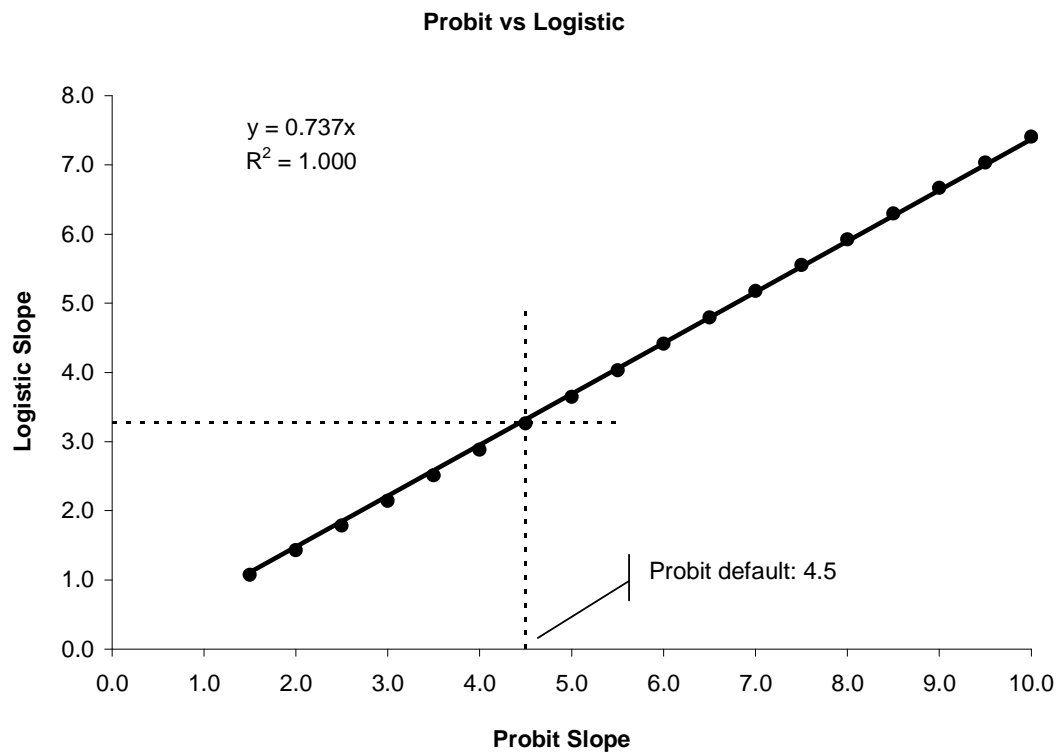
$$\text{Survival Probability} = \frac{1}{1 + \left( \frac{\text{conc}}{\text{LC50}} \right)^{\text{slope}}} \quad \text{Equation 4}$$

$$Z = (X - \mu) \cdot \text{probit\_slope} \quad \text{Equation 5}$$

where *Z* is the standard score (or the z-score); *X* is the log<sub>10</sub> of a concentration; and  $\mu = \log_{10}$  of the *LC50*. The probability of survival is the cumulative probability of the standard normal distribution up to the value *Z*<sup>7</sup>. This is easily calculated with the Excel function *NORMSDIST(Z)*.

<sup>6</sup> Based on Office of Prevention, Pesticides and Toxic Substances (OPPTS) test guidance numbers 850.1035 and 850.1350 for acute and chronic tests with mysids, respectively, these data should be readily available if acceptable tests were performed.

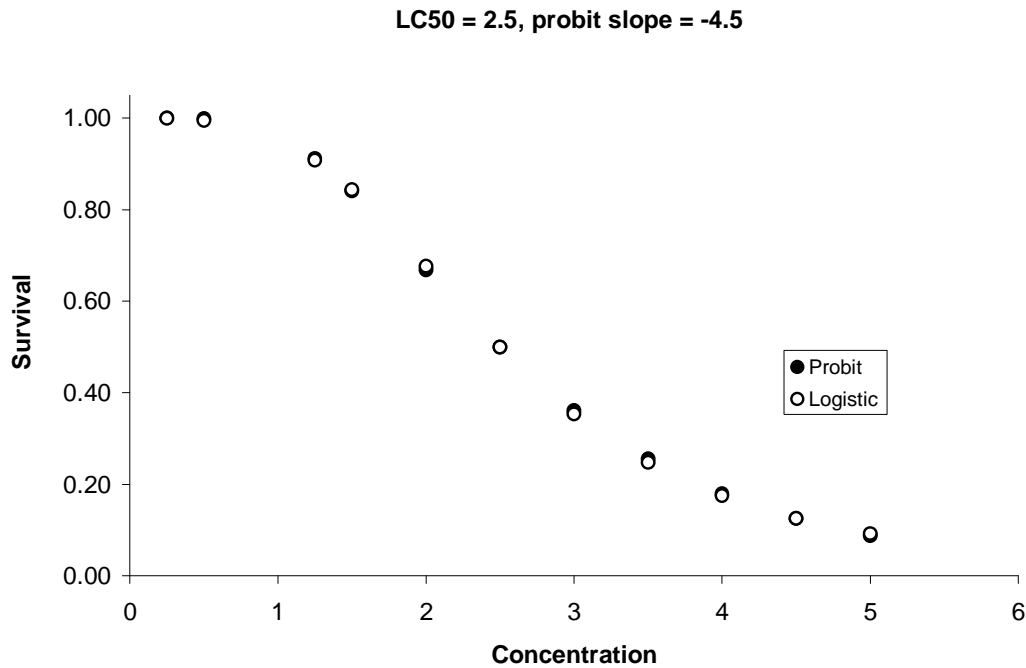
<sup>7</sup> Probits are actually *Z*+5 since the early user of this technique did not like to deal with negative numbers (see Bliss 1934, 1935, Finney 1971, Hubert 1980).



**Figure 3.** Relationship between the probit slope and the slope (shape factor) for the logistic equation.

Figure 4 shows a sample set of dose-response curves using an *LC50* of 2.5 and OPP's default probit slope of -4.5<sup>8</sup> and the corresponding logistic slope of -3.3 (calculated from the equation in Figure 3). The dose-response curves are essentially identical

<sup>8</sup> Note: the negative of the default slope was used so that survival (rather than mortality) was calculated.



**Figure 4.** Default survival curves using probit default OPP slope (-4.5) and the logistic equivalent (-3.3).

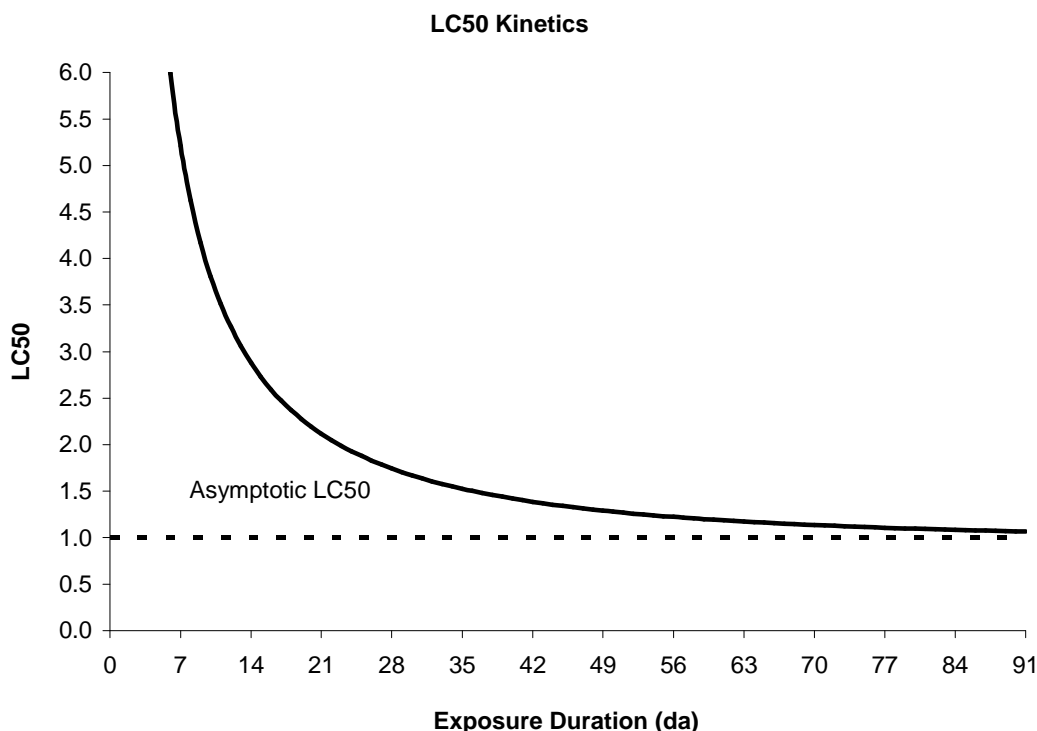
### *LC50 Versus Time*

The mysid model describe herein has a time-step of 7 days with 13 different weekly cohorts. Therefore, an estimate is needed of how the *LC50* changes with increasing exposure time. Figure 5 shows an idealized relationship between the *LC50* for a given toxicant and the duration of exposure. Equation 6 is a mathematical expression often used to model this relationship. An *LC50* at any *t* can be calculated, if the *LC50* with infinite exposure<sup>9</sup> is known (approached as an asymptote) and the kinetic parameter, *k*.

$$LC50_t = \frac{LC50_{\infty}}{1 - e^{-k \cdot t}} \quad \text{Equation 6}$$

Both the *LC50* at infinity and the kinetic parameter can be estimated if there are enough data from the acute test (e.g., 24, 48, 72 and 96 h *LC50*s), the chronic test (7, 14, 21 and 28 d), or a combination of both. However, the likely scenario is that there will not be enough survival data to directly use Equation 6. But a default value can be derived and used to estimate the *LC50*s for various exposures using historical data comparing *LC50*s for 28 d with those for 4 d. This is explained in detail below and in Appendix B.

<sup>9</sup> Also called the asymptotic *LC50* or incipient *LC50*. The latter appears to have been introduced in Sprague (1969).

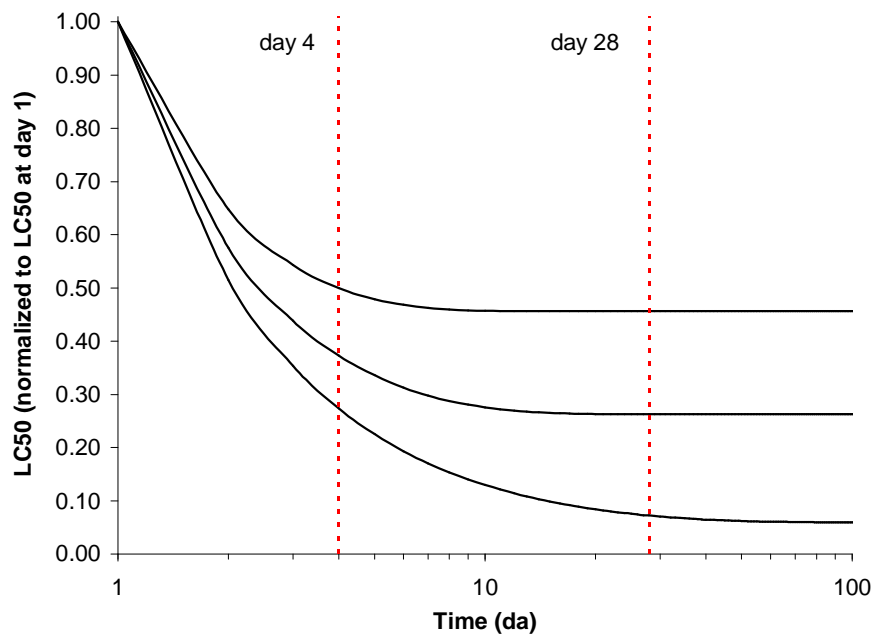


**Figure 5.** Idealized relationship of lethal concentration to exposure duration. Curve was created using Equation 6 with the asymptotic  $LC50$  ( $LC50_{\infty}$ ) set at 1.0 and the kinetic parameter,  $k$  set at 0.3045

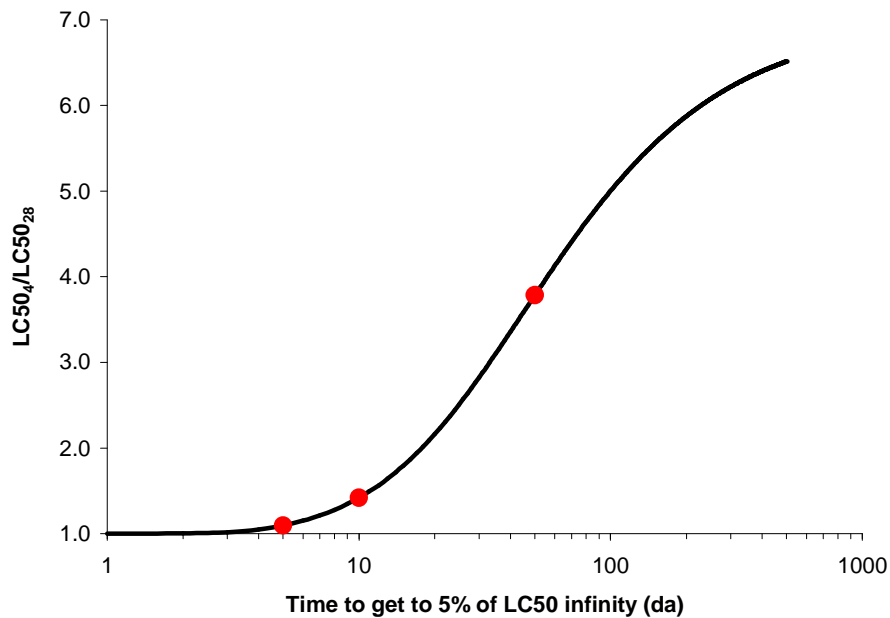
Figure 6 shows three different hypothetical curves relating  $LC50$  to duration of exposure using Equation 6. For convenience the  $LC50$  is set to 1.0 on day 1 for all three curves. The curves represent asymptotic  $LC50$ s reached in 5d, 10d and 50d. Two time durations have been marked, 4d and 28d. It is clear from the figure that as the time to reach an asymptotic  $LC50$  increases, the difference between the  $LC50$  after 4 days of exposure and that after 28 days get larger. Figure 7 shows this relationship in another manner. Here the x-axis is the time to reach the asymptotic  $LC50$ <sup>10</sup>. The y-axis is the ratio of the  $LC50$ s for days 4 and 28.

Figure 8 shows a cumulative distribution for  $LC50$  ratios from toxicity tests conducted with 24 compounds using *Americamysis bahia*. The actual data used are listed in Appendix C. The 95<sup>th</sup> percentile for the ratio of  $LC50$ s on days 4 and 28 is 5. This is used to determine the default number of days to reach an asymptotic  $LC50$ , as shown in Figure 9. The horizontal line labeled “A” is drawn at the above ratio of 5. A vertical line labeled “B” is drawn where “A” crosses the curve for the ratio of days 4 and 28. This second line crosses the x-axis at 100 days. Thus 100 days becomes the default time to reach an asymptotic  $LC50$ . Using Equation B3 (Appendix B), this yields a kinetic parameter ( $k$ ) for Equation 6 of 0.03045—which becomes the default kinetic value.

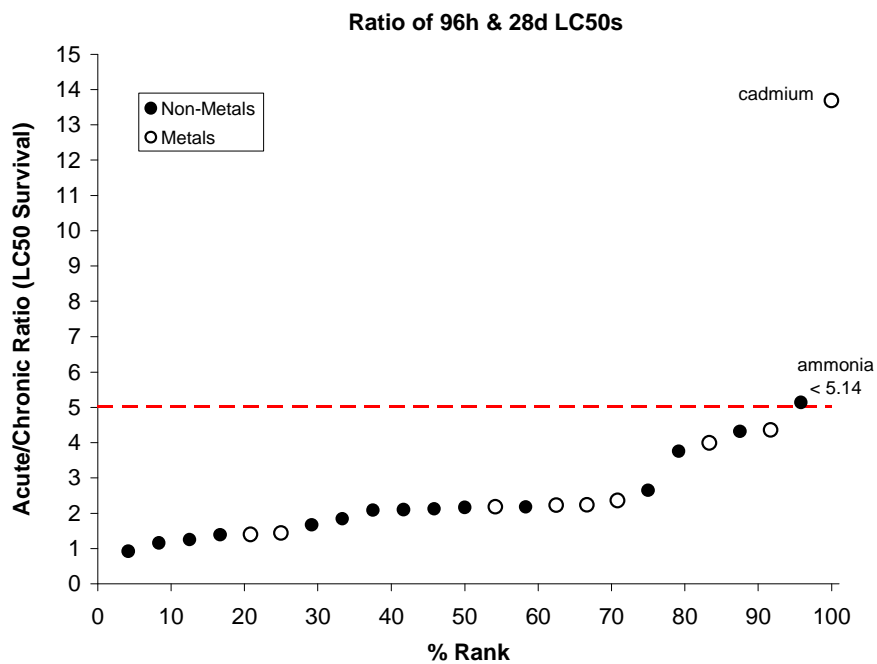
<sup>10</sup> This is actually set as the time to reach within 5% of the true asymptotic  $LC50$ . Because the true value is never reached an approximate value was selected that was close enough to this true value (see Appendix B for further explanation).



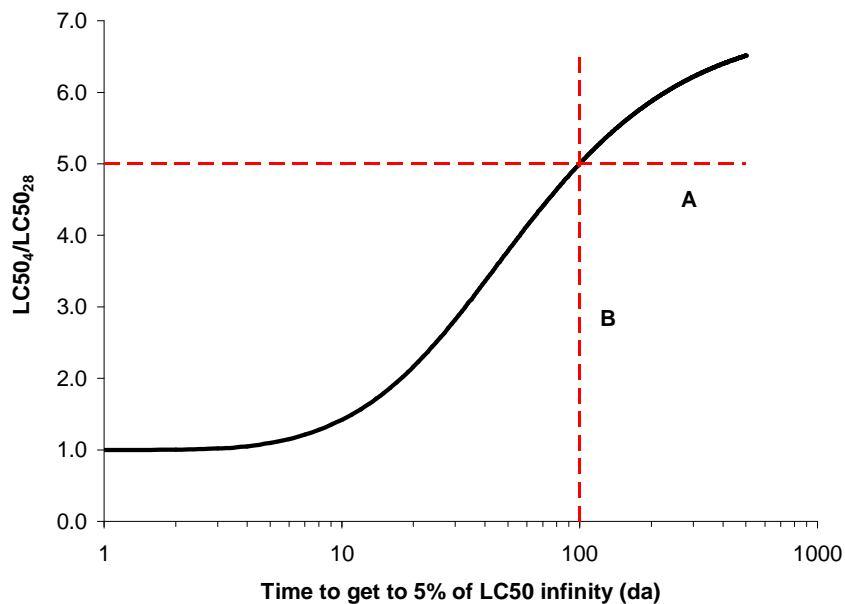
**Figure 6.** Three different curves representing the idealized relationship between  $LC50$  and duration of exposure. In the upper curve the asymptotic  $LC50$  is reached in 5d. In the middle curve, 10d. In the lower curve, 50d. The vertical lines show time at days 4 and 28.



