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SHORTCOMINGS OF THE LABORATORY-DERIVED MEDIAN LETHAL CONCENTRATION FOR PREDICTING MORTALITY IN FIELD POPULATIONS: EXPOSURE DURATION AND LATENT MORTALITY

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Abstract—Exposure duration and intensity (concentration or dose) determine lethal effects of toxicants. However, environmental regulators have focused on exposure intensity and have considered duration only peripherally. Conventional testing for toxicology tends to fix exposure time and to use the median lethal concentration (LC50) at that time to quantify mortality. Fixing the exposure duration and selecting the 50% mortality level for reasons of statistical and logistical convenience result in the loss of ecologically relevant information generated at all other times and ignore latent mortality that manifests after the exposure ends. In the present study, we used survival analysis, which is widely employed in other fields, to include both time and concentration as covariates and to quantify latent mortality. This was done with two contrasting toxicants, copper sulfate ($CuSO_4$) and sodium pentachlorophenol (NaPCP). Amphipods (*Hyalella azteca*) were exposed to different toxicant concentrations, and the percentage mortalities were noted both during and after the exposure ended. For $CuSO_4$ at the conventional 48-h LC50 concentrations, the predicted proportions dead after including latent mortality were 65 to 85%, not 50%. In contrast, only 5% or fewer additional animals died if the latent mortality was included for NaPCP. The data (including exposure time, concentration, and proportion dead at each time) for each toxicant were then successfully fit with survival models. The proportion of organisms dying at any combination of exposure concentration and time can be predicted from such models. Survival models including latent mortality produced predictions of lethal effects that were more meaningful in an ecological or field context than those from conventional LC50 methods.

Keywords—Survival analysis Latent mortality Median lethal concentration Complete median lethal concentration Toxicity

INTRODUCTION

The current wide use of the LC50 method to quantify chemical toxicity in ecotoxicology has its roots in mammalian toxicology. Researchers originally tried to quantify lethal thresholds of various chemicals and then shifted to quantifying the dose or concentration that killed 50% of exposed individuals [1]. Such a median lethal dose or median lethal concentration (LC50) was useful when quantifying relative toxicities of chemicals or change in the toxicity of one chemical under different exposure conditions. During the 1940s, environmental toxicologists adopted this approach for laboratory bioassays, using the results to imply environmental safety [2]. In these conventional LC50-based bioassays, the exposure duration is fixed based on convenience (e.g., 96 h fit within a workweek). The median effect level is used, because associated estimates of lethal concentrations at 50% generally exhibit less variability than those at higher or lower centiles [3].

Although convenient and statistically precise [4], the conventional LC50 method has shortcomings as a predictor of ecotoxic effects. First, noting effects only at one duration ignores the fact that toxic effect is a function of both exposure duration and intensity. Focusing on exposure intensity and considering duration only peripherally result in the loss of valuable information generated for other ecologically relevant times. Second, in mammalian toxicology, the LC50 method is designed for the measurement of toxic effects on individuals in the laboratory; in ecotoxicology, the primary intent is to predict the level of mortality expected in a field population. Adoption of the LC50 method compromises our ability to predict field-population consequences, because it does not routinely include mortality occurring after the exposure ends. This latent mortality is affected by several variables, such as toxic mechanism, former exposure concentration, exposure duration, life stage, and other experimental conditions [5–9]. To quantify latency (or lack of latency), observation must continue after the exposure ends.

The main goal of the present study is to modify existing methods to better include exposure duration and latent mortality into ecotoxicological models. Copper sulfate ($CuSO_4$) and sodium pentachlorophenol (NaPCP), which were expected to have contrasting latent effects, were used in the experiments. Mortality data for the amphipod *Hyalella azteca*, both during and after exposures, were analyzed with survival analysis, an approach applied to ecotoxicology and risk assessment [10,11] that can potentially resolve many shortcomings of the conventional LC50 method. By including exposure duration and concentration as covariates in models, the proportion dead at any toxicant concentration and any exposure time within the test range were predicted. Also, the conventional 48-h (during the exposure) LC50 values and the complete LC50 values (defined as the LC50 values calculated by including mortalities during and after exposure ends) of each toxicant were com-

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pared, and the latent effects of the two toxicants were contrasted.

MATERIALS AND METHODS

Amphipod culture and maintenance

The experimental amphipods (*H. azteca*) came from a population that had been maintained in our laboratory for more than two years and had never experienced contaminant exposure. Well water was used as the culturing water, and red maple (*Acer rubrum*) leaves were used as food. Test amphipods were one to two weeks old and were obtained by gently siphoning water from the cultures onto screens. The amphipods that passed through a 0.67-mm sieve but were retained by a 0.50-mm sieve were used as test organisms. They were maintained in the reformulated, moderately hard, reconstituted water (RMHRW) [12] with food at 23°C for at least 48 h before the exposures began.

