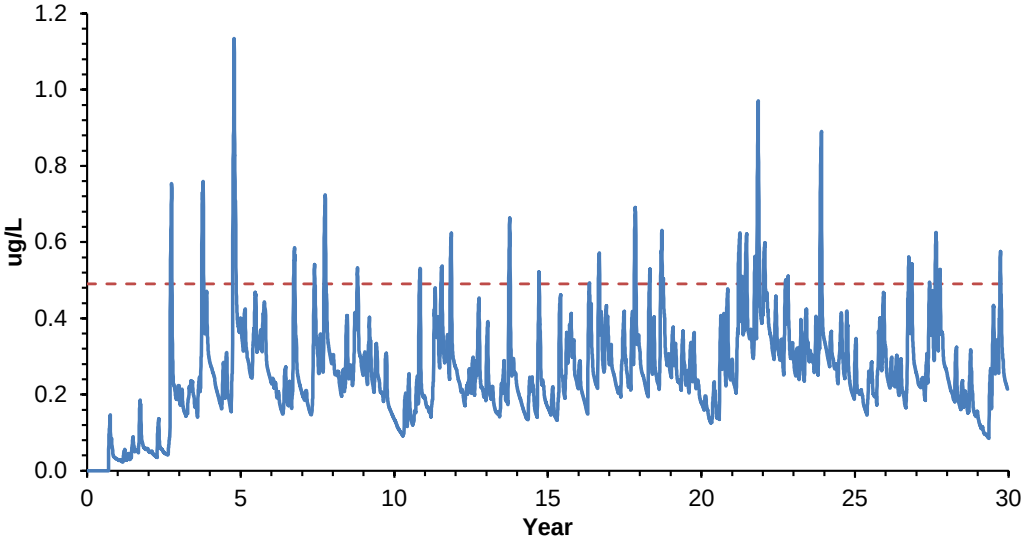
United States Environmental Protection Agency

EPA/600/R-16/062 | May 2016
www.epa.gov/ord

Matrix Population Model for Estimating Effects from Time-Varying Aquatic Exposures: Technical Documentation



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Notice

The research described in this report has been funded wholly by the U.S. Environmental Protection Agency. This report is contribution number ORD-016393 of the Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development. This document has been subjected to USEPA's peer review process and has been approved for publication. The mention of trade names of commercial products does not constitute endorsement for use.

Abstract

The Office of Pesticide Programs (OPP) models daily aquatic pesticide exposure values for 30 years in its risk assessments. However, only a fraction of that information is typically used in these assessments. The population model employed herein is a deterministic, density-dependent periodic matrix model for integrating time-varying pesticide exposure effects on the marine invertebrate *Americamysis bahia*. The external exposure concentrations are converted to time-varying scaled internal concentrations by coupling a one-compartment toxicokinetics-toxicodynamics model with the matrix model. Several exposure scenarios (each with the same risk as determined by OPP's traditional approach) were created within which population modeling documented different risk conclusions than assessments based on the traditional approach. Population modeling incorporates all available toxicological and exposure data, making a more complete assessment of the potential risk of time-varying aquatic concentrations.

Keywords: *Americamysis bahia*, matrix modeling, population level risk assessment, time-varying exposures

Acknowledgements

Dr. Keith Sappington from the US Environmental Protection Agency's Office of Pesticides Programs provided valuable insights into the application of these results for pesticide risk assessments. Drs. Giancarlo Cicchetti, Jason Grear and Diane Nacci provided technical reviews of the final report. Other comments from Marty Chintala, Joseph LiVolsi and Dr. Matthew Etterson also were helpful. Any remaining errors or misinterpretations of comments are the responsibility of the author.

Preface

Evaluation of different aquatic exposure scenarios is an integral part of EPA's risk assessment process for the registration or re-registration of pesticides. While the Office of Pesticide Programs (OPP) has research needs associated with exposure models (e.g, incorporation of spatial variability in exposure parameters or development of urban and residential aquatic exposure models), there is also a need to better link time-varying exposure to population-level effects. The successful application of population modeling would significantly reduce the uncertainty associated with OPP's risk assessments. Such modeling can account for cumulative impacts of multiple "low" exposures to the same chemical (minimizing potential for under protection), and can account for recovery after a short "high" exposure (minimizing potential for over protection).

One of the criticisms of population modeling is the limitation placed on this approach by its data requirements. Current toxicity data requirements for pesticide registration do not provide enough information for complex models. The specific model herein was created with this concern in mind. All toxicity parameters are derived from standard test data for the marine invertebrate *Americamysis bahia.* The model successfully integrates acute and chronic toxicity data, and uses all of OPP's time-varying modeled exposure data.

This report is a product for CSS 18.04.6, Integrated Modeling for Ecological Risk Assessment, Task 6: Case Study 3 - Pesticides Impacts to Aquatic Endangered Species.

Contents

INTRODUCTION

For assessing pesticide risks to aquatic organisms, the Office of Pesticide Programs (OPP) models pesticide spray drift, runoff, and erosion into an agricultural pond with specified water body and watershed characteristics. Numerous agricultural crop scenarios represent different crop, regional, climate, watershed, and agronomic specifications across the country. These crop scenarios are intended to capture agronomic and regional factors that greatly influence the delivery of pesticides to surface waters (e.g., precipitation patterns, soil characteristics, pesticide application timing). With each of these crop scenarios, an exposure model called the Pesticide in Water Calculator (PWC)[1] simulates daily concentrations for 30-year exposure distributions for surface water, sediment, and interstitial (pore) water. Because the PWC output depends in part on soil properties, soil and crop management practices and weather data, different regions of the country and different crop types will have different aquatic exposure time series for equivalent applications of the same pesticide. Typically, OPP derives a single acute and chronic estimated environmental concentration (EEC) from each 30-year time series that represents a conservative (high end) exposure concentration with an infrequent occurrence (e.g., once in 10 years). The acute and chronic EECs are then compared to available acute and chronic toxicity data to estimate risk to aquatic organisms. OPP's use of EECs reflects a tiered process whereby high end estimates of exposure are first used to identify potential risks, which identifies any need for additional refinements to risk estimates. It is clear that the EEC distills a large amount of information into a single value and thus greatly limits the ability to consider temporal aspects of organism exposure and life history which are known to influence risk. Population modeling, while not currently part of OPP's standard risk assessment, offers an additional higher tiered refinement—one that can use all of the exposure data, as well as combine acute and chronic assessment into a single estimate of risk. The use of population modeling assessment procedures will reduce the likelihood of over or under protection decisions that may occur due to an inability to incorporate time-varying exposures in the risk assessment process.

The Issue

OPP models daily aquatic pesticide exposure values for 30 years (10,350 values), yet only a fraction of that information is typically used in pesticide risk assessments. Time-varying exposure information is essentially not used. Depending on the type of analysis, the daily time series data may be used as is or converted to a 4-d running average (for comparison against acute toxicity data), or, if chronic toxicity data are used, converted to either a 21-d (invertebrates) or 60-d (fish) running average. Once

[1] http://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#aquatic

a time series is selected, the maximum value for each year is noted. The EEC is determined as the 90th percentile of 30 ranked values of annual maxima and is subsequently used to calculate a risk quotient (RQ), which is an EEC divided by a toxicity value of interest. In theory, this EEC reflects the annual maximum with an expected 1 in 10 year return frequency and roughly corresponds to the fourth highest annual maximum. In the case described in this report, the toxicity value is a mysid chronic value. Because such a small fraction of the available exposure data is used, one can easily imagine a situation whereby several time series have very similar EECs—thus very similar RQ values—yet the underlying pattern of daily exposures could be drastically different. In this situation, the currently estimated risk to the taxonomic group of interest would be similar, but the actual potential risk might be quite different among the different exposure series. Figure 1 shows a hypothetical set of exposure scenarios. The three scenarios were constructed so that each has the same third highest annual maximum. Visual inspection of these scenarios suggests they should have different effects on a population; however, the current risk assessment method would indicate the same risk. The challenge is to provide a procedure that can distinguish among these scenarios, yet be easy to understand and simple to implement. Ideally the procedure also would require no new data—that is, take full advantage of all of the currently required toxicity data for a given species.

The Potential Solution

One technique for incorporating effects of time-varying concentrations of pesticides is toxicokinetics-toxicodynamics modeling—TK-TD (Brock et al. 2010, Ashauer and Brown 2013). Toxicokinetics relates the time course of the concentration of a toxicant within an organism to the time course of that toxicant in the external medium. TK modeling is similar among many researchers. Most publications on the subject make the simplifying assumption that the organism is a one-compartment model[2] (i.e., the whole body concentration represents the target concentration), and assume uptake and elimination kinetics are first order (linearly related to external and internal concentrations, respectively). The biggest objection for using toxicokinetics has been the need for internal concentration data—not typically available from standardized tests. This can be overcome by using a scaled internal concentration (Ashauer and Brown 2013), which does not require estimation of the uptake kinetic parameter, and may even be the preferred method for determining uptake kinetic parameters over whole body measurements (Jager et al. 2011). Scaled internal concentration is described below in more detail in the MODEL STRUCTURE section.

[2] This may be because much of the TK-TD modeling to date has been with relatively small invertebrates.

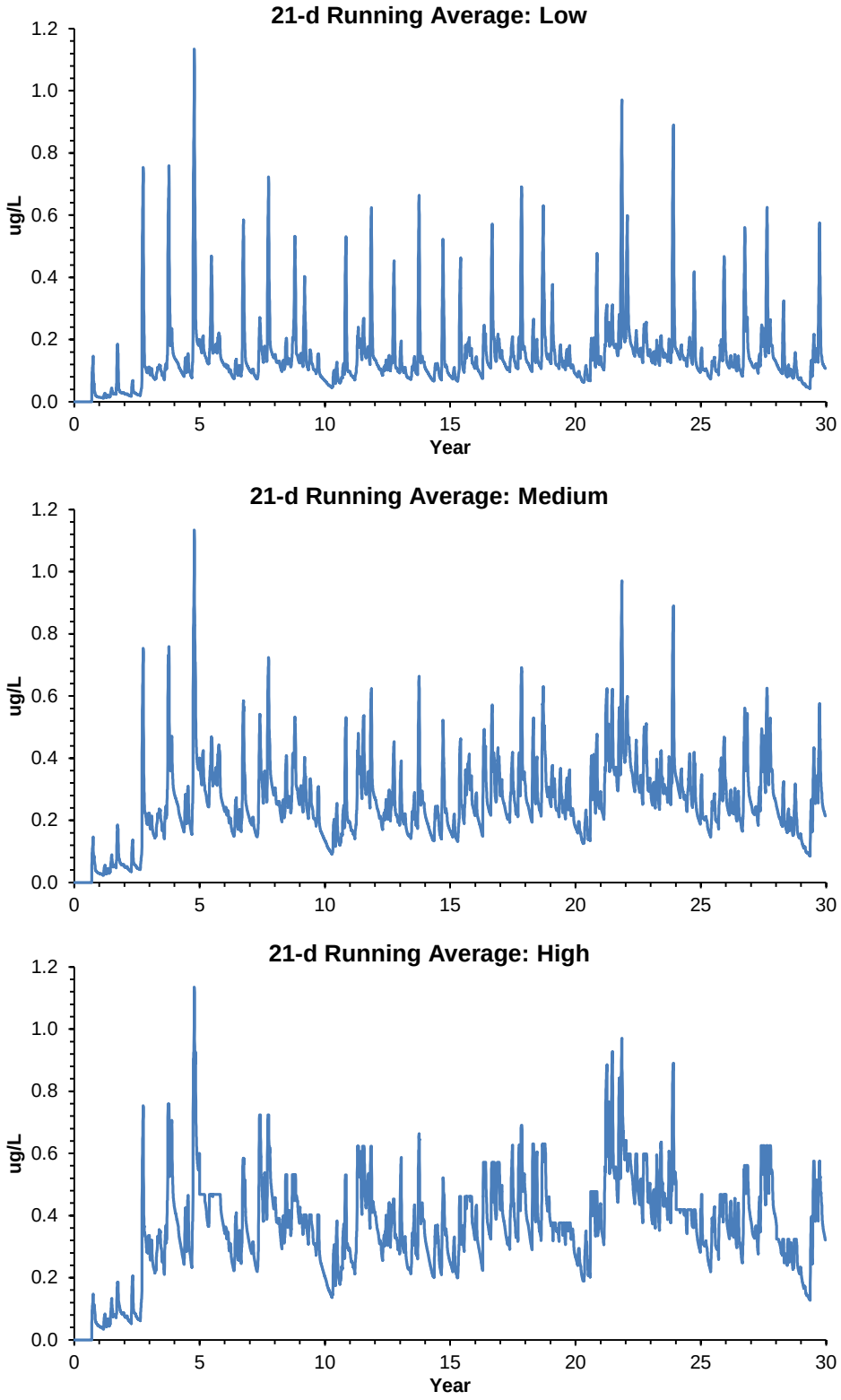

Figure 1. Three different time series, each with the same 30 annual maximum values. Time series differ only in the concentrations represented by the non-maximum values. For the "Low" time series, each of the "Medium" values were divided by 2, and for the "High" series, each of the "Medium" values were multiplied by 1.5. The plots are 21-d running averages and the original raw daily values are plotted in Appendix A.

While toxicokinetics is similar among many different TK-TD modeling efforts, toxicodynamics modeling can take several forms. Most of the TD approaches are different applications of hazard modeling[3]—as the internal concentration increases, the probability of an effect increases. The approaches for toxicodynamics differ primarily in the component to which the toxic effect (usually survival) is related—that is, whether it relates directly to the internal concentration or to some level of internal damage (Brock et al. 2010). In its simplest form the hazard rate is proportional to the internal concentration, and all concentrations, no matter how small, cause some degree of effect. This simple model also assumes any effect (or any recovery) is instantaneous and complete. The model described in this report uses this simplified form for toxicodynamics. Because of this simplification, the model can be parameterized using the toxicological data typically available to OPP during pesticide registration or re-registration.

Even though TK-TD models usually are applied to individuals, they can be directly coupled with population models (Brock et al. 2010), which is what is done herein. For this population approach, the report builds upon a matrix population model previously developed for the marine invertebrate *Americamysis bahia* (Thursby 2009). That model has a one week time step[4]. This new approach takes that into consideration and uses a periodic matrix technique (Caswell 2001), which creates 52 sub-matrices[5] to represent annual population activity. This method allows incorporating potential effects due to degree of seasonal exposures overlapping with seasonal biological events, such as reproduction. The purpose of this report, however, is not to promote a definitive population model, but rather to use this model to demonstrate the ability of such models to distinguish among various exposure time series, as well as among different toxicological features—such as seemingly minor differences in sensitivity.

