

US EPA ARCHIVE DOCUMENT

# Quantification of Toxic Effects for Water Concentration-Based Aquatic Life Criteria

## Part A

Section 1: Background – Aquatic Life Criteria Limitations and Needs

Section 2: Toxicity Model Formulations – Binary Endpoints

Section 3: Short-Term Copper Lethality To Juvenile Fathead Minnows

Russell J. Erickson  
Mid-Continent Ecology Division  
National Health and Environmental Effects Laboratory  
Office of Research and Development  
U.S. Environmental Protection Agency  
Duluth, Minnesota

FINAL  
April 15, 2007



## Executive Summary

This is the first of a series of reports that will present and evaluate methods for improving how toxic effect levels in aquatic organisms are addressed in the formulation and application of U.S. Environmental Protection Agency (U.S. EPA) water quality criteria for the protection of aquatic life. This work is being conducted in support of efforts by the Aquatic Life Criteria Guidelines Committee of the U.S. EPA Office of Water to develop new guidelines for derivation of aquatic life criteria.

Section 1 summarizes the current formulation of aquatic life criteria and identifies certain limitations regarding how well toxic effects on aquatic organisms are quantified in these criteria as a function of the magnitude and time-variability of exposures. It then broadly describes how better quantification of toxic effects could address these limitations and improve criteria utility.

Section 2 describes various models for the assessment of binary toxicity endpoints (yes/no responses such as death) that could be useful in better describing such effects in aquatic life criteria. It then broadly describes how these models can be parameterized based on standard toxicity test data and what considerations should go into selecting a model for actual use in criteria.

Section 3 presents a case study that establishes the feasibility of model parameterization and demonstrates that these models can adequately describe the observed time-variability of copper lethality to juvenile fathead minnows for relatively short ( $\leq 8$  d), constant and intermittent exposures. This section also demonstrates how these models can provide information useful to criteria and that this information might be adequate for criteria applications using relatively simple models rather than more complicated models that would be difficult to implement.

Subsequent reports will address model formulations for other endpoints; other case

studies regarding both acute and chronic exposures and both lethal and sublethal endpoints; and recommendations regarding the application of these models to aquatic life criteria, including minimum data requirements for model parameterization. These reports will provide the technical basis for developing the guidance for using these models to criteria, but are not intended to provide the actual guidance.

## **Section 1: Background – Aquatic Life Criteria Limitations and Needs**

### ***1.1 Introduction***

This is the first of a series of reports that will present and evaluate methods for improving how toxic effect levels to aquatic organisms are addressed in the formulation and application of U.S. Environmental Protection Agency (U.S. EPA) water quality criteria for the protection of aquatic life (“aquatic life criteria” or "ALC"). This work is being conducted in support of efforts by the Aquatic Life Criteria Guidelines Committee of the U.S. EPA Office of Water to develop new guidelines for derivation of aquatic life criteria.

This background section summarizes the current formulation of aquatic life criteria and identifies certain limitations regarding how well toxic effects on aquatic organisms are quantified in these criteria as a function of the magnitude and time-variability of exposures. It then broadly describes how better quantification of toxic effects could address these limitations and improve criteria utility. Section 2 will describe various models for the assessment of binary toxicity endpoints that could be useful for better describing such effects in criteria as a function of exposure magnitude and time-variability. Section 3 will present a case study evaluating how well such models describe the observed time-variability of copper lethality to juvenile fathead minnows over relatively short ( $\leq 8$  d) exposures.

Subsequent reports will address model formulations for other endpoints; other case studies regarding both acute and chronic exposures and both lethal and sublethal endpoints; and recommendations regarding the application of these models to aquatic life criteria, including minimum data requirements for model parameterization. It should be noted that the case studies in this series of reports are intended to provide the detailed technical basis for the development of guidance for the use of these models in ALC, but will provide neither this actual guidance nor

elementary explanations of statistical and other mathematical procedures that might be needed in such guidance.

### ***1.2 Overview of Current Criteria Formulation***

U.S. EPA aquatic life criteria are usually derived from laboratory toxicity test results using procedures described in “Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses” (Stephan et al.1985), hereafter referred to as the "Guidelines." These criteria consist of two concentrations – the Criterion Maximum Concentration (CMC) and the Criterion Continuous Concentration (CCC).

The CMC is determined based on available “acute values” (AVs) – median lethal concentrations ( $LC_{50}$ s) or median effect concentrations ( $EC_{50}$ s) from aquatic animal acute toxicity tests meeting certain data quality requirements. To compute a CMC, the Guidelines require that acceptable AVs be available for at least eight genera with a specified taxonomic diversity. For each genus, a Genus Mean Acute Value (GMAV) is calculated by first taking the geometric average of the available AVs within each species (Species Mean Acute Value, SMAV) and then the geometric average across the SMAVs within the genus. The fifth percentile of the set of GMAVs so obtained is calculated based on a specified estimation procedure, and designated the Final Acute Value (FAV). The FAV might be lowered to the SMAV for an important, sensitive species as appropriate. The CMC is set equal to half of the FAV to represent a low level of effect for the fifth percentile genus, rather than 50% effect. The CMC is used in criteria to limit peak exposures by requiring that 1-h averages of exposure concentrations not exceed the CMC more often than once in three years on average. It should be noted that use of a 1-h averaging period is not equivalent to a 1-h exposure, but rather to a longer exposure in which the worst hour is equal to the CMC.

The CCC is generally determined based on available “chronic values” (CVs), which are either (a) the geometric average of the highest no-observed-effect concentration (NOEC) and lowest observed effect concentration (LOEC) for effects on survival, growth, or reproduction in aquatic animal chronic tests or (b) in some recent criteria, the  $EC_{20}$  in such tests based on concentration/effect regression analyses. If CVs are available for at least eight genera with the required taxonomic diversity, the CCC is set to the fifth percentile of genus mean chronic values (GMCVs), by the same procedure used to derive an FAV from GMAVs. Otherwise, the CCC is set to the FAV divided by a “final acute chronic ratio” (FACR) that is based on acute:chronic ratios (the ratio of the AV to the CV from parallel acute and chronic tests) for at least three species with a specified taxonomic diversity. The CCC can also be based on plant toxicity data if aquatic plants are more sensitive than aquatic animals, or on other data as deemed scientifically justified. The CCC is used in criteria to limit more prolonged exposures by requiring that 4-d averages of exposure concentrations not exceed the CCC more often than once in three years on average.

### *1.3 Limitations of Current Criteria Formulation*

Criteria derived as described above are limited in the following ways regarding how well the likelihood and magnitude of toxic effects are quantified:

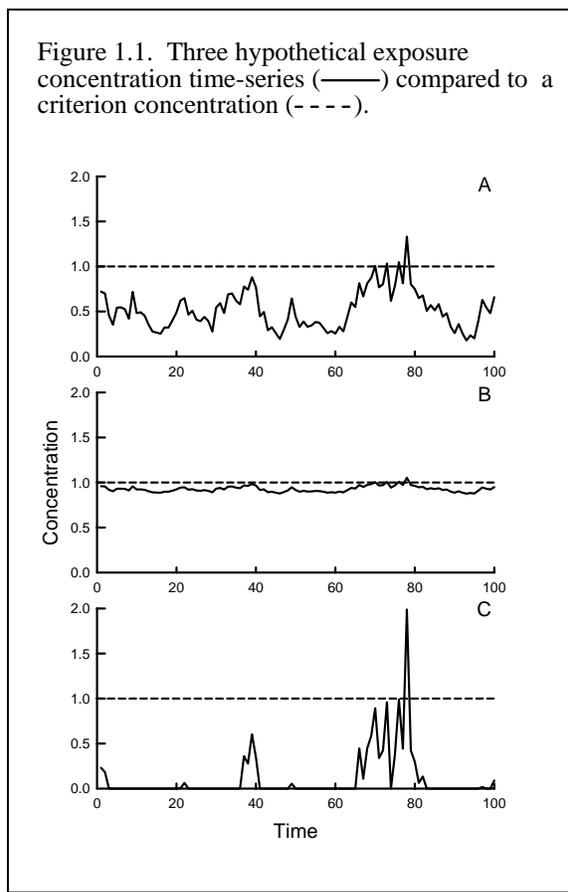
- (1) Only one level of effect is considered, rather than how levels vary with exposure. Acute toxicity analyses are based just on 50% effect, with no specific consideration of greater or lesser effects or of how rapidly the level of effect changes with concentration. Chronic toxicity analyses likewise consider only a single level of effect, based either on what is statistically significant or on some specified level of effect (e.g., 20%).
- (2) The actual level of effect represented by criteria concentrations is not well defined. For acute toxicity, the analysis does start with a specific level of effect (50%), but the division of the FAV by a factor of 2 results in the CMC corresponding to an unquantified low level of effect for the fifth percentile species, and unspecified levels of effects for more tolerant and sensitive species. For chronic toxicity, CVs based on statistically significant effects can represent a wide range of effect levels depending on the design and quality of the toxicity tests and the variability of responses.

(3) Whatever effect levels are represented by criteria concentrations, they correspond to laboratory exposures with roughly constant concentrations for fixed durations, unlike natural systems, where exposures generally have no specific duration and can vary markedly with time. For acute toxicity, the toxicity test duration is 48-96 h (depending on test species), and no assessment is made of how  $LC_{50}$ s differ for shorter or longer durations, or due to concentration variability within these time periods. For chronic toxicity, durations can vary from several days to several months or more, but effects for any one test represent a specific exposure regime that is unlikely to be close to those to which criteria are applied.

(4) Criteria address the issue of duration and concentration variability by requiring exposure concentrations to be below criteria concentrations when averaged over periods shorter than the durations of toxicity tests used to derive the criteria concentrations. Such an averaging period is intended to preclude exposure concentrations from being substantially higher than criteria concentrations for more than a small fraction of the test duration, thus ensuring effect levels stay within an acceptable range. However, the level of effect that criteria will then represent will depend on the magnitude and pattern of exposure variability, which constitutes another uncertainty regarding the effect levels actually represented by criteria conditions.

Some of these limitations are

illustrated in Figure 1.1. In this figure, three exposure time-series are compared to a hypothetical CCC based on chronic tests with a 30-d duration and implemented with a 4-d averaging period. In Figure 1.1A, a moderately variable exposure time-series is shown that satisfies the criterion concentration because, although concentrations on some days exceed the criterion, the maximum 4-d average does not. In Figure 1.1B, the exposure time-series also satisfies the criterion, but has much lower



variability. Its peak concentration is slightly lower than the first time-series, but its overall average is much higher and exposure concentrations are near the criterion concentration almost all of the time. For most toxicants, these two time-series should have different effect levels, but criteria treat them as being of equal concern. What level of effect, therefore, does satisfying the criterion actually represent? In Figure 1.1C, the exposure time-series is much more variable and violates the criterion, but the average concentration for the overall time-series is lower than in the two time-series that do not violate the criterion. Given that the peak concentrations in this time-series are only present for a short portion of the duration of the toxicity tests used to derive the criterion, does this time-series truly represent more severe effects than the other two?

These issues and questions can cause difficulties and uncertainties in applying criteria to the management of discharges and the interpretation of ambient monitoring data. Because criteria represent low, incompletely-defined levels of effect and because the magnitude of effects expected from concentrations above criterion concentrations are not quantified, it is difficult to assess the significance of occasional, minor-to-moderate exceedences of the criteria. To satisfy the requirement that criteria exceedences be rather rare, site assessments might be more conservative than actually needed to limit effects to acceptable levels. On the other hand, the prolonged effects of exposures being near, but not exceeding criteria, are also uncertain. In general, risk management is made more difficult when the risks associated with criteria are not well-quantified and are not comparable across different exposure scenarios.

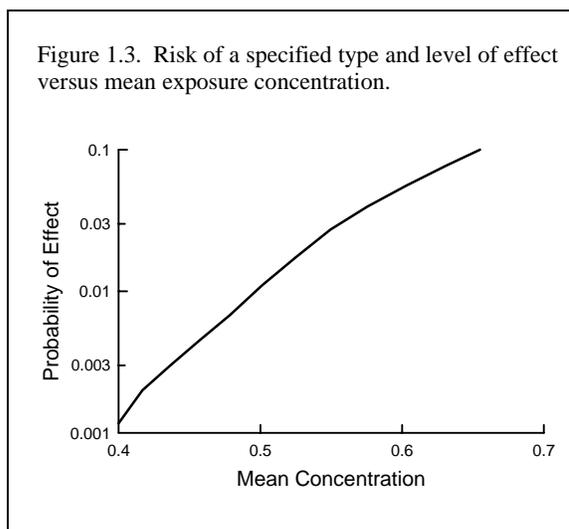
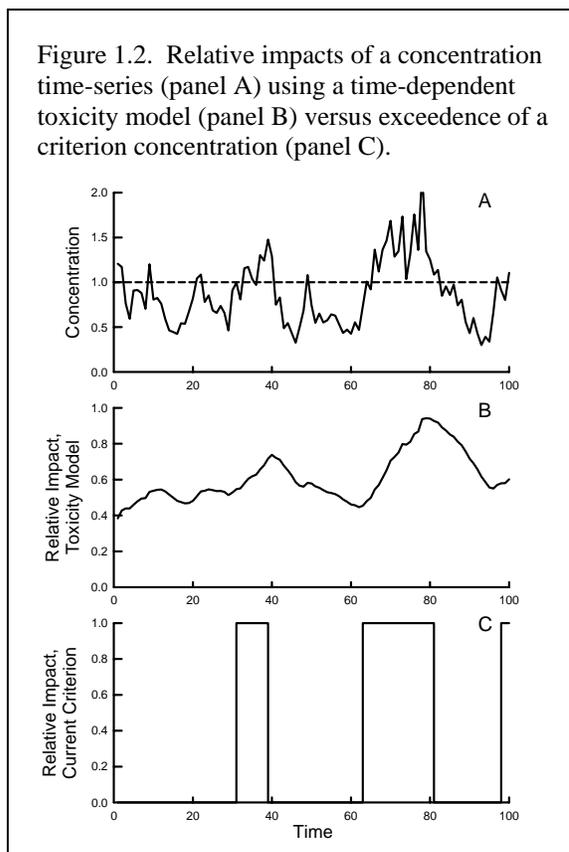
#### ***1.4 Expanded Criteria Formulation to Better Quantify Toxic Effects***

Improving the quantification of effects in aquatic life criteria will involve a variety of other issues, but it must start with better descriptions of the relationship of toxic effect levels to concentration and time. Toxicity test analysis often involves assessing just one level of effect at

the end of the test (e.g., 96-h  $LC_{50}$ ). This should be expanded to include a range of effect levels as a function of both concentration and time. Furthermore, this toxicity relationship should accommodate concentrations being time-variable, rather than roughly constant as is the case for most toxicity tests.

With such a toxicity relationship, the impact of any concentration time-series (e.g., Figure 1.2A) can be expressed as a quantitative function of time (Figure 1.2B). This is in contrast to current criterion formulations which treat any concentration (over a specified averaging period) below the criterion as being acceptable and any concentration above the criterion value being unacceptable (Figure 1.2C).

Furthermore, this time-series of toxicity levels can be used to specify the frequency of any given level of effect within an exposure time-series. This supports risk characterizations such as depicted in Figure 1.3, which shows the risk (=probability of occurrence) for a specified level of effect versus the mean exposure concentration. Figures 1.2 and 1.3 were developed for a particular type and level of



effect and for an exposure time-series with certain variability characteristics, using one of the toxicity models discussed later in this report. However, the specifics of these calculations are not important here, because this is intended to just exemplify the type of analyses that are possible, which could involve a variety of models, types of effects, and exposure scenarios. Such analyses could also address measures of exposure other than mean concentration and the uncertainty of such risk estimates.

With information such as that shown in Figure 1.3, a variety of risk assessment and management actions regarding water quality criteria could be improved. In general, there would be clearer meaning of the significance of exposures at or near the criteria limits and better separation of risk assessment and risk management. The level of exposure to be permitted from point or nonpoint sources could be related to specific levels of risk, and these levels of risk could be made more comparable across different exposure conditions. Ambient monitoring data could be assessed on a quantitative scale, rather than simply determining whether a semi-quantitative risk is exceeded. Such a quantitative scale could also be used in the interpretation of effects observed in natural and experimental ecosystems, and thus improve understanding of and decisions about risks represented by criteria. A significant aspect of all these improvements would be that criteria attainment need not be based on extreme value analysis of rare exceedences of criteria concentrations; rather, expected effects could be assessed based on more-easily measured characteristics of the exposure (e.g., the mean as in Figure 1.2), making implementation easier and more meaningful.

It should finally be noted that this approach eliminates the distinction between “acute” and “chronic” effects by addressing the effect of exposure time-series for any endpoint of interest. Therefore, rather than having separate criteria concentrations based on acute and

chronic tests, criteria would include toxicity relationships addressing different endpoints. Because the effect of time is included in these relationships, they could be merged into a single relationships based on whichever endpoint is most affected under particular exposure conditions, or based on population dynamics models that integrate these endpoints. Incorporating such expressions of risk into water quality criteria will also involve changes in how exposures are expressed (in particular, averaging periods would no longer be part of the criterion formulation) and how toxicity information across species is integrated. However, the work here will not consider the overall formulation of the criteria, but rather restrict itself to models for describing toxic effect levels for specific endpoints as a function of concentration and time, which is a necessary first step in criteria changes.

### ***1.5 References***

Stephan CF, Mount DI, Hansen DJ, Gentile JH, Chapman GA, Brungs WA. 1985. *Guidelines For Deriving Numerical National Water Quality Criteria For The Protection Of Aquatic Organisms And Their Use*. NTIS PB 85-227049, U.S. Environmental Protection Agency, Washington, DC, USA.

## Section 2: Toxicity Model Formulations – Binary Endpoints

This section will review a variety of models used in aquatic toxicology for describing the relationship of binary toxic effects (yes/no endpoints such as death) to concentration and time. Two broad classes of models will be presented – "deterministic" models for which an individual organism will or will not respond as a strict function of the exposure, and "stochastic" models for which an individual organism might or might not respond, the *probability* of the organism's response being a function of the exposure. For convenience, these models will be discussed in terms of mortality, but will apply to other binary endpoints as well.