$CuSO_4$ exposure and postexposure

Three $CuSO_4$ exposures were conducted in January, February, and July 2003, respectively. Copper sulfate was dissolved in the RMHRW to make five solutions with nominal dissolved Cu concentrations of 0.0, 0.2, 0.3, 0.4, and 0.6 mg/L. Each solution was delivered to four 12-well COSTAR 3513 cell culture clusters (Corning, Corning, NY, USA) with approximately 5 ml in each well. Two hundred and forty amphipods were then randomly assigned to the wells, with one animal per well. Each well contained a piece of red maple leaf as food (leaf weight in each well, 0.61 ± 0.32 mg; $n = 40$). Every amphipod exposed to the same concentration was considered to be a replicate. The cluster plates were then placed in a LAB-LINE AMBI-HI-LO Chamber (Lab-Line Instruments, Melrose Park, IL, USA). Mortality was checked at intervals of approximately 4 h. An amphipod was scored as dead and was removed from the well if no sign of appendage movement was discernible after gentle prodding. All the amphipods still alive after 48 h were carefully transferred to fresh RMHRW. Latent mortality was noted approximately every 4 h. The experiment ended at 112 h, when no more mortality was evident. All the amphipods still alive after that time were noted as right-censored (i.e., survivors).

NaPCP exposure and postexposure

Three NaPCP exposures were conducted in early June, mid-June, and late July of 2003, respectively. Sodium pentachlorophenol was dissolved in the RMHRW to make solutions with nominal NaPCP concentrations of 0.0, 0.2, 0.3, 0.5, and 0.8 mg/L. The exposure and postexposure procedures were the same as those described above for $CuSO_4$. The only difference was that the experiments ended at 85 h, when no more latent mortality was evident.

Water chemistry

The total alkalinity and pH of the RMHRW were measured before exposures began to ensure that they were within the expected ranges. The solutions were renewed during the experiments every 12 h. Both newly prepared and 12 h-exposed water samples were collected for measurements of pH and toxicant concentration. The pH values were measured with an ACCUMET Model-15 pH Meter (Denver Instrument, Denver, CO, USA) and PerpHect ROSS Electrode Model 8256 (Orion Research, Boston, MA, USA). Water samples for dissolved Cu measurement were acidified, stored at 4°C, and analyzed with a Perkin-Elmer AAnalyst 800 atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT, USA). Cupric ion concentrations were calculated with Visual MINTEQ Version 2.14 software [13] given the pH values and the nominal concentrations of ions in the RMHRW. Samples for NaPCP analysis were collected with glass bottles, stored in 4°C, and analyzed according to the method described by Carr et al. [14]. Each 25-ml water sample was mixed with 25 ml of deionized water and 0.5 ml of concentrated HCl. Ten milliliters of chloroform were added before the sample was shaken vigorously for 60 s. Five milliliters of the extract were collected in a polypropylene centrifuge tube. Two milliliters of 0.2 M NaOH were added to the extract, mixed vigorously for approximately 30 s, and centrifuged in an IEC HN-SII Centrifuge (International Equipment, Needham Heights, MA, USA) at 5,000 *g* for 5 min. The absorbance of the aqueous fractions was measured with a Beckman DU 650 spectrophotometer (Beckman Instruments, Fullerton, CA, USA) at 320 nm. Samples for temperature and dissolved oxygen concentration were taken periodically and measured with a Fisher mercury thermometer (Ever Read Thermometer, Dubuque, IA, USA) and YSI Model 57 oxygen meter (YSI, Yellow Springs, OH, USA), respectively.

Calculating conventional and complete LC50 values

The exposure concentration, total number of exposed amphipods, and number of dead amphipods were fit to a probit model using $\log_{10}$ concentration transformation with TOXSTAT Version 3.0 software [15] to calculate the conventional 48-h LC50 and the complete LC50 values, which included the mortality data of the postexposure period. The associated 95% fiducial limits were calculated as well.

Survival analysis modeling

Survival models were also used to model these data more completely. Survival analysis, also called time-to-event or failure-time analysis, was first developed in the medical sciences and engineering [16, 17], and only recently has it been applied to environmental risk assessment and ecotoxicology. The general approach involves exposing animals to specific toxicant concentrations and monitoring their mortality through time. Survivors are treated as statistically censored, because their exact times-to-death are unknown. Maximum likelihood methods are conventionally used to analyze the data because of this censoring. The general form of the survival model was the following hazard model:

$$h(t, x_i) = e^{f(x_i)} h_0(t, e^{f(x_i)})$$

where $h(t, x_i)$ is the hazard function, or the instantaneous death rate at time t conditioned on the amphipod's survival to time t for group x_i; $h_0(t, e^{f(x_i)})$ is the baseline hazard at time t for group x_i; $e^{f(x_i)}$ is a function that relates the hazard to the baseline hazard; and $f(x_i)$ is a function of either continuous variables, such as concentration, or class variables, such as sex [4].