Risk Analyses

A comparison of three different types of risk analyses demonstrated how population modeling provided a more quantitative distinction among different exposure time series. These analyses were deterministic, probabilistic, and population-level methods. The deterministic approach is currently the first tier of an assessment within an OPP regulatory risk determination. As mentioned above, this method uses a very small portion of a 30-year modeled exposure data series. The probabilistic approach is occasionally included in a risk assessment and includes all of the exposure data within a cumulative distribution. The same toxicological endpoints are

[3] Survival is considered a stochastic process. This is in contrast to an individual tolerance approach whereby there is the assumption of a distribution of thresholds for survival. In the latter approach, the individuals that die are the more sensitive ones; in the stochastic approach, the individuals that die are just the unlucky ones (Jager et al. 2011).

[4] The earlier model also is stochastic and density independent. The periodic version is not stochastic and is density dependent. Details of the model are in Appendix B.

[5] Note, this results in a 364-day year.

used as in the deterministic approach, except this approach calculates the probability that the exposure data exceeds that endpoint (based on a count of how many concentration values are greater than the endpoint value). The probabilistic approach makes no distinction about when a concentration occurs, it only determines how many times within 30 years that concentration occurs. For example, it does not distinguish between a high concentration occurring thirty times within a single year and the same concentration only occurring once a year.

As with the probabilistic approach, the population approach uses all of the exposure data—integrating potential time-varying effects on survival and reproduction into a single population response tracked through time. Population modeling also incorporates recovery when exposure concentrations decline. The approach eliminates the need to make separate acute and chronic risk determinations. In addition, it eliminates the need to rely on running averages because daily concentrations in the external medium are used to estimate daily scaled internal concentrations, which in turn track daily effects[6]. As such, and unlike the probabilistic method, the population modeling method can distinguish among exposure scenarios that may differ only by when a given concentration occurs. Finally, population modeling can evaluate other exposure-based issues, including such things as frequency of stressful exposures and the temporal distribution of stressful exposures (e.g., clumping vs. uniform spacing of exposure within a time series).

Population models can also distinguish among effects that differ from a toxicological perspective. For example, the relative ratio of 24 vs. 96 hr LC50 values. With the current pesticide risk assessment procedures, two species with similar 96 hr LC50 values (a standard acute assessment endpoint) for a given toxicant would be deemed to have similar acute risks. However, for some species the LC50 may stabilize after 24 hr, and for others, it may continue to change with increased duration of exposure. These are two different toxicokinetic parameters, and very likely result in two different population responses to the same exposure time series. Another toxicological factor is the relative response of survival vs. reproduction. Two species could have similar final chronic values based on reproduction, but the effect of the toxicant on survival could be very different. Again, two similar risks by current procedures, but likely very different population responses.

This report shows toxicokinetics-toxicodynamics modeling and periodic matrix population modeling are useful tools to account for a variety of exposure and toxicological factors. This assessment is based, in part, on the mysid's response to endosulfan, a compound already being removed from all uses within the United States.

[6] Each weekly survival probability is the minimum survival rate of that seven-day period—a mysid can only die once so the minimum within each week is selected.

MODEL STRUCTURE

Matrix Population Model

As stated above, the model builds upon an earlier matrix population model developed for *Americamysis bahia* (Thursby 2009). A matrix model allows independent tracking of effects on different subpopulation groups. This adaptation of the model retains the basic structure of the original matrix model—subpopulation groups are age classes and a one week time step. All age classes (ranging from 1 to 13 weeks) are assigned the same sensitivity, differing only in the length of time exposed. The earlier model is density independent, stochastic, and assumes constant exposure concentrations. The current model is density dependent[7] and deterministic[8]. For specifics on the derivation of control survival and reproduction demographics, refer to the earlier report.

The original mysid matrix model construct is insufficient to deal with time-varying toxicant concentrations. Because OPP uses modeled 30-year daily time series for estimating exposure to aquatic organisms, a different matrix configuration is needed. To accomplish this the original weekly time step was retained, but a separate matrix was created for each week of the year. This approach was patterned after periodic matrix models (Caswell 2001)[9]. In addition to providing a mechanism to track changes in effects due to variability in exposure through time, this approach also provided a means for incorporating variability in demographic parameters with time (e.g., spawning and non-spawning seasons). Figure 2 demonstrates pictorially how the model was constructed. Time-series exposure data occur one year at a time, and the beginning of subsequent years starts where the previous year left off until all 30 years are processed. Each matrix represents the population's status for its given week; however, each matrix retains a "memory" of its previous 12 weeks of the exposure series (see Appendix B for a more complete explanation). The endpoint was the proportion of weeks within the 30-year time series that the population declined to or below a given threshold based on weekly counts of total population size.

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[7] Populations in the natural environment do not grow indefinitely. There must be some sort of compensatory factor governing overall population growth. The current model uses density dependence as this factor. The mechanisms for density dependent growth are varied, and generally not specifically known for most species. Many population modelers default to one of several simplistic ways to incorporate density dependence. For this report, I have chosen to use the density dependent mechanism described by Leslie (1948).

[8] The demographic parameters (survival and reproduction) are fixed, changing only when exposed to a toxicant. Therefore, if you run the same exposure time-series more than once, the distribution of population size over time will be the same each time.

[9] Often, periodic matrix models are presented as a product of a sequence of sub-matrices which themselves represent different periods of time within an annual cycle—for example, spring, summer, fall and winter. The model presented in this report uses sub-matrices, but does not multiply them together for a single annual matrix.

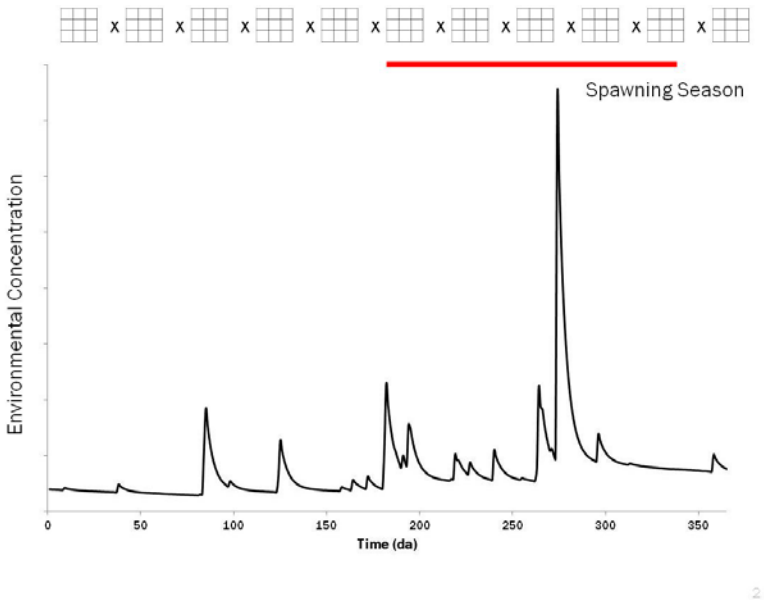

Figure 2. Diagram of how the population model interacts with the toxicant time series. The solid black line represents a hypothetical time series of toxicant concentration in the water column. The gridded squares across the top represent the positioning of the periodic sub-matrices in time. Note that there are only 11 matrices pictured for simplicity. In the model there are 52 such matrices covering each week of the year. The red line represents the spawning season—user defined.

The original mysid matrix model relies on Population Viability Analysis (PVA) to provide the effects endpoint (Ginzberg et al. 1982, Morris and Doak 2002). Population Viability Analysis estimates the probability that a population will fall below a given threshold within a given period of time (e.g., the probability of a 20% decline from the current population size within the next 10 years). Initially, the intent was to use a similar stochastic process to model the annual growth rate based on the periodic matrix—then apply PVA procedures. However, PVA was not practical after the analyses were expanded from one matrix to fifty-two[10]. Even very small changes in demographic parameters within the control matrices resulted in large variations in the annual growth rate of the population. For example, a weekly survival as high as 0.99 translated into an annual survival rate of 0.59 (0.99 raised to the 52nd power). Because of this, the periodic version of the matrix model was simplified to deterministic rather than stochastic—this also eliminated the need for hundreds, or even thousands, of runs for each annual series[11].

[10] In addition, PVA analysis assumes that the mean population growth rate is density-independent. Incorporating density dependence in the model complicates estimates of extinction risk (Morris and Doak 2002).

[11] PVA analysis requires not only an estimate of an average growth rate for a time period of interest, but also an estimate of the variability of that rate due to stochasticity of demographic parameters—thus the need for hundreds or even thousands of model runs.

The 2009 version of the model does not track population numbers, only changes in growth rate—so that model uses a simplifying assumption of no density dependence, that is, it allows exponential growth. The periodic model tracked population size on a weekly basis for 30 years. Exponential growth made tracking population size impractical since extremely large population sizes occurred. Density dependence within the model eliminated the possibility of these excessive population sizes. In addition, because the periodic model tracked exposures which vary over time, a mechanism for incorporating recovery was needed when exposure concentrations declined. Density dependence was one way to accomplish this[12]. The model applied the density dependent factor equally across each age class. Appendix B describes how this factor was calculated.

Effects Modeling Constraints

The Office of Pesticides Programs has specific test guidelines for acute and chronic tests for the mysid shrimp *Americamysis bahia* (OPPTS 850.1035 and OPPTS 850.1050). At least for the foreseeable future, ecological risk assessments will likely rely on the current suite of traditional tests. The approach in this report, therefore, restricted the parameter estimations for TK-TD modeling to data typically expected from these standard toxicity tests. This limited some of the types of TK-TD modeling to couple with the periodic matrix ensemble. Most notably damage and recovery/repair models were not considered. Damage and recovery models can account for delays in effects and different rates of recovery. By definition, damage is a reduction in the fitness of the organism (Brock et al. 2010), and the effect (usually survival) is proportional to the amount of damage. The rate of repair or recovery (also proportional to the amount of damage) has to be taken into account in "damage" TD models. However, the calculation of recovery rate constants requires measured internal concentrations (Jager et al. 2011). Recovery, by definition, also has to be related to sub-lethal endpoints—an individual cannot recover from mortality. Endpoints such as growth and reproduction often do not have enough time dependent observations to efficiently estimate organism recovery. For these reasons, the model herein did not consider a damage and recovery form of TK-TD modeling.

Estimating Time-Varying Survival Probability

To translate the effects of time-varying exposure concentrations into time-varying survival probabilities, there needs to be a way to estimate how the internal concentration changes with time. Often this is accomplished assuming a one-compartment model (Kooijman and Bedaux 1996a)—which treats any internal concentration as being uniformly distributed within the organism. The concentration in an organism at any given time depends on an increase based on the concentration in the water times the uptake rate and a decrease based on the internal

[12] The actual mechanism of density dependence is not modeled. For example, the approach does not distinguish between whether the dependence is a result of intra-specific competition for food or increase rate of predation as the population increases.

concentration times an elimination rate[13]. The elimination rate can be estimated from data that is frequently available from traditional toxicity tests. If the LC50 is calculated for different time intervals (e.g., 24, 48, 72 and 96 hr), the LC50 often shows an exponential decay in time with a decay constant that can serve as an estimate of the elimination rate constant[14] (Bass et al. 2010). See Appendix C for a full explanation. The other rate constant (uptake rate) is not so easily estimated, since concentrations within the animal's body are generally not available.

Kooijman (1983) and Mancini (1983) independently solved the issue by using what is now referred to as a scaled internal concentration (Jager et al. 2011). Kooijman (1983) scaled the unknown internal concentration by the bioconcentration factor (uptake rate divided by elimination rate) and related mortality to this value. Mancini (1983) scaled the internal concentration by dividing it only by the uptake rate constant, since it is possible to independently estimate the elimination rate constant based on LC50 vs. time. Mancini's explanation of the mathematics is easier to follow, and his explanation is apparently the first to apply the model to exposure scenarios where the concentrations both decrease and increase over time. A relationship between the scaled internal concentration and % mortality forms the basis for tracking survival probability. The slope of this relationship is what Kooijman and his colleagues more recently refer to as the "killing rate" (Kooijman and Bedaux 1996a, b; Jager and Zimmer 2012). Mancini's procedure forms the basis for the use of scaled internal concentration in this report—a full explanation is provided in Appendix D. The calibration procedure for fine tuning this rate is described in Appendix E, using chronic toxicity test results for endosulfan.

Estimating Time-Varying Reproduction Probability

Mancini (1983) and others provided ways to evaluate survival probability using data from standard toxicity tests—much of it relying on acute data. Simple TK-TD models for sublethal endpoints are not easily calibrated and generally require more data than provided by traditional test protocols (Ashauer and Brown 2013). Standard toxicity tests often do not have sufficient time series data for reproductive output in order to estimate directly the kinetic coefficient for reproduction (i.e., a reproductive "killing rate"). The most readily available reproduction data will be chronic end-of-test effects information. For the purpose of the model described herein, the ratio of chronic survival effect (e.g., 28-d LC50) to chronic reproduction effect (e.g., EC50) was used. A Survival to Reproduction Ratio (SRR) was calculated and the assumption

[13] The elimination rate accounts for many different processes, including actual excretion, internal binding, internal transformation to another chemical form, etc. Mancini (1983) uses the term detoxification rather than elimination.

[14] The elimination rate constant may not just represent whole body elimination. It is possible that a portion of the compensating process may represent repair or recovery's rate constant. Some researchers, therefore, refer to this as the "dominant rate constant" (e.g., Jager et al. 2011). For the purpose of the current model I will assume elimination constant refers to the combined actions that eliminate toxicity.

made that this ratio remains constant for any probability of survival. The reproduction rate factor (RRF) is related to survival probability as: RRF = EXP[LN(SP)*SRR]. Where SP is the survival probability for a given week. The model multiplies the control maternity rates by the RRF to estimate the maternity rate for a given exposure week. The RRF is assumed to be constant for all age classes. The calibration procedure for fine tuning the SRR, if needed, is described in Appendix E.

Minimum Data Requirements

The period matrix model toxicity data requirements are the same as the minimum data requirements for the earlier, non-periodic version of the model (Thursby 2009). Thursby (2009) provides a procedure for estimating default values even if these minimum data are not available. Briefly, the minimum data requirements are:

1. Acute LC50 values for different time periods—typically these will be 24, 48, 72 and 96 hr. These data are used to calculate the elimination rate constant. This constant is the only variable needed to convert time-varying external concentrations to time-varying scaled internal concentrations.
2. Survival probability over time for one or more "constant" concentrations. These data are often available from daily observations of mortality and are used to estimate the killing rate—which is assumed to be a constant for any concentration. The killing rate converts daily scaled internal concentrations to a daily survival probability. Calibration of the model output using chronic data can refine the killing rate constant.
3. Chronic LC50/EC50 ratio (SRR). This ratio is used to estimate the weekly reproduction adjustment factor.