### 2.1 Deterministic Models

#### 2.1.1 Single-Compartment, Lethal-Accumulation-Threshold Model

Although toxicity to aquatic organisms is typically referenced to chemical concentrations in exposure water, this is done with recognition that effects of chemicals nearly always depend on the chemical being accumulated into an organism. Studies on various chemicals, organisms, and endpoints have related effects to the extent of accumulation (see review by Jarvinen and Ankley, 1999). Toxicity models that relate effect levels to water concentrations can be developed by combining information on the relationship of effect levels to accumulation (toxicodynamics) with models or evaluations that address the rate and extent of accumulation (toxicokinetics) for exposures of interest (e.g., McCarty and Mackay 1993). The simplest such toxicity model for lethality can be formulated as follows:

- (1) An organism accumulates chemical by first-order, single-compartment kinetics (i.e., the accumulated concentration in the organism is described by a single, whole-body value, the gross uptake rate is proportional to the exposure concentration in the water, and the gross elimination rate is proportional to the accumulation):

$$\frac{dA(t)}{dt} = k_U \cdot C(t) - k_E \cdot A(t) \quad (2.1)$$

where  $A(t)$  is the accumulated concentration in the organism at time  $t$ ,  $C(t)$  is the exposure concentration in the water,  $k_U$  is an uptake rate constant, and  $k_E$  is an elimination rate constant. For this model, accumulation at time  $t$  can be calculated for any exposure concentration time-series by numerical integration of Equation 2.1, or by evaluation of the following integral:

$$\begin{aligned} A(t) &= \int_{x=t_0}^{x=t} (C(x) \cdot k_U \cdot e^{-k_E(t-x)}) dx \\ &= \frac{k_U}{k_E} \cdot \int_{x=t_0}^{x=t} (C(x) \cdot k_E \cdot e^{-k_E(t-x)}) dx \\ &= BCF_{SS} \cdot \bar{C}(t) \end{aligned} \quad (2.2)$$

where  $t_0$  is an earlier time at which accumulation is zero or low enough that it contributes negligibly to any accumulation at time  $t$ ;  $BCF_{SS}$  ( $=k_U/k_E$ ) is the steady-state bioconcentration factor; and  $\bar{C}(t)$  denotes a weighted running average of the water concentration, the weighting factor for this average decaying exponentially backward in time in accordance with the constant  $k_E$ . By integrating effects of exposure from  $t_0$  to  $t$ , Equation 2.2 reflects the fact that accumulation depends on both current and past exposure concentrations, with the relative importance of the concentrations decreasing the further back they are from the current time. If  $C$  is constant with time and exposure starts at  $t=0$ , the accumulation at time  $t$  would be:

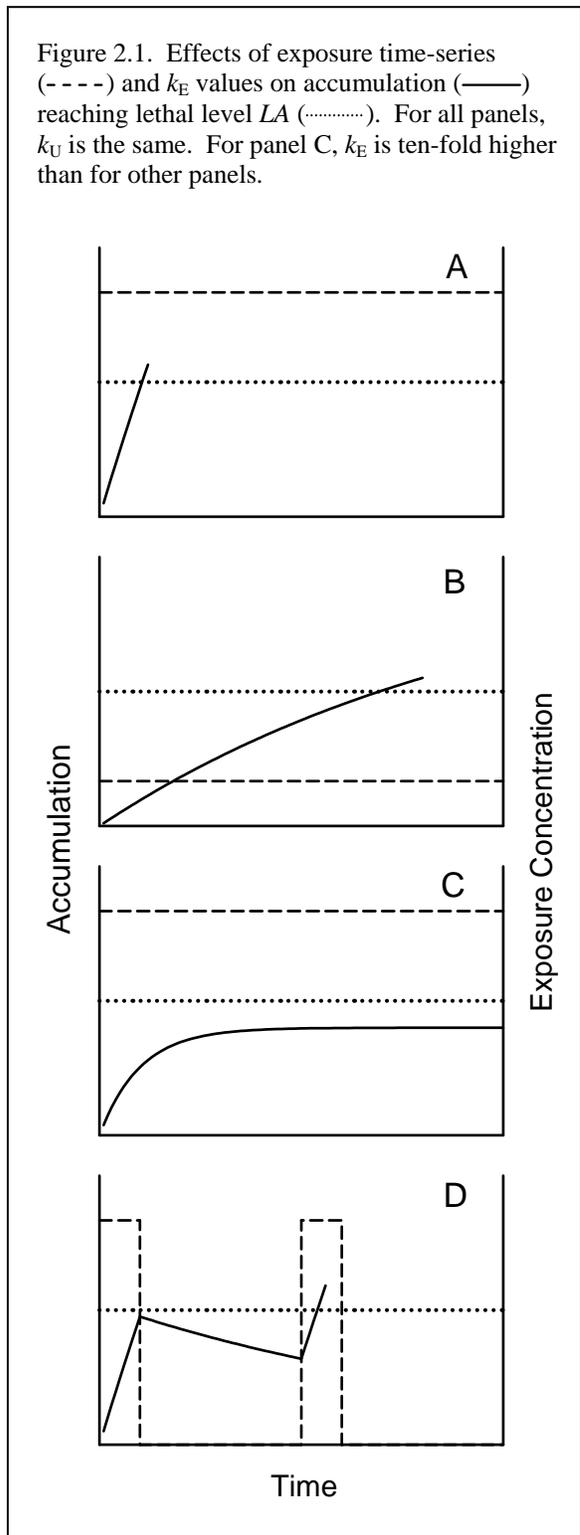
$$A(t) = BCF_{SS} \cdot C \cdot (1 - e^{-k_E t}) \quad (2.3)$$

(2) An organism dies when  $A(t)$  reaches a lethal threshold  $LA$ . Toxicity is assessed simply by tracking whether  $A(t)$  exceeds  $LA$ :

$$F(t) = \frac{A(t)}{LA} > 1 \quad (2.4)$$

where  $F(t)$  is the fraction of the lethal condition reached; i.e., the exposure is great enough to cause mortality if and when  $F(t)$  reaches 1.

Figure 2.1 illustrates how this model describes if and when mortality will occur. Panel A depicts a high enough exposure concentration that  $A(t)$  reaches  $LA$  quickly. Panel B uses the same model parameters as Panel A, but has a lower exposure concentration that requires more time to reach the lethal condition. Even lower exposure concentrations would further delay accumulation reaching the lethal level, and mortality would never occur if the exposure concentration is below that needed to reach  $LA$  at steady state ( $C \cdot BCF_{ss} < LA$ ). Panel C represents the same concentration as Panel A, but with a larger  $k_E$ , so that the  $BCF_{ss}$  is smaller, net accumulation rates decline rapidly with time, and  $A(t)$  at steady state is less than  $LA$ . Panel D denotes a pulsed exposure with the same model parameters and initial concentration as Panel A, but with the first pulse ending before  $A(t)$  reaches  $LA$ . During the cessation of exposure between pulses,  $A(t)$  declines, but then rises again during the second pulse, reaching  $LA$ .



The model described by Equations 2.1 to 2.4 strictly applies just to a single organism, and model parameters ( $LA$ ,  $k_U$ , and  $k_E$ ) would be expected to differ among organisms. For groups of organisms, these parameters would vary according to statistical distributions that need to be addressed to be able to evaluate statistics such as a median lethal concentration ( $LC_{50}$ ). Thus, to fit this model to actual toxicity data, parameter estimation involves not just these three "organism-level" parameters, but rather a greater number of "distributional" parameters (e.g., the mean and standard deviation of a distribution for each organism-level parameter).

Although this accumulation-based model allows effects to be expressed as a function of water concentrations, it requires explicit information on toxicokinetics and on the lethal accumulation threshold, which is often not well established. A study that addressed the effects of constant- and fluctuating-exposures of pentachloroethane on fathead minnows in relationship to accumulation will be presented in the second report in this series. However, requiring explicit information on accumulation and its relationship to toxicity precludes the use of abundant data that relate toxicity just to water concentration. Fortunately, this model can be adapted to describe the relationship of toxicity to exposure water concentration without explicitly quantifying accumulation.

The relationship of  $LC_{50}$ s to exposure duration for aquatic animals often is observed to follow a shape similar to Figure 2.2, with  $LC_{50}$ s declining exponentially from high values at short durations to a steady value ("asymptotic" or "threshold"  $LC_{50}$ ) at long durations. This curve is idealized and, in practice, its shape can be more complicated due to toxicity consisting of multiple steps or multiple mechanisms that operate on different time scales, and due to physiological responses to the toxicant which alter susceptibility. Nevertheless, a decline similar to that in Figure 2.2 is usually somewhat evident, provided measurements are made over a time

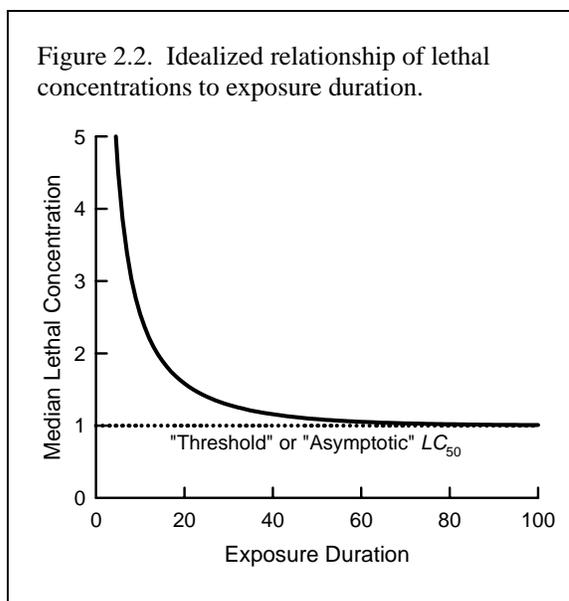
frame suitable for the chemical and test species.

Units for time or concentration are not given in Figure 2.1 because these will vary among chemicals and test organism – for some cases, this curve might span just minutes, and for other cases it might span months or more.

Zitko (1979), Mancini (1983), Neely (1984), and Chew and Hamilton (1985) noted that such an exponential decline is consistent with,

and plausibly attributable to, a model in which chemical is accumulated by first-order kinetics and in which death occurs when this accumulation reaches a lethal threshold (i.e., the model described in Equations 2.1 to 2.4). At short durations, high concentrations are needed to accumulate enough chemical fast enough to reach the lethal accumulation threshold quickly. As duration increases, lower water concentrations will cause mortality because there is more time for chemical to accumulate. With even greater duration, accumulation approaches steady state, and the lethal water concentration will approach an asymptotic value equal to the  $LA/BCF_{SS}$  (i.e., accumulation can never be high enough to elicit mortality if the water concentration is less than this). The rate at which this asymptotic lethal water concentration is approached will be equivalent to the rate at which steady-state accumulation is approached.

Explicit information on accumulation in the model represented by Equations 2.1 to 2.4 can be eliminated simply by dividing Equation 2.1 by  $LA$  so that it can be expressed in terms of the fraction of the lethal accumulation ( $F(t)$ ) that is present:



$$\begin{aligned}
 \frac{dA(t)}{dt} &= k_U \cdot C(t) - k_E \cdot A(t) \\
 \xrightarrow{\div LA} \frac{dF(t)}{dt} &= \frac{k_U}{LA} \cdot C(t) - k_E \cdot F(t) \\
 \rightarrow \frac{dF(t)}{dt} &= k_E \cdot \left( \frac{C(t)}{LC_\infty} - F(t) \right)
 \end{aligned}
 \tag{2.5}$$

where  $LC_\infty (=LA/BCF_{SS})$  is the threshold lethal concentration, the water concentration that would result in accumulation equal to  $LA$  at steady state. This equation simply states that the fraction of the lethal condition increases with time in proportion to water concentration and decreases with time in proportion to itself.

Whether mortality can be expected for any time-series can be assessed by numerical integration of Equation 2.5 or by the following general integral for Equation 2.5, analogous to Equation 2.2 for accumulation:

$$\begin{aligned}
 F(t) &= \int_{x=t_0}^{x=t} \left( C(x) \cdot \frac{k_U}{LA} \cdot e^{-k_E(t-x)} \right) dx \\
 &= \frac{k_U / k_E}{LA} \cdot \int_{x=t_0}^{x=t} \left( C(x) \cdot k_E \cdot e^{-k_E(t-x)} \right) dx \\
 &= \frac{\bar{C}(t) ?}{LC_\infty} > 1
 \end{aligned}
 \tag{2.6}$$

This equation embodies the perspective that toxicity at any time depends on both current and past exposure concentrations, with the relative importance of the concentrations decreasing the further back they are from the time in question. This is an intuitively reasonable concept, and Equation 2.6 simply provides an expression that describes this weighting.

Compared to Equations 2.2 and 2.4, the water concentration-based expressions of Equations 2.5 and 2.6 have the advantage of having just two parameters ( $LC_\infty$  and  $k_E$ ) rather than three because  $k_U$  and  $LA$  are not separable parameters when accumulation is not explicitly

addressed. For a constant exposure water concentration, the relationship between lethal concentration and time-to-death ( $t_D$ ) is:

$$LC = \frac{LC_{\infty}}{1 - e^{-k_E \cdot t_D}} \quad (2.7)$$

which provides the form of the curve presented in Figure 2.2. This equation uses both  $LC$  and  $t_D$  to emphasize that, whether time-to-death is examined as a function of concentration or lethal concentration as a function of exposure duration, the same relationship applies.

Again, these equations strictly apply just to single organisms, but can be easily extended to groups of organisms by treating each of the two organism-level parameters as a distribution rather than a single value. Thus, this model might involve four parameters consisting of means and standard deviations for both  $LC_{\infty}$  and  $k_E$ . These distributional parameters can be estimated from standard, constant-concentration, fixed-duration toxicity tests using Equation 2.7, provided that mortality is monitored for a sufficient number and range of observations times and test concentrations to encompass an adequate range of effect levels. Parameter estimation methods will be addressed in Section 2.3 and in the case studies presented in this and later reports. Once these parameters are estimated, they can be used to predict effect levels for any exposure time-series using Equation 2.5 or 2.6.

This toxicity model is very simplistic considering the variety of processes and compartments involved in the accumulation of chemical and the elicitation of toxic effects. However, for many organisms, chemicals, and exposure conditions of interest, this simple model might still be adequate for describing toxicity relationships to some acceptable approximation. For aquatic life criteria, even such an approximate model will provide valuable information on issues that are currently not well addressed. Nonetheless, consideration should be given to when

and how additional complexities might be appropriate. The following subsections will discuss some features that might be part of more complicated deterministic models.

### 2.1.2 *Damage-Repair Models*

One simplistic assumption in the single-compartment, lethal-accumulation-threshold model discussed above is that an organism will die immediately upon reaching a lethal accumulation threshold, but survive indefinite exposures just below the threshold. More realistically, once chemical is accumulated, any overt expression of toxicity involves a series of biochemical reactions with kinetic constraints that might affect time-to-death as much as, if not more than, accumulation kinetics. To address this issue, some toxicity models relate mortality to reaching a threshold level of biological damage rather than a threshold of chemical accumulation (e.g., Connolly 1987, Breck 1989, Ankley et al. 1995, Landrum et al. 2004).

A simple model for damage as a function of chemical accumulation is:

$$\frac{dD(t)}{dt} = k_D \cdot A(t) - k_R \cdot D(t) \quad (2.8)$$

where  $D(t)$  is the accumulated damage to the organism at time  $t$ ,  $k_D$  is a damage accrual rate constant, and  $k_R$  is a damage repair rate constant. Consideration of damage repair is necessary because otherwise damage incurred at some past time is considered to persist undiminished and be as important to effects as damage occurring more recently. Without assuming such repair in Equation 2.8, damage would increase indefinitely even at low exposures, resulting in no lower limit on effect concentrations as duration increases (i.e., no threshold effects concentration). Such zero-threshold models might be appropriate for some applications, and would be a subset of the more general treatment discussed here. An even more general treatment would be to assume that damage accrues only from accumulation that exceeds some threshold level; in such a

case, consideration of repair might be less important, but an additional parameter specifying this threshold accumulation would be needed.

If death occurs upon reaching a lethal damage threshold  $LD$ , then this model can be expressed in terms of the fraction of the lethal condition reached as follows:

$$\begin{aligned} \frac{dF(t)}{dt} &= \frac{k_D}{LD} \cdot A(t) - k_R \cdot F(t) \\ F(t) &= \int_{x=t_0}^{x=t} \left( A(x) \cdot \frac{k_D}{LD} \cdot e^{-k_R(t-x)} \right) dx \\ &= \int_{x=t_0}^{x=t} \left( \frac{A(x)}{LA_\infty} \cdot k_R \cdot e^{-k_R(t-x)} \right) dx \end{aligned} \tag{2.9}$$

where  $LA_\infty (=LD \cdot k_R/k_D)$  is the minimum accumulation for which the lethal damage can be reached at indefinite time. To estimate values for  $LA_\infty$  and  $k_R$  requires information on effects and accumulation over a range of exposure conditions, analogous to parameter estimation for the relationship of effects to exposure concentration described in Equations 2.5-2.7. Toxicity can then be related to water concentration by combining Equation 2.9 with Equation 2.2. However, this again requires considerable information on accumulation and the relationship of effects to accumulation, and cannot be applied to information that just relates toxicity to water concentration.

As for the lethal-accumulation-threshold model, this damage-repair model can be adapted to eliminate the need for explicit information on accumulation, by incorporation of the expression for accumulation from Equation 2.2 into Equation 2.9:

$$\begin{aligned} F(t) &= \frac{BCF_{SS}}{LA_\infty} \cdot \int_{x=t_0}^{x=t} \left( \bar{C}(x) \cdot k_R \cdot e^{-k_R(t-x)} \right) dx \\ &= \frac{\bar{C}(t)}{LC_\infty} > 1 \end{aligned} \tag{2.10}$$

where  $\bar{C}(t)$  denotes a weighted running average of the water concentration that reflects both  $k_E$  and  $k_R$ , and the threshold lethal concentration  $LC_\infty$  now equals  $LA_\infty/BCF_{SS}$ .

This model can also be expressed in terms of water concentrations by combining the first-order differential equations of Equations 2.1 and 2.9 into the second order differential equation:

$$\left(\frac{1}{k_E \cdot k_R}\right) \cdot \frac{d^2 F(t)}{dt^2} + \left(\frac{k_E + k_R}{k_E \cdot k_R}\right) \cdot \frac{dF(t)}{dt} + F(t) = \frac{C(t)}{LC_\infty} \quad (2.11)$$

For a constant exposure concentration, this differential equation has the following general solution:

$$\begin{aligned} F(t) &= P_1 \cdot e^{-k_E \cdot t} + P_2 \cdot e^{-k_R \cdot t} + \frac{C}{LC_\infty} \\ &= P_1 \cdot e^{-k \cdot t} + P_2 \cdot t \cdot e^{-k \cdot t} + \frac{C}{LC_\infty} \quad \text{if } k_E = k_R = k \end{aligned} \quad (2.12)$$

Where  $P_1$  and  $P_2$  are integration constants depending on initial conditions. For zero accumulation and damage at  $t=0$ ,  $P_1 = -C \cdot k_R / (k_R - k_E)$  and  $P_2 = -C \cdot k_E / (k_E - k_R)$  when  $k_E \neq k_R$ , and  $P_1 = -1$  and  $P_2 = -k$  when  $k_E = k_R = k$ . Substituting into Equation 2.12 the lethal condition  $F=1$ ,  $C=LC$ , and  $t=t_D$ , the relationship between lethal concentration and time-to-death for this model is:

$$\begin{aligned} LC &= \frac{LC_\infty}{1 - \frac{k_R}{k_R - k_E} e^{-k_E \cdot t_D} - \frac{k_E}{k_E - k_R} e^{-k_R \cdot t_D}} \\ &= \frac{LC_\infty}{1 - e^{-k \cdot t_D} - k \cdot t_D \cdot e^{-k \cdot t_D}} \quad \text{if } k_E = k_R = k \end{aligned} \quad (2.13)$$

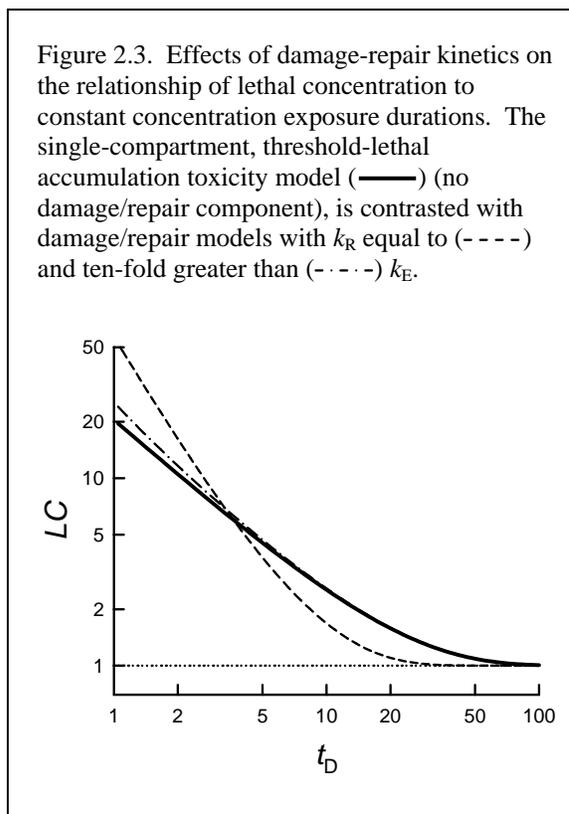
The formulation on the second lines of Equations 2.12 and 2.13, for  $k_E = k_R$ , is needed not only when this equality is exactly true, but also when these parameters are approximately equal, which causes parameter estimates to become uncertain, so that equating these parameters improves error estimates.

Although Equations 2.7 and 2.13 both involve exponential declines to a threshold lethal concentration, the equations represent different shapes (Figure 2.3) which can be discriminated with suitable data. The solid line in Figure 2.3 denotes the single-compartment, threshold-lethal-accumulation model of Equation 2.7 with  $LC_{\infty}=1$  and  $k_E=0.05$ . This is the same curve as Figure 2.2, except plotted on a log/log scale, on which the slope for this model at early times approaches -1 for all values of  $k_E$ .