The above function can be rearranged to the form of an accelerated failure time model:

$$\ln t_i = f(x_i) + \varepsilon_i$$

where t_i is the time, $f(x_i)$ is a function that relates the covariates to t_i, and ε_i is the error term, which equals $(\sigma \cdot L)$, where L varies with the proportion dead for which prediction is being made and can be obtained from Appendix Table 7 of Newman [10]. The scale parameter, σ, defines the shape and scale of the hazard curve. The t_i will have a Weibull, exponential, logit,

Table 1. The pH, dissolved oxygen concentrations (DOC), and water temperature of copper sulfate ($CuSO_4$) and sodium pentachlorophenol (NaPCP) exposure media[a]

	$CuSO_4$	NaPCP
pH (median)	8.10 (range, 7.89–8.27; $n = 240$)	8.19 (range, 8.13–8.28; $n = 100$)
DOC (mean ± standard deviation, $n = 20$, mg/L)	7.47 ± 0.15	7.57 ± 0.10
Water temperature (mean ± standard deviation, $n = 30$, °C)	22.97 ± 0.09	23.10 ± 0.29

[a] The pH values were measured for both newly prepared and 12 h-exposed water.

or log-normal distribution if ε_i is assumed to have either the distribution of extreme value with two parameters, extreme value with one parameter, logistic, or normal, respectively [18]. The exposure, postexposure, and entire (exposure + postexposure) survival data were fit to the accelerated failure model separately with SAS® procedure LIFEREG [19].

Akaike's information criterion (AIC) was used to select the best-fitting from the four candidate distributions above. The AIC is equal to $-2 \cdot$ (log likelihood statistics) $+ 2 \cdot$ (number of parameters) [20]. It favors parsimony in selecting among models. Lowest AIC values indicate the most parsimonious (best) model (i.e., the model with the most information per estimated parameter).

RESULTS

Water chemistry

The RMHRW for all solutions had an alkalinity of 59 ± 4 mg/L as $CaCO_3$ ($n = 10$) and a median pH of 8.15 (range, 8.12–8.16; $n = 30$). The pH value, dissolved oxygen concentration, and water temperature during the experiments are summarized in Table 1. The treatments with higher dissolved Cu concentration had lower pH values because of hydrolysis of the Cu^{2+}. Because both newly prepared and 12 h-exposed water pH values were measured, they have a relatively broad range. Table 2 summarizes the dissolved Cu and NaPCP concentrations during the 48-h exposures. The toxicant concentrations for controls and water during the postexposure period were less than the detection limits of the methods (Cu, 7 μg/L; NaPCP, 0.15 mg/L). The calculated percentages of mean Cu^{2+} concentration to dissolved Cu concentration in the exposures fell in the range of 2.7 to 3.1%.

Proportion dying during the time course

The cumulative proportions of dead amphipods at each observation time were plotted for the $CuSO_4$ and NaPCP experiments (Fig. 1). Minimal mortality was observed during the first several hours of exposure. No significant control mortality was observed in any experiment. After the $CuSO_4$ exposure ended, a large number of amphipods continued to die for a relatively long time. For NaPCP, only a few animals died during the postexposure period, and most of their deaths occurred soon after the exposure ended.

Conventional and complete LC50 values

The conventional and complete LC50 values with their 95% fiducial limits of $CuSO_4$ and NaPCP are shown in Figure 2. For $CuSO_4$, the conventional LC50 values were manifestly higher than the complete LC50 values. In experiments 1 and 2, their 95% fiducial limits did not overlap, and in experiment 3, the overlap was approximately 11%. For NaPCP, the complete LC50 values were only a little lower than the conventional LC50 values, and more than 60% of their 95% fiducial limits overlapped.

Survival analysis modeling

To predict the mortality during and immediately after the exposures ended and to determine any significant effect of former exposure concentration on the latent mortality, the 112-h survival data for $CuSO_4$ exposures and 85-h survival data for NaPCP exposures were first fit to the accelerated failure time models with the candidate survival time distributions of exponential, Weibull, log-normal, and logit (Tables 3 and 4). Natural log transformation of the concentration was used, both because this is the most common concentration metameter [10] and because the associated AIC values were lower than those without the transformation. For all the data sets, log-normal distributions proved to be the best based on the AIC. The survival data of exposure and postexposure were also fit separately to the accelerated failure time models. For data generated during the exposures, either Weibull or log-normal distribution displayed the best fit. When only the postexposure data were used, the best-fit models for $CuSO_4$ were log normal, whereas coefficients of concentration were not significantly different from zero for NaPCP exposures. The estimated intercept, coefficients for the concentration, and L values were all significantly different from zero ($p < 0.001$).