Model Operation

After the killing rate constant, the uptake rate constant, and the SRR are derived, the model operation is straightforward. An exposure time-series is selected and the spawning season defined. For this report each week was assigned a spawning factor of either 1 or 0. A factor of 1 meant that the original fecundity rates were used. A factor of 0 meant that all fecundity rates were set to zero. The default spawning season was 39 continuous weeks and began at week 10 (week 1 was the first week of January). For scenarios evaluating spawning season effect, six different scenarios were run—a 39-week season starting either week 10, 20 or 30, and a 26-week season, also starting either week 10, 20 or 30. Figure 3 demonstrates the first three years of a control population with spawning beginning on week 10 and lasting for 39 weeks. At the beginning of each modeling run, the model runs for several "years" with no exposure. This allows the population to reach stability with respect to annual cycling of weekly total population size, as well as stability with respect to the distribution of individuals among the different age classes. For each series with a different spawning scenario a separate 30-yr control was created. The modeling result is a time series showing the weekly change in population size as a percentage

of the control response. Figure 4 presents a partial time series as an example of how data are evaluated. Results are shown from the first ten years. The response is quantified by counting the number of times the weekly population size falls below a given threshold—expressed as a fraction of the total number of weeks in 30 years (1560).

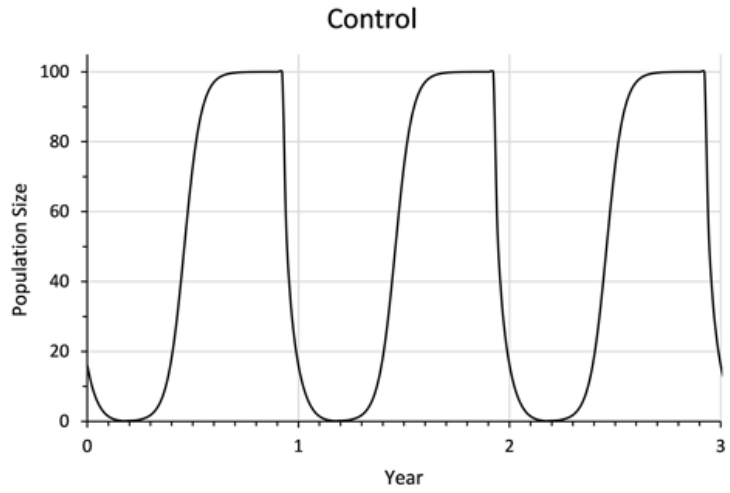

Figure 3. Example of a control population time series with spawning beginning in week 10 and lasting for 39 consecutive weeks. Only the first three years are shown—all 30 years are identical.

The risk of a population falling below a threshold obviously is a function of a specific threshold (the smaller the change from the control, the greater the potential for observing that change). A problem with this approach has been the selection of a population threshold (Thursby 2009). This can be overcome by the use of risk curves in which a range of population thresholds is used and the area under such curves calculated (Burgman et al. 1993)[15]. An alternate approach could use already established thresholds for different degrees of severity in population decline. For example, the World Conservation Union (IUCN 2012), defines a population as vulnerable if a 30% decline is observed over a specified amount of time or number of generations. A population is endangered if there is a 50% decline, and critically endangered if an 80% decline is observed or estimated[16].

[15] Risk definition in Burgman et al (1993) uses quasi-extinction, which is not the same as what is presented herein; however, the concept of total risk being related to area under the curve is similar.
[16] The World Conservation Union thresholds are for threatened and endangered species. They are presented here only as examples of how one might summarize the severity of risk to a population.

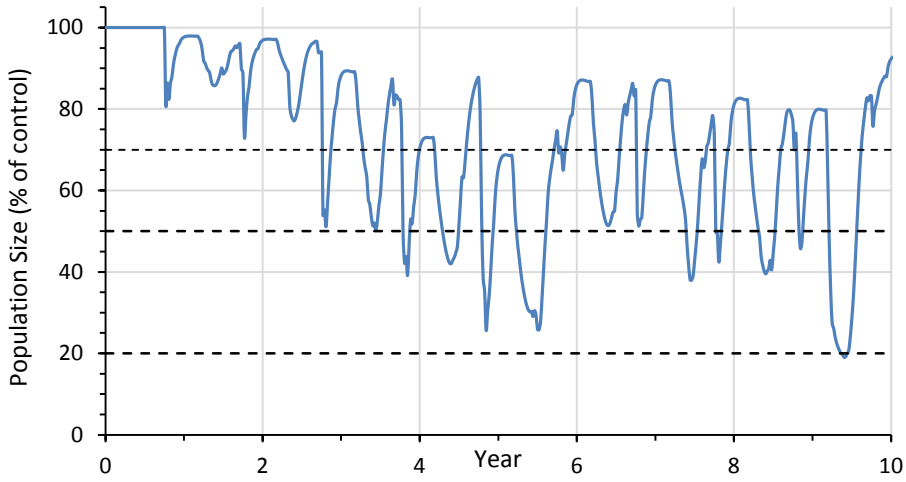

Figure 4. Sample model output showing population size as % of control for the first ten years of a model run. The horizontal dashed lines for the population thresholds for vulnerable (70%), endangered (50%) and critically endangered (20%). The number of weeks a population falls below a given threshold compared to the total number of weeks in 30 years (1560) is a direct estimate of the susceptibility of the population.

TOXICOLOGICAL FACTORS

Besides the above biological scenarios, several toxicological model scenarios also demonstrated the utility of population modeling. Figure 5 shows two sets of LC50 dynamics with time. These could represent two different species or two different toxicants with the same species. The point addressed is the effect of the rate at which mortality occurs as an organism responds to constant exposure. Both scenarios have the same 96-hr LC50—thus the traditional risk assessment would assign the same acute risk. However, one scenario clearly responds quicker to exposure (dashed line) than the other (solid line).

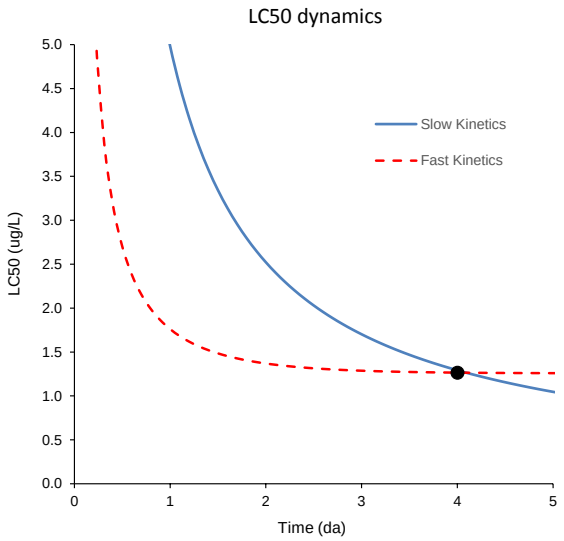

Figure 5. Acute toxicity scenarios. One in which the 24 hr and 96 hr LC50 values are very similar (dashed line) and the other where the LC50 changes more slowly with time (solid line). The elimination rate constant of the former is 1.25 d^{-1} and the latter, 0.025 d^{-1}. See Appendix C for a detailed explanation of the rate constant calculation.

A population model can distinguish between these acute scenarios. Figure 6 demonstrates why. The bottom panel displays a hypothetical 30-year time series of daily exposure concentrations. The top panel displays the daily probability of survival. The species whose LC50 value is similar at 24 and 96 hr ("fast kinetics") tracks more closely the peaks of daily exposure. The "slow kinetics" species buffers the exposure, such that sudden daily increases in exposure pass by before the effect of that exposure reaches its full potential. See Appendix D for a more detailed explanation.

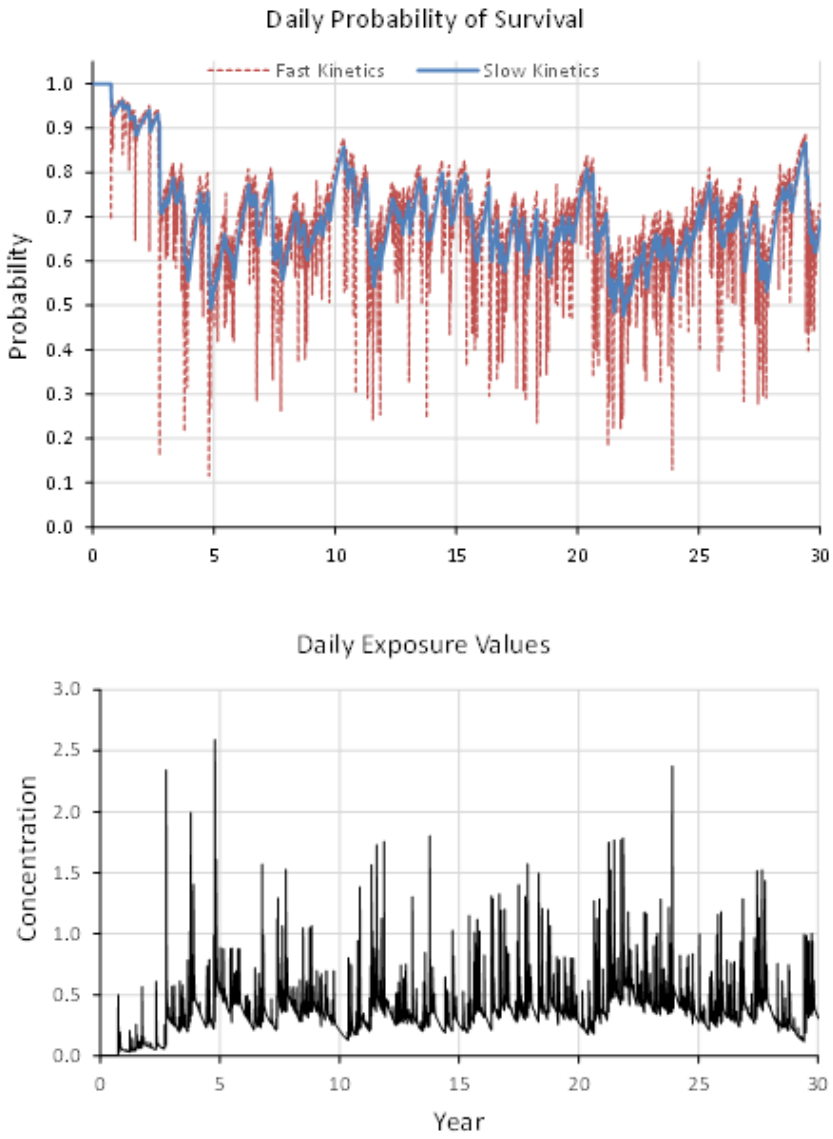

Figure 6. Daily survival probabilities (top) for two hypothetical species exposed to a daily exposure time series (bottom). See Figure 5 for description of fast and slow kinetics.

A second set of toxicological scenarios is shown in Figure 7. These hypothetical data represent dose response data from chronic tests. The chronic runs both used the same effect on reproduction, therefore both had the same NOAEC (no observable adverse effect concentration)—based on reproduction. The only difference was the relative sensitivity of survival compared to reproduction. In one scenario (Figure 7, top) survival was assumed to be insensitive relative to reproduction. In the other scenario, survival had a similar dose response to that for reproduction (Figure 7, bottom). Because both scenarios have the same NOAEC, both would result in the

same evaluation of chronic risk. Yet, logic would clearly dictate these two scenarios should not have the same level of risk. Again, population modeling will distinguish between these two chronic scenarios.

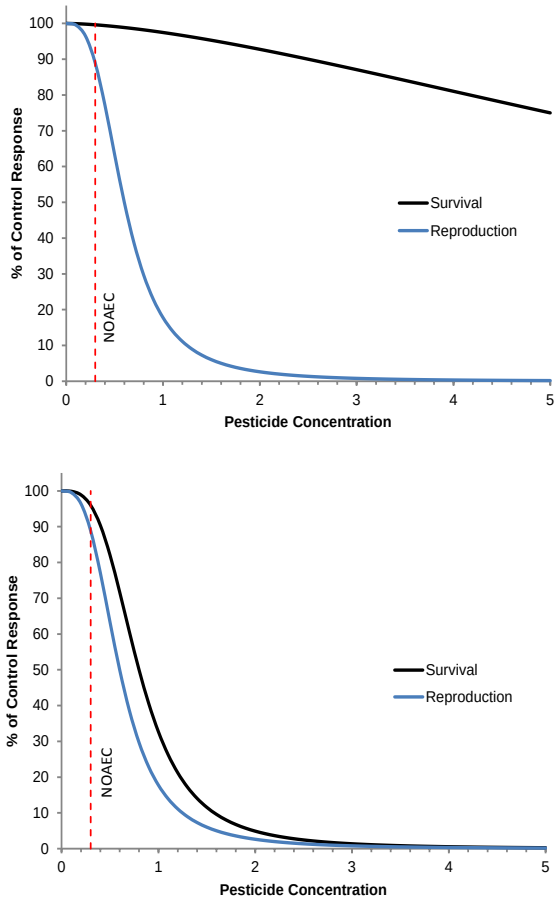

Figure 7. Chronic toxicity dose response scenarios. The scenario in the upper plot shows an NOAEC based on reproduction, and survival is significantly less sensitive than reproduction. The lower plots shows the same NOAEC; however, this time survival has a similar sensitivity to the pesticide as reproduction.

RESULTS AND DISCUSSION

Risk assessments using deterministic, probabilistic and population modeling techniques show the value of population modeling for exposure time series. The goal of this work was not to promote a particular model, but to promote the value of population modeling when specifically applied to risk assessments for pesticide time-varying exposures. The information presented below demonstrates the value of population modeling to distinguish among exposure and effects where the traditional assessment resulted in the same estimate of risk for each scenario. The analyses are based on the 21-d running averages presented in Figure 1 (daily values for each are in Appendix A).

Deterministic

All three time-series have the same annual maximum concentrations, and thus the same 90th percentile value. The summary of these values for the 21-d running averages (Figure 1) is presented in Table 1. The 90th percentile (0.772 ug/L) is used to calculate the risk quotient (RQ). For this example, the effect concentration is 0.49 ug/L[17], making the RQ 1.58, which is above the LOC of 1 for aquatic invertebrates. Based on this, we know there is the potential for acute effects on marine invertebrates—and that potential is the same for all three time series (by design).

Table 1. Annual maximum values from Figure 1.