**Figure 7.** Relationship between the ratio of  $LC50$ s on days 4 and 28 to time to reach the asymptotic  $LC50$  (represented by a value within 5% of the true asymptote). Data from the 3 curves in Figure 4 are indicated with solid circles.



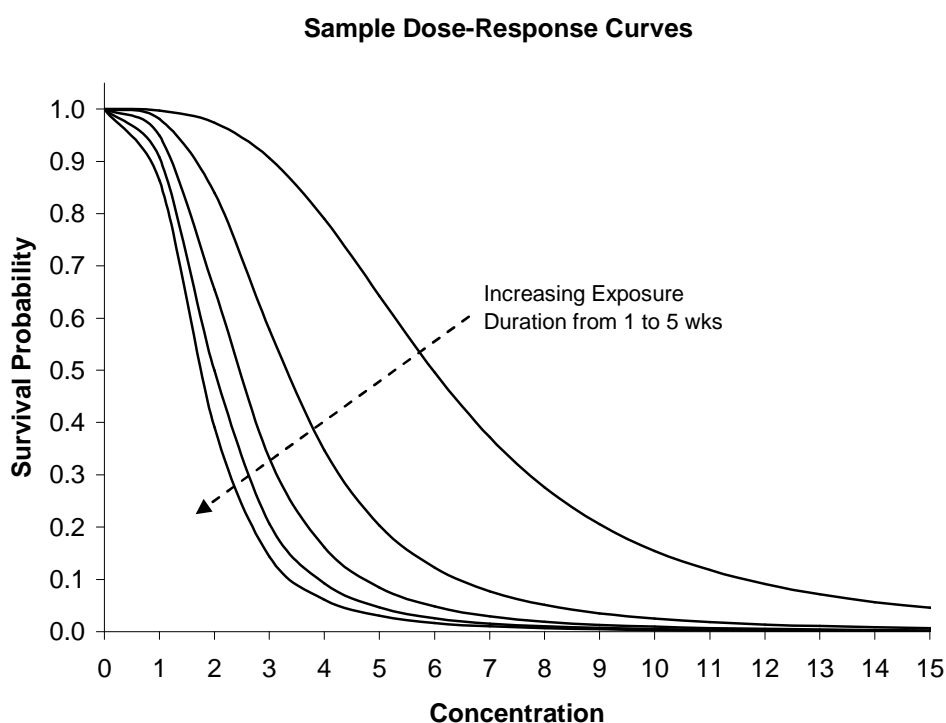
**Figure 8.** Cumulative distribution of survival acute/chronic ratios for mysids. Data are from tests listed in various EPA water quality criteria, and are shown in Appendix C. The horizontal dashed line is the 95<sup>th</sup> percentile.



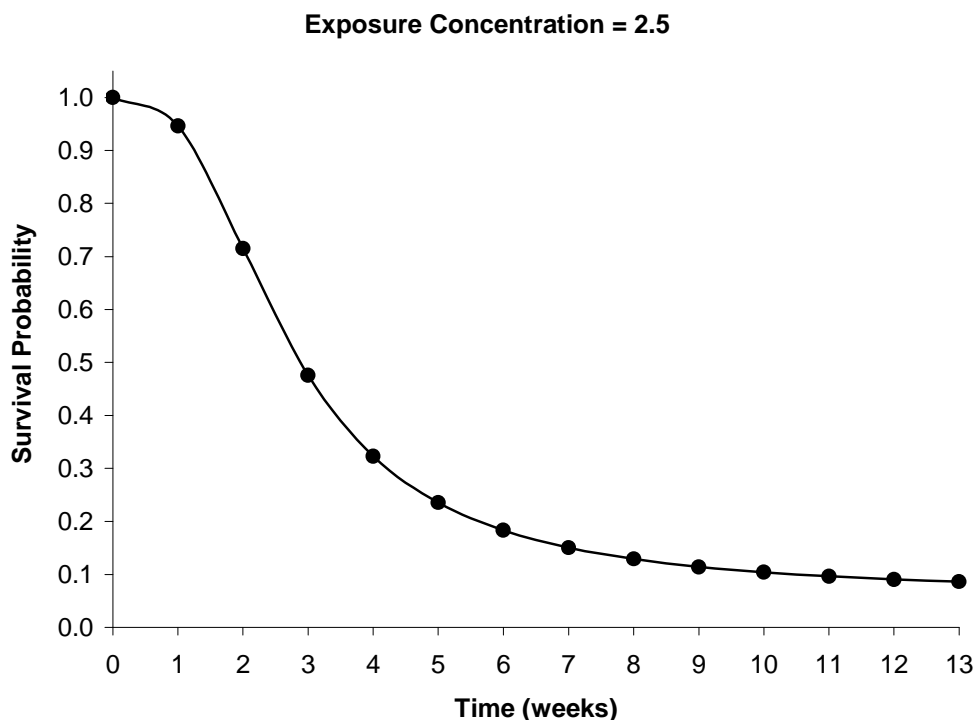
**Figure 9.** Relationships between  $LC50$  ratios and time to reach asymptotic  $LC50$ . The horizontal line labeled “A” is drawn at the ratio of 5. A vertical line labeled “B” is drawn where “A” crosses the curve.

There is an alternate method to estimate the kinetic parameter for Equation 6. If *LC50* values are available for two different periods of time (in days), then Equation B4 (Appendix B) can be used. You solve iteratively for the value of *k* that yields the correct ratio of the two *LC50* values.

Knowing the kinetics of how the *LC50* changes with time and assuming the “slope” in Equation 4 remains constant, a series of dose-response curves can be created for each age class (e.g. Figure 10). Therefore, the cumulative survival for any single exposure concentration can be calculated (e.g. Figure 11). This in turn allows the calculation of the probability of survival due to toxicity for each age class to the next by a simple ratio of two consecutive cumulative survival rates. The use of these values is explained in the Section 6 describing the Excel spreadsheets, Sheet: *Parameter Calculations*.



**Figure 10.** Sample set of dose-response curves for the first five weeks of exposure.



**Figure 11.** Survival probability based on a single exposure concentration, but allowing for the shift in dose-response curves as the duration increases.

### ***Reproduction Dose-Response Curve***

Table 3 shows the summary toxicity data from dose response curves for nine chemicals for which data were available for acute and chronic tests (survival and reproduction—number of young per available female reproductive day). The full dose-response curves for each chemical are shown in Appendix D. There is a relationship between the  $EC_{50}/LC_{50}$  ratio and the slope of the dose-response curve for reproduction (Figure 12). There is also a relationship between the  $EC_{75}/LC_{50}$  ratio and the reproduction effect slope (Figure 12—the  $EC_{75}$  was calculated from the equation for the dose-response for reproduction; Equation A1 in Appendix A). Figure 13 shows the relationship between the slope for the dose-response for reproduction and a range of  $EC_x/LC_{50}$  ratios (with 'x' ranging from 10% to 75% in 5% effect increments. Each of the curves in Figure 13 can be described with the same general equation:

$$repro\_slope = Y_0 + aR^b \quad \text{Equation 7}$$

where  $R$  is the  $EC_x/LC_{50}$  ratio,  $Y_0$  is the y-intercept, and  $a$  and  $b$  are shape factors for a particular curve. These latter three values are calculated using Equations 8-10, where 'x' is the % of the control response for a given concentration. Plots showing the relationship between each of these three factors and % of the control are shown in Appendix D.

$$Y_0 = 2.20 - 0.09 \cdot \ln(x - 8.62) \quad \text{Equation 8}$$

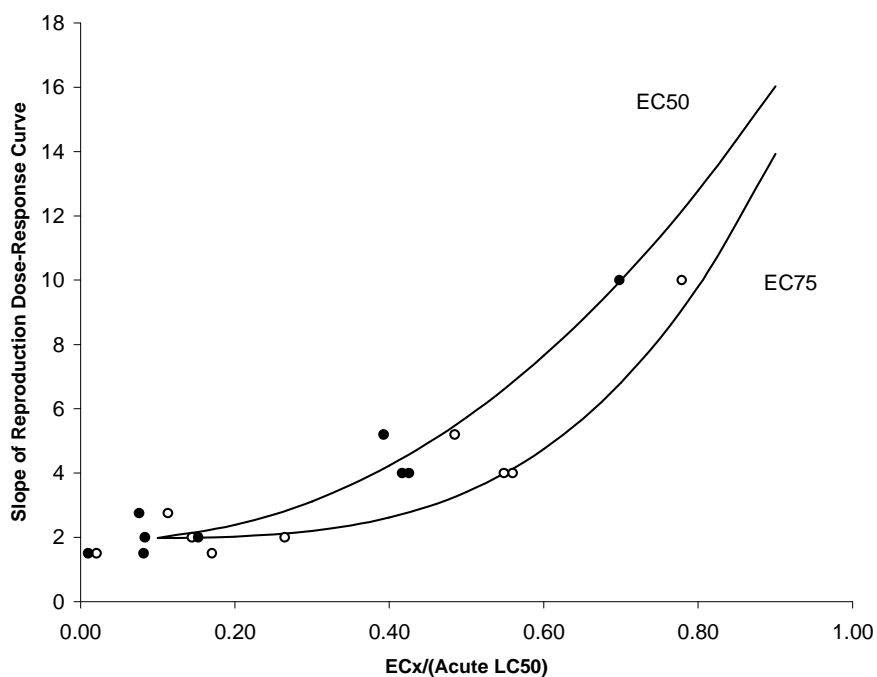
$$a = 16 + 0.6 \cdot x^{0.3} \quad \text{Equation 9}$$

$$b = 6.25 - 1.10 \cdot \ln(x - 9.00) \quad \text{Equation 10}$$

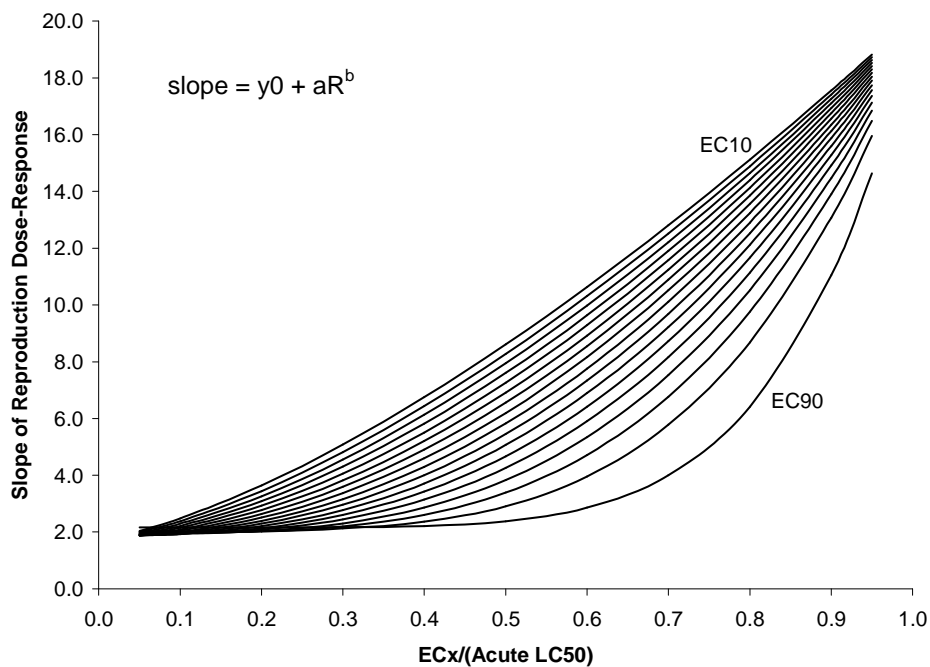
Why is the above important? In many data sets a dose-response curve for reproduction may not be available. However, the data requirements for a mysid chronic test will at least yield an *LOEC* value. The associated percent difference from the control for this value becomes the 'x' in Equations 8-10. These in turn give the values to use in Equation 7 for the slope of the reproduction dose-response curve. The *EC50* for reproduction is then calculated using Equation 4 (for % reproduction instead of survival). (Note: the *ECx* used to calculate *R* in Equation 7 is the *LOEC* associated with the % difference used in Equation 8-10.)

**Table 3.** Summary data from dose response curves in Appendix D.

Compound	Acute <i>LC50</i>	Repro <i>EC50</i>	<i>EC50/LC50</i>	Repro Slope
Acenaphthene	460	35	0.076	2.75
Aniline	2015	791	0.393	5.2
Carbofuran	5.29	2.25	0.425	4
Chlorpyrifos	42	3.5	0.083	2
Dichlorvos	13.9	9.7	0.698	10
Fluoranthene	29.3	2.4	0.082	1.5
Propoxur	36	15	0.417	4
Pyrene	31.7	4.84	0.153	2
Thallium	4830	48.6	0.010	1.5



**Figure 12.** Relationships between reproduction *EC50*/acute *LC50* and reproduction *EC75*/acute *LC50* with the slope of the dose-response curve for reproduction.



**Figure 13.** Relationships described in Figure 12 expanded to include *EC10* through *EC90* in 5% increments.

## Section 4: Sources of Stochasticity

The mysid model is a stochastic matrix model. During a model run there are several sources of stochasticity that are used to adjust the survival and reproduction parameters of the projection matrix. This includes variability in survival probability, maternity rate and sensitivity to a toxicant. Variability also can be categorized by demographic and environmental stochasticity, as well as what can be called toxicological stochasticity<sup>11</sup>. Demographic stochasticity is variation in vital rates (survival or reproduction) due to independent “chance variation in the actual fates of different individuals” within a time step of interest (Morris and Doak 2002, page 22; see also Lande et al. 2003, page 6). Environmental stochasticity, on the other hand, “describes temporal variation in vital rates driven by changes in the environment that are inherently erratic or unpredictable (unlike, for example, predictable seasonal changes)” (Morris and Doak 2002, page 18; and Lande et al. 2003, page 6). The term toxicological stochasticity describes the uncertainty associated with the true value of a toxicity endpoint—*LC50* for example. This latter uncertainty could be further influenced by demographic or environmental factors, or both.

### *Variability in Survival*

Table 4 shows the probability of survival for each of the first three age classes of mysids from the control treatments for each of the ten chronic tests used. These data yield three pairs of mean and variance values which were used to estimate the variance associated with any survival value. This variability comes from two main sources. The first is traditional demographic variance which can be based on the variance of the binomial distribution (Akçakaya 2002), which is

$$\text{var} = p \cdot (1 - p) / n \quad \text{Equation 11}$$

where  $p$  is the probability of survival and  $n$  is the number of individuals (which in the case of the mysid chronic test is 60). Demographic variance is usually an issue only for “fairly small populations” (Morris and Doak 2002, pages 23-25)<sup>12</sup>. This is demonstrated in Figure 14 for population sizes ranging from 10 to 100 individuals. The equation for any of these curves is

$$y = y_{\max} \cdot 4 \cdot (x - x^2) \quad \text{Equation 12}$$

Where  $y$  is the binomial variance,  $y_{\max}$  is the maximum variance (defined as  $0.25/n$ ), and  $x$  is the mean survival rate. This equation simplifies to

$$y = (x - x^2) / n \quad \text{Equation 13}$$

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<sup>11</sup> Because the model parameters are based on laboratory data the occurrence of what has been called observational or measurement error is assumed to be zero.

<sup>12</sup> Morris and Doak state: “As a rough guideline, we suggest that if there are at least 100 individuals in the population for a count-based PVA, or at least 20 individuals in the most important life stages in a demographic PVA (often the reproductive adults), it is usually safe to ignore demographic stochasticity.”—page 25.

The other source of variability is associated with the fact that the tests were conducted over a period of several years. Thus even though the same test procedures were used and the tests were conducted in the same laboratory, there are some expected differences in the water source, mysid population, and personnel conducting the tests. This might be thought of as the “environmental stochasticity” associated with laboratory tests. Table 4 lists the survival probabilities for the first three age classes from the chronic tests listed in Table 1 (the fourth age class is not included because, based on Equation 2, measured data is needed from the fifth age class, which is not available).

**Table 4.** Individual  $P_i$  values for each compound, calculated from the individual  $I(i)$  values presented in Table 1. Note: the mean  $P_i$  values in Table 4 do not exactly match the corresponding values from Table 2 because of Jensen’s inequality (see Ruel and Ayres 1999).

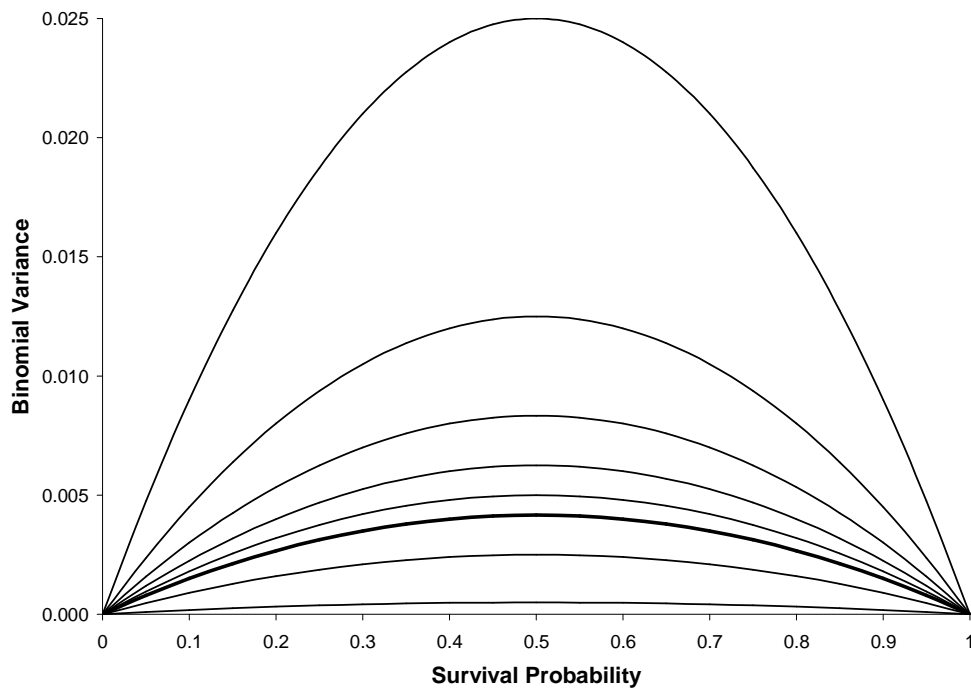
Compound	$P_1$	$P_2$	$P_3$
Acenaphthene 890703	0.966	0.860	0.776
Acenaphthene 890805	0.914	0.830	0.739
Aniline 871001	0.966	0.912	0.854
Carbofuran 921001	1.000	0.950	0.877
Dichlorvos 900101	0.948	0.972	0.915
Flouranthene 910702	0.929	0.971	0.902
Propoxur 900601	0.893	0.950	0.926
Pyrene 920701	1.000	0.925	0.874
Triethylene glycol/acetone	0.967	0.914	0.887
Thallium 880210	0.940	0.873	0.833
Mean	0.952	0.916	0.858
Var	0.0012	0.0023	0.0037

The mean and variance pairs for the  $P_i$  values in Table 4 are plotted in Figure 15 along with the binomial variance curves for  $n = 60$  and  $n = 35$ . The variance in these three pairs includes both demographic and “environmental” stochasticity. The portion of the variance associated with demographic stochasticity could be subtracted—as has been recommended by some (Kendall 1998, Akçakaya 2002)—because its inclusion can result in an overestimate of risks of population decline. However, this source of variance has not been eliminated for two reasons. First, the laboratory population size is large enough that this “extra” variance may not be significant. Second, the environmental variance that is currently represented by the data in Table 4 is likely to be small relative to the true variation expected in the field<sup>13</sup>. Leaving the demographic stochasticity in the model is not likely to be a significant overestimate of the true risk.