Table 2. Concentrations of dissolved Cu and sodium pentachlorophenol (NaPCP) during 48-h exposures

		Toxicant concentration (mean ± standard deviation, $n = 8$, mg/L)			
	Experiment No.	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Dissolved Cu	1	0.19 ± 0.02	0.28 ± 0.03	0.35 ± 0.04	0.53 ± 0.06
	2	0.13 ± 0.02	0.22 ± 0.03	0.30 ± 0.04	0.47 ± 0.06
	3	0.13 ± 0.02	0.21 ± 0.03	0.29 ± 0.04	0.44 ± 0.05
NaPCP	1	0.20 ± 0.02	0.36 ± 0.04	0.51 ± 0.03	0.77 ± 0.05
	2	0.20 ± 0.05	0.33 ± 0.03	0.51 ± 0.05	0.81 ± 0.03
	3	0.19 ± 0.02	0.32 ± 0.06	0.50 ± 0.02	0.79 ± 0.05

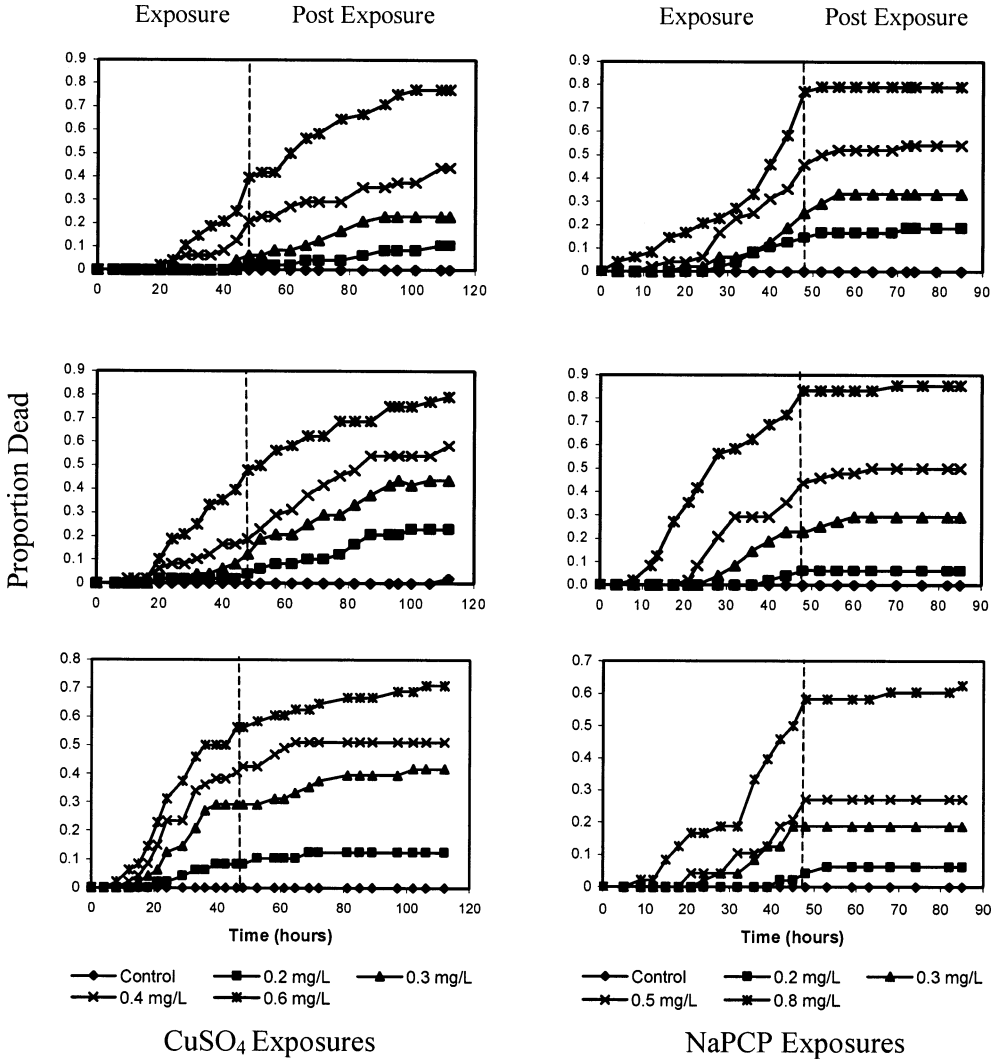

Fig. 1. Cumulative proportions of amphipods dead through time for the copper sulfate ($CuSO_4$) and sodium pentachlorophenol (NaPCP) exposures. The groups of lines indicate different nominal toxicant concentrations (for measured toxicant concentrations, see Table 2). The dashed lines at 48 h separate exposure and postexposure periods.