The dashed line denotes the damage-repair model of Equation 2.13 with  $LC_{\infty}=1$  and  $k_E=k_R=0.2$ . These values for  $k_E$  and  $k_R$  were arbitrarily set equal in this example and their shared value was selected so that the average  $LC$  over the time range shown was approximately the same for the solid and dashed lines. The

relationship for the dashed line is much steeper than for the solid line, approaching a log-log slope of -2 at early times, because it combines the kinetic constraints of both accumulation and damage-repair. At short exposure durations, concentrations must be especially high to cause both substantial chemical accumulation and quick accrual of lethal damage. As exposure duration increases, the  $LC$  drops more quickly than the single-compartment, threshold-accumulation model because damage accrual is accelerating as accumulation increases.

The dashed-dotted line denotes the model of Equation 2.13 with  $LC_{\infty}=1$ ,  $k_E=0.05$ , and  $k_R=5$ . This much larger value for  $k_R$  results in little shift from the solid line except at very early



times, although the slope for the dash-dotted line still approaches -2 at sufficiently small times off the scale of this graph. This simply indicates that very rapid damage-repair kinetics relative to accumulation kinetics ( $k_R \gg k_E$ ) will cause Equations 2.10 and 2.13 to be approximately equivalent to Equations 2.6 and 2.7, respectively, except at very early times. Furthermore, if the accumulation kinetics are much faster than the damage-repair kinetics ( $k_E \gg k_R$ ), Equations 2.10 and 2.13 also become approximately equivalent to Equations 2.6 and 2.7 except at very early times, with  $k_R$  substituted for  $k_E$ .

As such, Equations 2.6 and 2.7 do not just represent a toxicity model based on single-compartment toxicokinetics and lethal-accumulation-threshold toxicodynamics. Rather, they can be considered to represent a broader set of models in which the kinetic constant can represent any process regulating the effect of time on toxicity, not just accumulation (Connolly 1987, Breck 1989), or can represent the combined effect of multiple processes if this is approximately first-order. Subsequent use of Equations 2.6 and 2.7 will thus use a kinetic constant  $k$  without a subscript, indicating that the nature of the kinetic process(es) contributing to  $k$  are not necessarily known, and do not actually need to be known if this single constant provides a reasonable approximation for the toxicity relationships of interest. Similarly, Equations 2.10 and 2.11 can be more broadly interpreted as describing toxicity for which the kinetics can be reasonably approximated by two sequential processes, which do not need to be completely characterized for this model to be useful.

### ***2.1.3 Multiple Mechanisms of Action***

Another way in which the relationship of mortality to exposure can be more complicated than the simple model illustrated in Figure 2.2 is the existence of multiple mechanisms by which toxicity is elicited. Toxicants can act on multiple biochemical systems, and these actions can

differ with regard to both toxicokinetics and toxicodynamics, so that one mechanism might be most important for determining lethal concentrations within certain ranges of concentration and time, whereas other mechanisms would be important for other circumstances.

To extend the model described in Section 2.1.1 to two mechanisms simply requires applying Equations 2.5 and 2.6 to each mechanism as follows:

$$\begin{aligned}\frac{dF_A(t)}{dt} &= k_A \cdot \left( \frac{C(t)}{LC_{\infty,A}} - F_A(t) \right) \\ \frac{dF_B(t)}{dt} &= k_B \cdot \left( \frac{C(t)}{LC_{\infty,B}} - F_B(t) \right) \\ F_A(t) &= \frac{1}{LC_{\infty,A}} \cdot \int_{x=t_0}^{x=t} \left( C(x) \cdot k_A \cdot e^{-k_A(t-x)} \right) dx \\ F_B(t) &= \frac{1}{LC_{\infty,B}} \cdot \int_{x=t_0}^{x=t} \left( C(x) \cdot k_B \cdot e^{-k_B(t-x)} \right) dx\end{aligned}\tag{2.14}$$

where A and B refer to the two mechanisms, and, per previous discussion,  $k$  no longer has the subscript denoting elimination because it is being treated as a more general kinetic coefficient encompassing the entire toxicity process.

$F_A(t)$  and  $F_B(t)$  must be combined to specify how the two mechanisms jointly contribute to the overall toxic condition  $F(t)$ . One possibility for this is that the two mechanisms are completely independent, so that death occurs when either  $F_A(t)$  and  $F_B(t)$  exceeds 1.0, and thus  $F(t)$  will be equal to the larger of these two fractions. For constant concentration toxicity tests, the relationship of lethal concentration to time-of-death would therefore be:

$$LC = \min \left( \frac{LC_{\infty,A}}{1 - e^{-k_A \cdot t_D}}, \frac{LC_{\infty,B}}{1 - e^{-k_B \cdot t_D}} \right)\tag{2.15}$$

Another possibility is that the two mechanisms additively contribute to damage, so that  $F(t)$  is the sum of  $F_A(t)$  and  $F_B(t)$ . For a constant concentration toxicity test, the following relationship would therefore apply:

$$LC = \frac{1}{\frac{1 - e^{-k_A t_D}}{LC_{\infty,A}} + \frac{1 - e^{-k_B t_D}}{LC_{\infty,B}}} \quad (2.16)$$

Figure 2.4 illustrates how such models would deviate from the simple model of Equation 2.7. The bold solid line in Figure 2.4 again denotes the model of Equation 2.7 with  $LC_{\infty}=1$  and  $k=0.05$ . For the two-mechanism models, it is assumed that  $LC_{\infty,B}$  is 4-fold higher than  $LC_{\infty,A}$  and that  $k_B$  is 40-fold faster than  $k_A$ , and all parameters are scaled to produce  $LC_{\infty}=1$  and approximately the same average  $LC$  as the solid line. The dashed line denotes the model of Equation 2.15, for which the mechanisms are

independent. The portion of the dashed line below the solid line indicates how mechanism B results in lower lethal concentrations at short durations because of its faster kinetics. However, the high  $LC_{\infty,B}$  results in the dashed line crossing the solid line and causing higher  $LC$ s until intersecting the relationship for mechanism A, which controls toxicity at longer durations because of the low  $LC_{\infty,A}$ . The dash-dotted line denotes the model of Equation 2.16, for which the mechanisms are additive. The assumed additivity results in a gradual transition between the

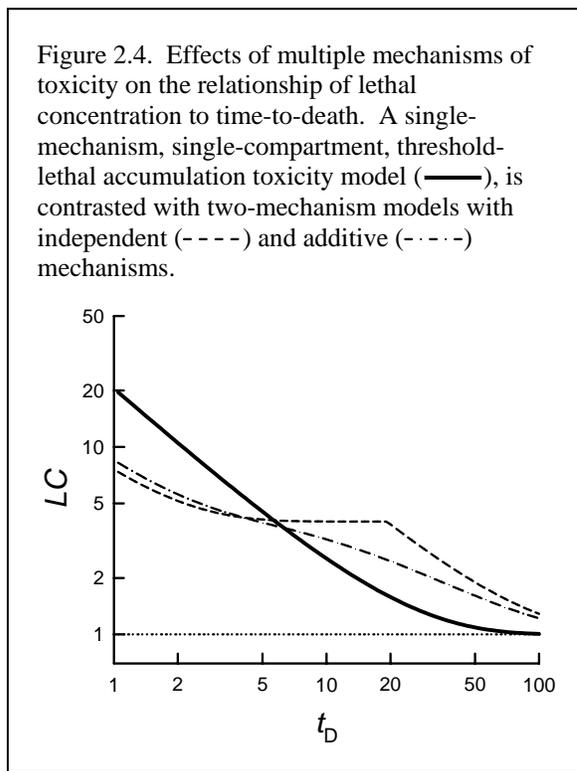


Figure 2.4. Effects of multiple mechanisms of toxicity on the relationship of lethal concentration to time-to-death. A single-mechanism, single-compartment, threshold-lethal accumulation toxicity model (—), is contrasted with two-mechanism models with independent (---) and additive (- · - · -) mechanisms.

two mechanisms, resulting in a smoother curve, but still with two phases reflecting the different kinetics of the two mechanisms.

#### **2.1.4 Multicompartment Toxicokinetics**

The first-order, single-compartment toxicokinetics model described by Equations 2.1 and 2.2 is a highly simplified approximation for chemical accumulation, which might or might not be an adequate approximation in a toxicity model for a specific chemical, organism, endpoint, and exposure scenario. An organism consists of various morphological compartments that accumulate and process chemicals at different rates, such that the concentrations in each department will have different time-dependencies. Such differences might be large enough to be important for the time-dependence of toxicity. Physiologically-based toxicokinetic (PBTK) models have been developed to describe the accumulation and speciation of chemicals in various compartments in aquatic organisms (e.g., Nichols et al. 1990), and can be a part of accumulation-based toxicity models. However, PBTK models require considerable physiological, morphological, and chemical partitioning information for parameterization, and their application to toxicity predictions requires relating effects to accumulation in a specific compartment. Their use in aquatic life criteria will be for specific circumstances and require special considerations, and they will not be addressed in this report.

However, to some approximation, multicompartment kinetics can also be addressed more empirically and more simply by extending Equation 2.1 to describe multiple compartments, which is well-established practice in toxicokinetics and pharmacokinetics research (e.g., Gibaldi and Perrier 1982). The simplest such modification is to treat an organism as consisting of two compartments, with first-order chemical exchange between the external environment (compartment 0) and compartment 1 and between compartments 1 and 2:

$$\begin{aligned}\frac{dM_1(t)}{dt} &= k_{01} \cdot C(t) - k_{10} \cdot M_1(t) - k_{12} \cdot M_1(t) + k_{21} \cdot M_2(t) \\ \frac{dM_2(t)}{dt} &= k_{12} \cdot M_1(t) - k_{21} \cdot M_2(t)\end{aligned}\tag{2.17}$$

where  $M_i(t)$  is the mass of chemical in compartment  $i$  and  $k_{ij}$  is a transfer rate coefficient from compartment  $i$  to  $j$  (e.g.,  $k_{01}$  is the coefficient for transfer from the external compartment to compartment 1).

For  $C$  constant with time, Equation 2.17 can be integrated to produce:

$$\begin{aligned}M_1(t) &= \frac{k_{01}}{k_{10}} \cdot C \cdot \left(1 - \frac{\beta - k_{10}}{\beta - \alpha} \cdot e^{-\alpha t} - \frac{\alpha - k_{10}}{\alpha - \beta} \cdot e^{-\beta t}\right) \\ M_2(t) &= \frac{k_{01}}{k_{10}} \cdot \frac{k_{12}}{k_{21}} \cdot C \cdot \left(1 - \frac{\beta}{\beta - \alpha} \cdot e^{-\alpha t} - \frac{\alpha}{\alpha - \beta} \cdot e^{-\beta t}\right) \\ A(t) &= \frac{M_1(t) + M_2(t)}{W}\end{aligned}\tag{2.18}$$

where  $W$  is the organism weight and  $\alpha$  and  $\beta$  are functions of  $k_{10}$ ,  $k_{12}$ , and  $k_{21}$  such that  $\alpha + \beta = k_{10} + k_{12} + k_{21}$  and  $\alpha \cdot \beta = k_{10} \cdot k_{21}$  (Gibaldi and Perrier 1982). With this empirical approach, the identity of the compartments is typically not completely characterized, so that  $M_1(t)$  and  $M_2(t)$  are not directly measured. Rather, Equation 2.18 (or comparable equations for other exposure scenarios) is used to analyze  $A(t)$  from accumulation and elimination experiments to estimate the four model parameters (transfer rate coefficients), which in turn can be used to estimate  $M_1(t)$  and  $M_2(t)$ .

To apply such a multicompartment toxicokinetics model to toxicity assessments would require information on the relationship of effects to accumulation that would allow specification of the lethal accumulation threshold in terms of either or both compartments. While such an application is plausible, and desirable when appropriate data are available, it would not address typical toxicity evaluations based just on water concentrations. As for the single compartment

model, these multicompartment models can be applied to interpretations of water-based toxicity evaluations without needing to quantify accumulation or the relationship of effects to accumulation, provided that the relative contributions of the two compartments to the lethal condition can be inferred. For a lethal accumulation threshold in compartment 1, the relationship of lethal concentration to time-to-death for a constant exposure concentration is:

$$LC = \frac{LC_{\infty}}{\left(1 - \frac{\beta - k_{10}}{\beta - \alpha} \cdot e^{-\alpha \cdot t_D} - \frac{\alpha - k_{10}}{\alpha - \beta} \cdot e^{-\beta \cdot t_D}\right)} \quad (2.19)$$

For a lethal accumulation threshold in compartment 2, this relationship is:

$$LC = \frac{LC_{\infty}}{\left(1 - \frac{\beta}{\beta - \alpha} \cdot e^{-\alpha \cdot t_D} - \frac{\alpha}{\alpha - \beta} \cdot e^{-\beta \cdot t_D}\right)} \quad (2.20)$$

Equations 2.19 and 2.20 provide shapes for the relationship of lethal concentrations to time that are different from the single-compartment toxicokinetics model of Equation 2.7 and from each other (Figure 2.5). The solid line in Figure 2.5 again denotes the model of Equation 2.7 with  $LC_{\infty}=1$  and  $k_E=0.05$ . The dashed line denotes the model of Equation 2.20 (lethal accumulation threshold in compartment 2) with  $LC_{\infty}=1$ ,  $k_{10}=0.2$ ,  $k_{12}=0.02$ , and  $k_{21}=0.2$ . The *relative* values for these parameters represent a situation for which the kinetics of exchange between the water and compartment 1 and between compartments 1 and 2 both are important to the kinetics of mortality in the time frame of

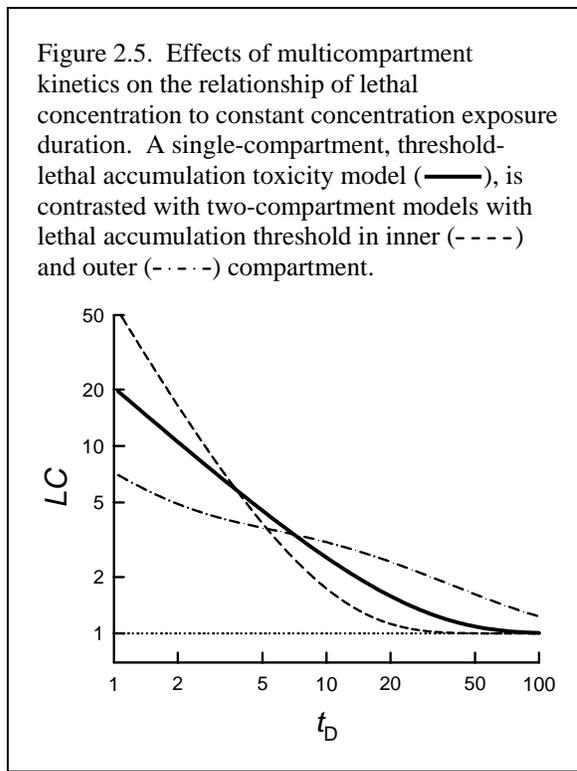


Figure 2.5. Effects of multicompartment kinetics on the relationship of lethal concentration to constant concentration exposure duration. A single-compartment, threshold-lethal accumulation toxicity model (—), is contrasted with two-compartment models with lethal accumulation threshold in inner (----) and outer (- · - · -) compartment.

interest, and the *absolute* values of these parameters are again scaled so that the average  $LC$  is

similar for the different lines. This produces a biexponential decline similar to the damage/repair model depicted in Figure 2.3; in fact, the model of Equation 2.20 cannot be distinguished from that of Equation 2.13, which reflects the fact that they both represent two sequential processes leading to toxicity (single-compartment accumulation followed by accumulation of damage, versus accumulation in an outer compartment followed by accumulation into an inner compartment). This reemphasizes the merits, when applying these models to water concentration-based toxicity data, of not attributing specific mechanisms to the processes causing toxicity and to recognize that various mechanisms might be responsible for data relationships.

In contrast, the model of 2.19 (lethal accumulation threshold in compartment 1) provides a two-phase relationship shown by the dash-dotted line in Figure 2.5, for which  $LC_{\infty}=1$ ,  $k_{10}=0.2$ ,  $k_{12}=0.5$ , and  $k_{21}=0.5$ . At early times, the  $LC$  decreases with time because accumulation in compartment 1 is increasing and the influence of uptake into compartment 2 is not yet significant. As time increases, the decrease in  $LC$  slows down because uptake into compartment 1 is largely offset by loss of chemical to compartment 2. At even greater times, compartments 1 and 2 approach steady-state with respect to each other, so that more of the uptake from water is retained in compartment 1, and the log-log slope of the  $LC$  thus becomes steeper again, until the asymptotic  $LC$  is approached. This biphasic relationship is similar to the two-mechanism model of Figure 2.4 and Equation 2.16, and, in practice, distinguishing these models would be difficult, and probably not important because they would produce similar results. However, Equation 2.19 would be more difficult and uncertain to parameterize because it does include one more parameter than Equation 2.16.

As for the models in Sections 2.1.1, 2.1.2, and 2.1.3, the parameters in Equations 2.19 or 2.20 can be estimated from the results of constant concentration toxicity tests provided that the

multicompartment kinetics are important enough to exert appreciable effects over the time-frame of the data. For the model represented by Equation 2.19, the parameters so estimated can be used to derive the kinetic constants of Equation 2.17 and 2.18, and then used to calculate toxicity under time-variable exposures. However, this is not true for the model represented by Equation 2.20, for which parameters estimated from constant concentration toxicity tests are not sufficient to uniquely specify all the kinetic constants, thus requiring additional assumptions or information to address time-variable exposures. Therefore, because the multiple compartment of Equations 2.19 and 2.20 provide relationships that (a) are not substantially different from the models of Equations 2.13 and 2.16 and (b) present more difficulties in parameterization and predictions of fluctuating exposure effects, they will not be used further in the efforts described in this series of reports.

## *2.2 Stochastic Models*

### *2.2.1 Distribution of Time-to-Death and Hazard Rate*

The models in section 2.1 are referred to as deterministic because they are premised on the assumption that any individual organism will either die or not die for any specified exposure conditions based on a fixed relationship for that individual. Variation between organisms arises from them having different values for the model parameters. Even if each parameter varies among different individuals in accordance with a statistical distribution, the response of any individual is still deterministic. An alternative approach for modeling mortality has its origin in the statistical analyses of time-to-event such as component failure, life expectancy, etc. Such statistical tools have been used in environmental risk assessment and toxicology for describing survival times under toxic chemical exposures (e.g., Dixon and Newman 1991, Newman 1995, Crane et al. 2002), and these sources provide the basis for the discussion here.

A basic variable of interest in an analysis of survival versus time is the "survivor function"  $S$ , an expression of the statistical distribution of the survival times of the organisms:

$$S(t, C) = \text{Probability of test organism survival to time } t \text{ when exposed to concentration } C \quad (2.21)$$

For a stochastic approach, the survivor function is a cumulative function of the "hazard rate"  $h(t, C)$ , the probability of death per unit time per surviving individual:

$$h(t, C) = -\frac{1}{S(t, C)} \cdot \frac{dS(t, C)}{dt} \quad (2.22)$$

The hazard rate represents a stochastic perspective because it specifies a probability that an organism will die in a given time interval, not a certainty based on the specifics of the exposure and the model parameters for the organism. Different organisms will die at different times, or not at all, based partly on random chance, not because of inherent differences between the organisms (although the hazard rates could be specified to depend on organism attributes if appropriate). The hazard rate also provides an effective basis for addressing time-variable exposures because it specifies the instantaneous risk to survivors at any time and therefore can be used to integrate this variability to estimate the survivor function, by the relationship:

$$S(t, C(t)) = e^{-\int_0^t h(t, C(t)) dt} \quad (2.23)$$

### 2.2.2 Specifying the Hazard Rate

If the hazard rate is constant with time for specific exposure conditions, the survivor function would have the simple exponential form  $e^{-\alpha t}$ , analogous to radioactive decay. However, the survivor function is usually not so simple because, even for a constant exposure concentration, the hazard rate can change with time. Surviving organisms might be more likely to succumb upon longer exposure because of increasing chemical accumulation, cumulative damage, etc., or be less likely to succumb because of compensatory mechanisms, greater

resistance in survivors, etc. The dependence of hazard rate on time must therefore be addressed. For constant exposure tests, this issue has been addressed by specifying a statistical distribution for the survivor function (i.e., a statistical distribution for survival times), which can then be related to the hazard rate using Equations 2.22.