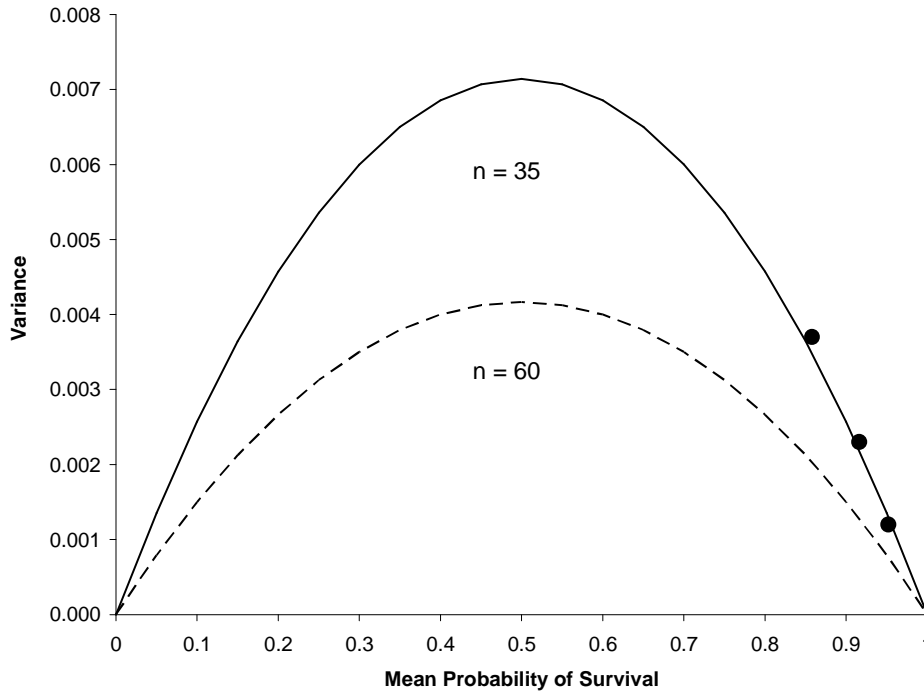
For model simulations, the relationship between the total laboratory variance and mean survival is described by Equation 11 with  $n = 35$ . Thirty-five was selected because it results in a curve that reasonably includes the three measured points. Although how accurately this curve represents the true relationship cannot be known, the variance has to be 0 when the survival rate

<sup>13</sup> This variance also is likely to be smaller than that associated with conducting the tests in different laboratories—recall that all of the data herein are from the same laboratory.

is 0 (all dead) and when survival rate is 1 (all alive). This relationship is assumed to hold no matter what the reason for a change in survival rate (e.g., age or toxicity).



**Figure 14.** Demonstration of the binomial variance for different population sizes. These are, from top to bottom, 10, 20, 30, 40, 50, 60, 100 and 500 individuals. The darkest line is the line for the typical mysid chronic treatment (60).



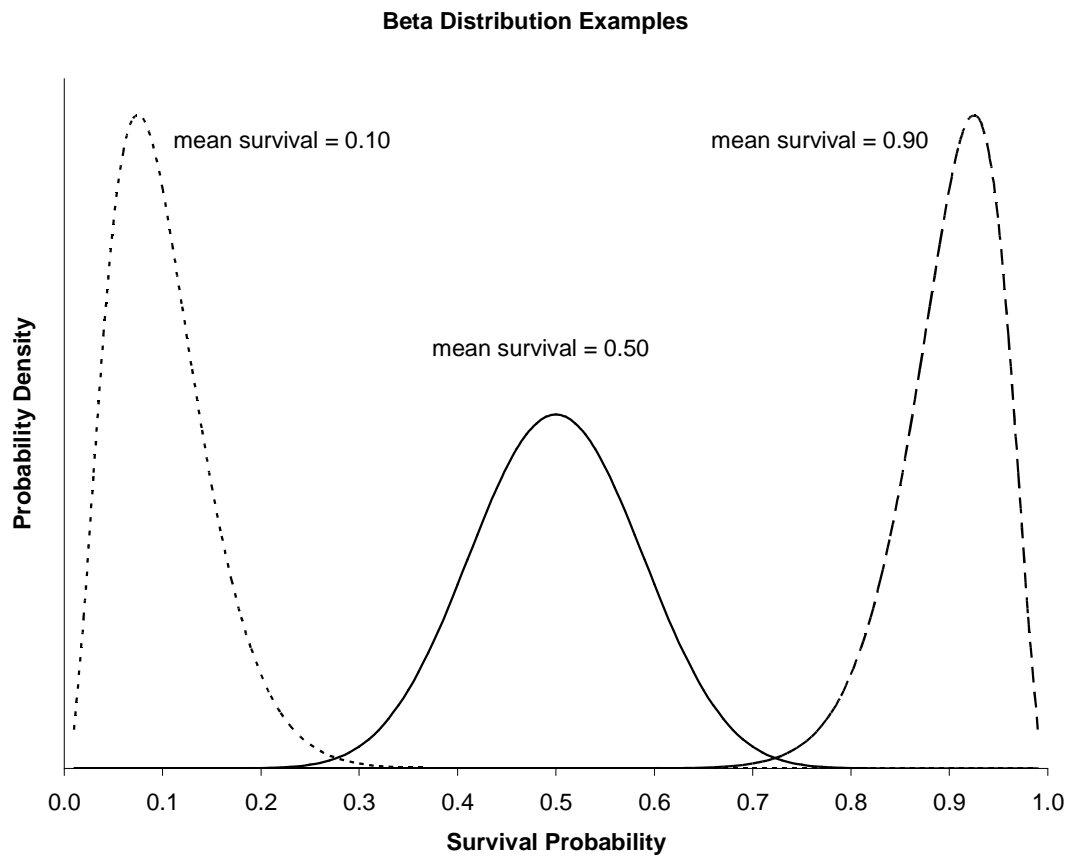
**Figure 15.** Plot of binomial variance for  $n = 35$  and  $n = 60$  along with the three pairs of mean and variance data points from Table 4.

The parameter for the binomial distribution is  $p$  (the probability of a successful event) which is usually fixed for a given situation—for example probability of a tossed coin being heads. However, in the modeling effort with the mysids  $p$  is assumed not to be fixed. At each time step of the model simulation survival values for each of the age classes is randomly chosen. A common continuous distribution for  $p$  values of a binomial distribution is the beta distribution. And the distribution recommended by Morris and Doak (2002, page 275) for survival rates is the beta distribution. All that is needed to establish the appropriate parameters for the beta distribution is the mean and variance of the survival rate for a given age class. There are two shape factors for the beta distribution called *alpha* and *beta* in order to be consistent with Microsoft® Excel. They are related to the mean and variance (Morris and Doak 2002, page 265; Evans et al. 2000) by the following equations

$$alpha = \bar{x} \left[ \frac{\bar{x} \cdot (1 - \bar{x})}{s^2} - 1 \right] \quad \text{Equation 14}$$

$$beta = (1 - \bar{x}) \left[ \frac{\bar{x} \cdot (1 - \bar{x})}{s^2} - 1 \right] \quad \text{Equation 15}$$

where  $\bar{x}$  = mean survival rate and  $s^2$  = variance of the survival rate. Beta distributions for three different mean survivals are presented in Figure 16 (variance was calculated using Equation 13).



**Figure 16.** Examples of three different beta distributions for mean survivals of 0.10, 0.50 and 0.90. The variance was calculated using Equation 12 with  $n = 35$  (based on the upper curve in Figure 13).

### Variability in Reproduction

Table 5 shows additional data for the maternity rate during chronic tests using the mysid *Americamysis bahia*. These data are from ORD's Gulf Ecology Division (GED—provided by Sandy Raimondo) and are summarized by different age classes than data from AED. These four means and variances along with the two means and variances from Table 2 are plotted in Figure 17. There are two significant conclusions from Figure 17. First, the variance increases as the mean increases. And second, the mean and variance are approximately equal. One appropriate distribution to use for maternity rate is the stretched beta distribution (Morris and Doak 2002, page 281). A stretched beta distribution is similar to a “regular” beta distribution except that it allows upper and lower limits other than 0 and 1. Equations 14 and 15 are modified slightly to accommodate these limits by substituting the following for the mean and variance (Morris and Doak 2002, page 281)

$$M = \frac{\bar{x} - \text{lower}}{\text{upper} - \text{lower}} \quad \text{Equation 16}$$

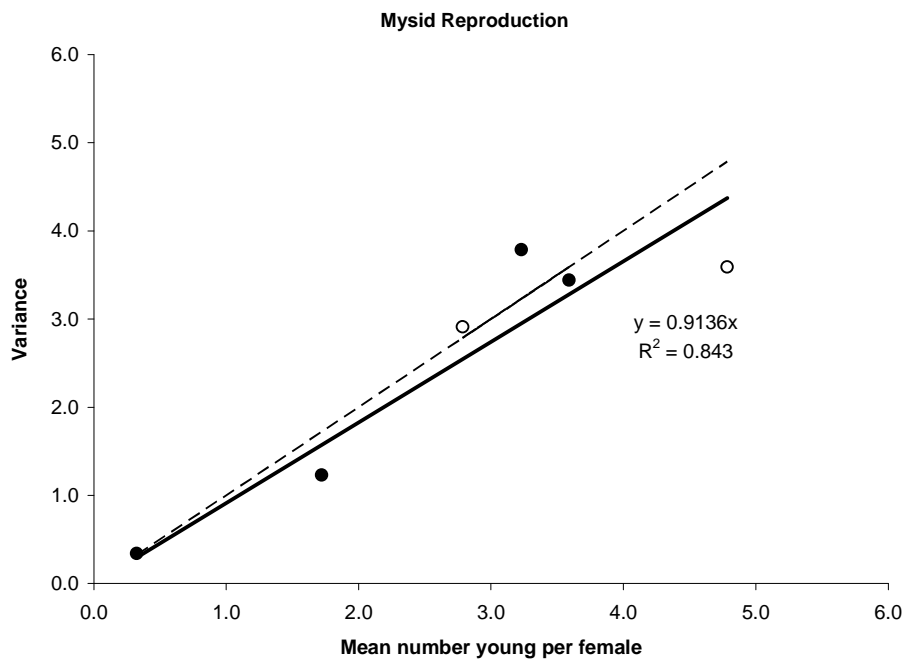
$$V = \frac{s^2}{(\text{upper} - \text{lower})^2} \quad \text{Equation 17}$$

where  $M$  and  $V$  are the “new” mean and variance used in Equations 13 and 14 to calculate the shape factors for the beta distribution.<sup>14</sup> Figure 18 shows several examples of stretched beta distributions based on the mean maternity rate for age class 4 from Table 1. Because of the relationship shown in Figure 17 the mean and variance are assumed equal. In the model simulations this will be true not only for the controls, but also for the means adjusted due to toxicity.

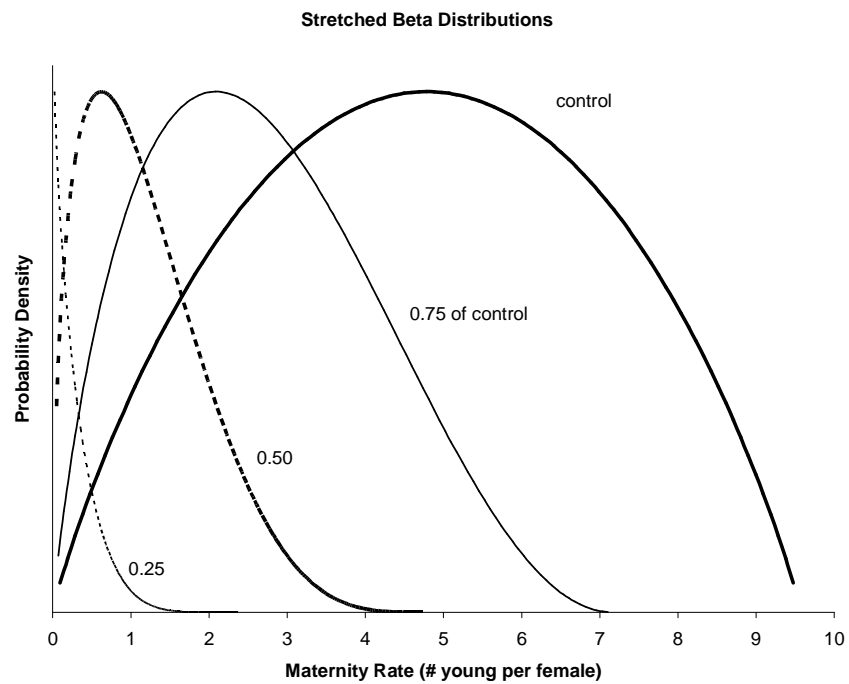
**Table 5.** Summary of mysid reproduction data in number of young per female of the given age class (original data provided by Sandy Raimondo from EPA's Gulf Ecology Division). Data column headings are the age classes that GED uses in their mysid chronic tests.

Chemical	16 days	20 days	24 days	28 days
DEF	1.44	1.36	1.71	0.00
Endosulfan	0.50	3.67	3.81	5.64
Fenthion	0	2.375	6.82	4.22
Methoprene	0	1.122	4.222	2.1
Silver Nitrate	0	1.13	2.90	3.75
Thiobencarb	0	0.67	2.07	3.67
mean	0.32	1.72	3.59	3.23
variance	0.34	1.23	3.44	3.79

<sup>14</sup> Note, minimum value of 0.3 is used for both shape factors.



**Figure 17.** Relationship between mean and variance for among test variability for mysid reproduction. Open circles are data from AED (Table 1) and closed circles are data from GED (Table 5).



**Figure 18.** Sample of stretched beta distributions used for maternity rates for mysids. Control is for mean of 4.785. For the other curves the mean was reduced to 0.75, 0.50 and 0.25 of the control. Variance was assumed to be equal to the mean.

### ***Variability in Sensitivity to Toxicants***

When assessing the risk of toxicants to population endpoints, the uncertainty associated with toxicity data can be as great as or greater than any other source of variability (Barnthouse et al. 1990). Minimum toxicity data requirements for the mysid acute test include the calculation of both the 96h *LC50* and its 95% confidence interval. This is sufficient information to create a distribution from which to incorporate the variability of toxicity into the stochastic mysid model.

Median lethal values (*LC50s*) are often assumed to have a lognormal distribution (Stephan et al. 1985). All that is needed is an estimate of the mean and standard deviation of the log (*LC50s*). As a start, an estimate of the arithmetic mean and its standard deviation are already available—reported 96h *LC50* and its 95% CI, from which the standard deviation can be estimated. Because the *LC50* has a lognormal distribution the 95% CI is often asymmetrical. A typical symmetrical 95% CI is calculated by subtracting and adding the following from the mean

$$1.96 \cdot \sigma / \sqrt{n} \quad \text{Equation 18}$$

where  $\sigma$  is the standard deviation and  $n$  is the sample size<sup>15</sup>. Even though the 95% CI is asymmetrical the confidence interval width is used to estimate the standard deviation by setting two times Equation 17 equal to this confidence interval width (CW) and solving for sigma

$$CW = 2(1.96 \cdot \sigma / \sqrt{n}) \quad \text{Equation 19}$$

$$\sigma \cong CW \sqrt{n} / 4 \quad \text{Equation 20}$$

With an estimate of the arithmetic mean and its standard deviation, they are converted into mean and standard deviations of the associated lognormal distribution. This is done using the following relationships (Evans et al. 2000)

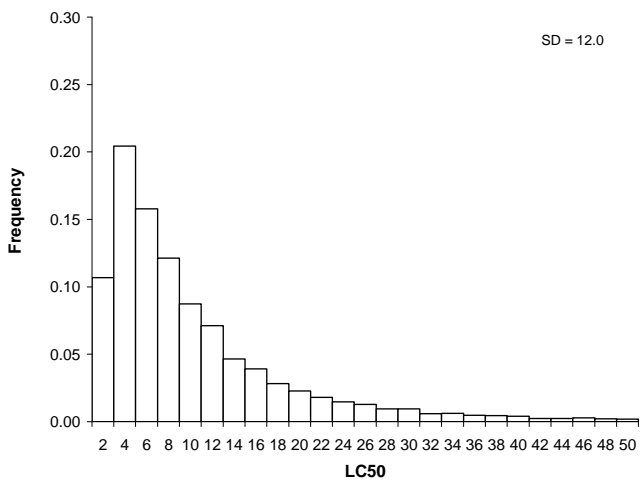
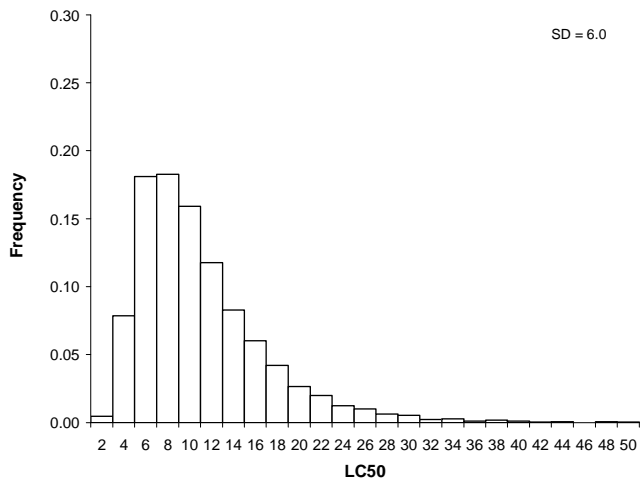
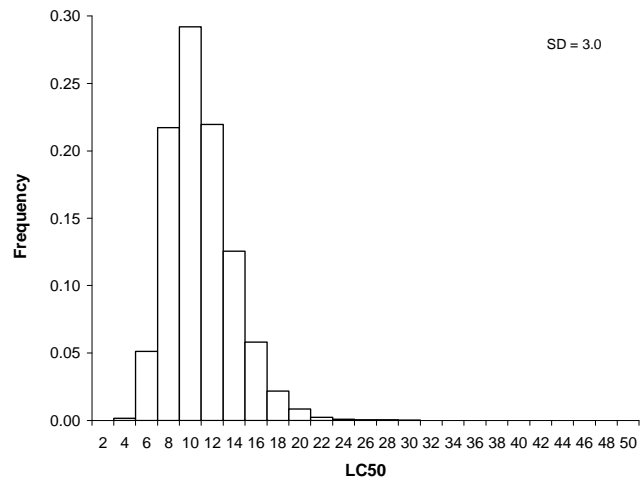
$$\mu = \ln(E(X)) - \frac{\ln\left(1 + \frac{\text{var}(X)}{E(X)^2}\right)}{2} \quad \text{Equation 21}$$

$$\sigma = \sqrt{\ln\left(1 + \frac{\text{var}(X)}{E(X)^2}\right)} \quad \text{Equation 22}$$

where  $\mu$  and  $\sigma$  are the mean and standard deviation of the lognormal distribution and  $E(X)$  is the expected value of the arithmetic mean and  $\text{var}(X)$  is its variance. Figure 19 shows three examples of a lognormal distribution. Each example has the same arithmetic mean (10.0—units are not relevant), only the arithmetic standard deviation is different. The sample size for Equation 20 is 12 since this is the minimum from the OPP guidance (two replicates, five treatments and a control).