DISCUSSION

Effects of the nature of toxicant on latent mortality

To illustrate the extent of latent mortality, the predicted proportion dead at the conventional LC50 concentrations and the proportion dead after including latent mortality are plotted in Figure 3. When latent mortality for $CuSO_4$ was considered, 65 to 85% of exposed animals died, not 50%. Therefore, any prediction of field-population mortality based on the conventional LC50 method would underestimate mortality by 15 to 35%. In contrast, only 5% or fewer additional animals died for NaPCP. The extent of latent mortality depends on several factors, such as nature of the toxicant, exposure concentration, and exposure duration. In the current study, the amphipod *H. azteca* displayed contrasting latent mortalities after the $CuSO_4$ and NaPCP exposures, mainly because these two chemicals have different modes of action. Gills are considered to be the primary target organ of Cu because of their high surface area in contact with the external medium. Changes in gill tissue of the tropical fish *Prochilodus scrofa* were investigated after 96-h Cu exposure [9]. Gills were damaged, with epithelial lifting, cell swelling, pavement, chloride and mucous cell proliferation, and blood vessel anomalies. Recovery of the gills did not become evident until 7 d after the fish had been transferred to clean water. In contrast to that of Cu, the toxicant effect of pentachlorophenols (PCPs) is considered to be reversible and causes little cumulative damage. Nuutinen et al. [21] quantified the *H. azteca* uptake, biotransformation, and elimination rates of PCP from water. Pentachlorophenol was metabolized directly by phase II conjugation reactions at a faster rate than contaminants transformed by oxidative metabolism with cytochrome P450. Those authors also found that both PCP and its metabolite had a rapid elimination rate. Therefore, animals will have a good chance of rapid recovery after exposure ends. Mortality of mosquitofish was reported to stop within 8 h after the PCP exposure ended [7].

Effects of previous exposure concentration on latent mortality

For certain toxicants, previous exposure concentration can affect latent mortality. In the postexposure models of $CuSO_4$, the effects of former exposure concentration were significant: The higher the concentration, the less time needed for a certain proportion of animals to die. Because the dissolved Cu concentration for each treatment was the lowest among all ex-

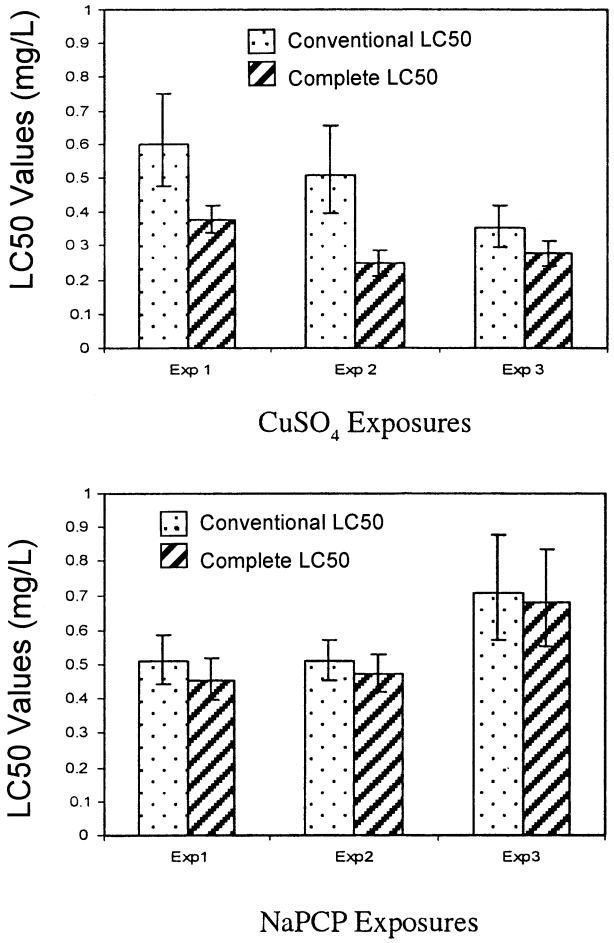

Fig. 2. Conventional (during the exposure) and complete (exposure + postexposure) median lethal concentrations (LC50s) for the copper sulfate ($CuSO_4$) and sodium pentachlorophenol (NaPCP) experiments. The error bars indicate the 95% fiducial limits.

periments in experiment 3, the cumulative gill damage caused by Cu might not have been so extensive, and accordingly, the latent mortality was not as evident as in the other two experiments. For the postexposure models of NaPCP, the coefficients of log concentration were not significantly different from zero, indicating no significant effect of former exposure concentration on latent mortality. It likely resulted from less cumulative damage occurring during the exposure, even at the highest concentration.

Incorporating exposure duration and latent mortality with survival models

The accelerated failure time models were generated for three different time periods (exposure only, immediately postexposure, and exposure + postexposure). The relationship among time, concentration, and percentage mortality was constructed. If the values of any two variables are given, the third can be estimated. For the purpose of illustration, the response surfaces combining these three factors based on the models during the exposures are shown in Figure 4. The conventional 48-h LC50 values and their 95% fiducial limits are also shown. Compared with the single LC50 value, the response surface allows the concentration that kills a certain proportion of amphipods at any time within the experimental range to be estimated. As for the postexposure models, not only the effect of recovery duration but also the effect of former exposure concentration can be quantified. When analyzed separately, especially for NaPCP, the AIC values were much lower than when a general model was assumed for the data sets. This indicates better fit of the separate models: Rates of mortality differed during and after the exposures.