Year	Maximum (ug/L)	Year	Maximum (ug/L)
1	0.147	16	0.462
2	0.185	17	0.572
3	0.754	18	0.691
4	0.759	19	0.631
5	1.135	20	0.377
6	0.468	21	0.478
7	0.585	22	0.971
8	0.724	23	0.598
9	0.532	24	0.890
10	0.402	25	0.419
11	0.531	26	0.468
12	0.623	27	0.561
13	0.453	28	0.625
14	0.663	29	0.324
15	0.522	30	0.575

[17] This is the mysid chronic value for endosulfan. A different chronic value would be used for freshwater invertebrates. A 60-day running average would be used for evaluating chronic effects to freshwater and saltwater fish.

Probabilistic

If we look at a cumulative distribution of the data from Figure 1, then differences among the data are more easily quantified (Figure 8). Although the deterministic approach shows the same risk analysis for each of the three exposure scenarios, the probabilistic approach clearly shows differences among the three. The probability of exceeding the chronic value for mysids (0.49 ug/L) is 3, 5 and 17%, for the low, medium and high series, respectively. However, we have no easy way to determine if any of these exposure scenarios are "bad" enough for concern.

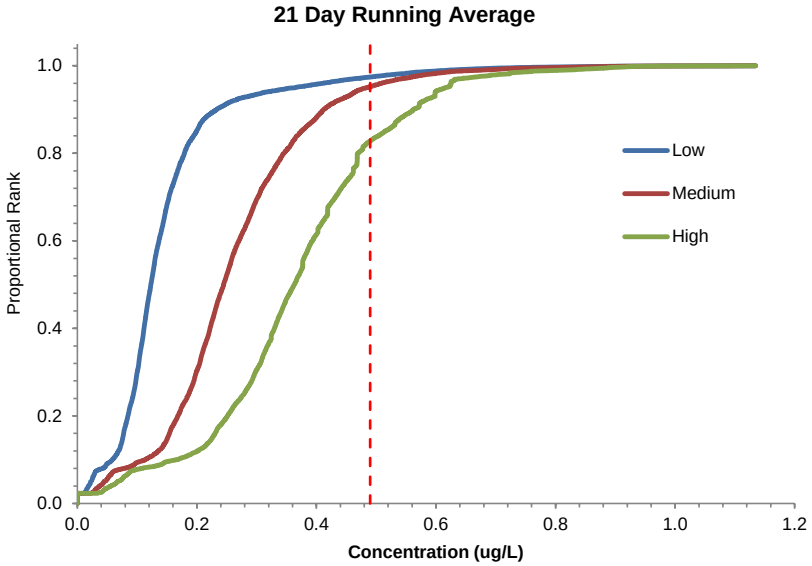

Figure 8. Cumulative distributions of the exposure data presented in Figure 6. The vertical dashed red line is the chronic value for mysids exposed to endosulfan (0.49 ug/L).

Population Modeling

Population modeling results are presented two ways, as the chance of decline below one of several thresholds, and as the total area under a risk curve. Figure 9 shows population results for endosulfan exposure which can be directly compared to the deterministic (Table 1) and probabilistic results (Figure 8). Whereas the deterministic and probabilistic methods only used the single chronic value for effect of endosulfan on mysids (0.49 ug/L), the population approach incorporates all of the dose response information for both acute and chronic exposures. Using all of the toxicological information available offers a more complete distinction among the three exposure scenarios. These distinctions are summarized in Table 2.

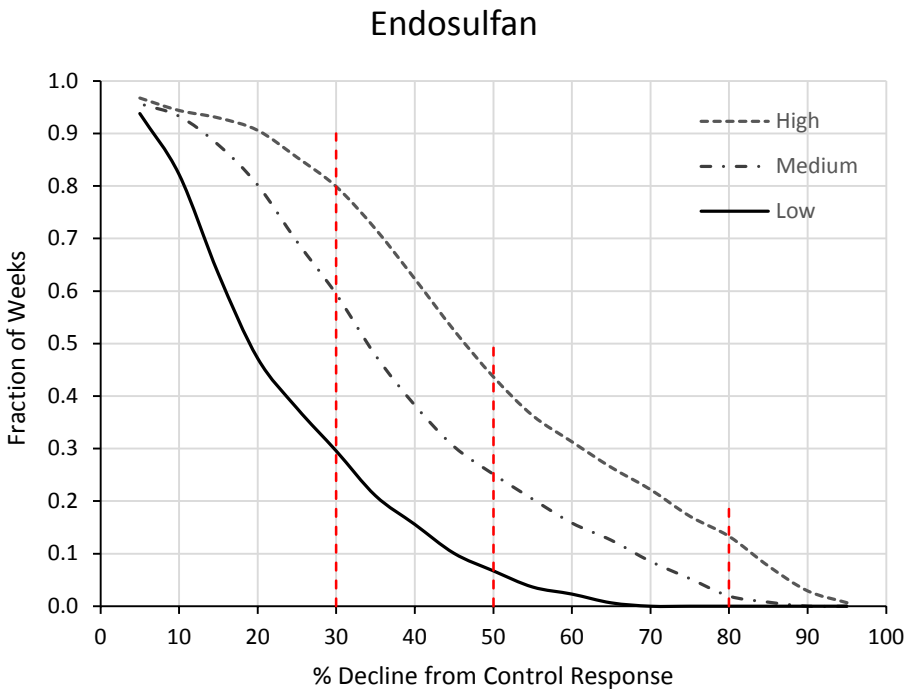

Figure 9. Summary of the chance of a given % decline in mysid population size relative to the control response for each of the three exposure scenarios when the exposures are assumed to be from endosulfan. Spawning season began on week 10 and lasted for 39 weeks. See Appendices C and E for summary of the endosulfan toxicity data. Vertical dashed lines represent % declines corresponding to IUCN categories of vulnerable (30%), endangered (50%) and critically endangered (80%).

Table 2. Summary statistics for Figure 9. AUC refers to the area under the curve.

	Threshold			
Exposure Scenario	30% Decline*	50% Decline	80% Decline	AUC
Low	0.30	0.07	0.00	20.4
Medium	0.59	0.25	0.02	35.8
High	0.80	0.44	0.13	48.9

*Decline data are fraction of time below the threshold.

Acute "Kinetics"

Figure 10 displays the population modeling results from the comparison with different acute toxicity dynamics—the acute dose responses for all model runs had the same 96 hr LC50. Model parameters assumed no direct effect on reproduction, and spawning season began on week 10 and lasted 39 consecutive weeks. The only difference among the runs were the values for elimination kinetics constant (see

Figure 5 for example, and Appendix C for explanation of kinetics constant). These ranged from 0.01 to 1.25 d^{-1} ("slow" to "fast" kinetics). Only the data from the "medium" exposure time series are displayed. The vertical dashed lines correspond to the declines associated with a population being labeled as vulnerable, endangered and critically endangered (IUCN 2012). The data for each kinetic scenario are summarized in Table 3. The area under each risk curve corresponds to the total expected relative decline in the population size. It is worth reminding that for all of these kinetic scenarios the risks determination by the deterministic and probabilistic methods are the same—the differences observed in Figure 10 are cause by alterations in the kinetics of acute toxicity only. The more similar a 24hr LC50 is to a 96 hr LC50 the quicker a species mortality rate responds to a change in the environmental concentration. The greater the ratio of 24 to 96 hr LC50s, the slower the mortality rate responds to changes in external toxicant concentrations and the more a species response is buffered against sudden, short-term changes in daily exposures.

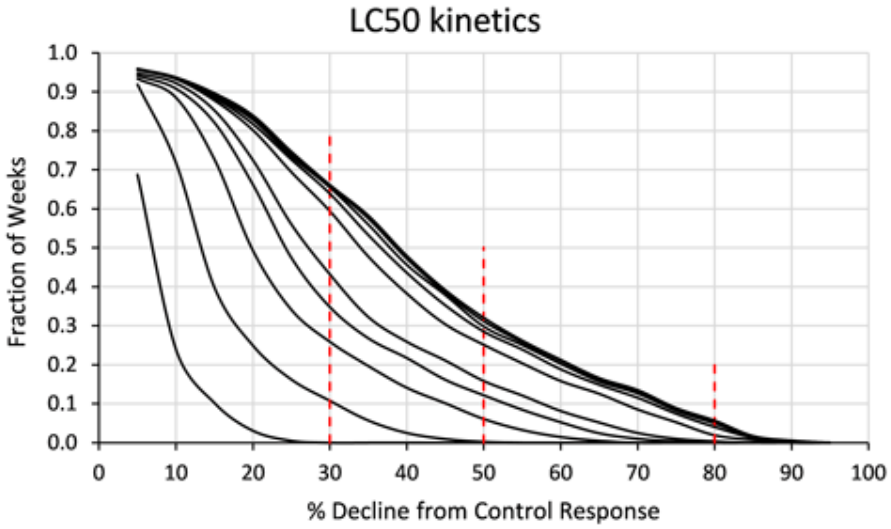

Figure 10. Summary of the chance of % decline in population size relative to the control response for ten different LC50 kinetic senarios. All population model runs used the medium exposure time series (see Appendix A). Elimination kinetic constants ranged from 0.01 d^{-1} (lowest curve) to 1.25 d^{-1} (upper most curve). Table 3 lists all of the kinetic contants, along with the summary data for each curve. Spawning season began on week 10 and lasted for 39 consecutive weeks. Vertical dash lines represent % declines corresponding to IUCN categories of vulnerable (30%), endangered (50%) and critically endangered (80%).

Table 3. Summary statistics for Figure 10. Kinetic constant refers to the value for the elimination constant. Only data for the medium exposure series are shown. Percentage declines correspond to IUCN categories of vulnerable (30%), endangered (50%) and critically endangered (80%). AUC is the area under the risk curve.

	Threshold			
Kinetic Constant	30% Decline*	50% Decline	80% Decline	AUC
0.010	0.00	0.00	0.00	3.67
0.025	0.11	0.00	0.00	12.19
0.050	0.26	0.06	0.00	20.66
0.075	0.35	0.12	0.00	25.74
0.100	0.43	0.16	0.00	28.95
0.250	0.59	0.25	0.02	35.83
0.500	0.64	0.29	0.04	38.42
0.750	0.65	0.30	0.05	39.48
1.000	0.66	0.31	0.05	40.11
1.250	0.66	0.32	0.06	40.49

*Decline data are fractions of time below the threshold.

Survival vs Reproduction Effects

Figure 11 is similar to Figure 10, except instead of displaying the results from different acute toxicity scenarios, it displays the results from two different chronic toxicity scenarios. All six model runs had a spawning season beginning on week 10 and lasting 39 consecutive weeks. In the first scenario (the upper plot in each of the three graphs), reproduction and survival have similar dose-responses to constant exposure concentrations (see Figure 7, bottom panel). In the second scenario for each time series, reproduction is significantly more sensitive to those exposures than survival (see Figure 7, top panel). The vertical dashed lines correspond to the declines associated with a population being labeled as vulnerable, endangered and critically endangered (IUCN 2012). The data are summarized in Table 4. As expected, the probability of decline for both kinetic scenarios increases as the exposure increases from low to high. Predictably, the model runs where both survival and reproduction are effected have a higher probability of decline than those where essentially only reproduction is influenced. Similar to the model runs for Figure 10, each pair of data is associated with the same probabilistic exposure summary.

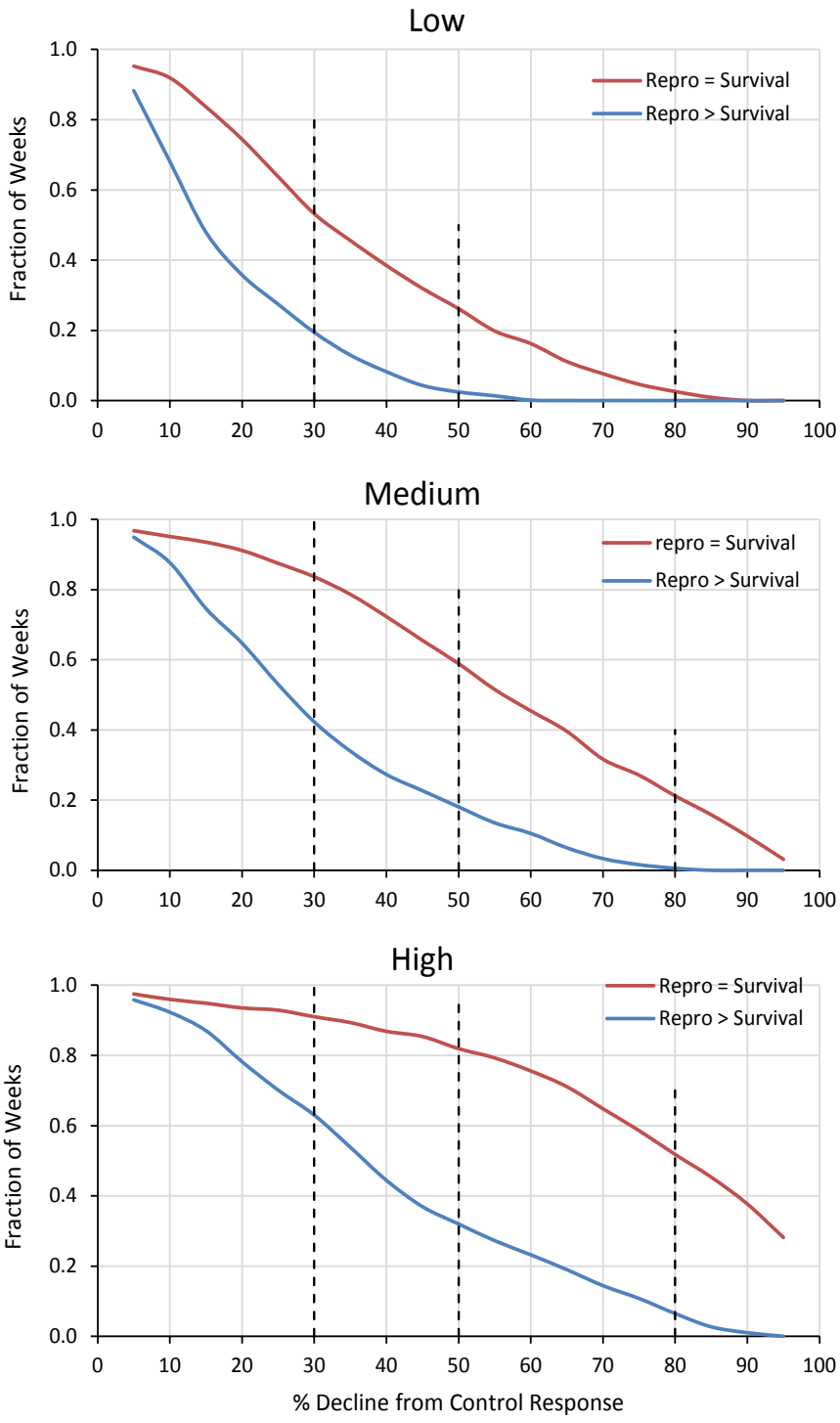

Figure 11. Summary of the chance of % decline in population size relative to the control response for each of the three exposure time series (see Appendix A). For each time series, two different toxicological scenarios were run. For the first (red line), the dose-response of reproduction and survival were set to be equal. For the second (blue line), reproduction was kept the same as the first run; however, survival sensitivity was significantly less than that of reproduction. The elimination constant was 0.25 d^{-1} (slow kinetics from Figure 5). Vertical dash lines represent % declines

corresponding to IUCN categories of vulnerable (30%), endangered (50%) and critically endangered (80%).