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<sup>15</sup> Sample size for the acute test is the number of replicates per treatment times the number of treatments (including controls).



**Figure 19.** Sample lognormal distributions for  $LC50$  values. Arithmetic mean was 10.0 for each with standard deviations of 3.0 (top), 6.0 (middle) and 12.0 (bottom). Confidence interval widths were 3.47, 6.93 and 13.86, respectively.

For the sake of simplicity the model couples the variability in reproduction (*EC50s*) directly to that for survival (*LC50s*). This is done by simply maintaining a constant *LC50/EC50* ratio. This is analogous to using a fixed acute-chronic ratio (ACR) in the derivation of water quality criteria (Stephan et al. 1985). Little, if any, data are available to describe the effect of exposure duration on the *EC50* for reproduction. Therefore, the *EC50* is assumed to remain constant for each age class.

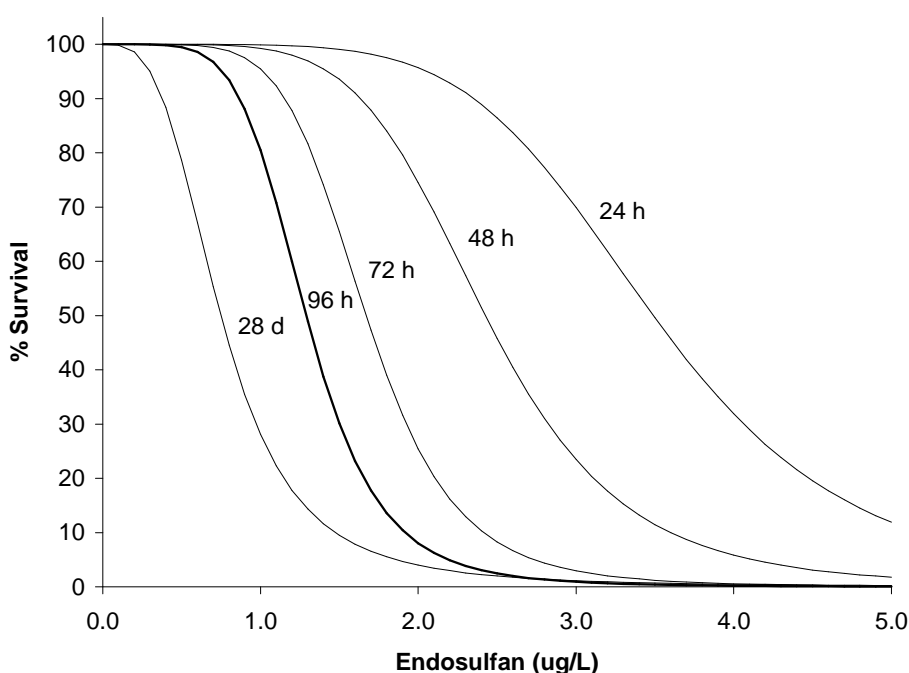
### ***Environmental Stochasticity***

The target population for this model is a typical laboratory population of *Americamysis bahia*. Therefore, this version of the model does not incorporate what might be considered traditional environmental stochasticity—variability associated with field populations having “good” or “bad” weeks for survival or reproduction. This source of stochasticity could be incorporated easily into the model. However, as of now, there is insufficient information on the magnitude of environmental stochasticity for mysids.

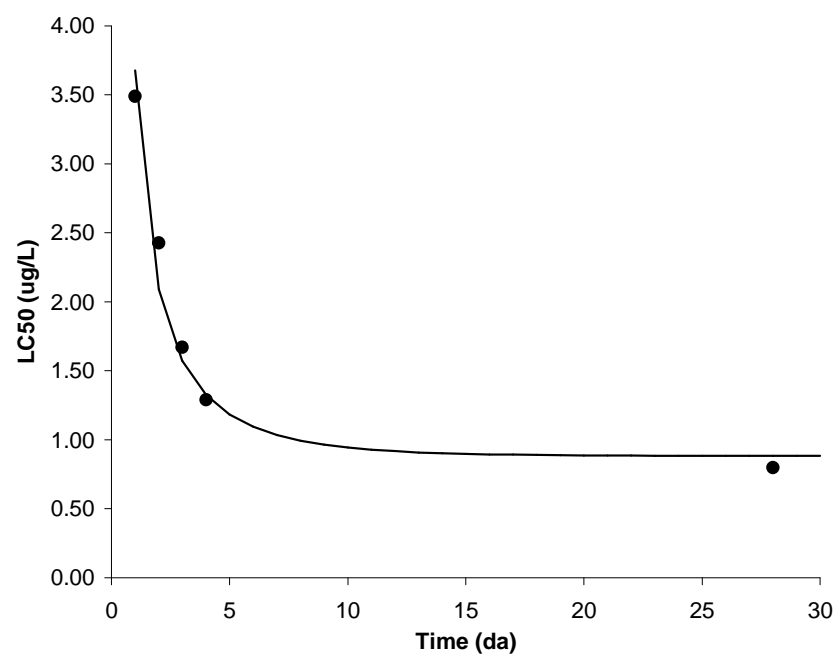
## Section 5: Model Validation of Default Values

As stated in Section 3, the use of the *Americamysis bahia* population model assumes that the minimum data available for a pesticide of interest will be the 96h acute *LC50* (along with its 95% confidence interval) and an *LOEC* (Lowest Observable Effect Concentration) for reproduction from a 28 d chronic—along with its response expressed as a percentage of control. Besides these data, the following additional information is desirable, but defaults are available: survival dose-response curve (probit slope needed); *LC50* vs time data; and reproduction dose-response curve (an *EC50* plus associated slope—based on Equation A1).

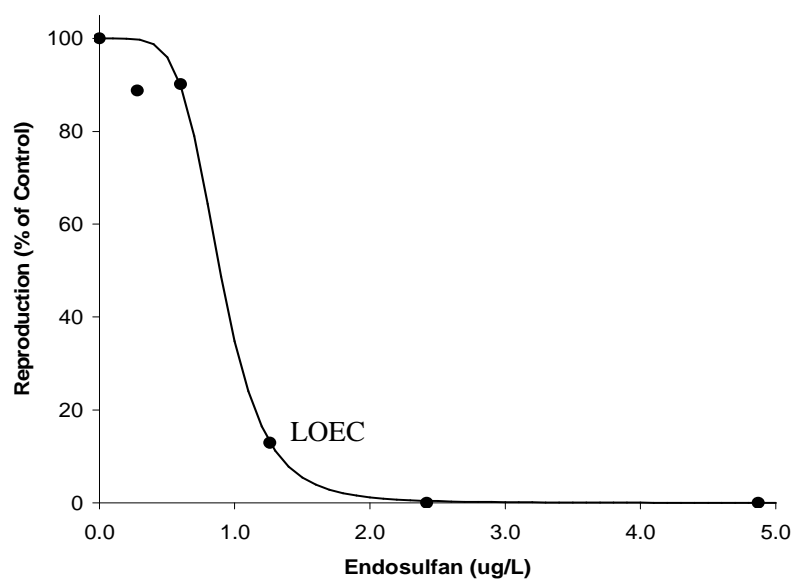
Data are available for the toxicity of the insecticide endosulfan to *A. bahia* that provide all of the above information, including the additional desired data. Thus the model can be run for endosulfan without needing to rely on the use of default parameters. This section compares the model output using this complete data set with two other runs of the model where some of the parameters were replaced with default values. Acute survival data were available from historical records at EPA's Atlantic Ecology Division and were summarized as part of an interlaboratory comparison (Schimmel 1981). Chronic survival and reproduction data were available from a similar interlaboratory comparison of chronic tests (McKenney 1982). The data from "laboratory 4" were used from this later study. The acute and chronic survival data are presented in Figures 20 and 21. The reproduction dose-response data are presented in Figure 22.



**Figure 20.** Dose-response curves from acute and chronic standard tests using *Americamysis bahia*. Curves were fit to the original data using Equation 4.



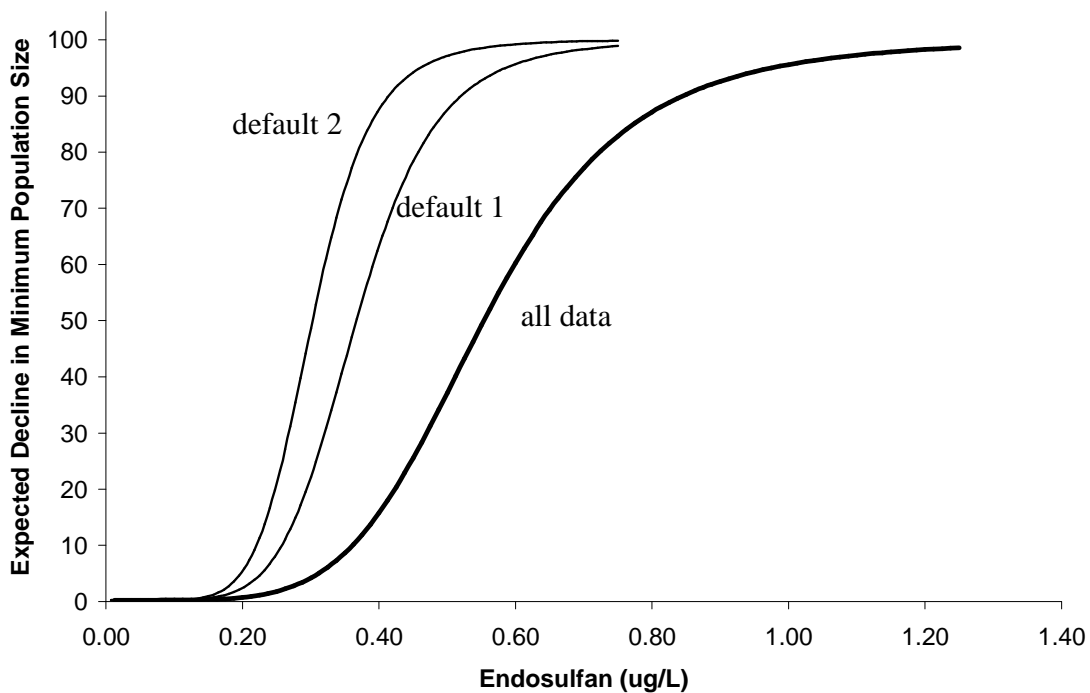
**Figure 21.** Plot of the LC50s from the data in Figure 20. Curve was fit using Equation 6.



**Figure 22.** Dose response curve for reproduction (young per available female reproductive day) expressed as percentage of the control response. Curve was fit using Equation A1.

The 96 h *LC50* for endosulfan is 1.29  $\mu\text{g/L}$  and the lower and upper 95% confidence limits are 1.00 and 1.75, respectively. The probit slope of the 96 h acute dose-response curve is 7.56. Equation 6 was fit to the *LC50* data in Figure 21 and the kinetic coefficient (*k*) is 0.27. Fitting Equation A1 from Appendix A to Figure 22 yields and *EC50* of 0.89  $\mu\text{g/L}$  and a slope of 5.47. The *LOEC* was 1.26  $\mu\text{g/L}$ , representing a response that is 13% of the control.

The above endosulfan data were used for a model run using 1000 time steps and 10 concentrations plus a control. Two other model simulations were run using default values for some of the data needs. In the first of these (default 1) OPP's default survival probit slope was used (4.5); the reproduction dose-response curve was calculated using the *LOEC* and % of control (*EC50* = 1.13  $\mu\text{g/L}$  and slope = 17.5); and a survival kinetic value calculated using a second *LC50* (2.43  $\mu\text{g/L}$ ) at 2 days. The second default run (default 2) used the same information as the first default run, except the survival kinetics were dictated by assuming the 100 day to incipient *LC50* default. The dose-response curves for all three model runs are shown in Figure 23. Note that, at least in this example, the use of default values results in more conservative output. The concentrations causing a 30% decline in populations are 0.47, 0.32 and 0.27  $\mu\text{g/L}$  for "all data", "default 1" and "default 2", respectively.



**Figure 23.** Model output from three separate runs. The line labeled "all data" was the run within which all of the input parameters were derived from the original toxicity tests data. The other two lines are from runs within which some data were ignored and default values used instead. See text for explanation.

## Section 6: Description of Excel Version of Mysid Matrix Population Model

The platform for this version of the mysid model is a series of seven Microsoft Excel spreadsheets. A limited amount of Visual Basic for Applications (VBA) code is used in various macros (Appendix G) and serves to automate the processing of the various spreadsheets. Technically one could run the model without this code; it just would be more tedious and time consuming. The spreadsheets are explained in detail in the sections below.

### Sheet: *Input Parameters*

The *Input Parameter* worksheet consists of three parts. The first is the area for inputs needed to run the model (Figure 24). This is the place for user supplied toxicity data and information on model runs to calculate the dose response curve.

Model Inputs for <i>Americamysis bahia</i>			
<b>Toxicant Information</b>			
Compound	Endosulfan		
units	ug/L		
<b>Survival</b>			
96 h LC50	1.29	Select one from the four buttons below for LC50 kinetics	
lower 95% CL	1	<input checked="" type="checkbox"/> Any other LC50: 2.43 at time 2 days	
upper 95% CL	1.75	<input type="checkbox"/> Estimated time to incipient LC50	
Probit Slope	7.56	<input type="checkbox"/> Use <b>DEFAULT</b> time to incipient LC50 (100 days)	
n	21	<input checked="" type="checkbox"/> "k" from any other source	0.27
		<b>Survival Curve Factor</b>	1
<b>Reproduction</b>			
LOEC	1.26	Select one from the two buttons below for reproduction	
% of control	13	<input type="checkbox"/> Estimated EC50	& slope
		<input checked="" type="checkbox"/> Actual EC50	0.89 & slope 5.47
<b>Model Parameters for Multiple Exposure Concentrations</b>			
# Time Steps per Conc	1000	weeks	View Input Summary Graphs
# of Tox Conc	10		
Initial Tox Conc	0	ug/L	
Tox Conc Increment	0.125	ug/L	
<div style="border: 2px solid black; padding: 5px; display: inline-block; background-color: #90EE90;"> <b>RUN</b> </div>			
% Completion of Current Run <span style="border: 1px solid black; padding: 2px;">100%</span>			
Run Time 2.03 Minutes			

**Figure 24.** Area of the *Input Parameter* worksheet requesting model inputs.

The cells in Figure 24 that are colored pale yellow are fields for data input. The green “RUN” button begins the simulation run. Click on the “View Input Summary Graphs” box and the view moves to the area that displays the survival and reproduction dose-response curves. The time and % completion locations at the bottom are just for information purposes so that the user can monitor the operation of a given simulation. Note that only the cells where data can be entered can be accessed.

There are four main sections of the input screen. The first, **Toxicant Information**, is used to identify the compound of interest. This information also is used to update the x-axis label in the output section of the *Input Parameter* worksheet (see below).

The second, **Survival**, is for the data associated with survival from the standard 96h toxicity tests. These include the *LC50*, the lower and upper 95% confidence limits, the probit slope<sup>16</sup> of the dose-response curve, and the sample size (*n*). The sample size, along with the confidence limits, is used in Equation 20 to estimate the standard deviation of the *LC50*. The right side of the *Survival* area has four “buttons”. One of these needs to be selected to indicate which method is being used to derive the kinetic parameter for the change in *LC50* with time (the “*k*” in Equation 6). The first button provides the data needed to use Equation B4 from Appendix B to calculate the kinetics factor based on known *LC50s* at two different durations of exposure. The next two buttons provide the information needed to use Equation B3. The final button provides *k* estimated directly from *LC50s* from a series of durations. The *Survival Curve Factor* is explained below in the section entitled, Sheet: *Survival Curve Graph*.

The third area on the input sheet is the area for data on **Reproduction** effects. The *LOEC* and % of the control response are needed if Equations 4 and 7 are going to be used to estimate the *EC50* and slope. This information is only needed if the first button on the right is selected. If there are enough data from the chronic test to directly calculate an *EC50* and the slope of the dose-response curve, then the second button should be selected and these data provided.