Importance of incorporating latent mortality and exposure duration into current ecotoxicology studies

The conventional LC50 methods tend to minimize the effects of covariates by controlling all the experimental conditions except concentration. Exposure duration, an important factor that affects mortality, is considered only peripherally and is often fixed. Consequently, information generated for all other times is lost, limiting the ability to predict toxicant effects on field populations. The survival analysis used in the present study is a better approach than point estimation for avoiding this shortcoming. Predictions from survival models are more useful than those from the conventional LC50 method, because effects of other covariates, such as exposure time, and effects of latent mortality and pulsed exposures can be quantified more efficiently. Combined with the life-table method and Leslie matrix approach [22,23], survival analysis could also be used to predict population qualities and fate through time. Addi-

Table 3. Akaike's information criterion (AIC) values and best-fit accelerated failure time models for copper sulfate experiments[a]

Experiment	Time period	AIC values for each distribution[b]				Model[c]
		Exponential	Logit	Log-normal	Weibull	
1	General[d]	315.6	282.0	*279.0*	287.2	$\ln T = 3.33 - 1.39 \ln C + 0.73 L$
	During[e]	173.2	123.0	124.6	*121.6*	$\ln T = 3.64 - 0.58 \ln C + 0.21 L$
	After[f]	232.6	231.0	*230.6*	232.2	$\ln T = 2.65 - 1.99 \ln C + 1.28 L$
2	General	395.2	361.0	*358.6*	369.6	$\ln T = 3.33 - 0.98 \ln C + 0.80 L$
	During	211.8	186.4	187.6	*186.2*	$\ln T = 3.37 - 0.89 \ln C + 0.40 L$
	After	320.2	320.8	*319.4*	322.2	$\ln T = 2.92 - 1.17 \ln C + 1.50 L$
3	General	421.2	411.2	*404.6*	423.0	$\ln T = 2.64 - 1.54 \ln C + 1.24 L$
	During	309.0	280.4	*275.4*	285.6	$\ln T = 2.92 - 0.90 \ln C + 0.76 L$
	After	151.2	152.0	*150.8*	152.4	$\ln T = 3.70 - 1.82 \ln C + 2.19 L$

[a] All the listed coefficients were significantly different from zero ($p < 0.001$).
[b] Values in italic denote the smallest AIC and the best-fitting distribution.
[c] Best-fit model is indicated by the AIC values C = concentration; T = time-to-death; L varies with the proportion dead for which prediction is being made.
[d] Model produced by fitting all data, including postexposure mortality.
[e] Model produced by fitting only data from the exposure phase of the experiments.
[f] Model produced by fitting only the data generated after the exposure ended.

Table 4. Akaike's information criterion (AIC) values and the best-fit accelerated failure time models for sodium pentachlorophenol (NaPCP) experiments[a]

Experiment	Time period	AIC values for each distribution[b]				Model[c]
		Exponential	Logit	Log-normal	Weibull	
1	General[d]	382.7	359.6	*358.6*	367.7	$\ln T = 3.50 - 1.09 \ln C + 0.51 L$
	During[e]	309.5	248.7	259.7	*238.3*	$\ln T = 3.67 - 0.56 \ln C + 0.34 L$
	After[f]	112.0	110.3	*108.8*	110.6	—
2	General	343.7	317.7	*313.2*	332.7	$\ln T = 3.18 - 1.53 \ln C + 0.83 L$
	During	286.2	224.0	*218.0*	229.1	$\ln T = 3.20 - 0.99 \ln C + 0.53 L$
	After	72.5	73.5	72.7	73.7	—
3	General	289.0	280.4	*276.1*	285.2	$\ln T = 3.89 - 1.29 \ln C + 1.04 L$
	During	240.1	190.7	191.2	*189.0*	$\ln T = 3.79 - 0.59 \ln C + 0.32 L$
	After	36.7	38.7	39.4	38.6	—

[a] All the listed coefficients were significantly different from zero ($p < 0.001$).
[b] Values in italic denote the smallest AIC and the best-fitting distribution.
[c] Best-fit model is indicated by the AIC values. C = concentration; T = time-to-death; L varies with the proportion dead for which prediction is being made. The best-fit models were not listed for the postexposure period, because the coefficients of the natural log of concentration were not significantly different from zero ($\alpha = 0.05$).
[d] Model produced by fitting all data, including post-exposure mortality.
[e] Model produced by fitting only data from the exposure phase of the experiments.
[f] Model produced by fitting only the data generated after the exposure ended.

tionally, the cubic spline methods can be used when addressing the question of multiphase exposure [24], in which all the data are included in one survival model while allowing different error distributions through time.