Table 4. Summary statistics for Figure 11. Low, Medium and High columns are data from three different exposure scenarios (see Figure 4). Repro = Survival corresponds to the model run where reproduction and survival dose-response were the same. Repro > Survival is the model run where reproduction was significantly more sensitive than survival (see Figure 3).

		Exposure Scenario		
Threshold	Dose-Responses	Low	Medium	High
		Fraction of Weeks Below Threshold		
30% Decline	Repro = Survival	0.53	0.84	0.91
	Repro > Survival	0.19	0.42	0.63
50% Decline	Repro = Survival	0.26	0.59	0.82
	Repro > Survival	0.02	0.18	0.32
80% Decline	Repro = Survival	0.03	0.21	0.52
	Repro > Survival	0.00	0.01	0.06
		Area Under Curve		
Total Risk	Repro = Survival	34.5	56.6	75.5
	Repro > Survival	15.2	28.3	39.5

Spawning Season

The effect of spawning season start date or length was not as dramatic as the effect of acute or chronic toxicity scenarios (Figure 12). Only the results for the "medium" time series are presented. There is little difference among the spawning start weeks (10, 20 or 30). It is worth noting, however, that the order of decline associated with the three different start weeks is different within the two different spawning season lengths. Also, the spawning season lasting 26 weeks generally had lower probability of decline values than the 39 week season.

Figure 13 displays the results from model runs using two different weekly population growth rates (lambda). Both runs used the medium exposure time series. The dashed lines corresponds to a run in which the weekly maximum growth rate was 1.61[18]—the growth rate for a control population during spawning season used in all previous model runs. The solid line represents a model run in which the growth rate of the control population was reduced to 1.41. This was accomplished by multiplying the spawning rates for each spawning season matrix by 0.6. The potential of a population decline increases substantially during an exposure time series when the underlying growth rate is smaller. This is likely for longer-lived species whose weekly growth rates should be substantially lower than those calculated for the short-lived mysid.

[18] This is the control's weekly growth rate using the mean demographic parameters from Thursby (2009).

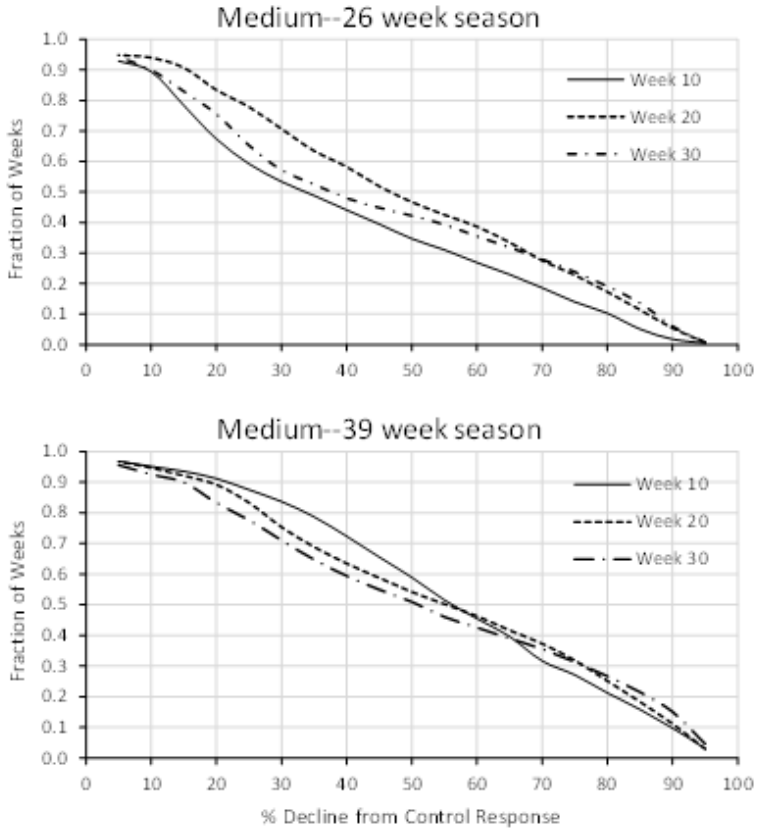

Figure 12. Summary of the effect of length and timing of spawning season on the % decline in population size relative to the control. Only the "medium" exposure scenario (see Figure 5) results are shown. The upper set of plots are for a spawning season lasting 26 consecutive weeks, beginning week 10, 20 or 30. The lower set are for a spawning season lasting 39 consecutive weeks.

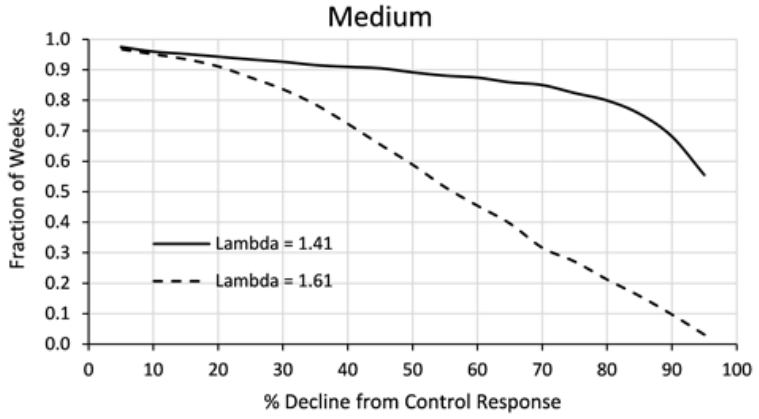

Figure 13. Summary of the effect of weekly population growth rate on the % decline in population size relative to the control. Only the "medium" exposure scenario results are shown. The length of spawning season was 39 weeks, with spawning begin on week 10.

CONCLUSIONS

Population models hold great promise for integrating exposure, toxicity and life history information into meaningful measures of risk. Several circumstances were presented within which population modeling would result in different risk conclusions than assessments based on the traditional approach. The traditional first tier procedure to risk evaluation (deterministic) relies only on the annual maxima. Three exposure scenarios were presented where the total effect of 30-year time-varying exposure series would have very different risks to a population, yet the deterministic assessment indicated the same risk for all series. The probabilistic approach was a little better. That approach at least uses all of the exposure data, and showed each exposure time series was distinct. This approach, however, can only indicate the proportion of time the expected environmental concentration exceeds the toxicological endpoint in question. The probabilistic method provided no information on the expected total consequence of those exceedences. Population modeling, on the other hand, provided an approach that allowed those consequences to be quantified. Once quantified, it becomes easier to determine whether the estimated effect is unacceptable or not. Clearly, population modeling provides a more complete assessment of the potential risk of a time-varying exposure than the traditional assessment methods.

The population model used in this report is certainly not the only mathematical model that could have been employed. The specific model used, however, was selected based on several constraints. These included having sufficient demographic data on which such a model could be based, using a commonly tested species, and being able to derive toxicity parameters for the model based entirely on standard test data. While other models may prove eventually to be more appropriate, the general conclusions about the relative value of population modeling should still stand.

The acute and chronic scenarios presented clearly indicate the potential for missing significant effects using only traditional endpoints such as LC50s, NOAECs and RQs. The model runs using different spawning seasons, however, did not show as great an effect on a mysids population's response to a given time series. The preliminary model runs using a reduced weekly growth rate suggest this lack of an obvious effect could be because mysid populations in general have a rapid growth rate. A short-term decline from a large exposure concentration can be rapidly overcome with a few weeks of lower concentration. It is likely that species with a significantly slower population growth rate—such as longer-lived invertebrates or fish—may display a more pronounced spawning season effect.

The selection of IUCN Red List thresholds for decline are consistent with their assessments for vulnerable, endangered and critically endangered species. These are presented as examples of how population modeling output might be evaluated based on observations of population size over 30 years. Red List categories can be avoided by using the area under a risk curve to establish the effect of a time series on a population. This, however, does not eliminate the need for judgement. Someone still

has to decide where to place the cut off between an acceptable and unacceptable area. Ultimately, the significance of any difference is a policy decision.

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Appendix A. Daily Modeled Pesticide Concentrations.

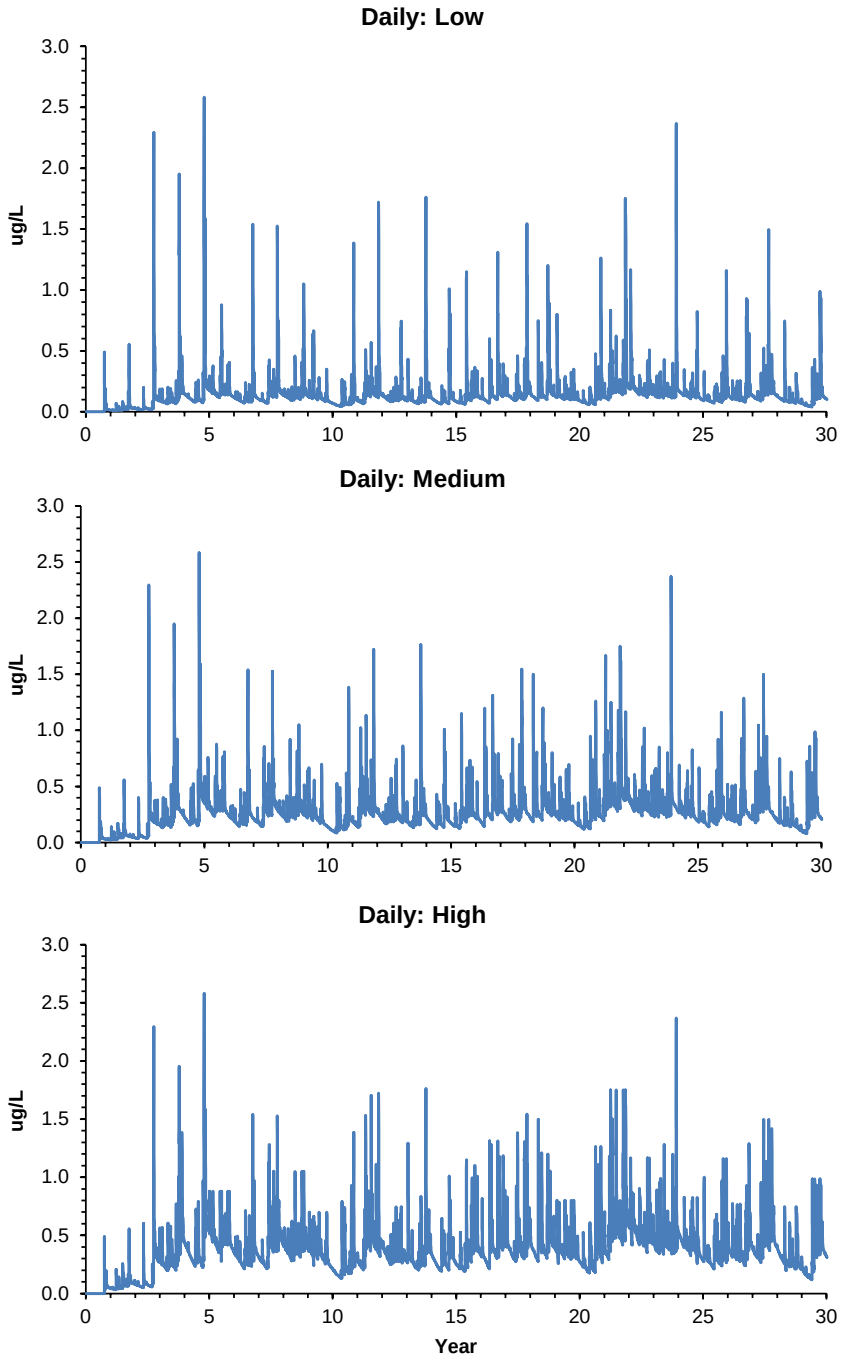

Figure A-1. Three different time series used for the model comparative runs. Each time series has the same maximum value for each of the 30 separate years, all other values were either multiplied by 0.5 (low), 1.0 (medium) or 1.5 (high).

Appendix B: Explanation of Periodic Model and Density Dependent Factor

A periodic matrix model is named as such because each cycle of the matrix (often expressed as a year) is divided into several periods. Periods can be seasons, months, etc.—and they do not have to be equal in length of time covered. Each period has its own matrix model with its own demographic parameters (see Caswell 2001, Chapter 13). The matrix product of all sub-matrices within a cycle is the projection matrix for that cycle, and its dominant eigenvalue is the annual growth rate for the population. While the model can provide annual growth rates via the eigenvalue method, its use herein is to following weekly population size.

Density dependence is incorporated into each sub-matrix using a density dependent factor:

$$[M_i \cdot n_i] \cdot DDF = n_{i+1} \qquad \text{Eq B-1}$$

Where:

M_i = a 13x13 sub-matrix for week *i* (*i* ranges from 1 to 52),

n_i = the population vector for week *i* (13 age classes),

DDF = a density dependent factor, see Equation B-2, and

n_{i+1} = the population vector for week *i+1*.

The density dependent factor is calculated as (based on Leslie 1948):

$$\text{DDF} = \frac{1}{[1+(\lambda-1)*\left({}^{n_i}/_{K}\right)]} \qquad \text{Eq B-2}$$

Where:

λ = maximum weekly growth rate in the absence of density dependence (1.64),

n_i = the total number of individuals in the i^{th} week,

K = carrying capacity (set at 100).

The use of density dependence requires a carrying capacity. Since the maximum size of field populations for mysids is not easily known, 100 was chosen as the maximum—100%, so the carrying capacity is a relative number. The weekly control population growth rate (lambda) for a spawning week was 1.64, for a non-spawning week, 0.49 [19]. As a population size approaches zero, the weekly growth rate approaches the maximum rate. As a population size approaches the carrying capacity, the maximum weekly growth rate approaches 1.0. If a population exceeds the carrying capacity, the maximum weekly growth rate is less than 1.0.