The relationship of hazard rate to exposure concentration can be determined based on the differences in survivor functions and hazard rates across different exposures. The combined effect of time and exposure concentration (and other factors such as organism attributes or physicochemical test conditions) sometimes has been described using a "proportional hazards" model:

$$h(t, C) = e^{g(C)} \cdot h_0(t) \quad (2.24)$$

which multiplies a baseline (control) hazard rate  $h_0(t)$ , which incorporates the basic form for the time-dependence of hazard, by a factor  $e^{g(C)}$  that is a function of the chemical exposure concentration. Another model commonly used is the "accelerated failure time" model,

$$\ln(t_D) = g(C) + \xi \quad (2.25)$$

where the function  $g(C)$  describes the relationship of the median log time-to-death to exposure concentration and the random variable ( $\xi$ ) describes the variability of log time-to-death around the median. This accelerated failure time model specifies how the survivor function distribution would vary with exposure concentration, so Equation 2.22 can be used to specify how the hazard rate varies with exposure.

Although Equations 2.24 and 2.25 incorporate an effect of time and thus might appear to be applicable to the time-variable calculations indicated in Equation 2.23, this actually is not the case. The basic difficulty regarding this is that the effect of time in Equations 2.24 and 2.25 is not some independent function of time, but depends on the exposure history and thus applies

only to the constant exposure in question. If the concentration changes with time, these equations do not reflect cumulative effects that occurred from the old concentration(s), but rather cumulative effects that would have occurred if the exposure had been at the new concentration all along. Equations 2.24 and 2.25 can be applied to time-variable exposures only if the hazard rate is time-invariant, which implies an instantaneous achievement of the hazard for any concentration. For any case in which hazard is time-dependent at a constant concentration, Equations 2.24 and 2.25 do not conceptually address the effects of time-variable concentrations, so that additional model specifications regarding the effect of time on hazard are needed.

One approach for applying the hazard rate concept to time-variable exposures was developed by Kooijman and coworkers (e.g., Kooijman and Bedaux 1996a, 1996b). Their model is based on chemical accumulation, using the same first-order, single-compartment kinetics model discussed in Section 2.1.1 (Equations 2.1 to 2.4). The hazard rate is assumed to be linearly proportional to the degree to which chemical accumulation exceeds a threshold accumulation value, and to change instantaneously as accumulation changes:

$$h(t) = \max(0, d \cdot (A(t) - A_0)) \quad (2.26)$$

where  $A(t)$  is measured or estimated as in Equations 2.2 and 2.3,  $d$  is a proportionality constant called the "killing rate" (Kooijman and Bedaux 1996a, 1996b), and  $A_0$  is an accumulation threshold for effects. It is thus the kinetics of accumulation that produce the time-variability of the hazard rate for constant exposures and allow extrapolation to time-variable exposures.

This model has four parameters [ $k_E$  and  $BCF_{SS}$  (or  $k_U$  and  $k_E$ ) for the toxicokinetics (Equations 2.1 to 2.3) and  $A_0$  and  $d$  for the toxicodynamics] if accumulation and the relationship of effect levels to accumulation are explicitly addressed. As was the case for the deterministic model, this stochastic model can also be formulated to be referenced to water concentrations

without explicitly including accumulation. When the water concentration ( $C$ ) is constant,

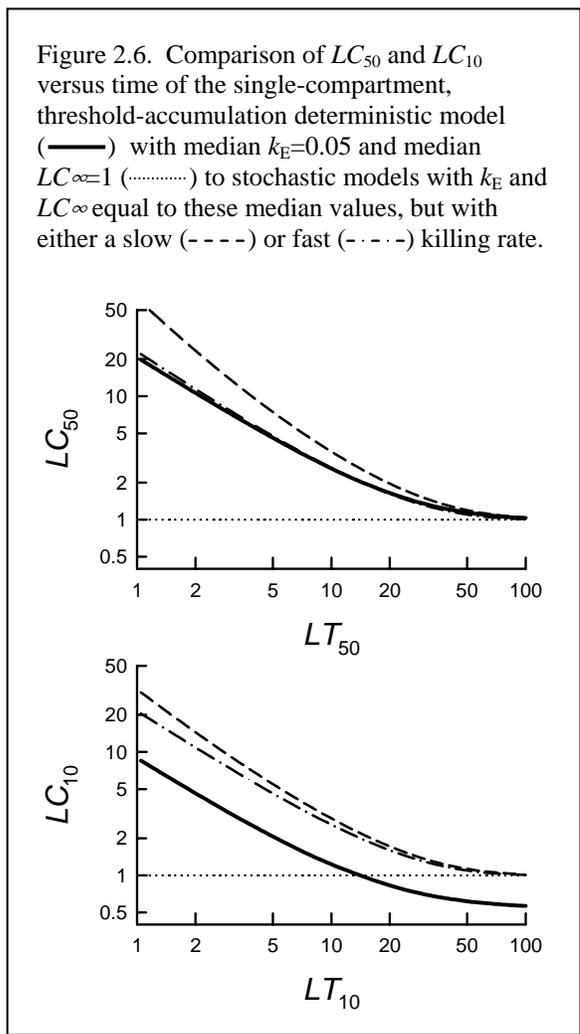
$$h(t) = \max\left(0, d' \cdot \left(C \cdot (1 - e^{-k_E \cdot t}) - C_0\right)\right) \quad (2.27)$$

where  $C_0$  is a threshold water concentration for effects ( $=A_0/BCF_{SS}$ ) and  $d'$  ( $=d/BCF_{SS}$ ) is a killing rate referenced to water concentrations rather than accumulation. For this formulation, the three parameters are  $k_E$ ,  $C_0$ , and  $d'$ , one fewer than the corresponding deterministic model of Equation 2.6. For any arbitrary time-series  $C(t)$ , the hazard rate would be:

$$h(t) = \max\left(0, d' \cdot \left(\bar{C}(t) - C_0\right)\right) \quad (2.28)$$

where  $\bar{C}(t)$  is computed as in Equation 2.6.

Unlike the deterministic model examples shown on Figures 2.2 to 2.5, an individual organism does not have a fixed relationship for lethal concentration versus time using this stochastic model. However, a model comparison similar to those in Figures 2.3 to 2.5 can be made based on  $LC_p$ , the concentration lethal to  $p$  percent of a group of organisms. In Figure 2.6, the bold solid lines are  $LC_{ps}$  for the single-compartment, lethal-accumulation-threshold deterministic model (Equations 2.7), with median  $LC_\infty=1$  and median  $k=0.05$ , and both parameters log-normally distribution with a  $\log_{10}$  standard deviation of 0.2. This line was computed based on Monte Carlo



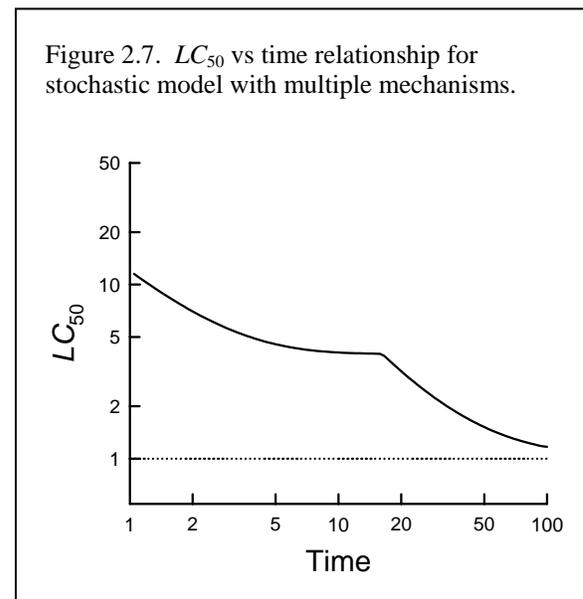
simulation, and for the  $LC_{50}$  is very close, but not identical, to the line on Figures 2.3 to 2.5 for the  $LC$  of an individual organism with  $LC_{\infty}=1$  and  $k=0.05$ . The dashed lines are the  $LC_p$  for the stochastic model of equation 2.28, integrated to predict survival using equation 2.23, with  $C_0=1$ ,  $k_E=0.05$ , and a slow killing rate,  $d'=1$ . The dash-dotted lines in Figure 2.6 represents the stochastic model when  $d'=100$ .

For the  $LC_{50s}$ , the stochastic model with the slow killing rate shows a steeper relationship than the simple deterministic model, similar to the damage/repair model of Equation 2.13 (Figure 2.3) or the two-compartment model of Equation 2.20 (Figure 2.4). This is understandable because, like these other models, this stochastic model includes two kinetic processes – the rate of accumulation and the rate of mortality for a given level accumulation. The similarity of the  $LC_{50s}$  for the deterministic model and the stochastic model with the fast killing rate simply demonstrates that this stochastic model is roughly equivalent to the single-compartment, lethal-accumulation-threshold deterministic model when the (deterministic) toxicokinetics are much slower than the (stochastic) toxicodynamics. Therefore, based on  $LC_{50s}$ , this stochastic model would not be readily distinguishable from deterministic models discussed previously.

However, some notable differences between the deterministic and stochastic models are evident when other  $LC_p$ s are examined. For the stochastic model,  $LC_{10s}$  are lower than the  $LC_{50s}$  at early times, but by a limited extent that depends on the killing rate. At longer exposure durations, the  $LC_{10s}$  approach the same value as the  $LC_{50s}$  because this stochastic model assumes that all the organisms have the same sensitivity, so that their effect concentrations become the same at durations long enough that stochastic differences have diminished. In contrast, for the deterministic model,  $LC_{10s}$  can be lower than the  $LC_{50s}$  both at early and later times, and the extent of this difference is independently determined by the standard deviations of the parameter

distributions. Therefore, the variation of  $LC_{PS}$  at long versus short durations and the variation of  $LC_{PS}$  at short duration relative to apparent killing rates can provide a basis for determining whether a deterministic or stochastic model best describes a data set. Of course, the stochastic model could be expanded to include differences among organisms (e.g.,  $C_0$  could be a distribution that varies among organisms, rather than a constant), but this would reduce the importance of the stochasticity and increase the number of parameters.

As with the deterministic model, this stochastic model also can be extended to address multicompartment kinetics, multiple mechanisms, and damage-repair, and could also include different concepts regarding the relationship of hazard rate to accumulation. A simple modification could be to repeat Equation 2.27 for two different mechanisms – perhaps one with high  $k$ ,  $d'$ , and  $C_0$ , so that organisms die quickly but only at high exposure concentrations, whereas another mechanism could have low  $k$ ,  $d'$ , and  $C_0$ , so that organisms continue to die at low exposures at extended durations. Figure 2.7 illustrates this



possibility, and shows how the stochastic model can produce results similar to the multiple-mechanism deterministic models in Figure 2.4.

## 2.3 Model Parameterization and Selection

### 2.3.1 Model Parameterization

This section will discuss general principles and approaches for parameterizing either the deterministic or stochastic binary endpoint models. This general information applies to all the

case studies that will be examined, and further details will be provided as needed in the specific sections on each study.

For mortality in any toxicity test, the fundamental observation is usually how many organisms subject to an experimental treatment die between one observation time and the next. A specific time-to-death for an individual organism is rarely determined; rather, it is only known that death occurred within the time interval between observations. Similarly, the concentration needed to kill an individual organism at a specific exposure duration is also not measured; rather, it is only known that this lethal concentration is between two treatment concentrations. Such a data set can be perceived as a matrix of treatment concentrations and observation intervals, with each element of the matrix containing the number of organisms dying during a specific interval in a specific treatment. If  $J$  is the number of experiment treatments (concentrations) and  $I$  is the number of observation times, let  $\mathbf{N}$  be an  $I+1$  by  $J$  matrix for which the element  $N_{i,j}$  ( $i \leq I$ ) is the observed number of deaths in the  $j$ th treatment between the  $i$ th-1 and  $i$ th observation time, and  $N_{I+1,j}$  is the observed number of surviving organisms in the  $j$ th treatment at the end of the test. This includes all the mortality observations made during the test, and each such observation is statistically independent.

The toxicity models discussed here are well suited to analysis of such data sets, because they can predict an expected probability of death (or survival)  $P_{i,j}$  for each element of such a matrix. For a constant concentration exposure starting at  $t=0$ , with observation times  $t_i$ , and with treatment concentrations  $C_j$ , the stochastic mortality model of Equation 2.27 provides estimates for  $P_{i,j}$  of:

$$\begin{aligned}
 P_{i,j} &= S_j(t_i) - S_j(t_{i-1}) && \text{for } i \leq I \\
 P_{I+1,j} &= 1 - S_j(t_I) \\
 \text{where :} &
 \end{aligned}
 \tag{2.29}$$

$$S_j(t_i) = e^{-\int_0^{t_i} h(t, C_j) dt} = e^{-\int_0^{t_i} \max\left(0, d' \left( C_j \cdot (1 - e^{-k_E t}) - C_0 \right) \right) dt}$$

For the deterministic mortality model described by Equation 2.7, an individual organism will still be alive for a given  $C_j$  and  $t$  if its  $LC_\infty$  is greater than  $C_j \cdot (1 - e^{-k_E t})$ . Thus, for a group of organisms for which  $f(k)$  and  $f(LC_\infty)$  are the density functions for the statistical distributions of  $k$  and  $LC_\infty$ :

$$\begin{aligned}
 P_{i,j} &= S_j(t_i) - S_j(t_{i-1}) \\
 P_{I+1,j} &= 1 - S_j(t_I) \\
 \text{where :} &
 \end{aligned}
 \tag{2.30}$$

$$S_j(t_i) = \int_{\min k}^{\max k} f(k) \left( \int_{C_j(1-e^{-k t_i})}^{\max LC_\infty} f(LC_\infty) dLC_\infty \right) dk$$

where max and min refer to the maximum and minimum variable values in the distributions of  $k$  and  $LC_\infty$ .

Whichever type of model is used, estimates of the model parameters can be obtained using computerized search algorithms to find the parameter values which maximize the likelihood  $L$  of the observed mortality observations  $\mathbf{N}$  as a function of the parameter set  $\Theta$  (Breiman 1973):

$$\ln(L(\mathbf{N} / \Theta)) = \sum_{j=1}^J \sum_{i=1}^{I+1} N_{i,j} \cdot \ln(1 - P_{i,j})
 \tag{2.31}$$

For the stochastic model of Equation 2.29, the parameter set  $\Theta$  is  $C_0$ ,  $k_E$ , and  $d'$ . For the deterministic model of Equation 2.30, the parameter set  $\Theta$  would be the parameters for  $f(k)$  and

$f(LC_{\infty})$ , which will typically be a mean and standard deviation for each distribution.

For the case studies presented in this series of reports, custom software developed with Intel Visual Fortran (Version 9.1, Intel Corporation) using IMSL library (Version 5.0, Visual Numerics Incorporated) routines were used for this likelihood maximization and for other data analysis. The search algorithm used was the Box Complex method (Box 1965). However, other commercial mathematical and statistical software and other algorithms are also suitable for such analyses. More computational details are provided as needed in the sections for each case study.

For the stochastic model, survivor functions for any time-variable exposure scenario of interest can be predicted using Equations 2.23. For the deterministic model, predictions are best conducted by Monte Carlo analysis. In such an analysis, a large number of organism-level parameter sets (e.g.,  $LC_{\infty}$ ,  $k$ ) would be randomly generated from the distributions [e.g.,  $f(k)$  and  $f(LC_{\infty})$ ] defined by the maximum likelihood parameter estimates. The model would then be applied to the exposure scenario of interest using each of these parameter sets to define if and when each organism was expected to die. These mortality estimates then would be aggregated to estimate the survivor function or other statistics of interest (e.g.,  $LC_{50}$ s at a specified time).

Model uncertainty can be addressed in various ways. Standard errors for the maximum-likelihood parameter values can be obtained from the parameter variance/covariance matrix estimated by inverting the information matrix for the maximum likelihood analysis (Breiman 1973). However, such standard errors generally underestimate actual uncertainties, especially for pooled analyses of multiple data sets, because structural model error and variation of parameters among data sets is not addressed. When data from multiple toxicity tests is available, an improved assessment of parameter uncertainty can be obtained by estimating separate parameters for each test, the variation of parameters among tests including both within- and

between-test sources of error. Another alternative is to conduct a pooled analysis of the data from all the tests, but to include between-test variability as part of the model and estimation procedure. For example, for the deterministic model, it might be assumed that the means for the distributions  $f(k)$  and  $f(LC_{\infty})$  for individual tests are in turn distributed with some overall mean and variance. Case studies later in this series of reports will illustrate how such a broader analysis might be conducted.

When appropriate estimates of model parameter uncertainty are available, the uncertainties for model predictions can be obtained by Monte Carlo analysis in which a large number of sets of parameter values are randomly generated from the parameter uncertainty distributions. For each of these sets of randomly-generated parameter values, the model calculations described previously would be conducted, providing a distribution of model results for any statistic of interest, from which confidence limits can be obtained. For example, for an  $LC_{50}$  at a specified time, if this process produced 999 estimates, the 25th highest value and 25th lowest value would provide approximate 95% confidence limits.

### ***2.3.2 Model Selection***

G.E.P. Box's dictum that "all models are wrong, some models are useful" is a useful perspective in selecting a toxicity model for use in aquatic life criteria. It must be recognized that any model provides just an approximation to the toxicity relationships of interest, and that the selected model need not be perfect to serve some need. Furthermore, the model need not even be the most complete and accurate available; rather, the most appropriate model would be one that provides acceptable performance with the lowest complexity and data requirements. The basic need of aquatic life criteria is for toxicity models which quantify the level of effect as a function of exposure concentration and duration, including exposures which have a certain

degree of time-variability. Any such quantification represents a fundamental improvement in defining the risks associated with criteria compared to current practice, and there will be a need to define specific performance criteria to determine model acceptability. What is the range of exposure time-series that the model will address? What uncertainty measures are desired and what level of uncertainty is adequate enough that more complex models are not needed? What is the minimal amount of data upon which models must be parameterized for a given species, and will this require that certain model parameters be based on default values derived from analyses on other species and/or chemicals? This report will not specify such requirements, but will present approaches and analyses that will assist in their development and application.

In choosing models for consideration, it is useful to examine how mortality varies with exposure concentration and time (e.g., Figures 2.3 to 2.7), in order to determine what properties the model should have. To serve this purpose, the case studies will include calculation of  $LC_{ps}$  and  $LT_{ps}$  (the time to kill a percentage  $p$  of the organisms at a specified concentration). These  $LC_{ps}$  or  $LT_{ps}$  can be used in simple figures and tables to illustrate data trends and to guide model formulation more effectively than the matrix of observed deaths within each time interval and treatment. However,  $LC_{ps}$  at different times are not statistically independent and do not represent the fundamental data upon which parameter estimation and model fit must be assessed. All parameter estimation will be done directly on the basic mortality observation matrix, using  $LC_{ps}$  or  $LT_{ps}$  only for exploratory data analysis or to illustrate model fit.

Once models are selected for consideration and are parameterized with the data, their relative goodness of fit will be based on the computed likelihood ( $L$ ) using the Akaike Information Criterion (AIC):

$$AIC = 2 \cdot n_p - 2 \cdot \ln(L) \quad (2.32)$$

where  $n_p$  is the number of model parameters and a lower AIC indicates a better model. This formula recognizes that higher values for the likelihood statistic indicate better model fit, and result in a lower AIC. However, models with more parameters (degrees of freedom) would be expected to have a better likelihood statistic, so the AIC is increased by the number of parameters to compensate for the effect of  $n_p$ . Therefore, a model with the lowest AIC would be considered superior, regardless of the number of parameters.

However, the AIC is not informative regarding the magnitude of deviations of model predictions from observations or the amount of data variability that is accounted for by the model, such as the  $R^2$  statistic (the fraction of the variance of the dependent variable explained by the regression) commonly used in regression analysis. Statistics such as  $R^2$  also can be used to illustrate how well data or statistics derived from the data are described by a model, although using  $R^2$  to address statistics such as  $LC_{PS}$  at different times must be done with recognition of the lack of statistical independence among observations. Various other measures of the deviation of observed and predicted  $LC_{PS}$  or mortality levels (e.g., mean deviation, mean of the absolute deviations, standard deviation of the difference) can also be used to describe model uncertainty.

However, whatever measures of fit are used, they should not be the sole basis for selecting a toxicity model for use in aquatic life criteria. A simpler, but less accurate, model might be preferred if the level of accuracy still satisfies specified performance goals for exposure regimes of interest. In general, model selection will involve various statistical and nonstatistical factors, and must be conducted in an *ad hoc* fashion.