When the LC50 metric was introduced into mammalian toxicology, the primary interest was toxic effect to individuals in a laboratory test. When the method was adopted by ecotoxicologists, the toxic effects should have been put into an ecological context. It is inappropriate for ecotoxicologists to focus on lethal effects during the exposures only. Latent mortality should be considered as well, especially when relating laboratory effects to those occurring in the field. For two chemicals with 48-h LC50 values that are the same for a certain species, their effects on field population may be quite different because of the different levels of latent mortality. The results

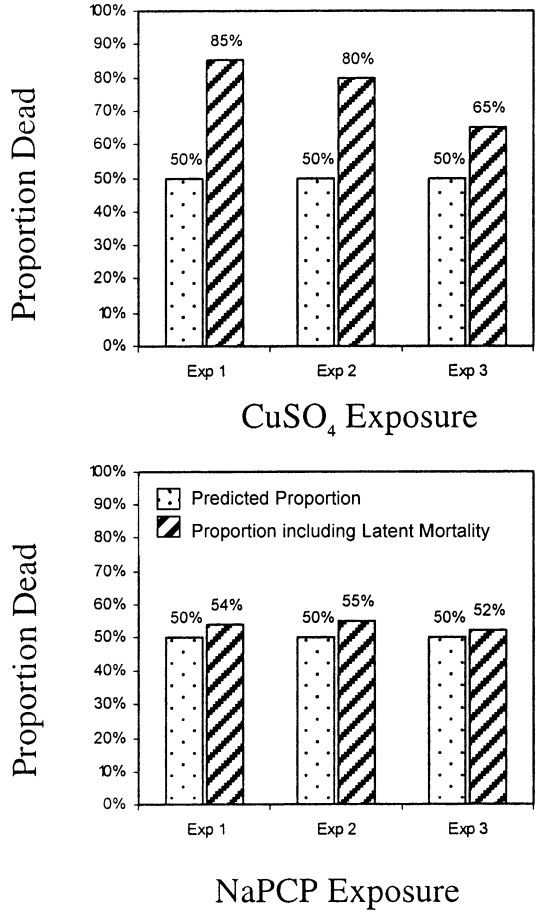

Fig. 3. Predicted proportion dead at the conventional median lethal concentrations (LC50s) and the proportion dead after including latent mortality for the copper sulfate ($CuSO_4$) and sodium pentachlorophenol (NaPCP) experiments.

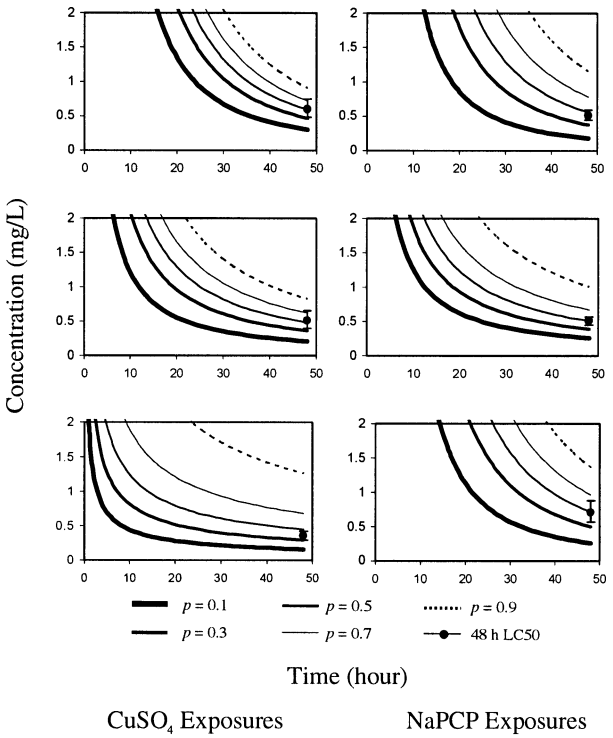

Fig. 4. Response surfaces generated from survival models of 48-h exposures to copper sulfate ($CuSO_4$) and sodium pentachlorophenol (NaPCP). The lines indicate different proportions dead. The 48-h median lethal concentrations (LC50s; ●) and their 95% fiducial limits (error bars) are also shown.

for $CuSO_4$ and NaPCP shown here illustrate this point. Recovery can be slow for toxicants like Cu that cause cumulative damage or have slow elimination. Therefore, if the Cu concentration was high enough to cause pronounced latent mortality, the proportion of exposed individuals dying will be much higher than the proportion predicted with the LC50 value, and the species population may be at a higher risk of local extinction than that suggested by the LC50 value. For toxicants with no significant latent mortality effect, such as NaPCP, a trivial difference is found between the conventional and the complete metric of mortality. There will be less possibility for a population going locally extinct, and less attention could be paid to its latent lethal effects. Therefore, we suggest that observation should be continued after exposure ends and that latent mortality information should be included in the estimates of lethal consequences to field populations. Survival analysis is a valuable means of quantifying mortality during and after exposure.