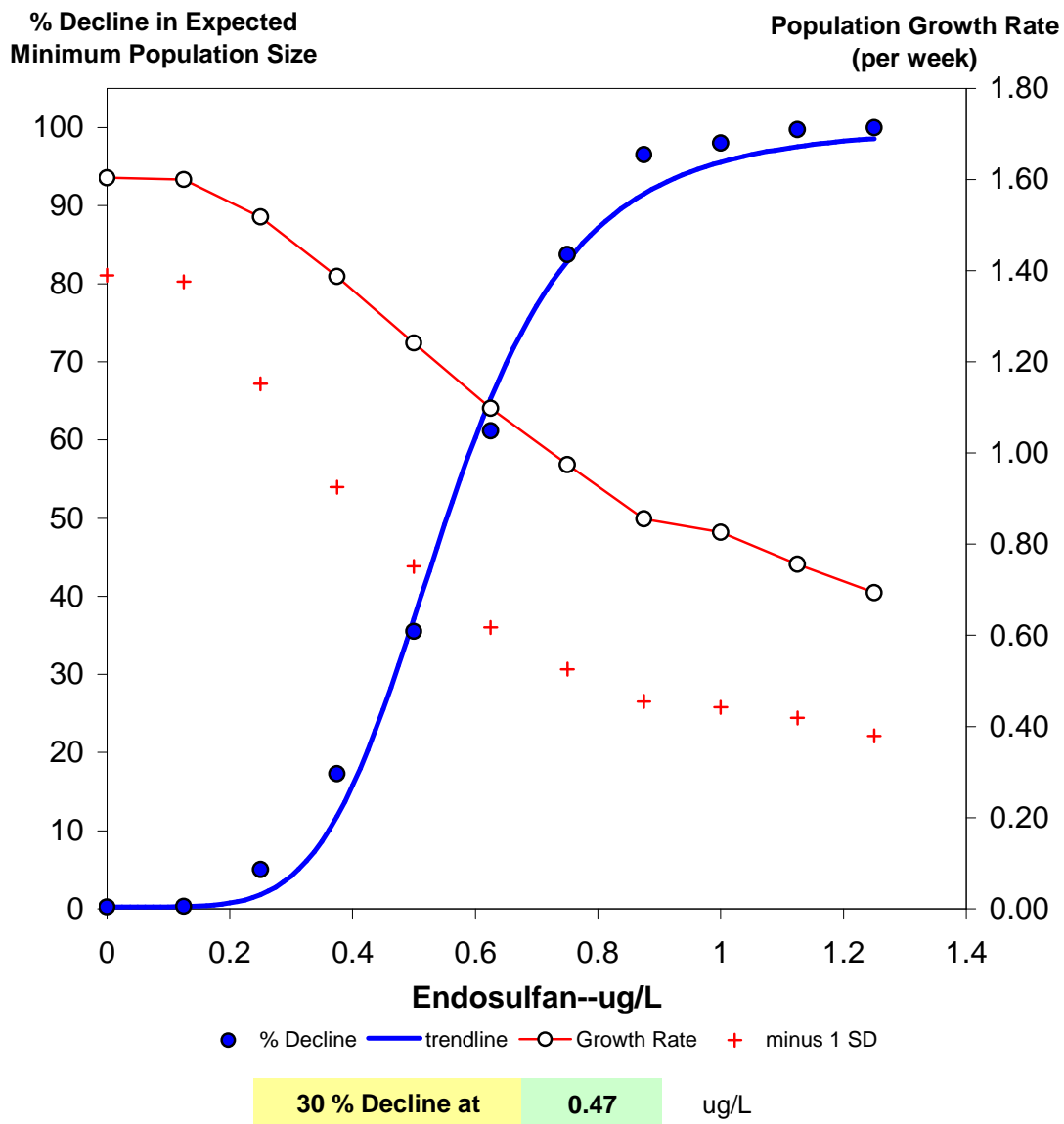
The last section on the input sheet is called **Model Parameters for Multiple Exposure Concentrations**. The first cell indicates the number of weekly time steps that each exposure concentration will be run. One hundred weeks is recommended for a “range finding” run to make sure that the dose-response curve covers a sufficient range of concentrations. Once the range of concentrations has been established, then the final run should be for at least 1000 weeks. At each weekly time step the growth rate for that week ( $\lambda$ ) is calculated and stored (both as the original  $\lambda$  and  $\ln(\lambda)$ ). At the end of each concentration the mean and variance of  $\ln(\lambda)$  are calculated and used in the PVA analysis (explained later). The next three cells in this area indicate how many toxicant concentrations are used and what they are. The final number of data points (currently fixed at 11) for the dose response will be the “# of Tox Conc” plus one for the control. The “Tox Conc Increment” is the amount by which the exposure concentration increases (linearly) for each set of exposures.

The second part of the *Input Parameters* worksheet displays the results from the most recent model run (Figure 21). This area has one additional place for user input, “**30% Decline at**”. Thirty is the default, but any value can be inserted (only the number is needed, the “% Decline

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<sup>16</sup> The probit slope is entered, but the logistic slope (Equation 4) is actually used—calculated from the probit slope. This was done because probit slope is what is traditionally supplied with test data received by OPP.

at” is part of the cell’s formatting). When a model run has been completed the concentration calculated for the given effect is displayed. However, the percentage can be changed after the model run and the calculation will be updated without rerunning the model. A “GREATER THAN” or “LESS THAN” appears if the calculated value is outside the range of exposure concentrations.

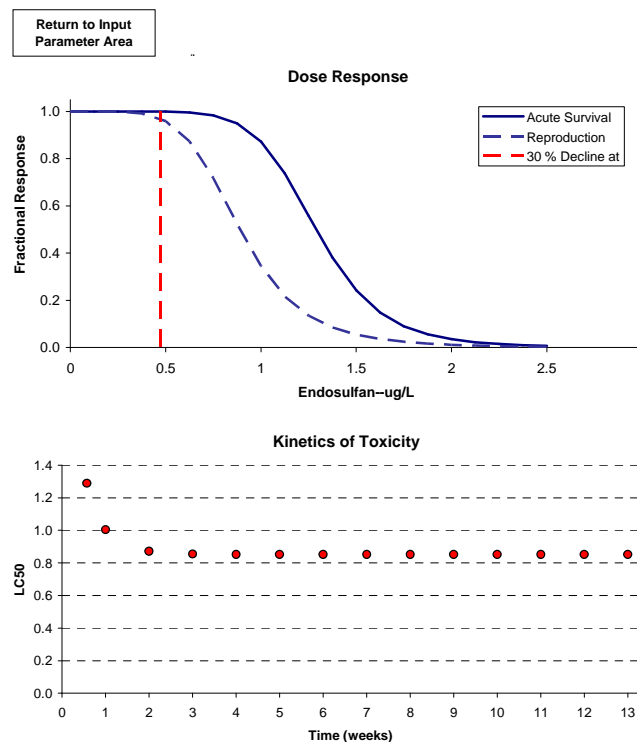


**Figure 25.** Example of output from a model run. The “% Decline at” box at the bottom is for user input of a specific effect of interest. This is automatically calculated at the end of a run. However, it can be changed without rerunning the model.

Figure 25 displays four sets of data. The solid blue circles are the calculated “% Decline in Expected Minimum Population” based on PVA analysis using changes in area under risk curves (McCarthy and Thompson 2001). The solid blue trend line is the best fit of Equation A5 (a more generic version of Equation 4). The solid red circles are the mean weekly population growth rates. And the red plus markers are these growth rates minus one standard deviation. A 30% decline in the expected minimum population size has been selected as a default cut off for calculating an exposure concentration that results in a biologically significant difference. Thirty-percent is the value used by the IUCN Red List (IUCN 2001) to categorize a species as vulnerable to extinction, and this can be applied to local extirpation. Red List categories are defined by consensus from the World Conservation Union. See Appendix F for more discussion of biological significance.

There is a small area just below the “Print” and “Save” boxes where the default pathway for the saved files to be placed. A new path may be entered into cell *Q43* and this will update the information in the macro that runs the save feature. If the pathway does not exist, then you will be asked if you want it created.

The final area on the *Input Parameter* page (Figure 26) is an area to the right of the other two areas that is used to display a summary of the dose response curves for the data that were entered (top). This plot also shows a vertical line for the population “effect” so that it can be compared to the original dose-response curves. The bottom plot displays the calculated *LC50*s for each of the weeks associated with the 13 age classes of the model.



**Figure 26.** Summary of the input dose-response curves (top) and the kinetics of the change in *LC50* over time for the current run of the model. These are based on the data entered into the Survival and Reproduction areas of the Input Parameter section.

### Sheet: *Control Survival Curve*

At the request of OPP, a method to modify the baseline survival curve for mysids was provided. This will allow a crude method to extrapolate the model from laboratory populations, with relatively high survival during the early weeks (i.e., a Type 1 survivorship curve—see Figure 13), to a population that may more accurately represent the field situation with presumably higher mortality during those early weeks (e.g., from predation). A survival curve factor of 1 gives the original data. A survival curve factor of 0.5 approximates a "Type 2" survivorship curve. Factors greater than 0.5 result in "Type 1" curves, and factors less than 0.5 "Type 3" curves. This worksheet is just for display purposes. The value for the factor is entered on the *Input Parameter* worksheet.

### Sheet: *Parameter Calculations*

The worksheet (Figure 27) has three primary areas used to calculate the  $P_i$  and  $F_i$  values for the mysid matrix model. **THERE ARE NO DIRECT USER INPUT CELLS ON THIS WORKSHEET.** All of the needed inputs for this page are linked to the *Input Parameter* worksheet; these are indicated by pale yellow file patterns in the appropriate cells. The three main areas are **Matrix: Survival**, **Matrix: Reproduction**, and **Toxicology**. The first calculates  $P_i$  values, the second calculates  $F_i$  values, and the third holds information related to dose-response curves.

*Matrix: Survival*—There is a cell for the computer to update the current exposure concentration. This value, along with the "slope", is used in Equation 4 to calculate the values in the *Toxicity Cumulative Survival* column. Below this value are cells that contain the current 96h  $LC50$  which is sampled from a lognormal distribution using Excel's *LOGINV* function. The " $k$ " is the kinetic parameter for Equation 2 which calculates how the  $LC50$  changes with time. The " $LC50_{inf}$ " is the calculated incipient  $LC50$  value—the  $LC50$  at infinite exposure duration. This is the other variable needed for Equation 6. It is calculated from a rearranged Equation 2 using the current 96 h  $LC50$ , " $k$ ," and  $t = 4$  days. The time scale for the kinetic factor is a day, therefore when the equation is used to fill in the values for the  $LC50$  column the age in weeks is multiplied by 7. The Weibull factors  $k_1$  and  $k_2$  are used in Equation 3 to fill out the column entitled, *Control Cumulative Survival*— $l(x)$ . This column is used to calculate the *Control  $P_i$*  column using Equation 1.

The column with the heading *Toxicity  $P_i$*  is calculated as the ratio of two consecutive values in the *Toxicity Cumulative Survival* column. *Product  $P_i$*  values are the product of the pairs of *Control  $P_i$*  and *Toxicity  $P_i$*  values for each age class. These values are used as the mean values for the beta distribution. The paired variances also needed for the beta shape factors are calculated using Equation 12. The shape factors, *alpha* and *beta*, are calculated with Equations 14 and 15 (the lower limit is set at 0.3 for both shape factors). These shape factors are in turn used to select *Random  $P_i$*  values using Excel's *BETAINV* function.

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The last information shown in the *Matrix: Survival* area is a boxed area to the right. The TRUE/FALSE values in this box correspond to the kinetic buttons on the *Input Parameter* worksheet. Whichever button is selected the appropriate cell in this boxed area is labeled TRUE<sup>17</sup>. The corresponding “*k*” value is selected for the *LC50* kinetics parameter mentioned above.

*Matrix: Reproduction*—The  $l(0.5)$  value is calculated from the survival area and is used in Equation 8 for the  $F_i$  values. There are two *EC50* values listed. The *EC50*s are based on the randomly selected *LC50* and the appropriate *EC50/LC50* ratio from the *Toxicology* area. The one used in the *fractional effect* calculation depends on which button is selected on the *Input Parameters* worksheet.

The *total maternity*— $m(x)$  column values within the reproduction area are multiplied by the *fractional effect* calculated from the dose-response curve. The maternity rate is the total number of young produced per female. As discussed in Section 4 under *Variability in Reproduction*, the variance is assumed to be equal to the mean. The lower limit and upper limit columns are the ranges used for the stretched beta distribution. The *Adjusted Mean* and *Adjusted Var* columns are calculated using Equations 16 and 17. The *alpha* and *beta* shape factors are from Equations 14 and 15—as with survival these have a lower bound of 0.3. The column entitled, *beta upper bound*, is included because on some occasions very large *beta* values are calculated causing an error in the *BETAINV* function. The upper bound is the same as the value in the *beta* column unless it exceeds 1000. In this case the upper bound is set to 1000. The *random m(x)* column are maternity rates (total young) selected using Excel’s *BETAINV* function. The  $Px \cdot m(x+1)$  column is a portion of Equation 2 (for convenience in calculation validation). Finally, the *Random  $F_i$*  column is based on Equation 2 (the units are now the number of females per female).

The third section on this worksheet is used to calculate parameters associated with the *toxicology* of the compound of interest. This area is subdivided into *survival*, *reproduction* and *LC50 versus time*. All of the pale yellow cells are data linked to the *Input Parameter* worksheet, and should be self explanatory. The last four cells in the survival row are calculated using Equations 20, 21 and 22, respectively. Within the reproduction row the *LOEC/LC50* value is the ratio (*R*) used in Equation 7. The values *Y0*, *a* and *b* are from Equations 8, 9 and 10, respectively. The *slope* is calculated using Equation 7. The *EC50* is calculated by rearranging Equation A1, solving for “*c*” (*EC50*). The last cell in the row is the ratio of this calculated *EC50* and the current *LC50*. Just below the end of the reproduction row are cells containing data for an *EC50* and its slope based on direct calculation from the actual test data. Finally, the TRUE/FALSE to the right of the reproduction data indicate which button was selected on the *Input Parameter* worksheet. The “TRUE” indicates which of the two *EC50*s and slopes will be used to calculate the *fractional effect* in the *Matrix: Reproduction* area.

The *LC50 versus time* area of the *Toxicology* section is the location where the *LC50* kinetics parameter is calculated only when *LC50*s from two different exposure durations are used. The value for 2<sup>nd</sup> time can be less than or greater than 4 days (the duration for the 96h *LC50*—which

<sup>17</sup> Note: the error “#DIV/0!” appears when any needed data are blank on the *Input Parameter* page.

will always be available). The values for  $2^{nd}$  time, 96h LC50 and  $2^{nd}$  LC50 are linked to the *Input Parameter* worksheet. The *Ratio* value is the largest LC50 (shortest duration) divided by the smaller LC50 (longer duration). The *Calculated Ratio* uses Equation B4 and iteratively adjusts the “*k*” using Excel’s *Goal Seek* command until this ratio is close to the actual ratio of the LC50s. If the ratio of the LC50 values is 1.05 or less (i.e., they are within 5% of each other), then the “*k*” defaults to 3.045 (see text for Equation B3) because the incipient LC50 is reached quickly. If the ratio of the LC50s is greater than the ratio of  $t_2/t_1$ , then the default “*k*” is 0.001 (see text for Equation B4). Note that  $t_1$  is always the earliest time. Whether the calculated “*k*” or one of the defaults is used is determined in the appropriate cell within the “boxed” area to the right of the *Matrix: Survival* area described above (using a formula in cell Q4).

### Sheet: *Mysid Matrix 13x13*

This worksheet contains the actual matrix model and an area for the summary of the current concentration’s simulation data. Figure 28 shows a screen shot of the model area. The cells of the matrix area are linked to the *Pi* and *Fi* columns just below the matrix. These two columns are linked to the *Parameter Calculations* worksheet and are reproduced on this sheet just for convenience. The area just to the right of the matrix has three columns of numbers, *t*, *t+1* and *normalized (t+1)*. The “*t*” column is the column vector of the current relative size of each of the age classes. The “*t+1*” column is the product of the matrix and the *t* vector—which results in the vector of the relative size of each age class at the next time step (one week). At the bottom of each of these two columns are their respective sums. The ratio of these sums is the growth rate (lambda) for the current weekly time step. This growth rate (along with ln (lambda)) is recorded for each time step. The “*normalized (t+1)*” vector is each value from the “*t+1*” column divided by the “*t+1*” total. At each new time step this normalized vector is copied and pasted to become the new “*t*” vector. Since this is a density independent model, this normalization step prevents the population size from exceeding the capacity for Excel to display numbers, allowing essentially an unlimited number of time steps.

The area entitled, **Summary information for current concentration**, is a location where the information at the end of each exposure is displayed. This information is copied and pasted into another area to the right of the matrix in Figure 28 (not shown). This latter area contains the summary information for the entire run. Some of this is used to update the endpoint graph on the *Input Parameter* worksheet. In addition to this summary information, there is also a location (not shown) on this worksheet where the growth rate for each time step for the current exposure concentration is recorded, and the mean and variance of ln (lambda) are calculated.

### Sheet: *Lambda Summary*

At the end of each exposure concentration for a given run of the model the growth rates mentioned above, along with their associated mean and variance, are copied and pasted into the *Lambda Summary* worksheet. This is done because these data are cleared from the *Mysid Matrix 13x13* worksheet at the start of each new concentration. The *Lambda Summary* worksheet provides a summary of the “raw” data for any give run of the model. **Note: these data are cleared at the beginning of each model run. It must be saved or printed if a record is desired.**

Age Class	1	2	3	4	5	6	7	8	9	10	11	12	13	t	t+1	normalized (t+1)
1	0	0.561	1.367	1.032	1.079	2.464	2.066	1.146	0.812	0.998	2.510	2.005	0.822	0.01948	1.69	0.7396
2	0.808	0	0	0	0	0	0	0	0	0	0	0	0	0.00130	0.02	0.0069
3	0	0.851	0	0	0	0	0	0	0	0	0	0	0	0.16606	0.00	0.0005
4	0	0	0.851	0	0	0	0	0	0	0	0	0	0	0.00000	0.14	0.0617
5	0	0	0	0.705	0	0	0	0	0	0	0	0	0	0.00663	0.00	0.0000
6	0	0	0	0	0.612	0	0	0	0	0	0	0	0	0.00024	0.00	0.0018
7	0	0	0	0	0	0.736	0	0	0	0	0	0	0	0.00031	0.00	0.0001
8	0	0	0	0	0	0	0.725	0	0	0	0	0	0	0.00578	0.00	0.0001
9	0	0	0	0	0	0	0	0.597	0	0	0	0	0	0.25499	0.00	0.0015
10	0	0	0	0	0	0	0	0	0.644	0	0	0	0	0.03858	0.16	0.0717
11	0	0	0	0	0	0	0	0	0	0.592	0	0	0	0.45169	0.02	0.0100
12	0	0	0	0	0	0	0	0	0	0	0.517	0	0	0.02199	0.23	0.1020
13	0	0	0	0	0	0	0	0	0	0	0	0.445	0	0.03296	0.01	0.0043
sum														1.000	2.290	1.000
														lambda	2.290	
														ln lambda	0.828	

Survival Fecundity				t-zero
Age Class	Random Pi	Random Fi		
1	0.808			0.44490
2	0.851	0.56053		0.26139
3	0.851	1.36704		0.14583
4	0.705	1.03234		0.07704
5	0.612	1.07901		0.03860
6	0.736	2.46381		0.01832
7	0.725	2.06571		0.00822
8	0.597	1.14632		0.00349
9	0.644	0.81200		0.00140
10	0.592	0.99794		0.00053
11	0.517	2.51043		0.00019
12	0.445	2.00490		0.00006
13	0.346	0.82225		0.00002

Summary information for current concentration			
conc	mean ln lambda	var ln lambda	Mean lambda
1.25	-0.3659	0.3654	0.694

**Figure 28.** Screen “shot” of *Mysid Matrix 13x13* worksheet. Note, the “summary” area displays the values from the end of the previous run.

### Sheet: *Quasi-Extinction*

This is a hidden worksheet. It can be viewed by selecting *Sheet* from Excel's *Format* menu. This sheet is called *Quasi-Extinction* because this is the phrase often used by conservation biologists in population viability analysis (PVA) to describe the risk of decline to a small population size, first used by Ginzberg et al. (1982). This sheet calculates the probability density function (pdf) and the cumulative density function (cdf) for the probability of a population falling below a given threshold during a given length of time. The threshold population size is expressed as a proportion of the initial population size (i.e., it can range between 0 and 1). These are based on Equations 3.4 and 3.5 from Morris and Doak (2002). The model currently only records the cdf value at 30 weeks<sup>18</sup>. However, both the pdf and cdf are calculated for time steps 1 through 250. This was done in part so that mean and variance values from Morris and Doak (2002) could be inserted in order to recreate their Figures 3.4 and 3.5 as a quality assurance step for the checking the accuracy of the Excel version of their equations<sup>19</sup>.

### Sheet: *Simpsons Rule*

This worksheet is named *Simpsons Rule* because this is the mathematical rule used for approximating the areas under the risk curves. It also is a hidden worksheet. The equation can be found in most any introductory calculus textbook. Even though the risk curves can account for threshold proportions from 0 to 1 (which has a maximum area of 1.0), the model only uses 0.05 to 0.95 in 0.05 increments. Therefore, the calculated area is normalized as a percent of the maximum area of 0.90.

