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Enhancing toxicity data interpretation and prediction of ecological risk with survival time modeling: an illustration using sodium chloride toxicity to mosquitofish (*Gambusia holbrooki*)

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Protocols for estimating toxic endpoints (e.g. 96-h LC_{50}) dominate research in aquatic toxicology to the near exclusion of equally appropriate techniques such as survival time modeling. By noting time-to-death during routine testing, statistical power, incorporation of covariates, prediction of toxic effect over time and linkage to life table characteristics can be improved. Consequently, prediction of ecological risk is enhanced. Survival time modeling is illustrated with acute NaCl toxicity data for the mosquitofish, *Gambusia holbrooki*. Extrinsic factors (salt concentration) and intrinsic factors (fish wet weight) influencing toxic impact are included in the model. A model assuming a Weibull distribution best fit these data. With such a model, median time-to-death can be estimated for any size fish at any toxicant concentration within the tested range. Survival time modeling extracts more information from toxicity data than routine endpoint methods but does not preclude estimation of standard endpoints. Survival time modeling should be considered as an adjunct to routine toxicity testing endpoints in many situations.

Key words: Toxicity; Modeling; Statistic; Freshwater; Fish; Sodium chloride

INTRODUCTION

Toxic endpoint methods comprise the cornerstone of current aquatic toxicology. Standard protocol and statistical techniques for analyzing both toxic endpoint and survival time data were clearly outlined in a series of seminal papers (e.g., Sprague, 1969; Stephan, 1977; Buikema et al., 1982). However, during standardization of testing techniques in the United States, methods for estimating toxic endpoint came to overshadow those associated with time-to-death estimation. This led to an undeniably uniform and statistically well-defined body of information with which sound

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regulatory decisions are made. As a consequence, endpoint techniques are so pervasive that most aquatic toxicologists use them to the near exclusion of equally or more appropriate techniques. Refinement of statistical techniques for analysis of survival time data in aquatic toxicology has lagged behind that of techniques for endpoint data.

Dominance of the endpoint approach in aquatic toxicology should not be taken as evidence of its superiority to other approaches. Sprague (1969) discussed the arguments of Gaddum (1953) and Finney (1964) regarding the loss of information that occurs when time-to-death data are abandoned in favor of percentage mortality data for a fixed endpoint. Cox and Oakes (1984) suggested that methods that focus on endpoint mortality waste information and make estimation difficult if some individuals are censored during the exposure period. Dixon and Newman's (1991) comparison of time-to-death to traditional endpoint approaches detailed the richness of information and enhanced statistical power that can be realized using survival time methods.

Survival analysis techniques are well established in epidemiology and engineering, and have an origin common to life table methodologies that were developed in the 1660s (Blackstone, 1986). Despite their statistical power, linkage to life table methods, prevalence in other fields and historical foundation, survival analysis techniques are rarely used by aquatic toxicologists. Some recent exceptions include work with genotype effect on mosquitofish tolerance to inorganic mercury and arsenate (Diamond et al., 1989; Newman et al., 1989; Dixon and Newman, 1991). Newman and Heagler (1991) briefly compared such techniques to those traditionally used to define animal size effects on toxicity also.

The purpose of the research described herein is to illustrate the clear advantages of noting time-to-death in research efforts employing routine toxicity testing protocol. This is done using data for sodium chloride toxicity to the Eastern mosquitofish (*Gambusia holbrooki,* Girard 1859). Incorporation of extrinsic factors (toxicant concentration) and intrinsic factors (fish size) are illustrated.

MATERIALS AND METHODS

Fish collection and maintenance

Mosquitofish were collected from Risher Pond, an abandoned farm pond on the Department of Energy's Savannah River Site near Aiken, South Carolina. This population of mosquitofish has been sampled in past toxicological studies (Chagnon and Guttman, 1989a,b; Diamond et al., 1989; Newman et al., 1989) and, to our knowledge, has never experienced significant exposure to contaminants.

Fish were collected by dip net in June 1991, (ambient water temperature: 30°C). They were maintained in a 520 l Living Streams™ chilled tank (22°C) until exposure

began (1 to 3 weeks after capture). Fish were fed Tetramin® tropical fish food twice daily during this holding period. They were not fed during the 96 h exposure period.

Sodium chloride exposure

A commercial proportional dilutor (Enviro-tox™ system, Specialized Environmental Equipment, Inc., Easley, South Carolina) was used for dosing fish. Thirty-eight to forty fish were randomly assigned to each of fourteen 11.4 l tanks. Water drawn from Upper Three Runs Creek was spiked to nominal concentrations of 0.0, 10.6, 11.0, 11.8, 13.2, 15.6 and 20.0 g NaCl/l, and delivered to the seven pairs of tanks at a rate of 34.5 l/tank/day. Water temperature was controlled by placing the exposure tanks into a chilled water bath.