CONCLUSION

Different levels of latent mortality occurred after 48 h of $CuSO_4$ or NaPCP exposure. Because the nature of the toxicant, exposure concentration, and former exposure time all affect latent effects, it is important to include latent mortality when comparing toxicities of chemicals and relating laboratory-derived metrics of toxicity to mortality in field populations. Survival analysis efficiently models such latent mortalities. Use of survival analysis to model both exposure and postexposure effects does not exclude calculating the conventional LC50. Furthermore, it can include several covariates in the model and, consequently, enhance our predictive capabilities for field populations. The current bioassay protocols could be extended to better include both exposure duration and latent mortality.

REFERENCES

1. Trevan JW. 1927. The error of determination of toxicity. *Proc R Soc Lond B Biol Sci* 101:483–514.
2. Cairns J Jr, Pratt JR. 1989. The scientific basis of bioassays. *Hydrobiologia* 188/189:5–20.
3. Finney DJ. 1947. *Probit Analysis. A Statistical Treatment of the Sigmoid Response Curve.* Cambridge University Press, Cambridge, UK.
4. 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, Boca Raton, FL, USA, pp 207–242.
5. Brooks G, Bourland BL, Matisoff G. 1993. Control of adult zebra mussels with chlorine dioxide. *Proceedings,* Third International Zebra Mussel Conference, Toronto, ON, Canada, February 23–26, pp 139–163.
6. Guadagnolo CM, Brauner CJ, Wood CM. 2000. Effects of an acute silver challenge on survival, silver distribution, and ionoregulation within developing rainbow trout eggs (*Oncorhynchus mykiss*). *Aquat Toxicol* 51:195–211.
7. Newman MC, McCloskey JT. 2000. The individual tolerance concept is not the sole explanation for the probit dose-effect model. *Environ Toxicol Chem* 19:520–526.
8. Arthur FH. 2001. Immediate and delayed mortality of *Oryzaephilus surinamensis* (L.) exposed on wheat treated with diatomaceous earth: Effects of temperature, relative humidity, and exposure interval. *J Stored Prod Res* 37:13–21.
9. Cerqueira CCC, Fernandes MN. 2002. Gill tissue recovery after copper exposure and blood parameter responses in the tropical fish *Prochilodus scrofa*. *Ecotoxicol Environ Saf* 52:83–91.
10. Newman MC. 1995. *Quantitative Methods in Aquatic Ecotoxicology.* CRC, Boca Raton, FL, USA.
11. Crane M, Newman MC, Chapman PF, Fenlon J. 2002. *Risk Assessment with Time to Event Models.* Lewis, Boca Raton, FL, USA.
12. Smith ME, Kazircgaj JM, Herrin LE, Brewer-Swartz S, Thoeny WT. 1997. A reformulated, reconstituted water for testing the freshwater amphipod, *Hyalella azteca*. *Environ Toxicol Chem* 16:1229–1233.
13. Kungliga Tekniska högskolan. 2003. Visual MINTEQ, Ver 2. 14. Department of Land and Water Resources Engineering, Stockholm, Sweden.
14. Carr RS, Thomas P, Neff JM. 1982. A simple spectrophotometric technique for the determination of pentachlorophenol in water. *Bull Environ Contam Toxicol* 28:477–479.
15. University of Wyoming. 1989. TOXSTAT, Ver 3. 0. Department of Zoology and Physiology, Laramie, WY, USA.
16. Allison PD. 1995. *Survival Analysis Using the SAS® System: A Practical Guide.* SAS Institute, Cary, NC, USA.
17. Elandt-Johnson RC, Johnson NL. 1980. *Survival Models and Data Analysis.* John Wiley, New York, NY, USA.
18. Cox DR, Oakes D. 1984. *Analysis of Survival Data.* Chapman & Hall, New York, NY, USA.
19. SAS Institute. 1999. *SAS/STAT® User's Guide,* Ver 8, Vol 2. Cary, NC, USA.
20. Atkinson AC. 1980. A note on the generalized information criterion for choice of a model. *Biometrika* 67:413–418.
21. Nuutinen S, Landrum PF, Schuler LJ, Kukkonen JVK, Lydy MJ. 2003. Toxicokinetics of organic contaminants in *Hyalella azteca*. *Arch Environ Contam Toxicol* 44:467–476.
22. Leslie PH. 1945. On the use of matrices in certain population mathematics. *Biometrika* 33:183–212.
23. Caswell H. 1996. Demography meets ecotoxicology: Untangling the population level effects of toxic substances. In Newman MC, Jagoe CH, eds, *Ecotoxicology: A Hierarchical Treatment.* CRC, Boca Raton, FL, USA, pp 255–292.
24. Harrell FE, Lee KL, Pollock BG. 1988. Regression models in clinical studies: Determining relationships between predictors and response. *J Natl Cancer Inst* 80:1198–1202.