In traditional PVA, a population threshold is selected for which the risk (or probability) of the current population declining to that threshold size is calculated. This risk is a function of this threshold (the smaller the change from the current population size, the greater the probability of observing that change), as well as the mean growth rate and the variability in that growth rate<sup>20</sup> (the greater the variability the greater the probability of decline). One problem with this approach has been the selection of a population threshold. This is overcome by the use of risk curves in which a range of population thresholds is used and the area between such curves calculated (Burgman et al. 1993).

For quasi-extinction risk curves, this area represents the change in the expected minimum population size. This is because quasi-extinction risk curves are cumulative probability distributions of the minimum population size, and the area to the left<sup>21</sup> of the curve is equal to the mean of the distribution (i.e. the expected

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<sup>18</sup> The probability of falling to or below a particular threshold has to be within a given time duration. Ten generations was chosen (30 weeks in the case of the mysid *Americamysis bahia*).

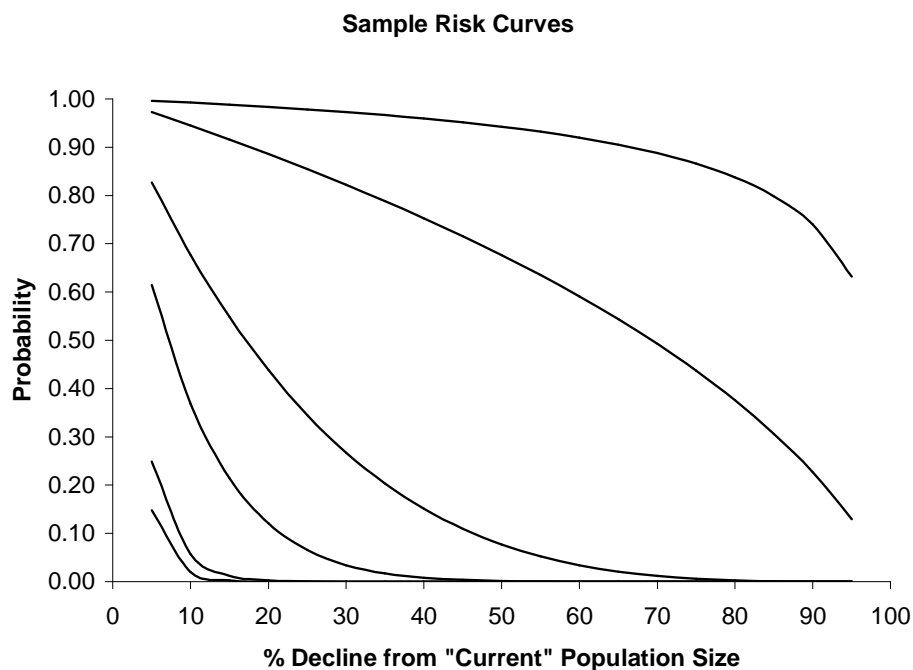
<sup>19</sup> The legends for Figure 3.4 and 3.5 in Morris and Doak (2002) both contain typos. The Equation numbers referred to should be Equation 3.4 and Equation 3.5, respectively.

<sup>20</sup> Actually what is used is the mean and variance of the ln of the growth rates.

<sup>21</sup> Note McCarthy's x-axis is the percentage of the initial population size. My calculations use an x-axis of % decline from initial population size (as does Inchausti and Weimerskirch 2004, and Akçakaya 1992), therefore my areas are those to the "right" of the curve rather than to the "left" of the curve—i.e., the x-axis is reversed. And the value is the expected minimum as a percentage of the current value.

minimum population size). Therefore, the area between two risk curves is equivalent to the change in the mean (McCarthy 1996, page 48, bottom of second column).

The area to the left (in the case of McCarthy 1996), or to the right in the mysid, case represents the expected minimum population size. If you take the area under the curve you have the proportional decline in this expected minimum—this is how the final results of the mysid model are expressed (it is analogous to the difference between expressing a survival curve as % survival or % mortality). Figure 29 shows some examples of risk curves for a control (curve on the bottom left) and 6 different hypothetical exposure concentrations. The greater the concentration the more the curves move towards the right, and the greater the percentage decline in the expected minimum population size. “Expected minimum” is referred to because by definition a risk curve is the cumulative distribution function for the minimum population size observed within a given period of time (McCarthy and Thompson 2001). The change in the expected minimum population size is equal to the area between two risk curves (McCarthy and Thompson 2001). Therefore, the total area under a given curve represents the expected percentage decline in the expected minimum populations. This is graphed in the summary plot on the *Input Parameter* worksheet of the model.



**Figure 29.** Sample risk curves. Bottom curve is the lowest exposure concentration and the top curve is the highest exposure concentration. As the magnitude of the decline increases, the probability for that amount of decline decreases.

### Sheet: *PVA Solver*

The hidden worksheet named *PVA Solver* uses Excel's Solver function to make the best fit curve to the dose-response curve for the percentage decline in the expected minimum population size. This curve is plotted on the summary graph on the *Input Parameter* worksheet. The Solver function selects the 50% effect concentration and slope for Equation A5 giving the best fit to the above dose-response curve (i.e., a non-linear least squares regression). Equation A5 is rearranged, using the above 50% effect concentration and slope, and solved for the concentration that yields the “% Decline at” from the *Input Parameter* worksheet. If the calculated value is greater than the highest concentration “tested,” then a “GREATER THAN” appears. Likewise, a “LESS THAN” appears if the value is less than the lowest concentration.

### Sheet: *Data Entry Error Check*

This is another hidden worksheet. It is linked to the *Input Parameters* worksheet, and checks for data entry errors. These included checking for missing data (blanks), and checking for non-numbers or negative numbers. It verifies that the confidence limits and the 96 h *LC50* are in the correct numerical order. This sheet also verifies that when the “two *LC50* method” is used to calculate the kinetics of the *LC50* that the *LC50* for the shorter duration is greater than the *LC50* for the longer duration. If errors in data entry are found, then an appropriate message is displayed and the model run terminated.

### Sheets Summary

The information in Section 6 summarizes the contents of all nine worksheets that are part of the Excel model's file—four hidden sheets and five visible sheets. Only the *Input Parameter* sheet remains open to the user. The other four visible sheets have to be selected to see their contents, but this is not necessary to either run the model or save the information at the end of a simulation run. Likewise the hidden sheets do not need to be seen for the model to be used. If you want to view the contents of any of the hidden sheets, then just select the **Format** menu, followed by **Sheet**, and then **Unhide**. You will be presented with a list of the hidden sheets; select the one you wish to view. Any unhidden sheets will be hidden again during the next model run—so it is not necessary to use the **Format** menu to hide the sheets.

## Appendix A: Equations used to model dose-response data.

There are a variety of sigmoid equations that have been used to model dose-response data (including both survival and reproduction endpoints). The one that is chosen here is a fairly simple one:

$$y = \frac{a}{1 + \left(\frac{x}{c}\right)^b} \quad \text{Equation A1}$$

Where:

- $y$  = proportional response of interest—for example survival or reproduction.
- $x$  = stressor concentration.
- $a$  = upper limit—for example 100 for percentage survival or percentage response relative to the control; or it can be the actual control response for reproduction or growth.
- $b$  = slope (or shape factor)—describes the steepness of the dose-response curve. If  $b$  is positive, then  $y$  decreases as  $x$  increases. If  $b$  is negative, then you get the opposite;  $y$  increases as  $x$  increases.
- $c$  = is the concentration that results in a 50% response—the inflection point of the sigmoid curve.

Because of the “ $a$ ,  $b$ , and  $c$ ”, this is often called a *three-parameter* logistic equation<sup>22</sup>. It frequently is reported in other forms:

$$y = \frac{a}{1 + \exp\{b[\ln(x) - \ln(c)]\}} \quad \text{Equation A2}$$

$$y = \frac{a \cdot x^b}{x^b + c^b} \quad \text{Equation A3}$$

If you apply mathematical rules for exponents and logarithms, Equation A2 simplifies to Equation A1 because:

$$\exp\{b[\ln(x) - \ln(c)]\} = \left(\frac{x}{c}\right)^b.$$

Equation A3 is the “familiar” Hill equation used in biochemistry<sup>23</sup>. If you divide the numerator and denominator of Equation A3 by  $x^b$  (same as multiplying by 1), the equation becomes:

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<sup>22</sup> There are other equations that are also called logistic equations and they are probably just as useful for fitting dose-response data as Equation A1 above. However, I prefer Equation A1 because it incorporates *EC50s* (or *LC50s*)—for which data are often available.

$$y = \frac{a}{1 + \left(\frac{c}{x}\right)^b} \quad \text{Equation A4}$$

Equation A4 is slightly different from Equation A1 because the Hill equation is most often used in situation where  $y$  increases as  $x$  increases. Mathematically Equations A1 and A4 are equivalent; however, in Equation A4 unlike Equation A1 if  $b$  is positive, then  $y$  increases as  $x$  increases. If  $b$  is negative, then you get the opposite;  $y$  decreases as  $x$  increases. This is true because:

$$\left(\frac{x}{c}\right)^b = \left(\frac{c}{x}\right)^{-b}.$$

The range for  $y$  in the above equations is from 0 to  $a$ . If there is a different lower limit, then Equation A1 is simply modified into a *four-parameter* logistic equation:

$$y = d + \frac{a}{1 + \left(\frac{x}{c}\right)^b} \quad \text{Equation A5}$$

Where  $d$  is the lower limit for  $y$ .

---

<sup>23</sup> Mouton, JW, MN Dudley, O Cars, H Derendorf and GL Drusano. 2005. Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: an update. J. Antimicrobial Chemotherapy 55:601-607.

## Appendix B. Calculation of kinetic coefficient for time-to-effect curves.

Traditionally changes in the median lethal effect as exposure duration increases have been the primary concern. However, the mathematics involved can be applied to any effect ( $ECx$ ). The following is based on the assumption that the kinetics of time-to-effect can be described by:

$$y_t = \frac{y_\infty}{1 - e^{-kt}} \quad \text{Equation B1}$$

Which is the same as Equation 4 in the main text, but where:

$y_t$  = a concentration that causes a specific percent effect (lethal or otherwise) at a given time  $t$  in days,

$y_\infty$  = a concentration for that same specific effect, but for a sufficiently long time such that no additional adverse effects occur (the threshold or asymptotic concentration at “infinity”),

$k$  = the kinetic coefficient that determines how quickly the effect concentration changes with time.

We can rearrange Equation B1 using simple algebraic steps and solve for  $k$ .

$$\begin{aligned} 1 - e^{-kt} = \frac{y_\infty}{y_t} &\rightarrow -e^{-kt} = \frac{y_\infty}{y_t} - 1 \rightarrow e^{-kt} = 1 - \frac{y_\infty}{y_t} \rightarrow -kt = \ln\left(1 - \frac{y_\infty}{y_t}\right) \\ &\rightarrow -k = \frac{\ln\left(1 - \frac{y_\infty}{y_t}\right)}{t} \quad \text{Equation B2} \end{aligned}$$

Note: as  $t$  approaches  $\infty$ ,  $y_t$  approaches  $y_\infty$ , and  $y_\infty/y_t$  approaches 1.

Let's assume that when  $t$  is “close enough” to  $\infty$  that  $y_t = 1.05 \cdot y_\infty$ . In other words, when  $y_t$  is within 5% of  $y_\infty$  it is close enough to the asymptote for toxicological purposes<sup>24</sup>. Substituting this value for  $y_t$  in the equation above results in:

$$\begin{aligned} -k = \frac{\ln\left(1 - \frac{y_\infty}{1.05 \cdot y_\infty}\right)}{t} &\rightarrow -k = \frac{\ln\left(1 - \frac{1}{1.05}\right)}{t} \rightarrow -k = \frac{\ln(0.0476)}{t} \\ &\rightarrow k = \frac{3.045}{t} \quad \text{Equation B3} \end{aligned}$$

<sup>24</sup> The equation for  $k$  can be solved using any percentage. Because the asymptotic  $LC50$  is just that, an asymptote, 5% gets close enough.

This means that if the approximate time in days at which the effect concentration nears the threshold is known the kinetic coefficient for the time-to-effect curve can be calculated. Sufficient data to estimate the number of days to the threshold effects concentration will not likely be available. It can be estimated, however, from a limited amount of data that are likely to be available. In fact the “ $k$ ” used in Equation B3 can be estimated given a few more mathematical relationships.

For any given kinetic coefficient  $y_t$  can be calculated for pairs of time values (e.g., days 4 and 28) and the ratio of those  $y$  values will be a constant no matter what the value of  $y_\infty$ . This is shown below. For  $y$  at  $t_1$  and  $t_2$  the following two equations result from Equation B1:

$$y_{t_1} = \frac{y_\infty}{1 - e^{-kt_1}} \quad \text{and} \quad y_{t_2} = \frac{y_\infty}{1 - e^{-kt_2}}$$

The ratio of  $y_{t_1}/y_{t_2}$  is:

$$\frac{\frac{y_\infty}{1 - e^{-kt_1}}}{\frac{y_\infty}{1 - e^{-kt_2}}}$$

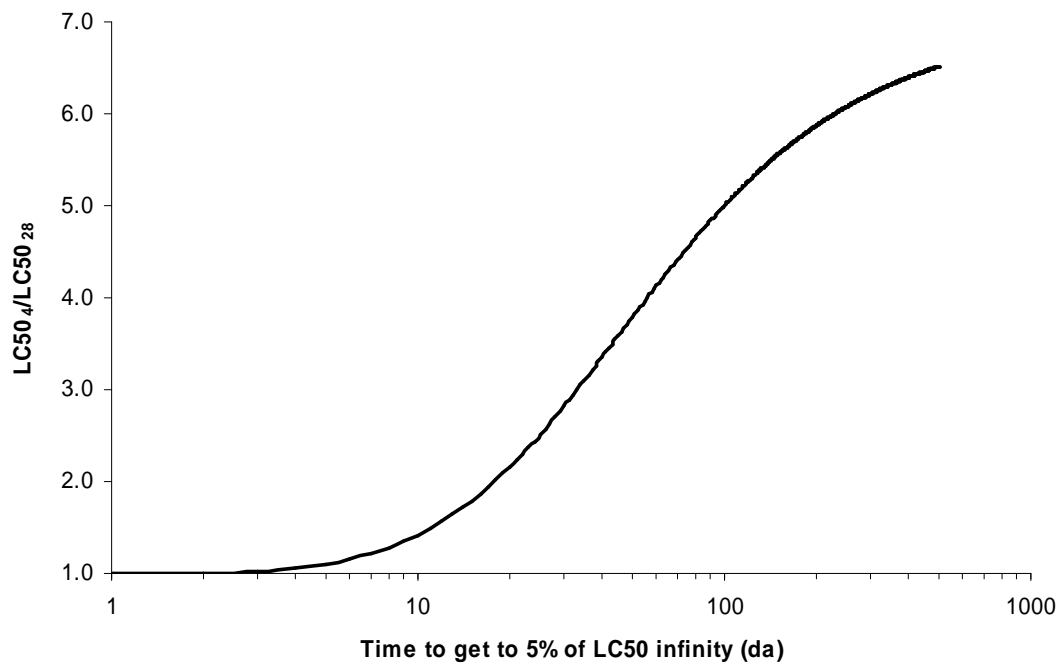
The  $y_\infty$ ’s cancel out:

$$\frac{\frac{1}{1 - e^{-kt_1}}}{\frac{1}{1 - e^{-kt_2}}}$$

The fractions rearrange, and the final ratio becomes:

$$y_{t_1}/y_{t_2} = \frac{1 - e^{-kt_2}}{1 - e^{-kt_1}} \quad \text{Equation B4}$$

The value of this ratio is independent of  $y_\infty$ . If a standard set of time interval pairs for the ratio is chosen, then the value of the ratio only depends on  $k$ . The relationship between this ratio and  $k$  (or between the ratio and time-to-threshold since  $k = 3.045/t$ ) can be examined. Figure B1 shows the complete relationship between the ratio of effects on day 4 and day 28 and the exposure duration needed to reach within 5% of the asymptotic  $LC50$ . Similar curves could be easily created for any pair of durations. The main text sets the default ratio at 5, this corresponds to an exposure duration of 100 days. The upper limit for the ratio in Equation B4 is the ratio  $t_2/t_1$ .

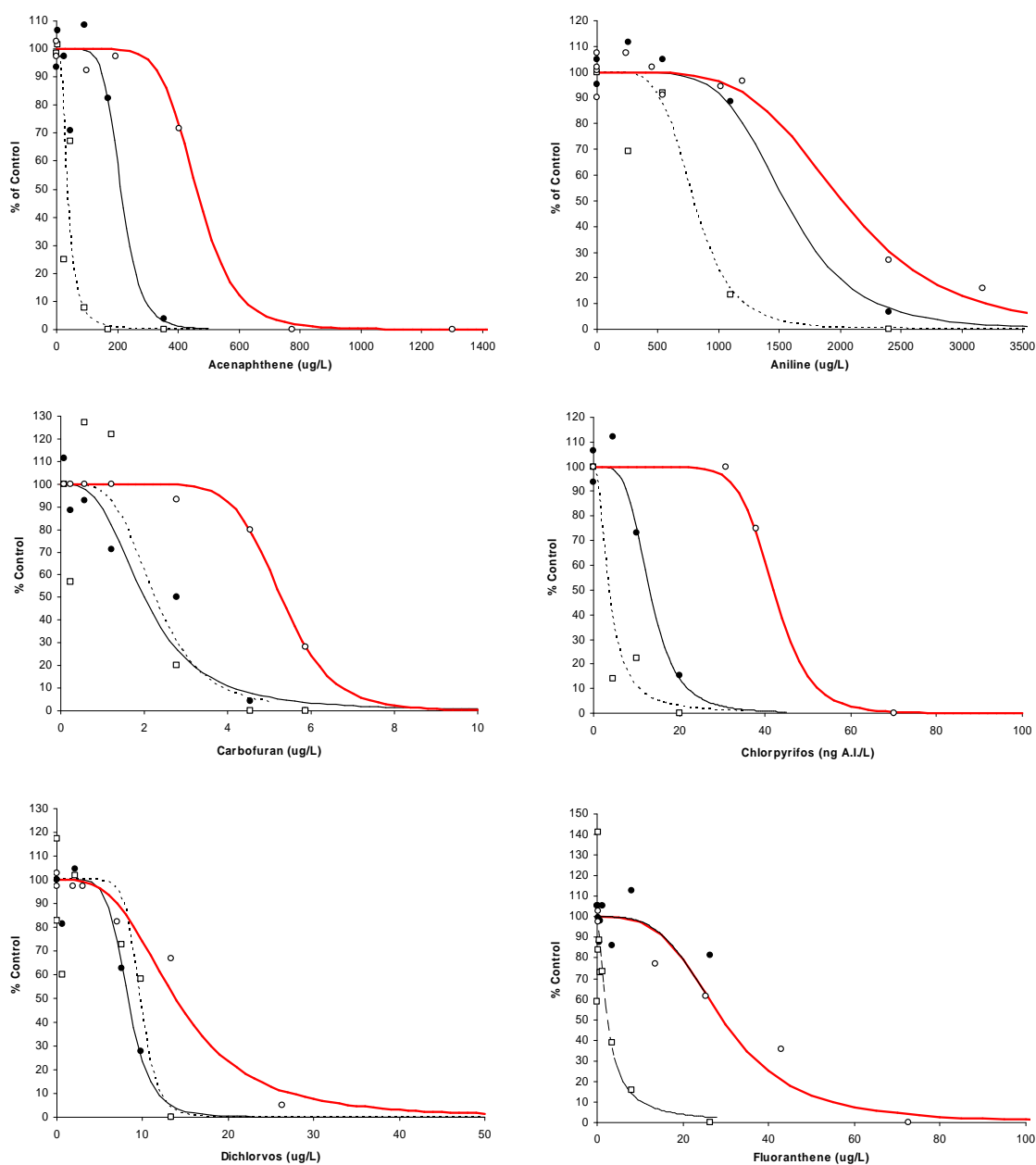


**Figure B1.** Relationship between the time to the asymptotic  $LC50$  and the ratio of the  $LC50$ s for 4 and 28 day exposure durations. This is the same figure as Figure 7 in the main text.