## 2.5 References

- Ankley GT, Erickson RJ, Phipps GL, Mattson VR, Kosian PA, Sheedy BR, Cox JS. 1995. Effects of light intensity on the phototoxicity of fluoranthene to a benthic macroinvertebrate. *Environ. Sci. Technol.* 29:2828-2833.

- Box MJ. 1965. A new method of constrained optimization and a comparison with other methods. *Computer J.* 8:42-52.
- Breck JE. 1988. Relationships among models for acute toxic effects: Applications to fluctuating concentrations. *Environ. Toxicol. Chem.* 7:775-778.
- Breiman L. 1973. *Statistics: With a View Toward Applications*. Houghton Mifflin, Boston, MA, USA.
- Chew, R.D. and M.A. Hamilton. 1985. Toxicity curve estimation: Fitting a compartment model to median survival times. *Trans. Amer. Fish. Soc.* 114:403-412.
- Connolly, J.P. 1985. Predicting single-species toxicity in natural water systems. *Environ. Toxicol. Chem.* 4:573-582.
- Crane M, Newman MC, Chapman PF, Fenlon J. 2002. *Risk Assessment with Time to Event Models*. Lewis Publishers, Boca Raton, FL, USA.
- Dixon PM, Newman MC. 1991. Analyzing toxicity data using statistical models for time-to-death: An introduction. In: Newman MC, McIntosh AW (eds). *Metal Ecotoxicology: Concepts and Applications*. Lewis Publishers, Boca Raton, FL, USA.
- Gibaldi M, Perrier D. 1982. *Pharmacokinetics*. Marcel Dekker, New York, NY, USA.
- Jarvinen AW, Ankley GT. 1999. *Linkage of effects to tissue residues: Development of a comprehensive database for aquatic organisms exposed to inorganic and organic chemicals*. SETAC Press, Pensacola, FL, USA.
- Kooijman SALM, Bedaux JJM. 1996a. *The Analysis of Aquatic Toxicity Data*. VU University Press, Amsterdam, The Netherlands.
- Kooijman SALM, Bedaux JJM. 1996b. Analysis of toxicity tests on *Daphnia* survival and reproduction. *Water Res.* 7:1711-1723.
- Landrum PF, Steevens JA, Gozziaux DC, McElroy M, Robinson S, Begnoche L, Chernak A, Hickey J. 2004. Time-dependent lethal body residues for the toxicity of pentachlorobenzene to *Hyalella azteca*. *Environ. Toxicol. Chem.* 23:1335-1343.
- Mancini JL. 1983. A method for calculating effects on aquatic organisms of time-varying concentrations. *Water Res.* 17:1355-1361.

- McCarty LS and Mackay D. 1993. Enhancing ecotoxicological modeling and assessment. *Environ. Sci. Tech.* 27:1719-1728.
- Neely WB. 1984. An analysis of aquatic toxicity data: Water solubility and acute LC50 fish data. *Chemosphere* 7:813-819.
- Newman MC. 1995. *Quantitative Methods in Aquatic Ecotoxicology*. Lewis Publishers, Boca Raton, FL, USA.
- Nichols JW, McKim JM, Andersen ME, Gargas ML, Clewell HJ, Erickso RJ. 1990. A physiologically based toxicokinetic model for the uptake and disposition of organic chemicals in fish. *Toxicol. Appl. Pharmacol.* 106:433-447.
- Zitko V. 1979. An equation of lethality curves in tests with aquatic fauna. *Chemosphere* 2:47-51.



## Section 3. Short-Term Copper Lethality To Juvenile Fathead Minnows

### 3.1 Description of Study and Exploratory Data Analysis

Lindberg and Yurk (1982, 1983a, 1983b) conducted a series of tests on the toxicity of copper to juvenile (ca. 30-day-old) fathead minnows that consisted of (a) 31 constant-exposure toxicity tests with durations ranging from 2.5 h to 192 h (including observations of mortality after termination of exposure when exposure duration was  $\leq 24$  h) and (b) 6 pulsed-exposure toxicity tests with pulse durations ranging from 2.5 to 12 h and pulse intervals ranging from 8 to 24 h. Five of the constant-exposure tests showed exposure concentrations which drifted with time or were otherwise uncertain, and will not be used here. The rest of this data set will be used here to demonstrate the use of constant-exposure tests for toxicity model parameterization and selection, to test the validity of model assumptions, and to evaluate model applicability to time-variable exposures.

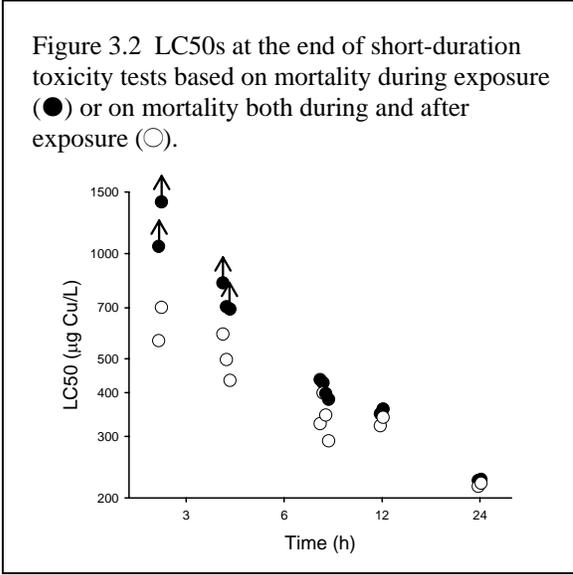
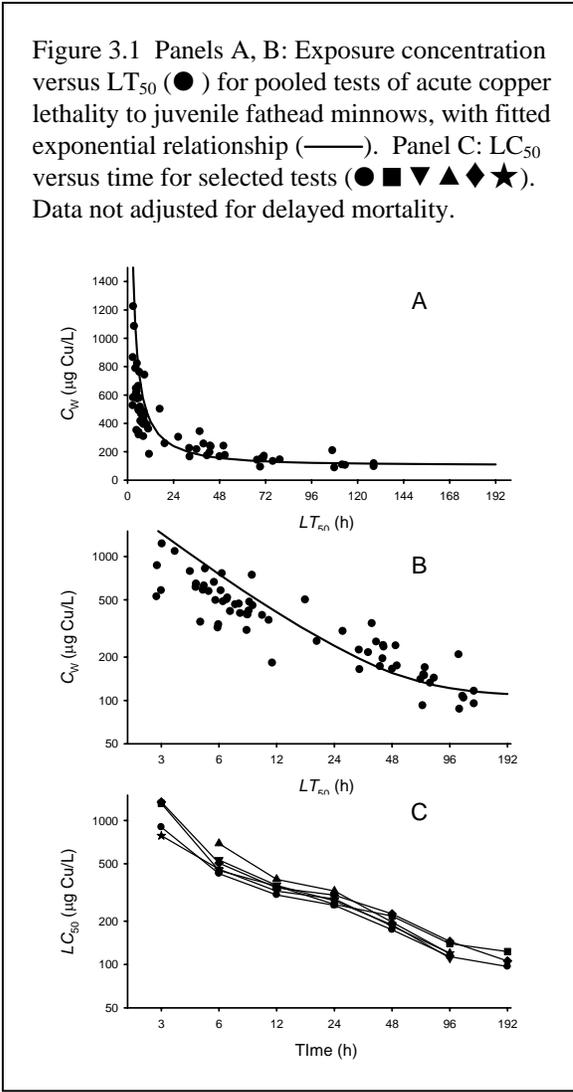
Exploratory data analysis of the constant-exposure tests showed three attributes important to model selection.

(1) For each test,  $LT_{50}$ s were estimated for each concentration at which mortality exceeded 40% by the end of the exposure. All the  $LT_{50}$ s so computed are plotted versus concentration on Figure 3.1A, and demonstrate a strong relationship to water concentration ( $C_w$ ) similar to that depicted in Figure 2-2. The solid line denotes a regression analysis for the relationship described in Equation 2-7 (conducted using Sigmaplot, version 9.01, SPSS, Chicago, IL, the graphics software used to create this figure) to illustrate that these data do approximately follow this relationship.

(2) However, closer inspection of the data shows certain deviations from the simple exponential relationship. This is more clear in the log/log plot of Figure 3.1B of the same  $LT_{50}$ s. For times  $< 24$  h, the  $LT_{50}$ s generally fall below the fitted regression line and, for times between 24 and 96 h, they generally fall above the line. These deviations are even

more clear in Figure 3.1C, which shows  $LC_{50}$ s calculated from just several tests for which  $LC_{50}$ s could be calculated for times from 6 h or less to at least 96 h. In contrast to a smooth single-phase exponential decline, Figure 3.1C suggests two phases to the response curve – a rapid mortality that is starting to level off at about 300  $\mu\text{g/L}$  by 24 h, followed by a second phase in which  $LC_{50}$ s drop again to level off near 100  $\mu\text{g/L}$ . This is the sort of behavior exhibited by the models presented earlier which either have two mechanisms (Figures 2-4, 2-7) or two-compartment kinetics in which toxicity reflects accumulation in an outer compartment (Figure 2-5).

(3) Several of the constant-exposure tests in this study had durations  $\leq 24$  h, with monitoring of mortality after the cessation of exposure. Figure 3.2 displays  $LC_{50}$ s plotted at the exposure duration, but either calculated (a) based just on mortality that had occurred by the cessation of exposure (filled symbols, arrows denoting  $LC_{50}$ s greater than the indicated values) or (b) based also on any mortality that occurred after the exposure (open symbols). Virtually no delayed mortality was present for 24 h exposures and the difference between  $LC_{50}$ s including and excluding delayed mortality was less than a factor of 1.1 for 12 h exposures; however, this difference was about a factor of 1.25 for 8 h exposures, a factor of at least 1.4 for 4 h exposures, and even greater for 2.5 h exposures. The smaller  $LC_{50}$ s when delayed



mortality is included further accentuate the two-phase behavior noted in Figure 3.1. Deterministic models that have both an accumulation and a damage-repair component, or that have effects related to accumulation in an internal compartment, could account for mortality persisting beyond the end of exposure. Such models are also suggested by the log/log slopes being greater than 1.0 at short durations for the solid symbols in Figure 3.2 (although the indeterminate  $LC_{50}$ s make the exact slopes uncertain). Stochastic models in which the hazard rate depends on past exposure (e.g., being a function of accumulation that persists for some time after exposure stops) could also explain the delayed mortality and steeper slopes.

These data therefore suggest a complicated toxicity relationship with more than one of the mechanistic possibilities discussed in Section 2. This therefore provides a good case study for not only discussing how models can be formulated and parameterized, but also for addressing the level of model complexity that can be justified and supported by constant concentration toxicity tests. Equally important, this is a good case study for exploring whether certain model complexities, even if they are supported by calibration data sets, are actually important for making model predictions for the time-variable exposures to which aquatic life criteria are applied.

### ***3.2 Evaluation of Deterministic Models***

#### ***3.2.1 Models Evaluated***

Based on the exploratory data analyses discussed in Section 3.1, three deterministic models were evaluated for this data set.

(1) Model D1 was the one-compartment, lethal-accumulation-threshold model of Equation 2.5-2.7. This is the baseline model that might typically be used in the absence of a demonstrated need for more complex models. The evaluation here will emphasize how much error is introduced by using such a model and not addressing the complexities of the toxicity relationship discussed in Section 3.1. The organism-level parameters for this model are designated as  $LC_{\infty,A}$  and  $k_A$ , to denote a single-mechanism "A" with one kinetic-constant and a threshold lethal accumulation.

(2) Model D2 was the two-mechanism, independent-action model of Equations 2.14-2.15. This model was included because it exhibits biphasic behavior (Figure 2-4) similar to that observed in the data (Figure 3.1). The organism-level parameters for this model are designated  $LC_{\infty,A}$ ,  $LC_{\infty,B}$ ,  $k_A$ , and  $k_B$ , "B" denoting the mechanism for the faster toxicity phase and "A" the slower phase.

(3) Model D2X extended Model D2 by applying the damage/repair model of Equations 2.10 and 2.13 to the fast toxicity phase, to address the delayed mortality associated with this phase (Figure 3.2). The organism-level parameters for this model are designated  $LC_{\infty,A}$ ,  $LC_{\infty,B}$ ,  $k_A$ ,  $k_{B1}$ , and  $k_{B2}$ . The two kinetic constants for the fast phase are differentiated by numbers to emphasize that, although one parameter might reflect chemical accumulation and the other damage/repair, it is uncertain what kinetic processes are associated with each constant. When an analysis indicated that  $k_{B1}$  and  $k_{B2}$  had the same or very similar values, the analysis was redone with the assumption that these values were the same, as described in Equation 2.12 and 2.13.

The multicompartment models of Section 2.1.4 were not part of this evaluation. As noted earlier, these models produce relationships that are not readily distinguishable from those of the multiple-mechanism and damage/repair models. In fact, as also noted earlier, the models used here should not be treated as definitely describing specific toxicity processes, but as general kinetic formulations that provide useful approximations to a variety of possible processes. Additionally, it is not possible to conceptually reconcile the toxicity relationships illustrated in Figures 3.1 and 3.2 with the two-compartment models discussed in Section 2.1.4. Because the first phase of toxicity involves delayed mortality and a steep log/log slope at earlier times, it must reflect toxicity occurring due to accumulation in an inner compartment (Equation 2.20), but the biphasic behavior would then require a third compartment as a sink for the inner compartment or as an additional site of toxicity. This creates complexities which are not needed to address the behaviors in Figures 3.1 and 3.2, and adds to the difficulties regarding parameterization and prediction already noted in Section 2.1.4 for the multicompartment models.

### 3.2.2 Model Parameter Distributions

The default assumption regarding the variation of organism-level parameters among different organisms was that they were log-triangular and independently distributed. Thus, fitting the models to toxicity data required estimating a mean and standard deviation for each organismal-level parameter, which would require four distributional parameters for Model D1, eight for Models D2, and ten for Model D2X (eight if the two kinetic constants for the fast toxicity phase are equal to each other). Two issues were addressed regarding this default assumption:

(1) Because estimation of a large number of parameters might be problematic for many toxicity data sets, some analyses using Models D2 and D2X assumed that the distributions for the organism-level parameters shared the same relative standard deviation, resulting in fewer distributional parameters and allowing an evaluation of whether individual standard deviations were important for model performance.

(2) Consideration was also given to the uncertainty that might be introduced by assuming independence among the organism-level parameters, when, in fact, there might be some correlation among these parameters (i.e., an organism with a higher-than-average  $LC_{\infty}$  might tend to have higher-than-average  $k$ , or vice-versa). Fully evaluating the extent and nature of the various correlations would be very difficult, if not infeasible, but some analyses with Models D1 and D2 assumed a correlation coefficient of either  $-1$  or  $+1$  between  $k$  and  $LC_{\infty}$  to determine whether the default assumption of a correlation coefficient of  $0$  might cause substantial errors.

### 3.2.3 Pooled Data Set Analyses

Analyses were conducted on the pooled data of all 26 constant concentration tests to provide the most complete information for assessing model attributes and overall parameter values. These pooled data include some tests (those with exposures  $\leq 24$  h) in which delayed mortality was monitored. Three versions of this pooled data set were used in the analyses to

variously address this delayed mortality information:

(1) Pooled data set "PE" (delayed mortality "excluded") consisted of all the observations up to the end of the exposure in each toxicity test, and thus did not include information on the observed delayed mortality. This dataset was analyzed using all three models and the various options concerning the parameter distributions, allowing the relative merits of the model formulations to be assessed using the type of data typically available from toxicity tests. Fitting Model D2X to such data still supports prediction of delayed mortality, and allowed evaluation of how well delayed mortality was predicted even when it was not included in the model parameterization.

(2) Pooled data set "PI" (delayed mortality "included") consisted of all the observations in each toxicity test, including those made of mortality after the end of exposure. This dataset was only used to parameterize Model D2X because Models D1 and D2 do not explicitly address such delayed mortality. This allowed comparison of how parameter values and model fit differed for Model D2X when delayed mortality was included or excluded in parameter estimation, and thus how well Model D2X actually describes the processes responsible for mortality both during and after exposure.

(3) Pooled data set "PA" ("adjusted" for delayed mortality) addressed the delayed mortality information so that Models D1 and D2 could be applied. This was accomplished by relating observed mortality to the exposure period needed to elicit the mortality, not to the time at which the actual mortality was observed. For exposures  $\leq 24$  h, for which delayed mortality was assessed, this required combining all the observations for each test and concentration (including the delayed mortality) into a single observation which specified the mortality resulting from that exposure duration and concentration. For exposures  $> 24$  h, observed mortality up to 24 h was combined, so that the first observation was the cumulative mortality at 24 h, thereby eliminating any appreciable effect of delayed mortality on the time course of toxicity; later observations were not modified. This still allows a meaningful expression of risks, because the mortality resulting from a particular exposure is addressed, albeit not the exact time sequence of the mortality. It also allows Models D1 and D2 to be used to address delayed mortality, because these models can be considered to describe the attainment of a lethal condition, with the delay in the observed mortality not being

explicitly modeled. This data set was not used to parameterize Model D2X because adjusting the data for delayed mortality already served the purpose of the second kinetic constant in the first phase of toxicity of this model; however, how well Model D2X predicted these adjusted data was still evaluated.

Table 3.1 summarizes the distributional parameter estimates for each model without (pooled data set PE) and with (pooled data set PI or PA) consideration of delayed mortality. Parameter estimation assumed that the logarithms of the organism-level parameters have a triangular distribution, so the table includes the estimated mean and standard deviation of the base 10 logarithm of the parameters. For example, for model D1 parameterized using pooled data set PE, the  $\log_{10}(LC_{\infty,A})$  is estimated to be distributed with mean 2.042 and standard deviation 0.152. The table gives the standard error for each such distributional parameter estimate, this uncertainty being very small because of the large amount of data in the pooled data sets. The table also lists the antilog of the mean estimates for the log parameters, which provides a median estimate for each organism-level parameter on its original scale. For model D1 parameterized using pooled data set PE these median estimates are 110 ug Cu/L for  $LC_{\infty}$  and 0.0284/h for  $k$ .

This table provides three columns for the AIC, corresponding to the three different pooled data sets used in the analyses. The AIC in bold text indicates the data set to which the likelihood was maximized for each analysis; however, AICs for the other data sets also can be computed, and are provided where useful for discussing model performance below. For example, if Model D2X is parameterized using data set PE, how well does it predict the delayed mortality data in set PI, compared to when it is parameterized using set PI? If the model is sufficient, such cross predictions should be good, and discrepancies can help identify model limitations. However, AICs can only be compared within columns because the different

Table 3.1. Deterministic model parameter estimates based on pooled constant-concentration toxicity tests (26 tests, 2.5-192 hr). Parentheses denote standard error of parameter estimates. Bold AIC values denote AIC for data set used in parameterization.)