[19] Based on average control demographic parameters from Thursby (2009).

Each year of exposure data is evaluated in 91-d running blocks—this is the expected duration of *Americamysis bahia* life history (13 weeks). This is visualized in Figure B-1. Each weekly sub-matrix is a 13x13 matrix, consisting of 13 age classes (Thursby 2009). Age class 1 is only exposed to the concentrations represented in the current week. Age class 2, however, has been exposed for 2 weeks—the current week and "reaches back" into the previous week. That is, the current concentration within individuals that are 2 weeks old is an integration of the previous 14 days. Each age class reaches back a different number of days. Age class 13 reaches back the full 91 days. This results in each age class having a different scaled internal concentration at any given time since they are integrating over a different number of days. Each sub-matrix covers a different portion of the annual exposure time series (Figure B-2).

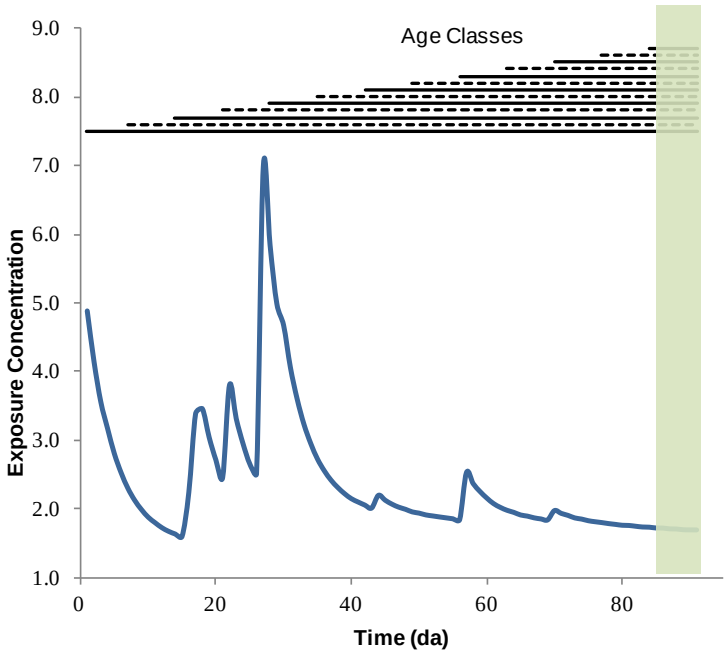

Figure B-1. Demonstration of "reaching back" for each age class. The light green vertical bar represents the current exposure week. The horizontal black lines (solid and dashed) represent the time period to which each age class has been exposed.

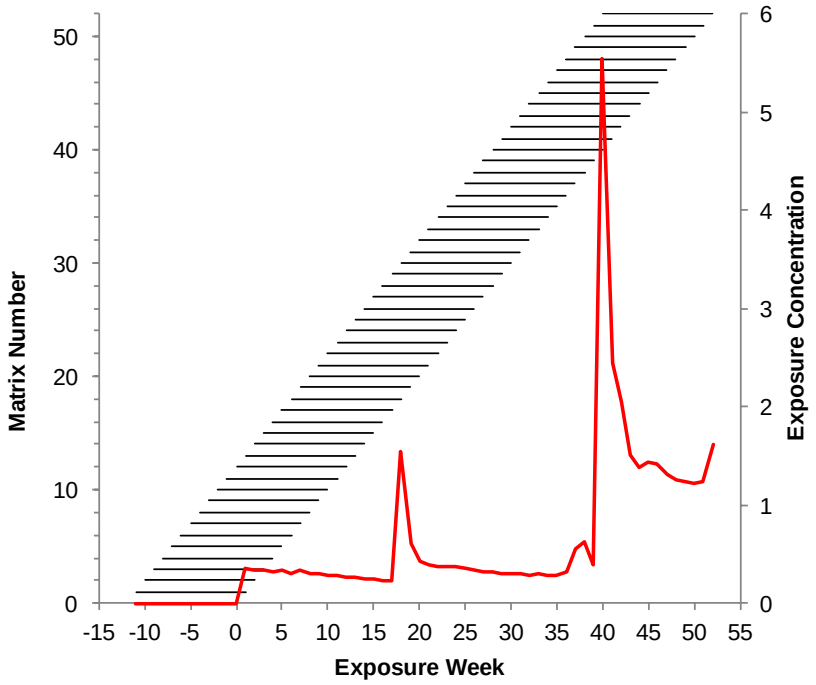

Figure B-2. Picture of a single year's worth of exposure evaluation within the model. Each sub-matrix (represented by a horizontal black bar) integrates different, but overlapping, portions of the time series.

Figure B-3 demonstrates the cumulative internal concentration (represented by $Q_{(t)}/k_{in}$—see Appendix C for a detailed explanation) for each age class for a hypothetical 91-d exposure block. Survival for a given week is estimated as the lowest calculated survival probability from among the 7 days represented by the current week's exposure. The lowest value is used since individuals can only die once. This survival value is the one used for a given age class within a given weekly sub-matrix. For each year, 676 survival values are calculated—one for each age class (13) for each week of the year.

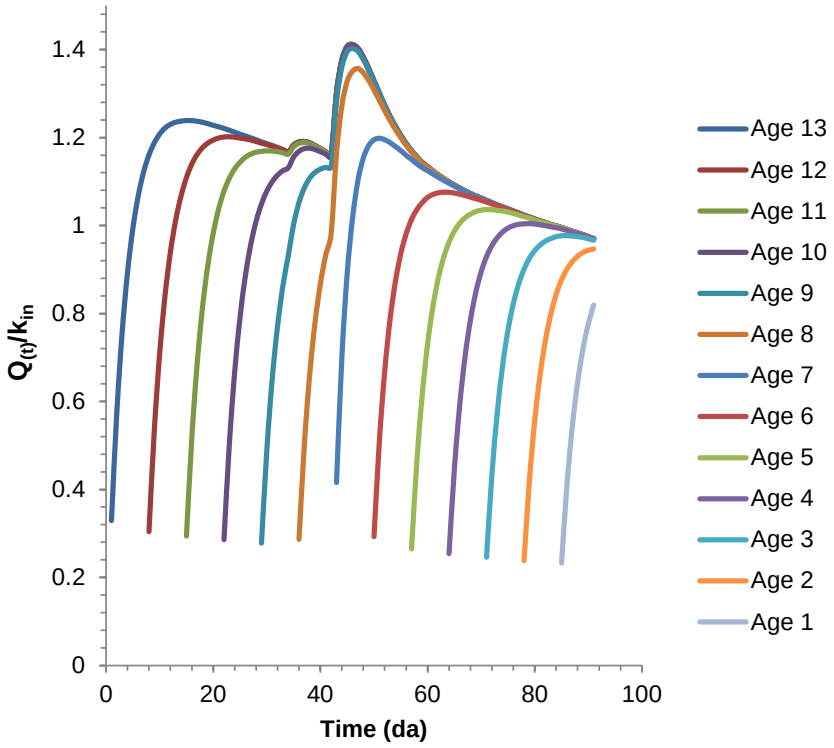

Figure B-3. Calculated $Q_{(t)}/k_{in}$ values for each age class over the entire length of their exposure. Note each curve does not begin a 0 because the calculations begin with the end of day 1. As long as there is an in water concentration greater that 0 on day 1 there will be a value for $Q_{(t)}/k_{in}$ greater than 1 on day 1.

Survivorship Calculations

The "hazard function" or "hazard rate" approach is used by the model to estimate survival. Terms like "time-to-event" or "accelerated life testing" are also used in the literature. In this model there are no sensitivity sub-groups; every individual has the same probability of dying for any given exposure. Whether or not a particular individual dies is a random process. For example, if during a particular time interval the stressor exceeds some threshold, then a portion of the population will die. This portion is determined by the "killing rate" or proportionality constant. Because the sensitivities of the individuals that die are the same as the sensitivities of the individual that do not die, there is no change in the range of sensitivity within the population of living organisms. Thus, if during the next time interval the stressor level does not change, then more individuals will die (the same proportion as the previous time interval).

To understand how we apply the hazard function approach to a series of time-varying exposures, we begin with raw data such as that shown in Figure B-4. This figure is hypothetical survival data from a single toxicant concentration monitored for 10 periods (e.g., over 10 days).

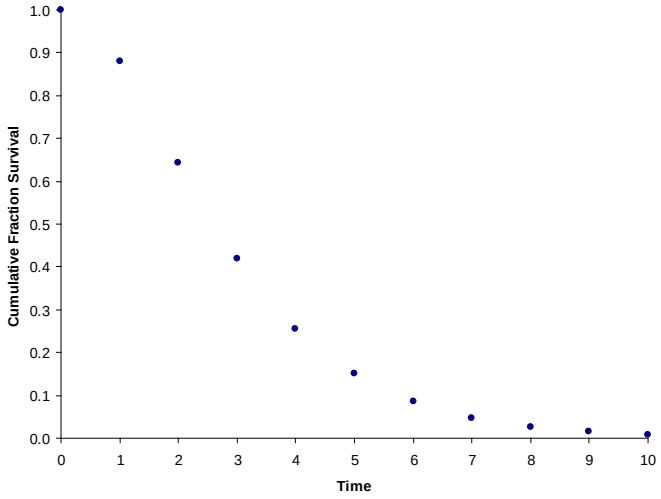

Figure B-4. A hypothetical time to death curve for a single toxicant exposure concentration.

To use the hazard function we need to calculate a new constant that Kooijman and coworkers (e.g., Kooijman and Bedaux, 1996a,b) call the killing rate, k_{kill}. We fit a survivorship curve to the above set of data that has k_{kill} as the only unknown. The development of this survivorship curve is explained below.

A good explanation for the relationship between the following is in Lee (1992). There are three main functions that we need to understand:

S(t) = *Survival Function*—defined as the probability that an individual survives longer than *t.* Also defined as 1-*F(t)* where *F(t)* is the cumulative density function (*cdf*) for mortality;

f(t) = *Probability Density Function (pdf)*—probability of dying in a given time interval divided by the duration of that interval (also defined as the derivative of *F(t)*;

h(t) = *Hazard Function*—probability of dying at time *t* assuming that the individual has survived to time *t* (also referred to as the age specific mortality rate.

These values are interrelated.

$$h(t) = \frac{f(t)}{1 - F(t)} = \frac{f(t)}{S(t)} = \frac{pdf}{1 - cdf} \qquad \text{Eq B-3}$$

Since the *pdf* is the derivative of the *cdf:*

$$f(t) = \frac{d}{dt}\left[1 - S(t)\right] = -S'(t) \qquad \text{Eq B-4}$$

By substitution into *h(t)* equation above we get:

$$h(t) = \frac{S'(t)}{S(t)} = \frac{d}{dt}\ln[S(t)] \qquad \text{Eq B-5}$$

Integration from zero to *t* and using *S(0)* = 1 , we have

$$-\int_0^t h(x)\,dx = \ln[S(t)] \qquad \text{Eq B-6}$$

Solving for *S(t)*

$$S(t) = \exp\left[-\int_0^t h(x)\,dx\right] \qquad \text{Eq B-7}$$

Widianarko and Van Straalen (1996) use the term hazard rate, which is just the probability of dying. They define hazard rate as being directly proportional to the internal concentration of a toxicant. They introduce a proportionality constant θ, and write the hazard rate as:

$$h(t) = \theta Q_{(t)} \qquad \text{Eq B-8}$$

[20]

Their θ is similar to the killing rate k_{kill} used by Kooijman and his coworkers. The two values are related by:

$$k_{kill} = \theta \frac{k_{in}}{k_{out}} \text{ or, rearranging: } \theta = k_{kill}\frac{k_{out}}{k_{in}} \qquad \text{Eq B-9}$$

Using the above definition of θ, and Eq C-2 (from Appendix C) and B-6 from above we get:

$$h(t) = \left(k_{kill}\frac{k_{out}}{k_{in}}\right)\frac{k_{in}}{k_{out}}C_w\left(1 - e^{-k_{out}\cdot t}\right) \qquad \text{Eq B-10}$$

Which simplifies to:

$$h(t) = k_{kill}C_w\left(1 - e^{-k_{out}\cdot t}\right) \qquad \text{Eq B-11}$$

Substituting this equation back into Eq B-4 we get:

$$S(t) = \exp\left[-\int_0^t k_{kill}C_w(1 - e^{-k_{out}\cdot x})\,dx\right] \qquad \text{Eq B-12}$$

[20] Others (e.g., Kooijman group) essentially use $Q_{(t)}$-Q_{NEC} where Q_{NEC} is the no effect concentration of the toxicant—in other words the hazard rate is proportional to the degree to which the internal concentration exceeds some no effect concentration.

The solution to this integral is:

$$S(t) = \exp\left[- k_{kill} C_w \left(t \cdot k_{out} \cdot e^{k_{out} \cdot t} + 1 \right) \frac{e^{-k_{out} \cdot t}}{k_{out}} + \frac{k_{kill}}{k_{out}} C_w \right] \qquad \text{Eq B-13}$$

This equation is fit to the % survival vs. time data (e.g., Figure B-4) to determine k_{kill} which will be the only unknown in the above equation—C_w will be known (and assumed a constant) for a particular data set. The constant k_{out} is determined independently (see Appendix C). Once we have an estimate for k_{kill}, we can relate survival to $Q_{(t)}/k_{in}$.

If we just substitute the definition of θ into Eq B-8 we have:

$$h(t) = \left[\frac{k_{out}}{k_{in}} k_{kill} \right] Q(t) \qquad \text{Eq B-14}$$

Rearranging we get:

$$h(t) = k_{out} k_{kill} \left[\frac{Q(t)}{k_{in}} \right] \qquad \text{Eq B-15}$$

Applying Eq B-7:

$$S(t) = \exp\left(- \int_0^t k_{out} k_{kill} \left[\frac{Q(t)}{k_{in}} \right] dx \right) \qquad \text{Eq B-16}$$

Integrating:

$$S(t) = \exp\left[- k_{out} k_{kill} \left[\frac{Q(t)}{k_{in}} \right] t \right] \qquad \text{Eq B-17}$$

Since the time step is 1 (a single day), we substitute t = 1 in to Eq B-17 and apply this to the time series for $Q_{(t)}/k_{in}$ (see Appendix D) and derive the time series for *S(t).*

Reproduction

Data sufficient to evaluate reproduction in the same manner as survival—that is, enough information to establish an empirical rate constant for reproduction analogous to k_{kill} (e.g., k_{repro})—is seldom, if ever available. The model assumes a fixed relationship (user defined) between survival and reproduction.