## Appendix C: List of compounds used for Figure 8 in the main text.

Chemical	Chronic (28 da) LC50	Acute (96 hr) LC50	Ratio	Comment	References
Acenaphthene	211	460	2.18		Thursby et al. 1989
Ammonia	> 331	1700	< 5.14	EC40 at 260	Cardin 1986
Aniline	1547	1930	1.25		Thursby and Berry 1987b
Arsenic III	818	1740	2.13		Lussier, et al. 1985
Atrazine	232	1000	4.32		Ward and Ballantine 1985
Cadmium	8.04	110	13.68		Lussier, et al. 1985; Gentile, et al. 1982
Carbaryl	9.7	9.0	0.92	Essentially a ratio of one	Thursby and Champlin 1991
Carbofuran	2.0	5.3	2.65		Champlin and Poucher 1992b' acute from day 4 of chronic
Chlorpyrifos	0.020	0.042	2.10	Chronic estimated: test lasted 35 da	OPP 1993, 1994
Chromium VI	> 909	2030	< 2.23	NOEC at 909, EC10 at 68, no EC15 or above	Lussier, et al. 1985
Copper	126.00	181	1.44		Lussier, et al. 1985
Dichlorvos	8.3	13.9	1.67		Thursby, et al. 1990
Fluoranthene	27	31	1.15		Champlin and Poucher 1991a,b
Mercury	> 2.51	3.50	< 1.39	EC40 at 2.11	Lussier, et al. 1985
Nickel	117	508	4.36		Lussier, et al. 1985
Phenanthrene	7.2	27.1	3.76		Kuhn and Lussier 1987
Phenol	3924	8500	2.17		Kuhn et al. 1987; Thursby and Berry 1987a
Propoxur	14.2	36.0	2.54		Thursby, et al. 1990
Pyrene	23	32	1.39		Champlin and Poucher 1992a; acute from day 4 of chronic
Selenium IV	376	1500	3.99		Ward, et al. 1981
Silver	64	141	2.22		McKenney 1982
Thallium	2050	4830	2.36		Thursby and Berry 1988
1,2,4-Trichlorobenzene	235	490	2.08		Springborn 1988 a,b
Zinc	229	499	2.18		Lussier et al. 1985

**Appendix D.** Graphs from chronic tests used to establish the relationship between acute toxicity and the slope of the reproduction dose response curve. Equation 4 was fit to each set of data.



**Figure D1.** Dose response data from chronic tests using the mysid *Americamysis bahia*. Open circles are survival data from 96h acute tests, closed circles are 28d chronic survival, and open squares are reproduction data from 28d tests. All of the data, with the exception of chlorpyrifos, are from AED's historical test data. Chlorpyrifos data were provided by OPP.

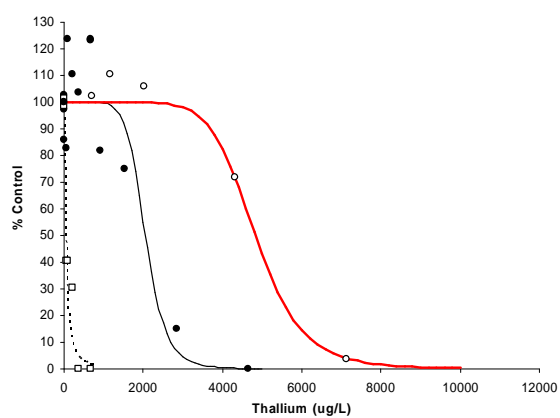
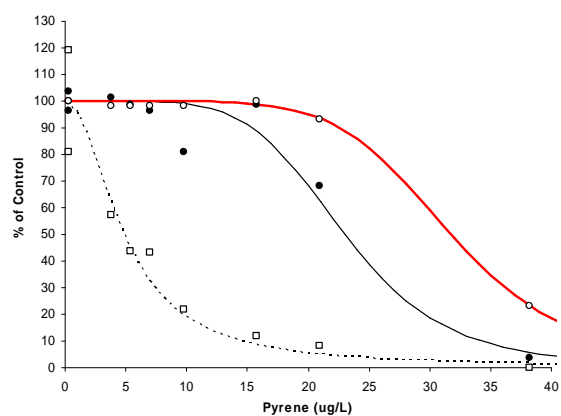
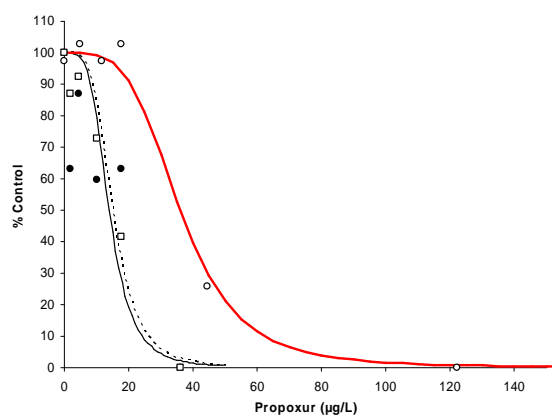
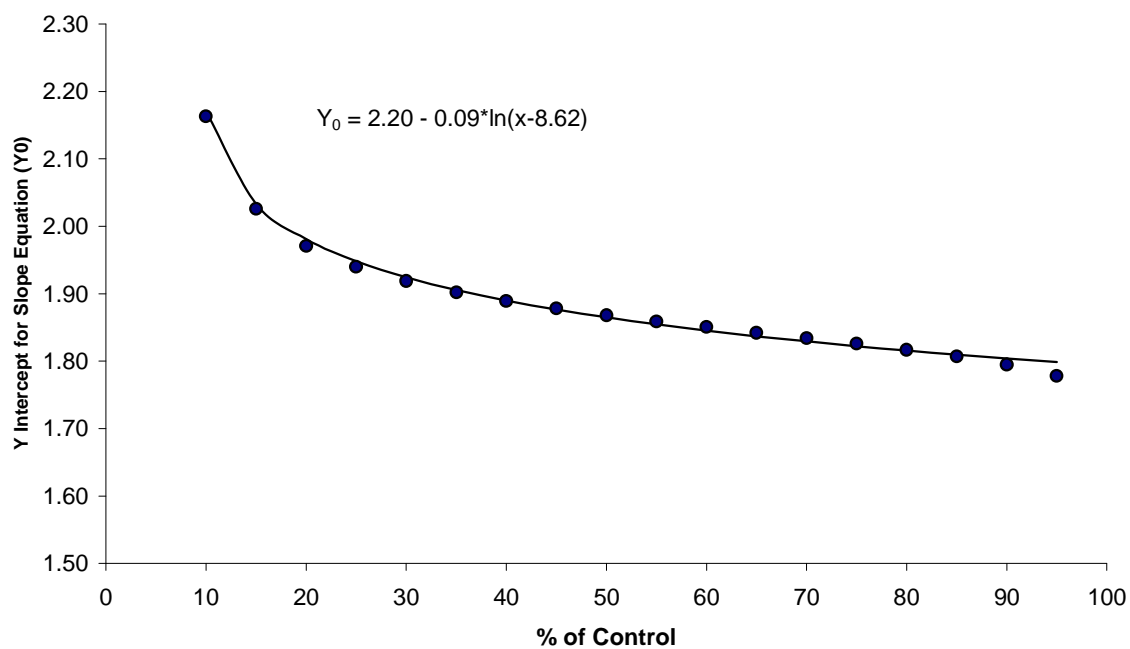
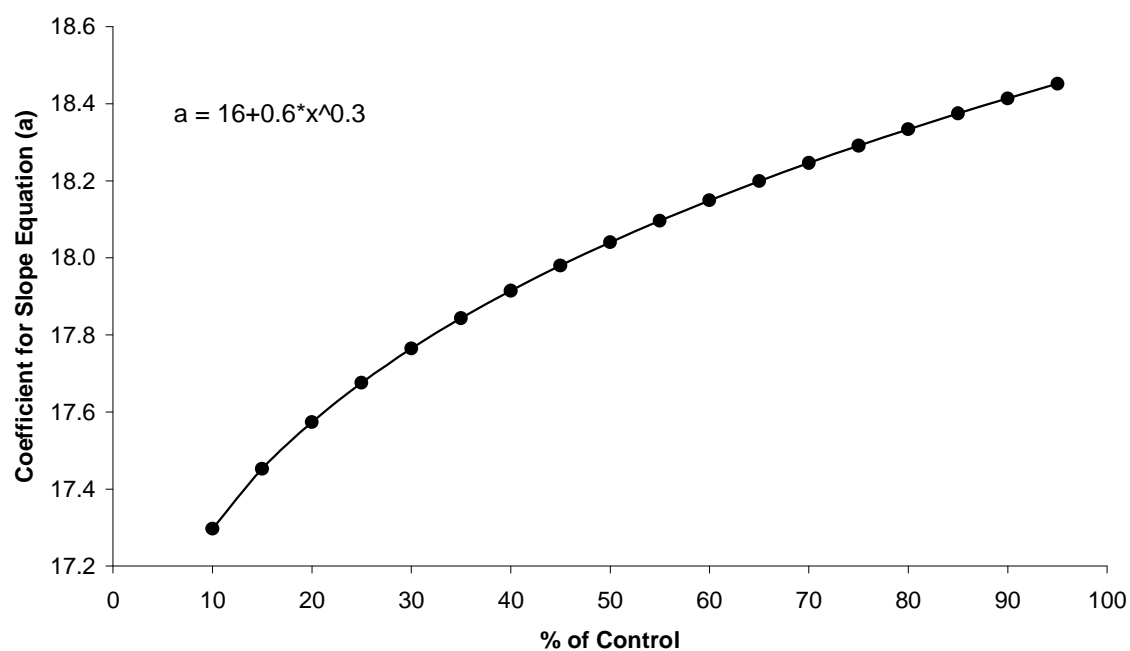


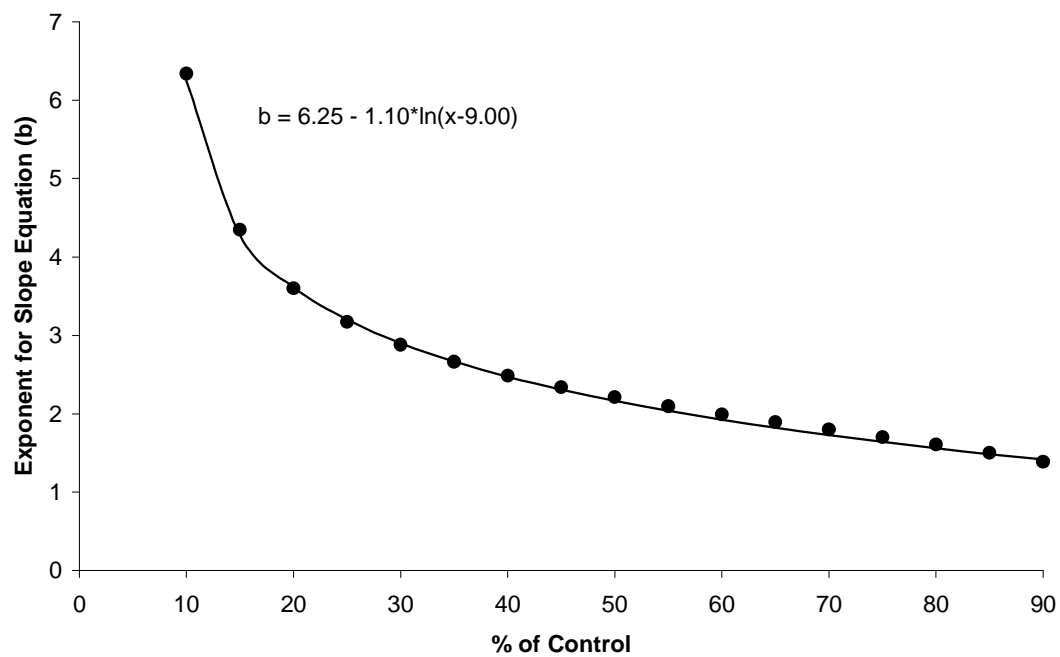
Figure D1 continued.



**Figure D2.** Relationship between the y-intercept for Equation 7 and the % of the control response for a given concentration.



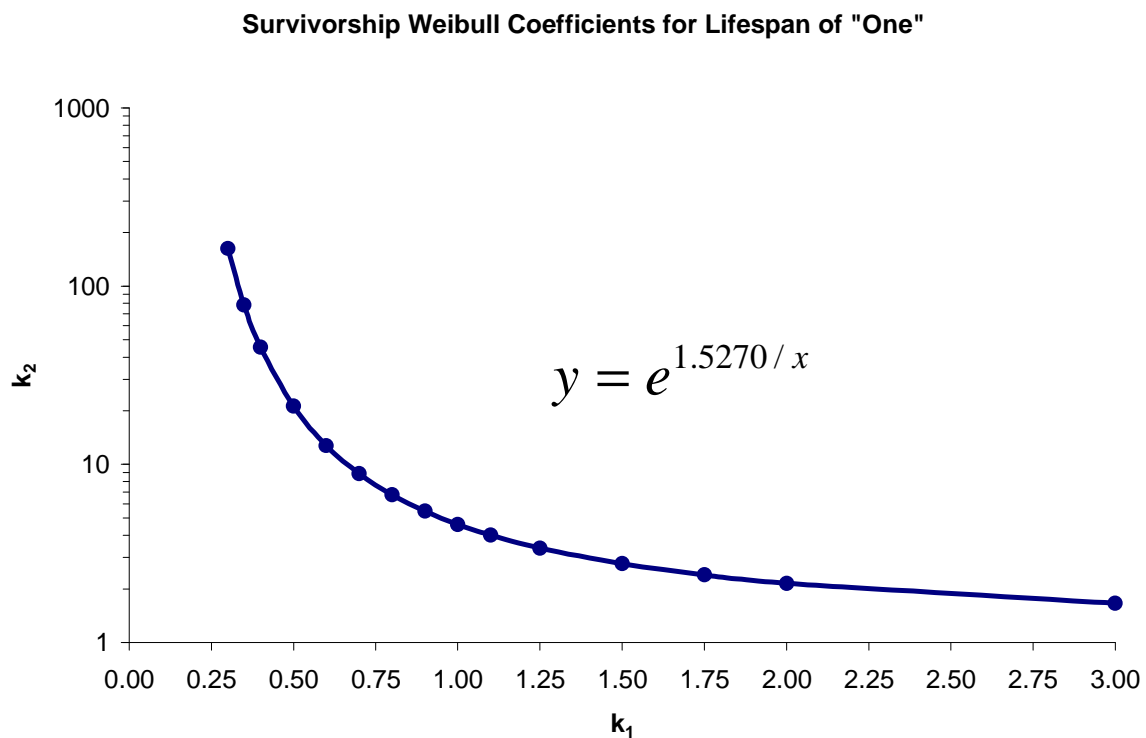
**Figure D3.** Relationship between the shape factor 'a' for Equation 7 and the % of the control response for a given concentration.



**Figure D4.** Relationship between the shape factor 'b' for Equation 7 and the % of the control response for a given concentration.

## Appendix E. Supplemental information for Weibull survivorship curve for *Americamysis bahia*.

Data in Figure E1 represent the general relationship between the two shape parameters of the Weibull equation (Equation 3). If the lifespan is fixed at a given length, then there is a mathematical relationship between the two coefficients ( $k_1$  and  $k_2$ ). These values are easily derived by selecting a series of  $k_1$  values<sup>25</sup> and then, through an iterative process, selecting a  $k_2$  value for each  $k_1$  so that the survivorship reaches the desired minimum at the end of the lifespan. This can be “zero”, or any other value. The equation in Figure E1 represents a value of 0.01 (1%) at the end of a lifespan of one (this can represent 1 day, 1 week, 1 month, etc.).



**Figure E1**

The equation for Figure E1 can be modified to allow for any lifespan duration. The most general form of the  $k_2$  equation is:

<sup>25</sup>  $k_1$  greater than 1 is a Type I survivorship, equal to 1 is Type II, and less than 1 is Type III.

$$k_2 = \frac{e^{1.5270/k_1}}{\omega} \quad \text{Equation E1}$$

Where *omega* is the lifespan duration.

Equation E1 assumes that 1% of the population will still be alive at the end of the normal lifespan. The equation can be easily adjusted to accommodate other probabilities of survival by just changing the value of the constant. Table E1 lists several other constants.

**Table E1.** Constants for Equation E1 using different end of lifespan ( $\omega = 13$ ) probabilities of survival.

Probability of Survival	Constant
0.00001	2.438
0.00005	2.292
0.0001	2.220
0.0005	2.028
0.001	1.932
0.005	1.668
0.01	1.527

## Appendix F. Biological Significance

We have selected a 30% decline in the expected minimum population size as our default cut off for calculating an exposure concentration that results in a biologically significant difference. Thirty percent is the value used by the IUCN Red List (IUCN 2001) to categorize a species as vulnerable to extinction, and this value can be applied to regional extirpation. Red List categories are defined by consensus from the World Conservation Union.

Most scientists recognize that the goal in any experimental design is not to find a “statistically significant difference”. If this were the case, then with a sufficiently large number of replicates any difference can be statistically significant. Kirk (1996) summarizes:

As John Tukey (1991) wrote, “the effects of A and B are always different—in some decimal place—for any A and B. Thus asking ‘Are the effects different?’ is foolish.” Because the null hypothesis is always false, a decision to reject it simply indicates that the research design had adequate power to detect a true state of affairs, which may or may not be a large effect of even a useful effect.

What we really want to know is: “are the differences we observe among treatments, stations, etc. different enough to be a cause of concern?” In other words, are they biologically significant (or in the case of education or medical research—practically significant or clinically significant). Biological significance is not easy to define, but we usually know it when we see it.<sup>26</sup> The decision of what constitutes a biologically significant difference requires judgment. And as Kirk (1996) further wrote relative to practical significance:

...judgment inevitably involves a variety of considerations, including the researcher’s value system, societal concerns, costs and benefits, and so on.