Analysis Options	Median Value for Parameter					Mean of Log <sub>10</sub> Parameter					Standard Deviation of Log <sub>10</sub> Parameter					Akaike Information Criterion		
	LC <sub>50,A</sub> μgCu/L	k <sub>A</sub> 1/hr	LC <sub>50,B</sub> μgCu/L	k <sub>B1</sub> 1/hr	k <sub>B2</sub> 1/hr	LC <sub>50,A</sub>	k <sub>A</sub>	LC <sub>50,B</sub>	k <sub>B1</sub>	k <sub>B2</sub>	LC <sub>50,A</sub>	k <sub>A</sub>	LC <sub>50,B</sub>	k <sub>B1</sub>	k <sub>B2</sub>	AIC-PE	AIC-PI	AIC-PA
Model D1 (Separate SD) (Pooled Set PE)	110	0.0284				2.042 (0.007)	-1.546 (0.009)				0.152 (0.003)	0.220 (0.003)				<b>6896</b>		
Model D2 (Shared SD) (Pooled Set PE)	91	0.0136	229	0.088		1.957 (0.011)	-1.865 (0.019)	2.368 (0.007)	-1.056 (0.012)		0.150 (0.002)					<b>6597</b>	11570	
Model D2X (Shared SD) (Pooled Set PE) (Unequal k)	87	0.0131	263	0.224	0.341	1.941 (0.010)	-1.884 (0.012)	2.420 (0.004)	-0.649 (0.082)	-0.467 (0.099)	0.163 (0.002)					<b>6437</b>	9315	4562
Model D1 (Separate SD) (Pooled Set PA)	104	0.0326				2.017 (0.007)	-1.486 (0.012)				0.112 (0.005)	0.372 (0.008)						<b>4552</b>
Model D2 (Shared SD) (Pooled Set PA)	90	0.0137	264	0.187		1.956 (0.011)	-1.862 (0.018)	2.422 (0.005)	-0.726 (0.011)		0.153 (0.002)							<b>4018</b>
Model D2X (Shared SD) (Pooled Set PI) (Equal k)	88	0.0192	174	0.165		1.943 (0.010)	-1.716 (0.016)	2.240 (0.006)	-0.783 (0.006)		0.194 (0.002)					6793	<b>8835</b>	4771

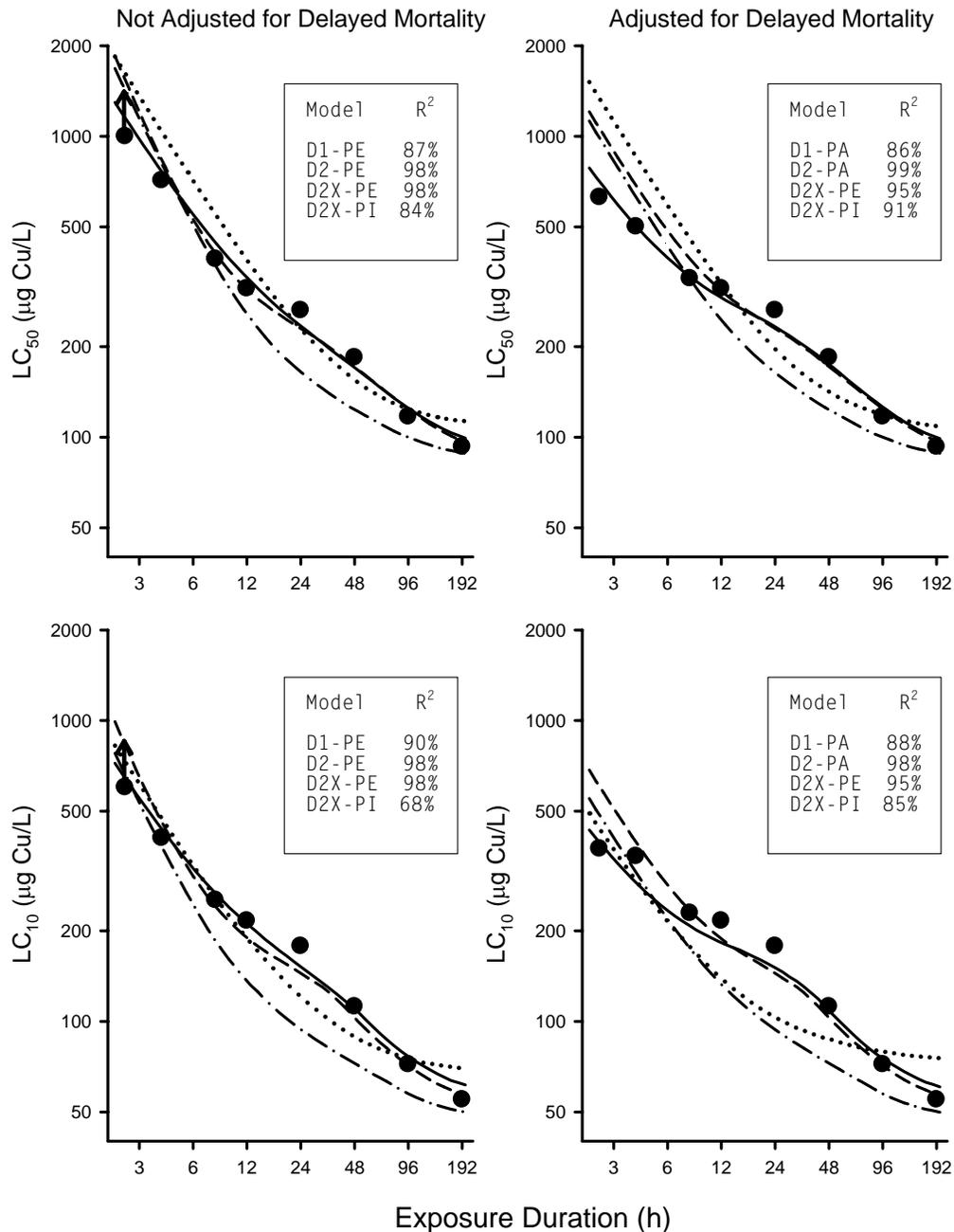
columns have different general magnitudes depending on the amount of data in each data set.

For Models D2 and D2X, the analyses shown in Table 3.1 are those in which the organism-level model parameters share the same standard deviation. Analyses in which separate values for these standard deviations were estimated (*not shown*) provided little or no improvement in the AIC and produced similar values for the standard deviations of the different organism-level parameters. For Model D1, the analyses shown in Table 3.1 are those with separate values for the standard deviation of  $k$  and  $LC_{\infty}$  because this did result in appreciably better fit than the analyses with a shared standard deviation (*not shown*). The different values for the standard deviations of  $k$  and  $LC_{\infty}$  for Model D1 are symptomatic of this model ignoring the biphasic nature of the model, which causes the standard deviation of the  $k$  to be inflated because it is a compromise value for the different kinetic constants of the two phases.

Analyses in which the organism-level model parameters were assumed to be correlated rather than independent are also not shown in Table 3.1 because this resulted in poorer model fits. Such poorer fit provides support for the assumption of parameter independence in the analyses, although it is still possible that some limited correlation exists.

Figure 3.3 illustrates the relative fits of the analyses shown in Table 3.1 by comparing predicted  $LC_{50s}$  and  $LC_{10s}$  to average observed  $LC_{50s}$  and  $LC_{10s}$  at 2.5, 4, 8, 12, 24, 48, 96, and 192 h. The left panels show observed  $LC_{ps}$  for mortality occurring up to the designated exposure duration; the model prediction lines are for Models D1 and D2 parameterized with data set PE, and Model D2X parameterized with both data sets PE and PI. The right panels show observed  $LC_{ps}$  adjusted for the mortality occurring after the end of exposure; the model prediction lines are for Models D1 and D2 parameterized with data set PA, and Model D2X parameterized with both data sets PE and PI. The predicted  $LC_{ps}$  were calculated by Monte-Carlo simulations.

Figure 3.3.  $LC_{50}$  and  $LC_{10}$  versus time for toxicity of copper to juvenile fathead minnows for constant exposures. The left panels address  $LC_p$ s at the end of the specified exposure periods (not adjusted for mortality after the exposure) while the right panels address  $LC_p$ s adjusted for mortality occurring after the specified exposure periods. Circles (●) denote average observed  $LC_p$ s from 26 constant-exposure toxicity tests. For model D1 (.....) and model D2 (—), parameterization used pooled data set PE for the left panels and pooled data set PA for the right panels, because predictions of  $LC_p$  with and without delayed mortality required the different data treatments. For model D2X, parameterization with pooled data set PE (---) and pooled data set PI (- · - · -) are included on both left and right panels because this model can do predictions of  $LC_p$  with and without delayed mortality with either parameterization. The inset boxes provide the relative sum of squared deviations ( $R^2$ ) of the predicted and observed  $LC_p$ s for each model; the  $R^2$  is identified according to the model used for the prediction and the data set used to parameterize the model.



9999 sets of organism-level model parameters were generated by random selection from the maximum likelihood estimates for the distributions of these parameters (Table 3.1). The model was then applied to each parameter set to estimate  $LC$ s for exposure durations from 2 to 200 h (for Model D2X, both including and excluding mortality expected after exposure). The  $LC_P$  for each duration was set to the appropriate percentile within these sets of  $LC$ s. The fits of the model-estimated  $LC_P$ s to the observed  $LC_P$ s were summarized using the  $R^2$  statistic (Figure 3.3), although this not statistically rigorous because the  $LC_P$ s are not statistically independent.

The analyses summarized in Table 3.1 and Figure 3.3 support the following observations regarding the relative merits of the models which were evaluated:

(1) Adding the second mechanism of toxicity results in appreciably improved fit relative to a single mechanism. When parameterized using data set PE, the AIC-PE for Model D2 is 4.5% less than that for Model D1 (Table 3.1). When parameterized using pooled data set PA, there is even a greater improvement of 13% in AIC-PA (Table 3.1), because adjusting for delayed mortality further increases the biphasic nature of the toxicity. This better fit is evident in Figure 3.3, in which Model D2 closely follows the biphasic nature of the average observed  $LC_{50}$ s and  $LC_{10}$ s, whereas the simple exponential decline of Model D1 shows substantial deviations. This is reflected in the  $R^2$  for the deviation of model predictions from the  $LC_{50}$ s and  $LC_{10}$ s. Although Model D1 has respectable  $R^2$ s (86-90%), Model D2 is much better (98-99%).

(2) Adding the second kinetic constant to the fast mechanism of toxicity in Model D2X results in additional improvement in fit relative to Model D2. When parameterized using pooled data set PE, the AIC-PE for Model D2X is 2.5% lower than that for Model D2 (Table 3.1). The importance of this second kinetic constant is most evident in the AICs in Table 3.1 that included the delayed mortality (data set PI). For Model D2 parameterized using data set PE, AIC-PI, which includes the delayed mortality, is 4973 greater than AIC-PE; this increase is the maximum possible because this model predicts no delayed mortality. For Model D2X parameterized using pooled data set PE, this increase is only 2878, indicating it predicts a large fraction of the delayed mortality, in addition to the improved fit during the exposure period. The importance of the second kinetic constant is also suggested in the left panels of Figure 3.3, where Model D2X

shows steeper slopes than Model D2 in the first phase of toxicity, such steeper slopes also being evident in the observed  $LC_{50}$ s and  $LC_{10}$ s. (This benefit of the second kinetic constant is not reflected in better  $R^2$ s for Model D2X than Model D2 because these  $R^2$ s do not consider the "greater than" values at 2.5 h and thus do not adequately account for the steep slopes at short durations)

(3) Including the delayed mortality in the parameterization of Model D2X has mixed effects on model performance. There was a 5% decrease in AIC-PI when Model D2X was parameterized using data set PI compared to the AIC-PI when the model was parameterized using data set PE. Although this decrease is appreciable, it is not much of an improvement considering that the better fit was based on a lot of additional information. Furthermore, improved fit for the delayed mortality was at the expense of a worse fit to the data within the exposure periods (AIC-PE in Table 3.1) and resulted in a loss of the biphasic behavior of the model and much poorer  $R^2$  for predicting  $LC$ p during the exposure period (Figure 3.3). These problems with fit suggest that the mechanisms causing the delayed mortality are not accurately described by the model. This inadequacy of the model is also indicated by  $k_{B1}$  and  $k_{B2}$  being equal when parameterized using data set PI. When these constants are equal, the delayed mortality is at its maximum relative to the mortality within the exposure period, and the fact that the model parameterization was pushed to this limit indicates an inadequacy for describing the relationship of mortality during and after exposure.

(4) Model D2 can effectively describe delayed mortality if the data can be adjusted for these delays as with data set PA. As noted above, Model D2 parameterized to data set PA results in high prediction  $R^2$ s for the average observed  $LC_{50}$ s and  $LC_{10}$ s adjusted for delayed mortality (right panels of Figure 3.3). In contrast, Model D2X parameterized based on pooled data set PE resulted in higher AIC-PA than Model D2 (Table 3.1) and smaller  $R^2$ s for the delay-adjusted  $LC_{50}$ s and  $LC_{10}$ s in Figure 3.3. Although this poorer fit for Model D2X is due to the advantage Model D2 has in being parameterized based on the adjusted data, it still indicates that Model D2X does not completely reflect the processes producing delayed mortality. Nonetheless, Model D2X parameterized with data set PE still produced good  $R^2$  for predicting the  $LC_p$ s in the right panels of Figure 3.3, and thus would still be useful for data sets without explicit information on delayed mortality.

The pooled analyses thus showed importance for addressing both the biphasic nature of the toxic response and the existence of delayed mortality. If appropriate information is available on the delayed

mortality to adjust the data to reflect time-to-lethal-exposure rather than time-to-death, using such adjusted data with Model D2 provides the best performance (provided the risk predictions do not need to explicitly address time-to-death). In the absence of such information, Model D2X provides a means to address much, but not all, of the delayed mortality based on inference from the mortality patterns within the exposure period. However, although these analyses have shown certain differences in model performance for describing constant-concentration tests, it is not possible to state how important this will be for addressing fluctuating exposures. Therefore, all three models will be considered in section 3.2.5 regarding pulsed exposures, using the parameter values from Table 3.1.

### ***3.2.4 Individual Data Set Analyses***

The pooled data addressed above has much more information than typically will be available for model parameterization, and the standard errors of the parameters are consequently misleadingly small because the analyses assume that whatever model is being evaluated is absolutely correct and that the true parameter values are the same for all the toxicity tests. Under such assumptions, this large amount of data results in small standard errors that do not reflect model formulation error or variations among tests, and thus do not provide a basis for reasonably assessing the uncertainty of model estimates. To address the issue of parameter differences among tests, and thus better describe uncertainty, the models were also parameterized based on individual toxicity tests within the pooled data set which were of sufficient duration to include both phases of toxicity (seven 96-hr and five 192-hr tests). Because these tests had no delayed mortality information, this parameterization was analogous to pooled data set PE regarding the type of information available. Based on the findings of the pooled analyses, Model D1 was parameterized with separate standard deviations for the two organism-level model parameters and Models 2 and 3 were parameterized with shared standard deviations.

Table 3.2 provides the averages (across tests) of the maximum likelihood estimates

Table 3.2. Deterministic model parameter estimates based on analysis of individual constant-concentration toxicity tests (seven 96-hr tests, five 192-hr tests). (Parentheses denote standard deviation of individual estimates.)

Analysis Options	Median Value of Parameter					Mean of Log <sub>10</sub> Parameter					Standard Deviation of Log <sub>10</sub> Parameter					Akaike Information Criterion
	LC <sub>∞,A</sub>	k <sub>A1</sub>	LC <sub>∞,B</sub>	k <sub>B1</sub>	k <sub>B2</sub>	LC <sub>∞,A</sub>	k <sub>A1</sub>	LC <sub>∞,B</sub>	k <sub>B1</sub>	k <sub>B2</sub>	LC <sub>∞,A</sub>	k <sub>A1</sub>	LC <sub>∞,B</sub>	k <sub>B1</sub>	k <sub>B2</sub>	
Model D1 (Indiv SD) (96 hr Tests)	104	0.0317				2.016 (0.029) [0.083]	-1.499 (0.055) [0.118]				0.109 (0.021) [0.041]	0.278 [0.031] (0.055)				194-491
Model D1 (Indiv SD) (192 hr Tests)	105	0.0205				2.020 (0.026) [0.089]	-1.688 (0.039) [0.224]				0.135 (0.016) [0.025]	0.175 [0.026] (0.040)				350-446
Model D2 (Shared SD) (96 hr Tests)	61	0.0087	211	0.117		1.784 (0.127) [0.069]	-2.062 (0.171) [0.224]	2.324 (0.021) [0.085]	-0.933 (0.039) [0.079]		0.125 (0.008) [0.017]					176-455
Model D2 (Shared SD) (192 hr Tests)	95	0.0125	279	0.091		1.980 (0.030) [0.107]	-1.903 (0.048) [0.174]	2.446 (0.029) [0.092]	-1.039 (0.056) [0.110]		0.128 (0.007) [0.012]					337-415
Model D2X (Shared SD) (96 hr Tests)	64	0.0095	225	0.200	0.703	1.804 (0.099) [0.060]	-2.026 (0.135) [0.206]	2.350 (0.018) [0.108]	-0.701 (0.083) [0.163]	-0.157 (0.237) [0.291]	0.131 (0.008) [0.020]					173-456
Model D2X (Shared SD) (192 hr Tests)	95	0.0123	303	0.192	0.502	1.981 (0.030) [0.107]	-1.906 (0.055) [0.170]	2.480 (0.022) [0.089]	-0.720 (0.076) [0.112]	-0.304 (0.240) [0.322]	0.131 (0.007) [0.011]					337-407

and standard errors for the  $\log_{10}$  distributional parameters (mean and standard deviation) of each organism-level model parameter, with separate entries for each test duration. This table also provides the standard deviations of these distributional parameters across the individual tests. The magnitude of these standard deviations among tests relative to the average of the standard errors estimated for the individual tests is indicative of whether there are important sources of uncertainty not included in the model analyses (e.g., variability of organism sensitivity across tests). These analyses of individual toxicity tests support the following observations regarding the models:

(1) On average, the AIC was reduced by Model D2 substantially (9%) in comparison to Model D1, reinforcing the importance of describing the biphasic nature of the data and illustrating how this is evident even in the individual data sets. Model D2X provided no further reduction of the AICs (<0.5%), which was likely due to these individual toxicity tests not having sufficient information at shorter durations and high concentrations to discriminate Model D2 and Model D2X, in contrast to the more diverse set of tests used for the pooled analysis. This inadequate information to parameterize Model D2X resulted in large standard errors for  $k_{B2}$ .

(2) In general, the average distributional parameter estimates for the individual toxicity tests were similar to the estimates for pooled data set PE (Table 3.1) and were also similar between the two different test durations in Table 3.2. A notable exception to this similarity is that, for Models D2 and D2X, the  $LC_{\infty,A}$  and  $k_A$  were substantially smaller on average for the individual 96-h tests than for either the pooled data or the individual 192-hr tests. This represents a limitation of addressing two phases of toxicity from such short tests, in which evaluating the second phase of toxicity depends on data from 24 to 96 h, a limited time range which is unlikely to contain enough information to extrapolate well to longer durations. Providing the best fit to data at <96 h can thus result in erroneous extrapolations to longer durations. This indicates the need for integrating analyses of acute toxicity tests with longer exposures, which was satisfied at least to some degree by the 192 h exposures.

(3) As expected, the average standard errors of the distributional parameters from the analyses of individual toxicity tests (Table 3.2) are much greater than the standard errors for the pooled data analyses (Table 3.1). This simply reflects the greater uncertainty of

any estimates from more limited data. More importantly, the average standard errors (in parentheses in Table 3.2) are generally smaller than the standard deviations across individual tests (in brackets in Table 3.2), indicating the presence of between-test variability that isn't addressed in the error estimation portion of model parameterization. An exception to this is again the  $LC_{\infty,A}$  and  $k_A$  estimates for the 96-hr tests, which had high standard errors because of the lack of sufficient information from longer times needed to adequately characterize this phase of the toxicity.

These analyses of individual level tests thus further indicated some importance of addressing the biphasic nature of the data, indicating the advisability of using Model D2 and D2X. However, these analyses also showed potential problems in parameterizing either Model D2 or D2X based just on 96-hr tests and in parameterizing Model D2X (and thus inferring some of the delayed mortality) without sufficient data on deaths at early times at high concentrations. These analyses also demonstrated the potential problems with basing model prediction errors on standard errors estimated as part of model parameterization procedures that do not address between-test variability. The next subsection will further explore how important these performance and uncertainty issues are in the prediction of the effects of pulsed exposures, including consideration of how parameter variability among different toxicity tests can provide uncertainty information for model predictions.

### ***3.2.5 Predictions of Pulsed Exposures***

Although analysis of constant exposure tests provides some basis for evaluating the appropriateness of different model formulations, the ultimate measure of the utility of a model will be how well it predicts effect levels for a range of exposure scenarios with fluctuating concentrations. In this section, such predictions will be examined for some intermittent exposure toxicity tests.