Appendix C: Calculation of Elimination Constant

The equation used for estimating internal concentration assumes a one-compartment model where the likelihood of death is associated with the concentration of the toxicant in some critical compartment. One-compartment models are assumed by most of the references on this topic. The rate of change of the quantity of the toxicant in the organisms is defined by:

$$\frac{dQ_{(t)}}{dt} = k_{in}C_w - k_{out}Q_{(t)} \qquad \text{Eq C-1}$$

Where $Q_{(t)}$ = concentration of toxicant inside organism at time t (µg/g);
C_w = concentration of toxicant in external medium (e.g., water—µg/L);
k_{in} = uptake rate into organism (L/g·t) [21];
k_{out} = elimination or detoxification rate (1/t);
t = time.

Simply put, this means that the amount that the internal concentration increases by in a given time period is equal to a constant proportion of the external concentration minus an elimination (or detoxification) rate that is proportional to the current internal concentration. The solution to this differential is:

$$Q_{(t)} = \frac{k_{in}}{k_{out}}C_w\left[1 - e^{-k_{out}\cdot t}\right] \qquad \text{Eq C-2}$$

Since k_{in} and k_{out} are considered constants for a given organism, the only variables that influence $Q_{(t)}$ in the above equation are the concentration of the toxicant in the water and the duration of exposure to that concentration. If we assume C_w is a constant, and that exposure is infinite, then the maximum internal concentration for the toxicant is $(k_{in}/k_{out})C_w$ [22]. Note that k_{in}/k_{out} is the steady state bioaccumulation factor.

Let's assume that we are exposing a group of individuals to a concentration that will kill 50% of them. For this case, let's define x as the median threshold value. Since x is the median internal threshold, this makes the external concentration that results in x an LC50 value. If we rearrange Eq. C-2, then we can show:

$$C_w\left[1 - e^{-k_{out}\cdot t}\right] = \frac{k_{out}}{k_{in}}Q_{(t)} \qquad \text{Eq C-3}$$

[21] Units of rate constants are somewhat arbitrary—established so that units of final value are correct.
[22] Mathematically as t approaches ∞, the "e" term in the above equation approaches 0.

Which at t = ∞, becomes:

$$C_w\left[1-e^{-k_{out}\cdot\infty}\right]=\frac{k_{out}}{k_{in}}Q_{(\infty)} \qquad \text{Eq C-4}$$

This, because we have defined C_w as an LC50, simplifies to:

$$C_w=\frac{k_{out}}{k_{in}}Q_{(\infty)}=LC50_{\infty} \qquad \text{Eq C-5}$$

Substituting back into Eq B-4 and rearranging, we can calculate the LC50 for any time *t*.

$$C_w=LC50_t=\frac{LC50_{\infty}}{1-e^{-k_{out}\cdot t}} \qquad \text{Eq C-6}$$

In Figure C-1 the water concentration to achieve a given toxic event is plotted vs. time to that event. Although this can be any measure of toxicity, frequently we will be plotting median lethality as the event (i.e., tracking the change in LC50 over time). By fitting Eq B-6 to the data, we can estimate the asymptote (the LC50 at infinity—or the incipient LC50[23]) and the value for k_{out}—which is the shape parameter for the curve, related to the steepness of the curve. Jager et al. (2011) make the point that determination of the elimination constant using toxicity data is actually preferred to basing that calculation on measured internal concentrations. Internal concentrations may include toxicant not located at the primary action site, whereas, effects data are always responding to the concentration at the action site—even if we do not know the location of the specific site. Figure C-2 shows the application of Eq C-6 to data for endosulfan LC50 values for *Americamysis bahia*. Data for days 1 through 4 are from Schimmel (1981). Datum for 28 day exposure is from (McKenney 1982).

[23] This term appears to have been introduced by Sprague, JB. 1969.

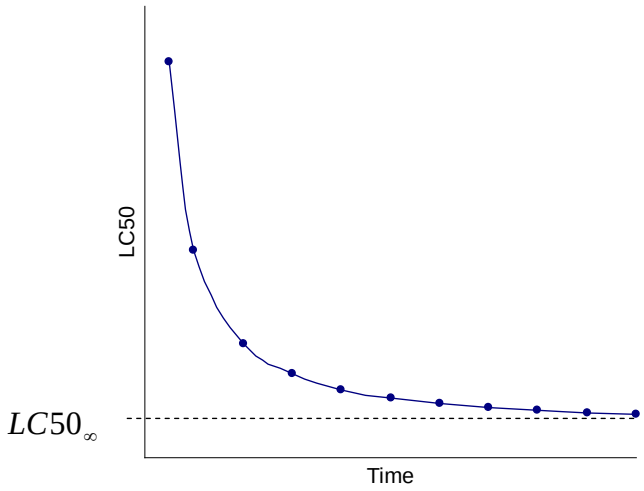

Figure C-1. Hypothetical relationship between time and LC50 values.

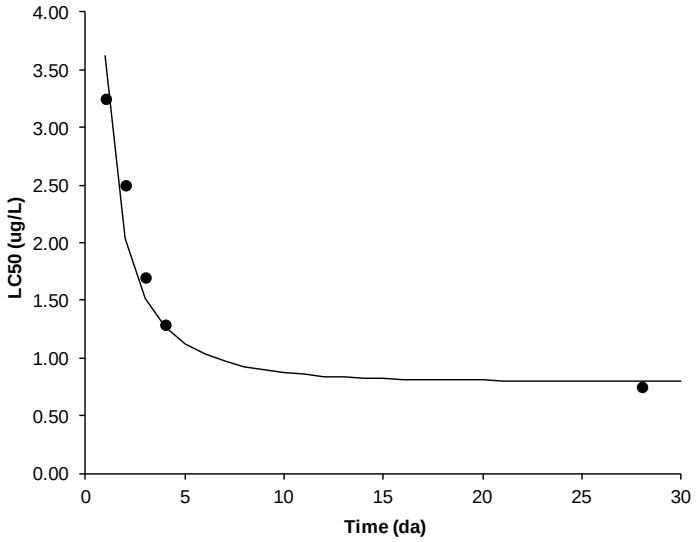

Figure C-2. Plot of mysid LC50 values for endosulfan. Curve was fit using Eq C-6. The asymptotic LC50 is 0.80 ug/L and the kinetic parameter k_{out} is 0.25 t^{-1}.

Appendix D: Explanation of Mancini Calculation of Scaled Internal Concentration

Toxicity is more closely related to internal toxicant concentration than external concentration. But, internal concentrations are rarely measured as part of traditional toxicity testing procedures. However, toxicity can be related to what has been recently called a scaled internal concentration (Jager et al. 2011). Because of this, all that is needed in order to estimate the internal kinetics of a given toxicant (using the one-compartment model) is the external concentration time series and k_{out}. The latter is calculated using traditional toxicity data (see Appendix C), and the former is from either direct measurements or, as in our case, modeled.

Even though Mancini (1983) was not the first to introduce the equations for the single-compartment model, he seems to have been the first to show how we can calculate internal values based on "environmental" time-varying concentrations. Ultimately what is needed is an estimate of the % survival for a group of organisms for each time period of interest. Ideally we would establish a relationship between internal concentration and % survival (or calculate rates of uptake and elimination: k_{in} and k_{out}). However, that would require that we actually measure the internal concentrations, which may be cumbersome and expensive. Alternately we can establish a relationship between survival and some estimate of internal kinetics. In the case of Mancini's paper that kinetic estimator is $Q_{(t)}/k_{in}$. Recall from Appendix C that the $LC50_{\infty}$ and k_{out} can be estimated using standard toxicity data. The $LC50_{\infty}$ is the same as $k_{out} \cdot Q_{(\infty)}/k_{in}$. So if we divide the $LC50_{\infty}$ by k_{out} we get $Q_{(\infty)}/k_{in}$. This means survival can be related directly to $Q_{(t)}/k_{in}$, so only how $Q_{(t)}/k_{in}$ varies with time is needed and not $Q_{(t)}$.

Mancini's equation 5 shows us how to calculate the time-varying quantity $Q_{(t)}/k_{in}$. To do this, the previous internal "quantity" loss is calculated for the current time interval:

$$decay\_of\_previous\_amount = \frac{Q_{(t-1)}}{k_{in}} \cdot e^{-k_{out} \cdot t} \qquad \text{Eq D-1}$$

And to this add the amount that this "quantity" would increase by from the current external concentration—based on Eq C-2 (Appendix C):

$$new\_amount = \frac{C_w(t)}{k_{out}}\left[1 - e^{-k_{out} \cdot t}\right] \qquad \text{Eq D-2}$$

Where $C_w(t)$ is the concentration in the external environment at time *t.* At the end of the current time interval *t* the new value is:

$$\frac{Q_{(t)}}{k_{in}} = \frac{Q_{(t-1)}}{k_{in}} \cdot e^{-k_{out} \cdot t} + \frac{C_w(t)}{k_{out}}\left[1 - e^{-k_{out} \cdot t}\right] \qquad \text{Eq D-3}$$

Using Eq D-3, the daily values for exposure concentrations are converted into daily values for $Q_{(t)}/k_{in}$. For a time step of 1 day Eq D-3 simplifies to:

$$\frac{Q_{(t)}}{k_{in}} = \frac{Q_{(t-1)}}{k_{in}} \cdot e^{-k_{out}} + \frac{C_w(t)}{k_{out}}\left[1 - e^{-k_{out}}\right] \qquad \text{Eq D-4}$$

Note: The "*t*" in $Q_{(t)}$, $Q_{(t-1)}$ and $C_w(t)$ is part of the value name and not a mathematical value.

Appendix B shows survival can be expressed as a function of $Q_{(t)}/k_{in}$, therefore, once the time series for $Q_{(t)}/k_{in}$ is known, the time series for survival is known.

Appendix E: Mysid Chronic Data for Endosulfan and Model Calibration (Quality Control)

In order to set the survival kinetic parameter (k_{kill}), survival data over time are needed for the same concentration. The acute data available for endosulfan shown below does not provide a lot of these data (Figure E-1). I could have just used the 3.02 ug/L data—and that might have been okay. Instead, to increase the amount of data, the dose response data for each day, along with the 28-d survival dose response data (Figure E-2) were used to create a new data set of survival vs. time using four different concentrations (1.5, 2.0, 2.5 and 3.0 ug/L) that more evenly cover the effect range. These are plotted in Figures E-3 and E-4.

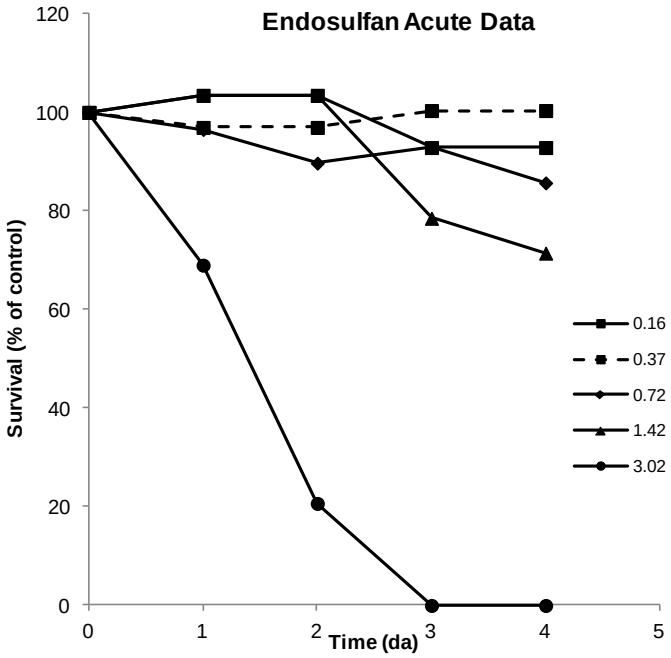

Figure E-1. Acute data for endosulfan from raw data available from Schimmel (1981).

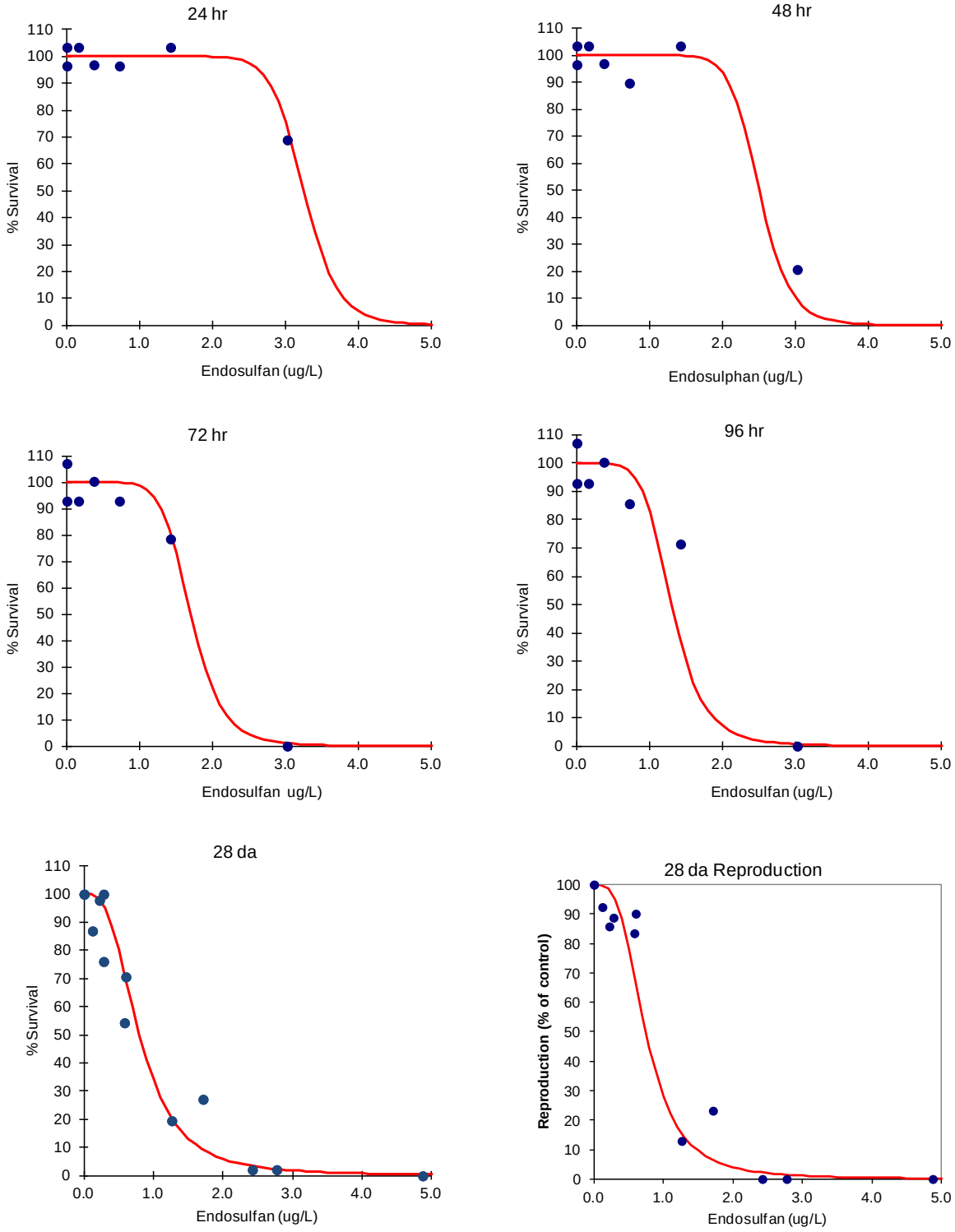

Figure E-2. Dose-response data for endosulphan survival (1, 2, 3 4 and 28 da) and 28-d reproduction dose response.