The lure of “statistical significance” is that it gives the appearance of being totally objective, turning a “continuum of uncertainty in to a dichotomous reject-do-not-reject decision” (Kirk 1996). I say “appearance of objectivity” because the common reliance on an  $\alpha = 0.05$  is entirely arbitrary, used routinely only because of historical convention. Cohen (1990) wrote:

[It] is not a cliff but a convenient reference point along the possibility-probability continuum. There is no ontological basis for dichotomous decision making in psychological inquiry.

There have been numerous reviews of the fallacy of continued sole reliance on statistical significance for evaluating results (e.g., Cohen 1990, Kirk 1996, Campbell 2005, Di Stefano 2005). Although there is some logic for abandoning—or at least supplementing—statistical significance for a different evaluation of what magnitude of difference among treatments is of value (e.g., practical significance or biological significance), there appears to be some reluctance on the part of researchers to embrace any other evaluation other than statistical significance—perhaps because the former requires the insertion of judgment. Kirk (1996) continues:

...focusing on  $p$  values and rejecting null hypotheses actually distracts us from our real goals: deciding whether data support our scientific hypothesis and are

---

<sup>26</sup> With apologies to Justice Potter Stewart

practically significant or useful....It is true that an element of subjectivity is introduced into the decision process when researchers make this kind of judgment....It is a curious anomaly that researchers are trusted to make a variety of complex decisions in the design and execution of an experiment, but in the name of objectivity, they are not expected or even encouraged to decide whether data are practically significant.

In fact an essential ingredient in the “research process is the judgment of the scientist” (Cohen 1990).

The issue of “what is ‘biologically significant’ is a major problem in conservation biology and does not seem to have easy solutions” (Reed and Blaustein 1997). It is a “policy decision” and should be selected by consensus (Reed and Blaustein 1997). We are recommending the Red List definition of “vulnerable” as the default value to be used to define a biologically significant difference. A 30% decline over some time interval of interest<sup>27</sup> is the value used by the IUCN Red List (IUCN 2001) to categorize a species as vulnerable to extinction. Red List categories are defined by consensus from the World Conservation Union.

---

<sup>27</sup> The Red List uses 10 years or 3 generations, which ever is longest. However, the model applies the 30% decline over a duration of 10 generations—30 weeks in the case of mysids.

## Appendix G. List of VBA macro code

### THIS FIRST SET ARE THE MACROS CONTAINED WITHIN THE MODULE NAMED: Mysid\_Model

```
Private Function StaticRand()
```

```
    'this is a function that allows the creation of a random number that does
```

```
    'not change until the model calls for a new number--saw this in several places on web, but also in
```

```
    'Walkenbach, John.1999.Microsoft Excel 2000 Power Programming with VBA
```

```
    'Published by Hungry Minds, Inc, New York, NY. 869 pp.
```

```
StaticRand = Rnd
```

```
End Function
```

```
-----  
Sub RUN_MYSID()
```

```
    'this is the master macro for running the simulation
```

```
Application.ScreenUpdating = False
```

```
'This section checks for data entry errors
```

```
    Sheets("Data Entry Error Check").Visible = True
```

```
    Sheets("Data Entry Error Check").Select
```

```
    Range("I1").Select
```

```
    If ActiveCell.Value > 0 Then
```

```
        Date_Entry_Error_Check 'data entry error check macro
```

```
        Sheets("Data Entry Error Check").Visible = False
```

```
        Sheets("Input Parameters").Select
```

```
        Exit Sub
```

```
    End If
```

```
Sheets("Data Entry Error Check").Visible = False
```

```
Application.ScreenUpdating = True
```

```
Sheets("Input Parameters").Select
```

```
ActiveSheet.Unprotect
```

```
Range("I23").Select 'hides "selected" cell behind object--cosmetic only
```

```
    'the "N" cells below are used to calculate time elapsed--font is white to hide these cells
```

```
Range("N3").FormulaR1C1 = "=now()"
```

```
Range("N4").FormulaR1C1 = "=now()"
```

```
Application.ScreenUpdating = False
```

```
Range("N3").Copy
```

```
Range("N3").PasteSpecial Paste:=xlValues "'freeze" start time
```

```
    Dim NumSteps, NumConc As Integer
```

```
    Dim InitConc, IncrConc
```

```
    NumSteps = Range("D22") 'number of time steps in weeks
```

```
    NumConc = Range("D23") 'number of treatments in addition to initial concentration
```

```
    InitConc = Range("D24") 'usually zero
```

```

IncrConc = Range("D25") 'the amount of increase for the concentration for each new exposure

ClearPrevious 'calls macro that clears the data from the previous run

LC50_kinetics_gooseek 'calls macro that finds survival kinetic parameter when 2 LC50s are
used

'LOOP FOR EXPOSURE CONCENTRATIONS
For k = 0 To NumConc
Sheets("mysid matrix 13x13").Select
Range("Z2") = (IncrConc * k) + InitConc 'sets current exposure concentration

'set initial conditions
Range("F21:F33").Copy 'copies the t-zero population numbers
Range("P3").PasteSpecial Paste:=xlValues
Application.CalculateFull 'new set of random numbers
Range("Q19").Copy 'starting ln lambda
Range("X2").PasteSpecial Paste:=xlValues
'LOOP FOR NUMBERING TIMESTEPS
Range("W2").FormulaR1C1 = "0"
For i = 1 To NumSteps
Range("W2").Offset(i, 0).Select
ActiveCell.Value = i
Next i
'LOOP FOR LN LAMBDA
For j = 1 To NumSteps
Range("R3:R15").Copy 'copies the normalized t+1 population
Range("P3").PasteSpecial Paste:=xlValues 'paste the normalized t+1 into the "t" location
Application.CalculateFull 'new set of random numbers
Range("Q19").Copy 'copies the new lambda value
Cells(j + 2, 24).PasteSpecial Paste:=xlValues 'paste the new lambda into the data list
Next j
'at end of conc loop, copy raw ln lambda values
Columns("W:AA").Copy
Sheets("Lambda Summary").Select
Range("A1").Offset(0, (k * 5) + k).PasteSpecial Paste:=xlValues
Sheets("mysid matrix 13x13").Select
'copy summary statistics for conc
Range("B39:E39").Copy
Cells(k + 2, 30).PasteSpecial Paste:=xlValues
Range("U5") = (k + 1) / (NumConc + 1)

Sheets("Simpsons Rule").Visible = True 'unhides the worksheet while it is needed
Sheets("Simpsons Rule").Select 'to calculate the area below the risk curves
For p = 1 To 19 'the number of theta values
Range("A27").Select

```

```

ActiveCell.Value = p * 0.05 'updates current threshold value ("theta")
Range("A29").Copy 'copies the cdf values associated with the current theta
Cells(p + 1, 2).PasteSpecial Paste:=xlPasteValuesAndNumberFormats 'paste cdf into column B
    Next p
Range("C25:D25").Copy 'copies current concentration and normalized area value
Cells(k + 2, 7).PasteSpecial Paste:=xlPasteValuesAndNumberFormats
Range("C27").Copy 'copies conc header
Cells(1, 13 + k).PasteSpecial Paste:=xlPasteValuesAndNumberFormats
Range("B2:B20").Copy 'copies threshold curve data
Cells(2, 13 + k).PasteSpecial Paste:=xlPasteValuesAndNumberFormats
Sheets("Simpsons Rule").Visible = False 'hides the worksheet
Sheets("Input Parameters").Select
Range("N4").FormulaR1C1 = "=now()" 'updates the elapsed time at the end of each conc.
exposure
'Sheets("Input Parameters").Select
Application.ScreenUpdating = True 'allows the screens to update between each exposure conc
Application.ScreenUpdating = False

Next k

PVA_SOLVER 'calls macro that fits curve to dose-response data

Range("I23").Select 'hides "selected" cell, just cosmetic
ActiveSheet.Protect
End Sub

```

---

```

Sub ClearPrevious()
    'this macro clears the data from the previous run
    Sheets("mysid matrix 13x13").Select
    Dim Count
    Count = Range("U2") 'the count of the number of lambda values from the previous run
    Range("W3").Resize(Count, 2).ClearContents
    Range("AD2:AH51").ClearContents 'clears previous lambda values
    Range("U5").ClearContents 'zeros out the % complete datum

    'these next lines enable the "risk curve area" data to be cleared from the previous run
    Sheets("Simpsons Rule").Visible = True
    Sheets("Simpsons Rule").Select
    Range("B2:B20").ClearContents 'clears last curve's information
    Dim Col_Count
    Col_Count = Range("J3") 'the number of risk curves from previous run
    Range("G2").Resize(Col_Count, 2).ClearContents 'clears concentration vs area information
    Range("M1").Resize(20, Col_Count).ClearContents 'clears all of the curves' data
    Sheets("Simpsons Rule").Visible = False

```

```

Sheets("Lambda Summary").Select
Cells.Clear 'clears all of the lambda summary data from the previous run

Sheets("Input Parameters").Select
'Change line color of PVA trendline to match background
ActiveSheet.ChartObjects("PVAchart").Activate
ActiveChart.SeriesCollection(3).Select
With Selection.Border
    .LineStyle = xlNone
End With
'ActiveSheet.ChartObjects("DR_Curve").Activate
'ActiveChart.SeriesCollection(3).Select
'With Selection.Border
'    .LineStyle = xlNone
'End With
Range("P25").Font.ColorIndex = 35 'change PC20 color to match background
Range("P26").Font.ColorIndex = 2 'color to match background

Range("I23").Select 'hides selected cell, just cosmetic
Application.ScreenUpdating = True
Application.ScreenUpdating = False
End Sub

```

---

```

Sub PVA_SOLVER()
    'this macro runs the solver function to fit the dose-response curve
    Sheets("PVA-Solver").Visible = True 'unhides worksheet
    Sheets("PVA-Solver").Select
    Range("K1").FormulaR1C1 = "1.00" 'makes sure each run starts from same value
    Range("K2").FormulaR1C1 = "-1.00" 'makes sure each run starts from same value
    SolverOk SetCell:="$H$5", MaxMinVal:=1, ValueOf:="0", ByChange:="$K$1:$K$2"
    SolverSolve (True)
    'this portion of the macro reset colors of endpoints to make them visable
    Sheets("Input Parameters").Select
    'Change line color of PVA trendline to match markers
    ActiveSheet.ChartObjects("PVAchart").Activate
    ActiveChart.SeriesCollection(3).Select
    With Selection.Border
        .ColorIndex = 5
        .Weight = xlMedium
        .LineStyle = xlContinuous
    End With
    Range("N4").FormulaR1C1 = "=now()" 'sets the ending time so that duration of run
        'can be calculated
    Range("N4").Copy "'freeze" end time
    Range("N4").Select

```

```

With Selection
.PasteSpecial Paste:=xlValues
End With
Range("P25:P26").Font.ColorIndex = 1 'change PCx font color to black
    Sheets("PVA-Solver").Visible = False 'hides worksheet
    Sheets("Input Parameters").Select 'just to make sure that model ends on correct sheet

End Sub

```

---

```

Sub LC50_kinetics_goalseek()

'this macro sets the kinetic factor when two different LC50s are known
Sheets("parameter calculations").Select
Range("Q4").Select
If ActiveCell.Value = True Then
    Range("B51").Select
    Range("B51").FormulaR1C1 = "1.0"
    Range("G53").GoalSeek Goal:=Range("G51"), ChangingCell:=Range("B51")

End If

End Sub

```

---

#### **THIS MACRO IS CONTAINED WITHIN THE MODULE: ERROR\_Check**

```

Sub Date_Entry_Error_Check()

Range("I9").Select
If ActiveCell.Value = "ERROR" Then
    MsgBox "There is at least one data entry error in the main set of five" _
        & vbCr & "survival parameters. Make sure that all are positive numbers," _
        & vbCr & "and that the lower CL and upper CL are less than and greater" _
        & vbCr & "than the 96h LC50, respectively."
    Exit Sub
End If

Range("I11").Select
If ActiveCell.Value > 0 Then
    MsgBox "There is at least one survival kinetics data entry error." _
        & vbCr & "Make sure there are no blanks and that all are positive numbers." _
        & vbCr & "If you selected the 'two' LC50 method, then make sure that the" _
        & vbCr & "earlier LC50 is greater than the later one."
    Exit Sub
End If

```

```

Range("I22").Select
If ActiveCell.Value > 0 Then
    MsgBox "There is at least one reproduction data entry error." _
        & vbCrLf & "Make sure there are no blanks and that all are positive numbers."
    Exit Sub
End If

```

```

Range("I35").Select
If ActiveCell.Value = "ERROR" Then
    MsgBox "There is at least one exposure parameter data entry error." _
        & vbCrLf & "Make sure there are no blanks and that all are positive numbers."
    Exit Sub
End If

```

```

End Sub

```

---

**THESE MACROS ARE CONTAINED WITHIN THE MODULE: Move\_Macros**

```

Sub gotoDose_Response_Input_Graphs()
Application.ScreenUpdating = False
LC50_kinetics_goalseek 'resets kinetic parameter--only needed with "2 LC50" method
    Sheets("Input Parameters").Select
    ZoomToRange ZoomThisRange:=Range("AA1:AN1"), PreserveRows:=False
    Range("AA1:AN32").Select
    ActiveWindow.Zoom = True
    Range("Ac3").Select
End Sub

```

---

```

Sub gotoInput_Parameter_Area()
    Range("A1:S32").Select
    ActiveWindow.Zoom = True
Range("I23").Select
End Sub

```

---

```

Sub ZoomToRange(ByVal ZoomThisRange As Range, _
    ByVal PreserveRows As Boolean)
    'This procedure was adapted from "Zooming On A Range"--www/cpearson.com/excel/topic.aspx
    'The author of this code has declared it "Public Domain" (see
    'www/cpearson.com/Excel/LegaleseAndDisclaimers.aspx)
ActiveSheet.Unprotect
Dim Wind As Window
Set Wind = ActiveWindow
Application.ScreenUpdating = False

```

' Put the upper left cell of the range in the top-left of the screen.

```
,  
Application.GoTo ZoomThisRange(1, 1), True  
With ZoomThisRange  
    If PreserveRows = True Then  
        .Resize(.Rows.Count, 1).Select  
    Else  
        .Resize(1, .Columns.Count).Select  
    End If  
End With  
With Wind  
    .Zoom = True  
    .VisibleRange(1, 1).Select  
End With  
ActiveSheet.Protect  
End Sub
```

---

### **THESE MACROS ARE CONTAINED WITHIN THE MODULE: Print\_Save\_Macros**

```
Function PathExists(MyPath) As Boolean  
    'returns TRUE if the path exists  
    '--saw this in several places on web, but also in  
    'Walkenbach, John.1999.Microsoft Excel 2000 Power Programming with VBA  
    'Published by Hungry Minds, Inc, New York, NY. 869 pp.  
    Dim x As String  
    On Error Resume Next  
    x = GetAttr(MyPath) And 0  
    If Err = 0 Then PathExists = True _  
    Else: PathExists = False
```

End Function

---

```
Sub printSummary()  
'prints Input Parameter and endpoint graph to the current default printer  
Application.ScreenUpdating = False  
    ActiveSheet.PageSetup.PrintArea = "A1:T31"  
    ActiveWindow.SelectedSheets.PrintOut From:=1, To:=1, Copies:=1, Collate _  
        :=True  
    ActiveSheet.PageSetup.PrintArea = ""  
        With ActiveSheet.PageSetup  
            .FitToPagesWide = 1  
            .FitToPagesTall = 1  
        End With  
End Sub  
Sub CopyPasteValues()  
    ActiveSheet.Unprotect
```

' this macro clears out the extraneous materials from the parameter summary sheet  
' which being saved as a summary of a model run

```
Cells.Select  
Selection.Copy  
Selection.PasteSpecial Paste:=xlPasteValuesAndNumberFormats
```

```
ActiveSheet.Shapes("ViewBox").Cut  
ActiveSheet.Shapes("RunBox").Cut  
ActiveSheet.Shapes("ReturnBox").Cut  
ActiveSheet.Shapes("PrintBox").Cut  
ActiveSheet.Shapes("SaveBox").Cut
```

```
ActiveSheet.ChartObjects("DoseResponse").Activate  
ActiveChart.ChartArea.Select  
ActiveWindow.Visible = False  
Selection.Delete
```

```
ActiveSheet.ChartObjects("LC50kinetics").Activate  
ActiveChart.ChartArea.Select  
ActiveWindow.Visible = False  
Selection.Delete
```

```
Columns("Z:AK").Clear  
Range("M3").Select
```

End Sub

---

Sub CopyPasteChart()

'the macro converts the endpoint chart to a picture

Dim TheChart As Chart

Application.ScreenUpdating = False

```
ActiveSheet.ChartObjects("PVAchart").Activate
```

```
Set TheChart = ActiveChart
```

```
TheChart.CopyPicture appearance:=xlScreen, Size:=xlScreen, Format:=xlPicture
```

```
ActiveWindow.Visible = False
```

```
ActiveSheet.Paste
```

```
Selection.Left = TheChart.Parent.Left
```

```
Selection.Top = TheChart.Parent.Top
```

```
ActiveSheet.ChartObjects("PVAchart").Delete
```

```
Range("M3").Select
```

End Sub

---

```

Sub Save_Previous_Run()
Dim modelWB As Workbook
Set modelWB = ActiveWorkbook 'This assigns the model filename to a variable--allows this
                              'macro to still operate even if the filename is changed
Dim default_Path
default_Path = Range("Saving_Default")
                              'This is the default path to which the results will be saved.
                              'It's location is on the "Input Parameter" sheet off screen
                              'below the save box.
Dim MyFile, MyPath
MyPath = InputBox("Pathname for Summary Information", "Enter Pathname--don't forget the
backslash at the end", _
default_Path)
'You can accept the default path or enter a new one.
If PathExists(MyPath) = False Then
ans = MsgBox("Path does not exist. Create?", vbYesNo, "Path Selected")
If ans = vbNo Then Exit Sub
If ans = vbYes Then Mkdir MyPath 'If the path does not exist, it will be created.
End If
MyFile = InputBox("Filename for Summary Information", "Enter UNIQUE filename",
"PVA_Summary.xls")
Dim MySummary As Workbook
Set MySummary = Workbooks.Add 'adds a workbook to serve as the summary file
MySummary.SaveAs (MyPath & MyFile) 'names the new workbook

Application.ScreenUpdating = False

modelWB.Activate
Sheets("Lambda Summary").Copy before:=Workbooks(MyFile).Sheets(1)
modelWB.Activate
Sheets("Input Parameters").Copy before:=Workbooks(MyFile).Sheets("Lambda Summary")
Sheets("Input Parameters").Name = "Summary of Model Parameters" 'renames sheet

'These next two macros clean up the summary file
CopyPasteValues
CopyPasteChart
Sheets("Sheet1").Visible = False
Sheets("Sheet2").Visible = False
Sheets("Sheet3").Visible = False
MySummary.Save
MySummary.Activate

Application.ScreenUpdating = True

End Sub
-----

```

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