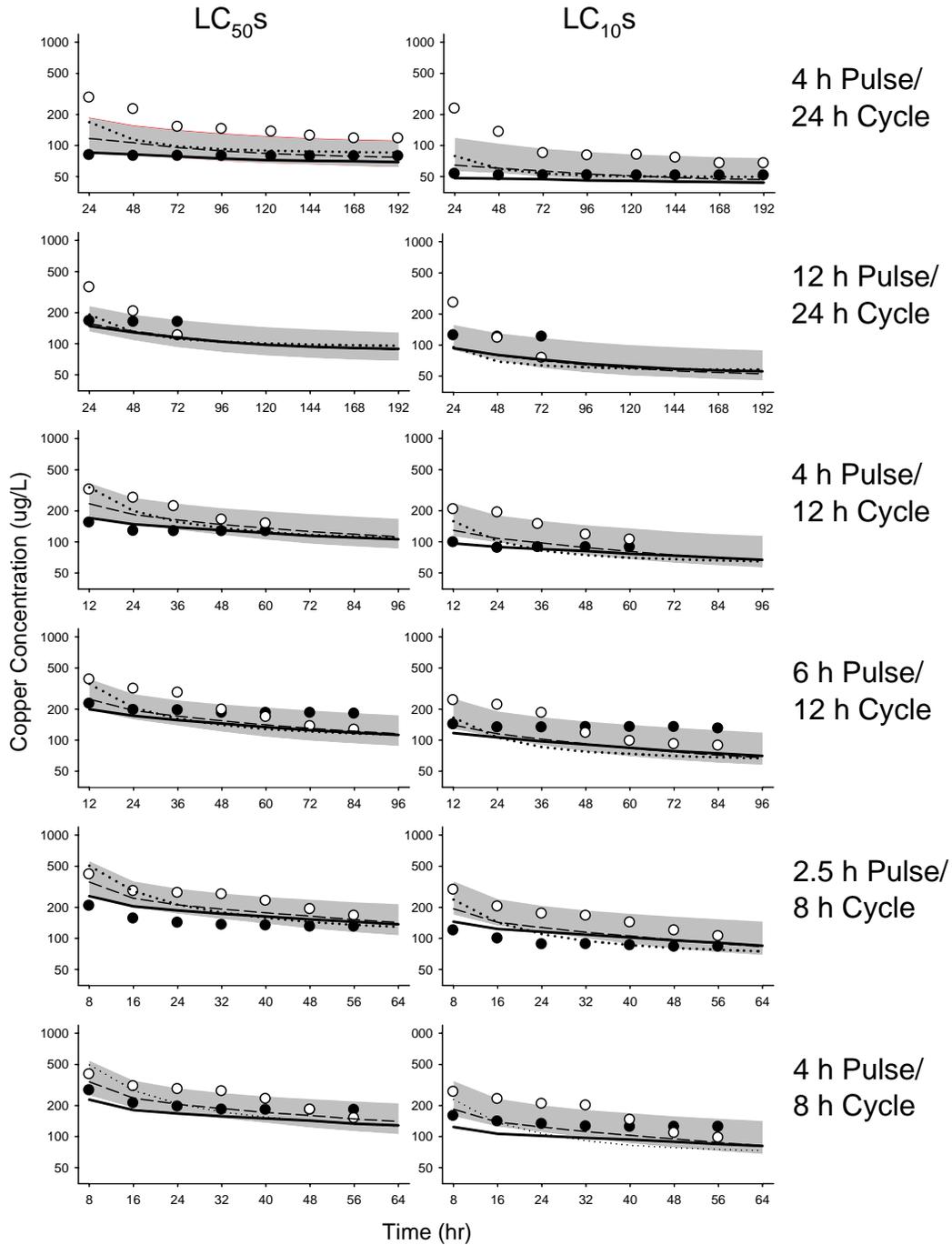
Lindberg and Yurk (1982, 1983a, 1983b) conducted six intermittent exposures consisting of 2.5- to 12-h exposures to copper separated by 5.5- to 20-h exposures to control water (8 to 24

hr total cycle time for copper plus control exposure periods). Experimental procedures resulted in a rapid enough transition between the exposure and control periods that these experiments can be treated as “on-off” or “rectangular” pulses for the purposes of model predictions. Figure 3.4 shows measured  $LC_{50}$ s and  $LC_{10}$ s (filled circles) at the end of the control period following each pulse (thus including any delayed mortality after the pulse), based on the average concentration over the entire cycle. The average concentration is used here rather than the pulse concentration because it provides a more useful comparison among different exposure scenarios, including constant exposures. Figure 3.4 also shows the  $LC_{10}$ s and  $LC_{50}$ s (empty circles) from constant exposure tests run simultaneously with each pulsed-exposure test.

For the pulsed exposures tested, the measured  $LC_p$ s (filled circles) show only small effects of time. Relative to the first pulse,  $LC_{50}$ s changed by less than 5% for the once-daily pulses (24-hr exposure cycle), by about 20% for the twice-daily pulses (12-h exposure cycle), and 35% for the thrice-daily scenarios (8-h exposure cycle). This is in contrast to the much greater time-dependence of the constant exposure  $LC_p$ s (empty circles), where the changes were 60-70%. Such reduced time-dependence of pulsed-exposure  $LC_p$ s versus constant-exposure  $LC_p$ s is expected when constant-exposure  $LC_p$ s decline less than proportionately with time, so that high pulses averaged over the exposure cycle are more damaging than a constant exposure with the same average over that period. The initial fast phase of toxicity and the delayed mortality also contribute to the limited time-dependence of the pulse  $LC_p$ s.

Another feature of the observed data is that the pulsed exposures  $LC_p$ s (filled circles) at the end of the tests are higher than for the companion constant exposure tests (empty circles) when the pulse period is 50% of the pulse cycle, but lower when the period is only 17-33% of the cycle. This effect is rather small, but noteworthy because pulsed exposures generally exert

Figure 3.4. Toxicity of copper to juvenile fathead minnows in pulsed exposures. Solid circles (●) denote observed LC<sub>5</sub>s for each pulse based on average concentration over pulse cycle and mortality at end of pulse cycle. Open circles (○) denote observed LC<sub>50</sub>s at the same times in companion constant exposure tests. Model predictions are provided for models D1 (.....) and D2X (- - -) parameterized with pooled data set PE and model D2 (—) parameterized with pooled data set PA. Gray band denotes 10-90th percentile range of predictions of Model D2X parameterized using individual 192-hour toxicity tests.



greater effects than constant exposures when compared on the basis of average concentrations, regardless of the nature of the pulses. This effect is also noteworthy in that it is not predicted by the models considered here, so it might reflect unidentified processes in the pulsed exposure that ameliorate effects, such as physiological recovery/adaptation during the control period exposure between pulses. Such processes would also contribute to the lack of time-dependence in the observed pulsed  $LC_{pS}$ .

Figure 3.4 also includes model predictions of the pulsed-exposure  $LC_{50S}$  and  $LC_{10S}$ . The dashed and dashed-dotted lines represent predictions based on Models D1 and D2X, respectively, parameterized with pooled data set PE. These predictions for Models D1 thus do not reflect delayed mortality and for Model D2X only reflect expected delayed mortality inferred from the mortality patterns within the exposure periods of the constant concentration tests. The bold solid line denotes prediction based on Model D2 parameterized with pooled data set PA, and thus includes expectations based on more complete information regarding delayed mortality. For each model, 999 sets of organism-level parameters were randomly generated from the distribution parameters in Table 3.1 and each such set was used to generate an expected organism  $LC$  (including mortality delays) for each pulse of the intermittent exposures sequences shown in Figure 3.4. The predicted pulse  $LC_{10S}$  and  $LC_{50S}$  were set to the 10th and 50th percentiles of the resultant  $LC$  sets.

The shaded areas on Figure 3.4 denote the 10th and 90th percentile predictions based on parameters estimated from the individual 192-h toxicity tests. The average and the standard deviation of the distributional parameters across the 192-h toxicity tests for Model D2X in Table 3.2 were used to randomly generate 999 sets of distributional parameters for this model, which in turn were each used to generate 999 sets of organism-level parameters. Each of these 999 sets of

organism-level parameters was used to predict the pulse  $LC_{10s}$  and  $LC_{50s}$ , and the 10th and 90th percentiles of these  $LC_p$ s bound the shaded areas on Figure 3.4. The width of the shaded area thus represents the general magnitude of the uncertainty that would arise in  $LC_p$ s due to variation among tests.

The models predict more time-dependence of the pulsed  $LC_p$ s than actually observed (Figure 3.4, Table 3.3). The degree of the time dependence in these predictions decreases from Model D1 to Model D2 to Model D2X when parameterized using pooled data set PE, reflective of the importance of the initial fast toxicity phase and of delayed mortality for the low  $LC_p$ s in the initial pulses. The predictions of Model D2 parameterized using data set PA, for which the delayed mortality is fully reflected in the initial fast toxicity phase, show even less time-dependence, the predicted ratio of the first to the last pulse only being 10-20% higher than observed (Table 3.3). This predicted time dependence indicates the models (as parameterized) do not fully describe all the processes important for this pulsed toxicity. Nevertheless, the predictions are still within a factor of 2 of observations and, if the initial fast toxicity phase is addressed, the limited time-dependence of the observed pulsed  $LC_p$ s is predicted well enough for these models to have considerable utility.

Table 3.3. Observed and predicted ratios of  $LC_{50}$  of first pulse to  $LC_{50}$  of last pulse .

Pulse Duration/ Recovery (h)	Ratio of $LC_{50}$ of first pulse to last pulse				
	Observed	Model D1-PE	Model D2-PE	Model D2X-PE	Model D2-PA
4/20	1.03	2.0	1.6	1.5	1.2
12/12	1.02	1.7	1.5	1.4	1.2
4/8	1.2	2.7	2.0	1.7	1.4
6/6	1.3	3.0	2.3	2.0	1.6
2.5/5.5	1.5	3.7	2.5	2.3	1.7
4/4	1.6	3.7	2.6	2.3	1.7

### 3.3 Evaluation of Stochastic Models

#### 3.3.1 Models Evaluated

Based on the exploratory data analyses discussed in Section 3.1, two stochastic models were evaluated:

(1) Model S1 was the single-compartment and single-mechanism model of Equation 2.27-2.29. As for deterministic Model D1, this is a baseline model that might typically be used in the absence of a demonstrated need for more complex models. The parameters for this model are  $C_{0A}$ ,  $d_A$ , and  $k_A$ , where the subscript A designates the mechanism.

(2) Model S2 included two independent mechanisms to address the biphasic nature of the data discussed in Section 3.1. For the stochastic model being used here, multiple, independent mechanisms simply involve summing the hazard rate expressions (Equation 2.28) for the separate mechanisms. For the second mechanism, the model parameters are designated as  $C_{0B}$ ,  $d_B$ , and  $k_B$ .

#### 3.3.2 Pooled Data Set Analyses

Parameters for Models S1 and S2 were estimated using both data set PE (excluding delayed mortality observations) and pooled data set PI (including delayed mortality) as described in Section 3.2.3. Table 3.4 provides the maximum likelihood estimates and standard errors for the log-transformed parameters, the untransformed parameter values, and the AIC scores for each analysis. Figure 3.5 shows the model predictions for  $LC_{50}$ s and  $LC_{10}$ s under constant exposure, both unadjusted and adjusted for delayed mortality, analogous to Figure 3.3.

The analyses summarized in Table 3.4 and Figure 3.5 support the following observations regarding the relative merits of the models which were evaluated:

(1) As for the deterministic model, adding the second mechanism of toxicity results in appreciably improved fit relative to a single mechanism because of the biphasic nature of the data. Including the second mechanism for parameterization using pooled data set PE resulted in a 6% reduction of AIC-PE and, for parameterization using pooled data set PI,

Table 3.4. Stochastic model parameter estimates based on pooled constant-concentration toxicity tests (26 tests, 2.5-192 hr). (Parentheses denote standard error of log parameter estimates.)

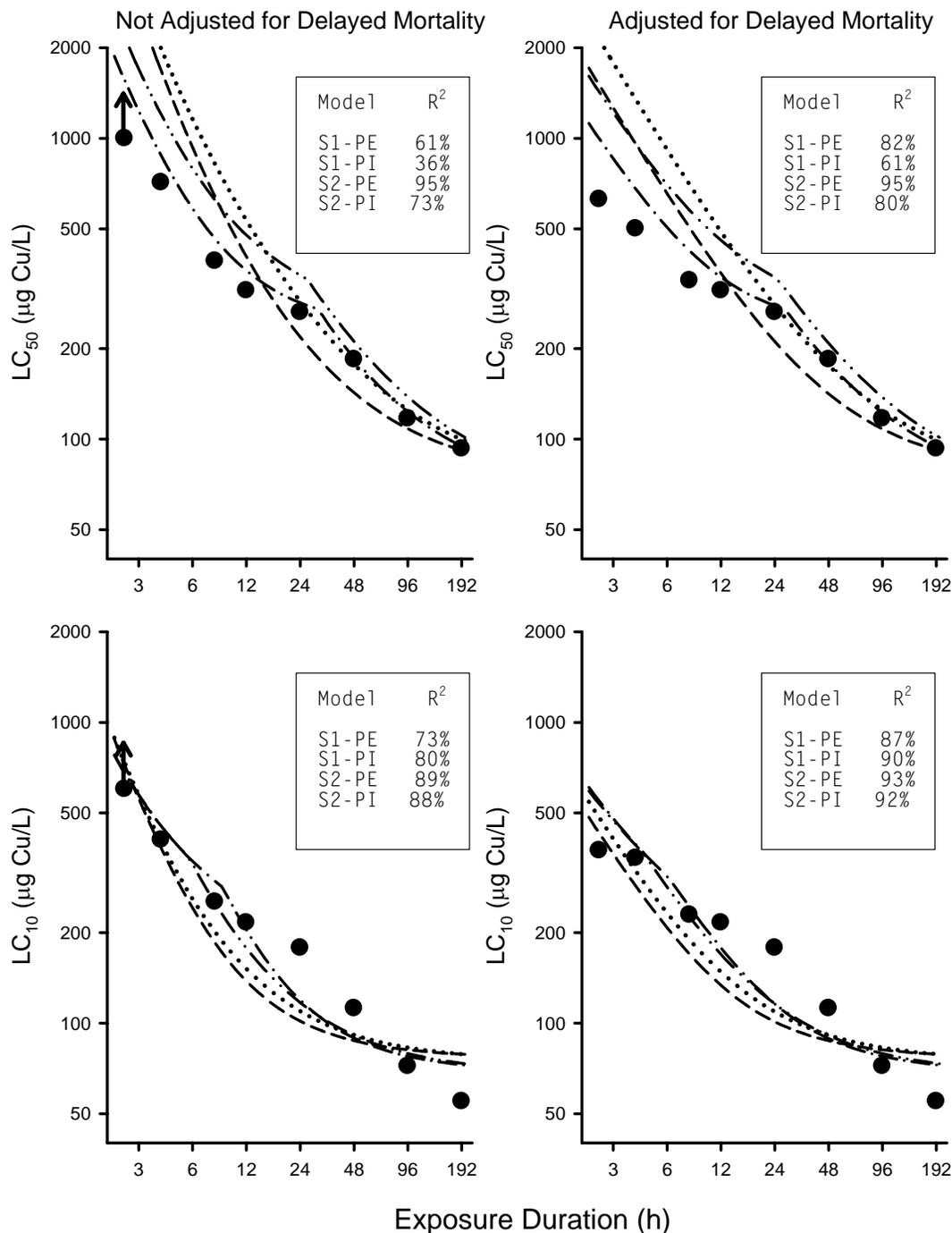
Analysis Options	Parameter Values						Log Parameter Values						AIC	
	C <sub>0A</sub>	d <sub>A</sub> × 10 <sup>3</sup>	k <sub>A</sub>	C <sub>0B</sub>	d <sub>B</sub> × 10 <sup>3</sup>	k <sub>B</sub>	C <sub>0A</sub>	d <sub>A</sub>	k <sub>A</sub>	C <sub>0B</sub>	d <sub>B</sub>	k <sub>B</sub>	PE	PI
Model S1 (Data Set PE)	76	0.244	0.356				1.883 (0.002)	-3.613 (0.011)	-0.448 (0.019)				<b>7085</b>	9709
Model S1 (Data Set PI)	75	0.150	0.597				1.876 (0.003)	-3.824 (0.008)	-0.224 (0.029)				7252	<b>9418</b>
Model S2 (Data Set PE)	69	0.148	0.184	269	0.743	0.393	1.836 (0.003)	-3.831 (0.016)	-0.734 (0.024)	2.430 (0.009)	-3.129 (0.022)	-0.406 (0.018)	<b>6687</b>	10360
Model S2 (Data Set PI)	68	0.107	0.489	340	0.222	0.719	1.834 (0.004)	-3.969 (0.013)	-0.311 (0.040)	2.532 (0.012)	-3.653 (0.031)	-0.143 (0.051)	7079	<b>9291</b>

resulted in a 2% reduction in AIC-PI (Table 3.4). For the  $LC_p$ s in Figure 3.5, the  $R^2$  for Model S2 is always higher than for Model S1.

(2) As for the deterministic model, including the delayed mortality in model parameterization had mixed effects, again indicating that the nature of the relationship of the delayed mortality to the mortality during the exposure period was somewhat different than assumed by the models. When the model was parameterized using the delayed mortality (data set PI), the AIC-PI was, as expected, lower than when the models were parameterized using data set PE (Table 3.4); however, this was at the expense of a poorer fit during the exposure period (AIC-PE). This is reflected in Figure 3.5, in which  $R^2$ s are worse for the models parameterized based on data set PI.

(3) In general, the stochastic models showed poorer fit to the data than the deterministic models. Whether parameterized to data set PE or PI, the stochastic models had higher AICs than the comparable deterministic model analyses (Table 3.4 versus Table 3.1). Similarly, the  $R^2$  for the predicted  $LC_{50}$ s and  $LC_{10}$ s also were slightly to substantially poorer for the stochastic models (Figure 3.5) than for the deterministic models (Figure 3.3).

Figure 3.5.  $LC_{50}$  and  $LC_{10}$  versus time for toxicity of copper to juvenile fathead minnows for constant exposures. The left panels address  $LC_{p,s}$  at the end of the specified exposure periods (not adjusted for mortality after the exposure) while the right panels address  $LC_{p,s}$  adjusted for mortality occurring after the specified exposure periods. Circles (●) denote average observed  $LC_{p,s}$  from 26 constant-exposure toxicity tests. Model predictions are for model S1 parameterized with pooled data set PE (----) and pooled data set PI (.....) and model S2 parameterized with pooled data set PE (-.-.-) and pooled data set PI (-.-.-). The inset boxes provide the relative sum of squared deviations ( $R^2$ ) of the predicted and observed  $LC_{p,s}$  for each model; the  $R^2$  is identified according to the model used for the prediction and the data set used to parameterize the model.