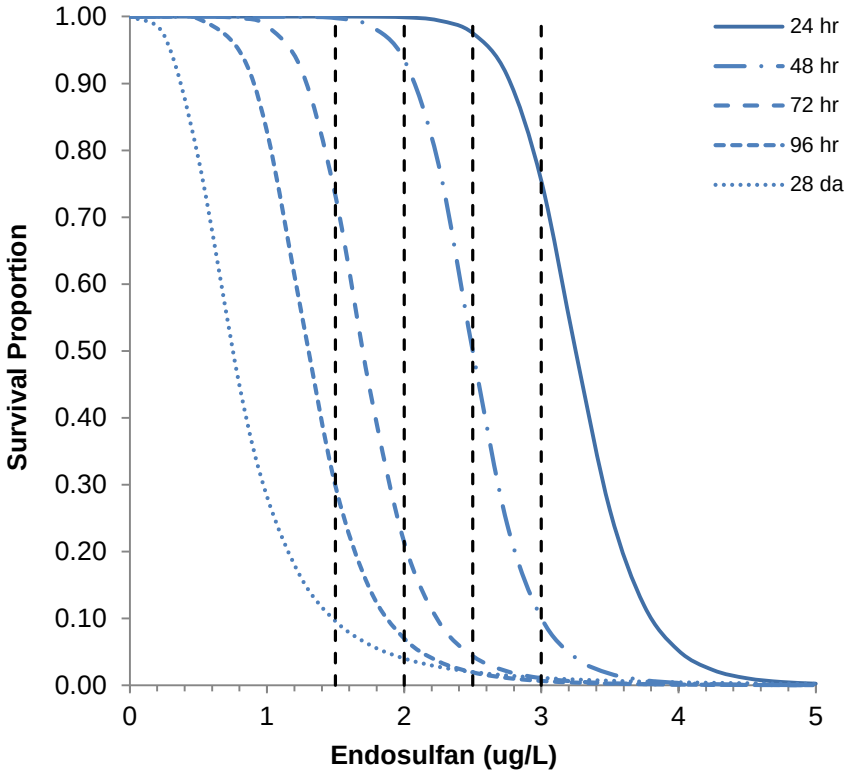

Figure E-3. Calculated survival dose-response data for endosulfan.

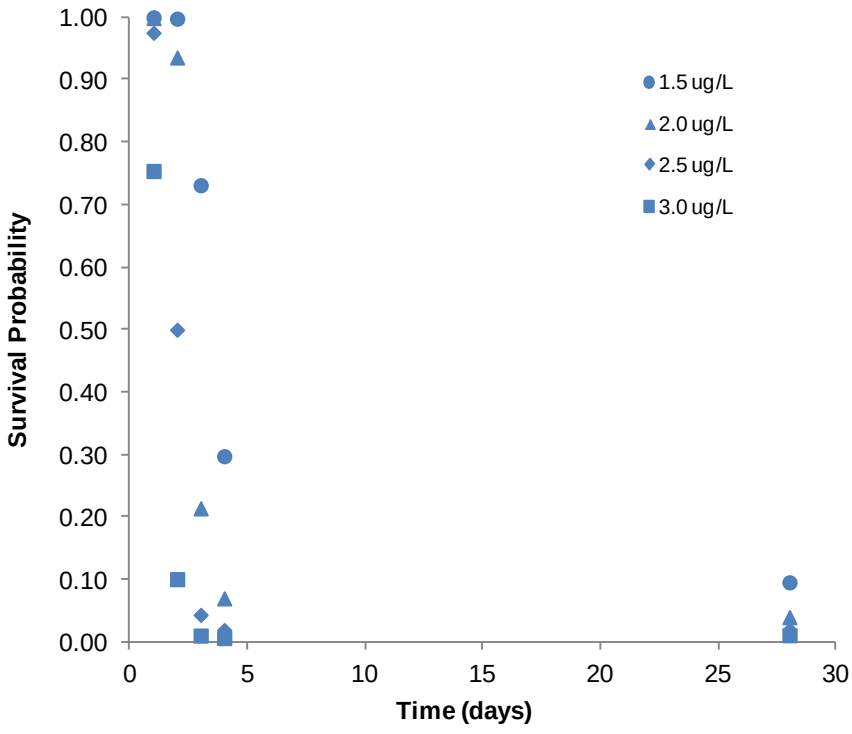

Figure E-4. Plot of data from Figure E-3 for 1.5, 2.0, 2.5 and 3.0 ug/L.

The data from Figure E-4 were combined into a single regression (Figure E-5). Equation B-17 was used to fit the data. The concentration (*Cw*) used was the average for the four data sets (2.25 ug/L). The kinetic parameter for elimination (k_{out}) was from Equation B-6 fit to data in Figure C-2 (0.25 t^{-1}). An estimate of 0.60 t^{-1} for k_{kill} gave the best fit (r^2 = 0.77).

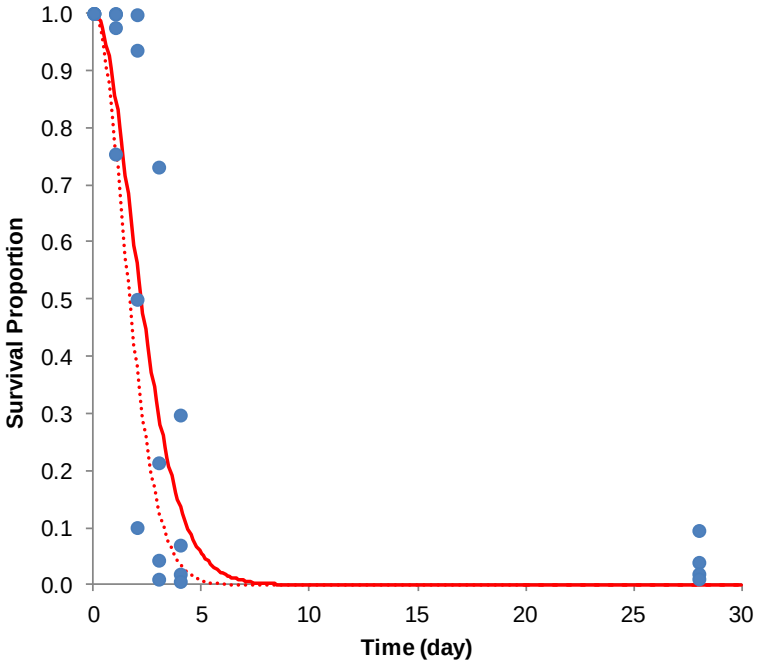

Figure E-5. Plot of survivorship versus time using data from Figure 15. Solid red line uses average concentration (2.25 ug/L) and all of the data (k_{kill} = 0.60 t^{-1}). Dashed red line is fit using a k_{kill} = 1.00 t^{-1}.

Setting kinetic parameters for reproduction is not as straightforward as for survival. This is primarily because there is not as much time-to-reproduction data available so that $Q(t)/k_{in}$ can be related to reproduction kinetics. As a compromise, reproduction was considered to be a function of the survival kinetics through the ratio of survival LC50 to reproduction EC50 from the chronic test. This assumes that the short-term exposure relationship of survival to reproduction is the same as that relationship in the chronic data set. Figure E-2 (bottom 2 plots) shows that the chronic dose responses of survival and reproduction are similar. The survival to reproduction ratio is 0.89.

For calibration purposes, the model was run using constant concentrations covering the range of measured values within the available 28-d chronic tests (McKenney 1982). A special density independent version of the model was created for conducting the calibration[24]. This version of the model tracks population size;

[24] The assumption is that chronic tests have sufficient food and low enough density of individuals to make density dependent factors minimal.

however, because only the first four weeks of the model are used (corresponding to a chronic test's 28 days) there was no problem with the model output exceeding Excel's capacity for number size. As with a chronic test, the model run began with all individuals (100) assigned to the youngest age class. Calibration run only tracked this initial cohort through each week as a measure of survival (consistent with a chronic test). For reproduction, total number of young produced during the four weeks was totaled separately.

The survival kinetic parameter of 0.60 was used as the starting point in the periodic model. However, the model output (for the first 4 weeks) under estimated the effect of endosulfan on mortality. Changing the kinetic parameter from 0.60 to 1.00 resulted in a better fit between the model and the laboratory data (see Figure E-6). Note that a survivorship curve using 1.00 is not that different from the curve using 0.60 (Figure E-5).

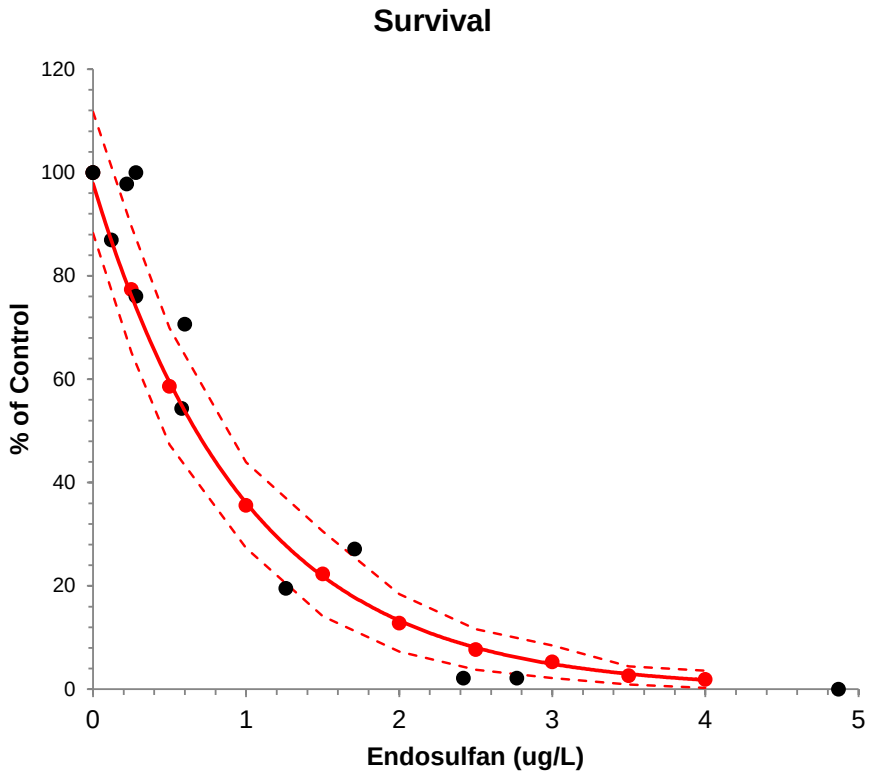

Figure E-6. Comparison of model survival output with data from 28-d laboratory chronic tests. Solid black points are the data from the 28-d endosulfan chronic tests. Solid red line is the model output using the 1.00 kinetic parameter. Dashed red lines are plus and minus one standard deviation from the model runs.

The model calibration runs used 0.89 as the survival-to-reproduction ratio. The reproduction calibration results (number young per female) are shown in Figure E-7. The match between test data and model run was reasonably good enough to keep the 0.89 ratio for use in the final model runs for time-varying exposures.

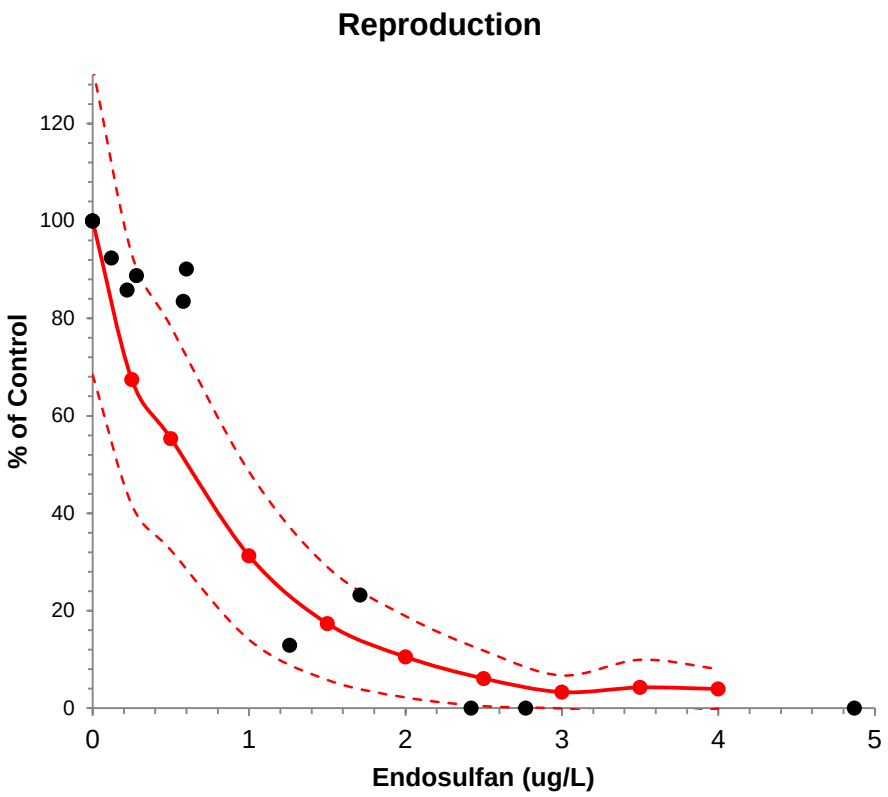

Figure E-7. Comparison of model reproduction (based on number young per female) output with data from 28-d laboratory chronic tests. Solid black points are the data from the 28-d endosulfan chronic tests. Solid red line is the model output using the 0.89 SRR. Dashed red lines are plus and minus one standard deviation from the 100 stochastic model runs.