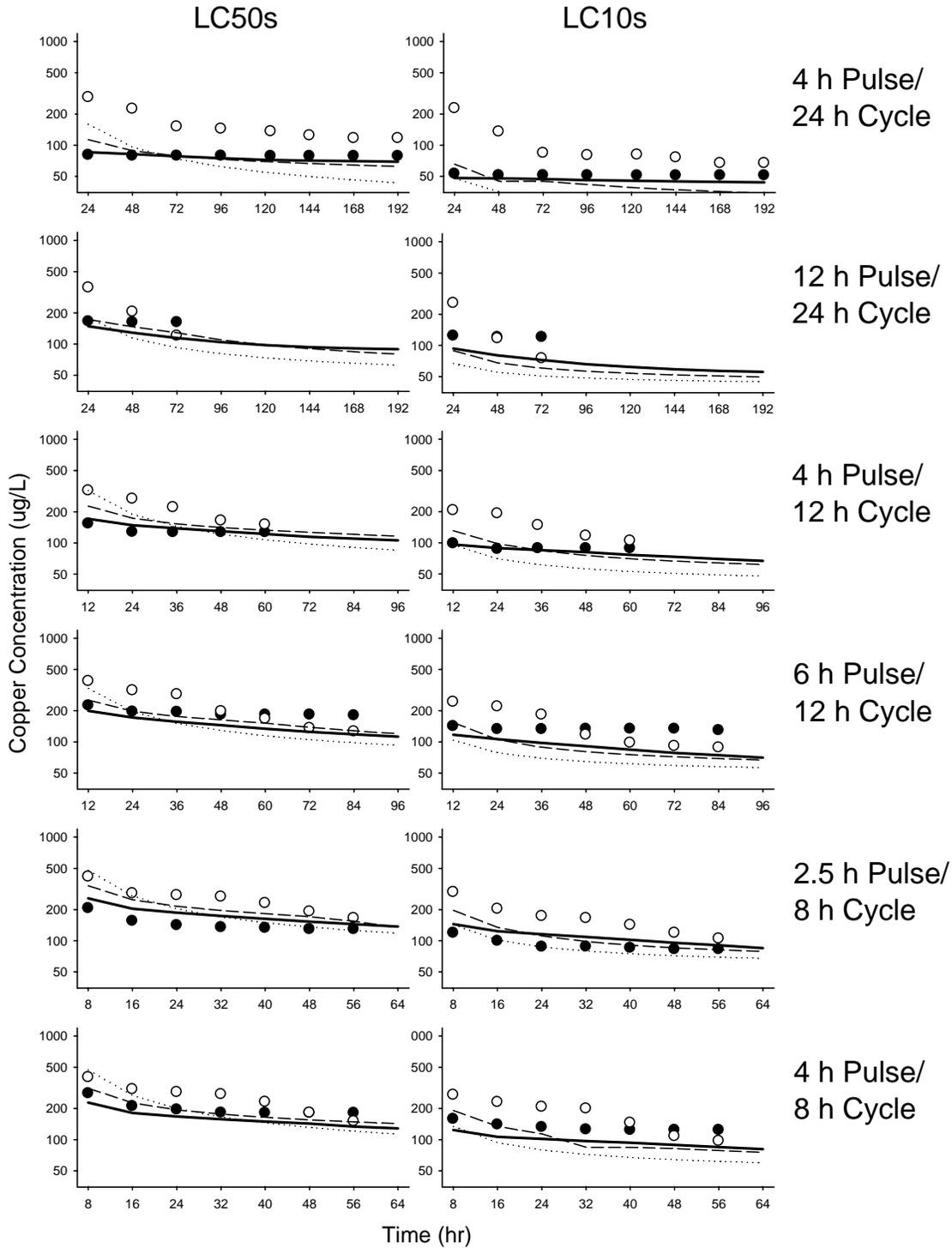
(4) One aspect of the poorer fit of stochastic models is that they overestimate the difference between the  $LC_{50}$ s and  $LC_{10}$ s (Figure 3.5) at short durations and underestimate the difference for the longer durations, in contrast to the deterministic models (Figure 3.3). This is again due to the difference between  $LC_p$ s being tightly linked to the effect of time (killing rate) for the stochastic model, whereas for the deterministic model the difference between  $LC_p$ s is independent of the time effect. If the time course of toxicity indicates the need for a certain killing rate, this can cause such inappropriate estimates of the difference between  $LC_{50}$ s and  $LC_{10}$ s. In addition, Model S2 also did not show the strong observed biphasic behavior for  $LC_{10}$ s (Figure 3.5), whereas the Models D2 and D2X did (Figure 3.3).

### 3.3.3 Predictions of Pulsed Exposures

Figure 3.6 provides predictions for the pulsed exposures using Models S1 and S2 parameterized using data set PE, contrasted with the predictions for Model D2 parameterized with data set PA. Like the deterministic models parameterized without information on delayed mortality, these stochastic models predict more time dependence of the pulse  $LC_{50}$ s and  $LC_{10}$ s than was observed, but stochastic model S2 still provides reasonable predictions. For some exposure durations, the stochastic models also tend to give poorer predictions of the difference between the  $LC_{50}$ s and  $LC_{10}$ s, as was true for the constant concentration exposures (Figure 3.5).

By assuming that all organisms have identical sensitivities, the stochastic models also require that, if a certain percentage of organisms are killed in the first pulse, at least this percentage of the survivors will be killed in the next pulse, and so on until all the organisms have died after a sufficient number of pulses. The data in these experiments contradict this, with various exposures showing partial kills in the first pulse with little or no subsequent mortality in subsequent pulses. This aspect of the stochastic models also would predict a convergence of the  $LC_{50}$ s and  $LC_{10}$ s at later pulses, which, while not always evident in the time span of these tests, is also contradicted by the observed  $LC_p$ s. This indicates the importance of having some

Figure 3.6. Toxicity of copper to juvenile fathead minnows in pulsed exposures. Solid circles (●) denote observed LC<sub>10</sub>s for each pulse based on average concentration over pulse cycle and mortality at end of pulse cycle. Open circles (○) denote observed LC<sub>50</sub>s at the same times in companion constant exposure tests. Model predictions are provided for models S1 (.....) and S2 (----) parameterized with pooled data set PE, compared to model D2 (—) parameterized with pooled data set PA.



differences in sensitivity among the organisms to explain both the time-dependence of the pulse  $LC_{pS}$  and differences between the  $LC_{50S}$  and  $LC_{10S}$ , which is just not addressed by the stochastic models as formulated. The stochastic models could be modified to include differences among the organisms; however, this would increase model complexity and make the stochastic aspect of these models less important, if not superfluous.

### ***3.4 Summary and Model Application to Aquatic Life Criteria***

The above analyses of the data of Lindburg and Yurk (1982, 1983a, 1983b) demonstrated that the toxicity models discussed in Section 2 can be effectively parameterized and, in some cases, used to make useful predictions for both constant and pulsed exposures. For this data set, the stochastic models (Models S1 and S2) and the single-mechanism deterministic model (Model D1) do not perform well, lacking the ability to describe certain features of the observed mortality. In particular, Models S1 and D1 do not address the biphasic nature of the data and the stochastic models do not address sensitivity differences among organisms that are evident in the data. The deterministic models which address the biphasic nature of the data and delayed mortality, and which include differential sensitivity among the organisms (Model D2X and Model D2 parameterized with data adjusted for delayed mortality), provide better performance that approximates observed mortality reasonably well.

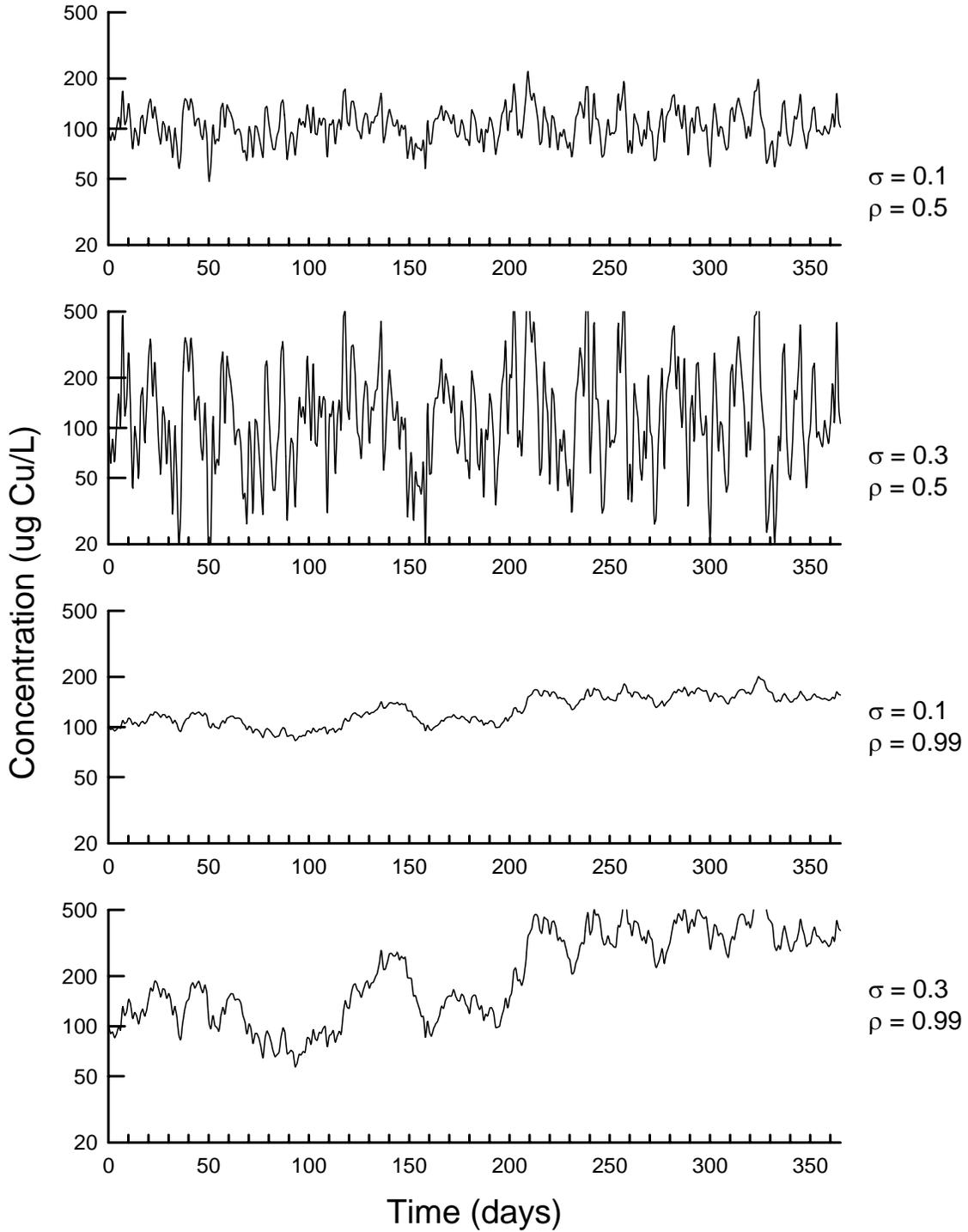
However, the applicability of any of these models to aquatic life criteria is still not resolved without comparing their predictions to the types of exposure time-series that aquatic life criteria must handle, and the poorer performing models identified above still might have acceptable utility. Pulsed-exposures such as those examined above represent an extreme in time-variability, which would tend to maximize prediction errors for the models used here and magnify differences between the models. In particular, the low  $LC_{pS}$  of the initial pulses and the

limited time-dependence of  $LC_{ps}$  of later pulses are in large part due to the abrupt, high exposure of previously unexposed organisms to the first pulse, which increases the importance of the initial fast toxicity phase and the delayed mortality associated with this phase. This would be relevant to spill situations or short, intermittent exposures preceded by low concentrations, but might not be important to more typical exposure time-series of concern to aquatic life criteria.

The sensitivity of predictions to model formulations will be further examined here using some hypothetical exposure time-series more germane to aquatic life criteria. Ten-year exposure time-series were generated by randomly selecting base 10 logarithms of mid-day concentrations from normal distributions with different standard deviations ( $\sigma=0.1$  or  $0.3$ ) and auto-correlation coefficients ( $\rho=0.5$  or  $0.99$ ). Concentrations between these mid-day concentrations were assigned by linear interpolation. For a median concentration of  $100 \mu\text{g Cu/L}$ , Figure 3.7 shows the first year of these time-series, illustrating how they differ both in variability and smoothness. It should be noted, however, that these exposure time-series are still relatively simple and do not incorporate seasonality and intermittency that might be important for aquatic life criteria. Other types of time-series will need to be examined as new criteria procedures are developed, before conclusions about these models are finalized. The examples given here are intended just to demonstrate how these models can be applied to exposure scenarios of concern and how the merits of different model formulations can be assessed.

These time-series cannot be analyzed like the constant and pulsed exposure toxicity tests discussed previously, which have a definite beginning and end and use a single set of previously-unexposed organisms. For the deterministic models, once organisms of a given sensitivity die, the impacts of the rest of the exposure time-series on organisms with that sensitivity are not assessed. By random chance, the deaths for this sensitivity level can be early or late in the

Figure 3.7. Portions of time-series used for comparing risk levels predicted by different toxicity models under fluctuating log-normal concentration scenarios. These panels show the time-series with log mean = 2, log standard deviation = 0.1 or 0.3, and daily autocorrelation coefficient = 0.50 or 0.99.

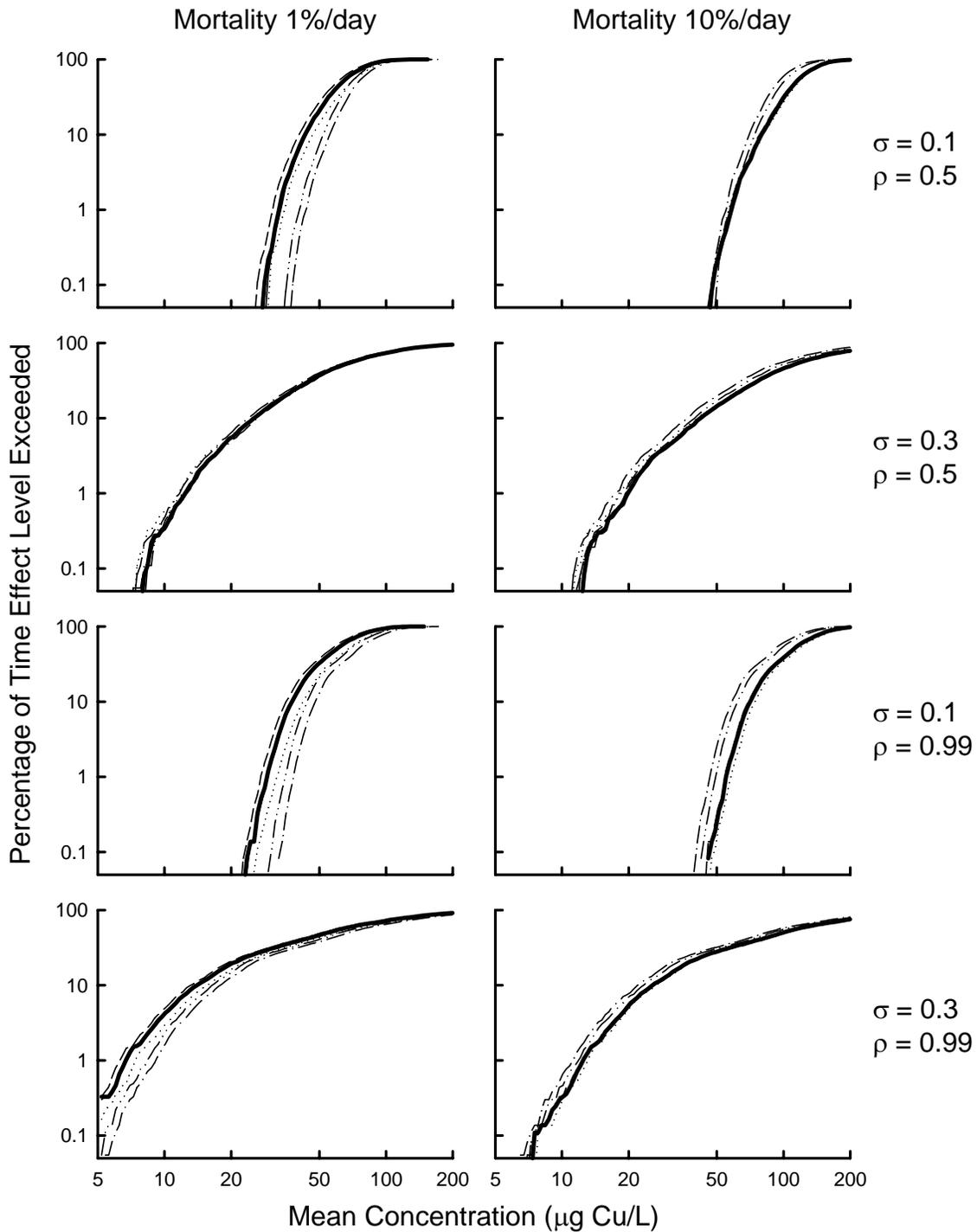


time-series, which does not provide a meaningful measure of risk; rather, risks can be meaningfully quantified only by testing multiple sets of organisms with different time-series or different starting points. For the stochastic models this is not as serious of an issue, because the assumption that all organisms having the same sensitivity allows risks to be computed for the surviving organisms; however, these risks will vary depending on the starting point of exposure, so there is still a need for different sets of organisms with different time-series or exposure starting points within a series. The use of multiple sets of organisms has the added advantage of allowing some consideration of the exchange of organisms in natural populations between areas of low and high exposure.

The analyses conducted for the time-series of Figure 3.7 therefore involved a new cohort of organisms being introduced each day and evaluating the combined effect on all such cohorts. To avoid an indefinite buildup of the number of organisms and needing to computationally track so many organisms, the cohorts were gradually removed over a period of 50 d, emulating migration out of the exposure area. The effect measure used was the percentage mortality each day of the combined organisms for all cohorts at the start of the day. This mortality rate was evaluated for each of the four time-series at 100 median concentrations ranging from 5 to 200  $\mu\text{g}$  Cu/L. Six models were used in this analysis, including Models D1, D2, D2X, S1, and S2 parameterized with data set PE, and Model D2 parameterized using data set PA (which provided the best predictions for the pulsed exposures and is used here as a reference).

The risks of specific mortality rates were computed as the percentage of days the rate was exceeded over the ten-year time-series. Figure 3.8 shows the risks for 1% and 10% mortality per day as a function of mean exposures concentration for each time-series and model. This figure supports the following observations:

Figure 3.8. Risk curves for 1% and 10% mortality per day versus average concentration for four exposure scenarios of log normal concentration distributions with different standard deviations ( $\sigma$ ) and daily autocorrelation coefficient ( $\rho$ ). Curves are given for model D2 parameterized using pooled data set PA (—), for models D1 (.....), D2 (—), D2X (- - - -), S1 (- · - · -), and S2 (- · - -) parameterized using pooled data set PE. The bold and narrow solid lines for model D2 are generally indistinguishable.



(1) These models can be used for providing useful information regarding a range of effect levels for a variety of exposure scenarios, allowing the risks of aquatic life criteria to be more meaningfully defined. This includes relating effect levels to more easily monitored and controlled measures of exposure such as mean concentrations, rather than extreme values.

(2) Figure 3.8 illustrates how mean concentrations must be lower for more variable exposures, which is expected because it is necessary to limit the high end of the concentration distribution responsible for most of the toxicity. In contrast, the different daily autocorrelations examined have little effect on risk, because the effects for this endpoint and toxicant do not require high exposures for prolonged times.

(3) For the deterministic models, there is very little effect of model formulation and parameterization. The risk curves for the two different parameterizations of Model D2 are not distinguishable on Figure 3.8, and differ only slightly from Model D2X. Model D1 risk curves are also very similar to those for Model D2, predicting at most 20% higher median concentrations for the same probability of an effect, despite using only a single toxicity mechanism to describe the biphasic data. The lack of sensitivity to model formulation is attributable to mortality for these exposure time-series being largely due to the slower toxicity mechanism, which is nearly identical for Models D2 and D2X and which is also reasonably approximated by the single mechanism of Model D1 (Table 3.1). In contrast, the pulsed exposures examined earlier were highly affected by the faster toxicity mechanism.

(4) The stochastic models produce risk curves somewhat different than the deterministic models, tending to overestimate the risk of the larger effect levels and underestimate the risk of the smaller effect levels relative to Model D2. This again reflects the lack of independence in these models between the effect of time and the number of organisms affected, which contributed to the poorer performance of these models for the pulsed and constant exposures examined earlier. However, the deviations from the deterministic models are never large, being at most 50% and usually much smaller. Thus, the deficiencies of these models do not keep them from having considerable utility for these types of exposure time-series.

As noted earlier, the risk curves given here are just one example of how these models can be applied to aquatic life criteria. Various other possibilities exist for toxicity endpoints, exposure measures, and structuring the population of organisms being evaluated. More simple model applications are also possible. For the stochastic models, risk curves similar to Figure 3.8 would result from applying the models to a single cohort of organisms. For the deterministic models, the lethal condition variable  $F(t)$  could be tracked without removing organisms when  $F(t)$  exceeds one to simply provide a "meter" for the severity of the exposure time-series. These models could also be used merely to select better averaging periods under the current aquatic life criteria framework, without explicitly addressing the magnitude and time-variability of effects. Any final analysis needs to be tailored to the total framework being used for criteria (e.g., are these results to be fed into a population model?) and a more complete description of the exposure time-series to be addressed. Nonetheless, the analyses presented here do demonstrate the feasibility of analyzing toxicity data with relatively simple models which then can provide information on risks useful for aquatic life criteria.

### ***3.5 References***

Lindberg C, Yurk J. 1982, 1983a, 1983b. Progress in studies to determine effects of intermittent dosing of copper on fathead minnows. ***In:*** Second-, third-, and fourth-quarter reports to U.S. EPA, Cooperative Agreement CR809234020, *Aquatic Pollutant Hazard Assessments and Development of a Hazard Prediction Technology by Quantitative Structure-Activity Relationships*. Center for Lake Superior Environmental Studies, University of Wisconsin, Superior, WI.