All tanks were checked for mortality at 8 h intervals. A fish was scored as dead and removed if no signs of ventilation or other movement were discernible after gentle prodding. Wet weight of each dead fish was measured upon removal. Fish surviving 96 h of exposure were killed, weighed and their times-to-death noted as right-censored (>96 h).

Water quality

Temperature and dissolved oxygen concentrations were measured daily with a Hydrolab Surveyor II™. Total alkalinity (potentiometric titration) and pH were measured at 0 and 88 h of exposure in all tanks. Samples for major cation and anion analyses were also taken at these times. A Dionex Model 4020i ion chromatograph was used to measure sodium and chloride concentrations in the exposure water. Calcium, magnesium and potassium concentrations were determined using an Hitachi 180-80 polarized Zeeman atomic absorption spectrophotometer. Details of the analyses are outlined elsewhere (Newman et al., 1989; Diamond et al., 1991). Sulfate was not measured accurately because of peak overlap with chloride in these spiked waters. Sulfate concentrations ranged from 1.9 to 2.3 mg/l in Upper Three Runs Creek waters (Diamond et al., 1989; Newman et al., 1989; Diamond et al., 1991).

Survival time modeling

Miller (1981) and Cox and Oakes (1984) discussed details of the survival time methods used herein. Relative to aquatic toxicology, these techniques and pertinent software were reviewed by Dixon and Newman (1991).

Generally, the hazard (proneness to fail, force of mortality or instantaneous death rate) was modeled over the course of the toxicity test. If the natural logarithm of the cumulative survival of a group of individuals [ln $S(t)$] were plotted over time, the hazard function [$h(t)$] would be the negative of the slope, i.e., $-\mathrm{d} \ln S(t)/\mathrm{d}t$. Assuming

a proportional hazard model, the hazard for any individual or class of individuals can be estimated using an arbitrary baseline hazard, exposure time and pertinent covariates (salt concentration and size, in this case).

$$h(i,t) = h(\text{ref.},t)e^{x\beta}$$

where $h(i,t)$ = the hazard of an individual at time, t; $h(\text{ref.},t)$ = hazard for the arbitrary, base-line at time, t; x = a matrix of covariates (salinity and size in this study); and β = a vector of regression coefficients that estimate the influence of each covariate on the hazard.

A nonparametric model (Cox proportional hazard model) that assumes no specific underlying distribution but, instead, uses a family of Lehmann alternatives for the hazard function, can be used if the underlying distribution for the hazard function is unknown (Miller, 1981). The assumption is maintained that the regression coefficients scale the hazard functions between individuals or classes of individuals. Alternatively, a variety of underlying distributions can be examined as potential distributions describing the hazard (see Tables 2.1 and 2.2 of Cox and Oakes, 1984; Table 6, Dixon and Newman, 1991).

Goodness of fit for various distributions to the data set can be evaluated visually using a series of transformations. Plots of appropriately transformed data for a given distribution result in a straight line. The most frequently examined distributions are the exponential (ln of cumulative survival vs. time), Weibull (ln[–ln of cumulative survival] vs. ln time), log normal (probit of cumulative mortality vs. ln of time), and log logistic (ln[cumulative survival/(1–cumulative survival)] vs. ln of time) distributions. Relative goodness of fit can also be estimated using the log likelihood value associated with the candidate models (Dixon and Newman, 1991). If the same number of parameters is being estimated for each model, the model with the larger log likelihood value fits the data better. Further details regarding goodness of fit techniques are given in Chapter 7 of Miller (1981).

The SAS procedure LIFEREG (SAS, 1985) was used to develop survival time models in this study. The covariates, fish wet weight (g) and salt concentration (g NaCl/l) were included in the model as continuous variables.

RESULTS

Water quality

Tables 1 and 2 summarize water quality during the 96 h exposure period. Variables that could potentially display significant daily variation, e.g., temperature and dissolved oxygen concentration were measured most frequently. Measured concentrations of sodium chloride added to the spiked tanks were 10.3, 10.8, 11.6, 13.2, 15.8

TABLE I

Overall water quality in exposure tanks. See Materials and Methods for more details. Temperature and dissolved oxygen concentrations were measured in each of the fourteen tanks daily. The remaining variables were measured in each tank at the onset and end of the exposure period.

	Mean	Standard deviation	*N*
Temperature (°C)	20.4	0.8	138
Dissolved oxygen (mg/l)	6.1	0.3	136
pH (median, range)	6.21	6.07–6.43	28
Titration alkalinity (mg/l as $CaCO_3$)	11.0	0.9	28
Calcium (mg/l)	3.5	1.2	27
Magnesium (mg/l)	0.6	0.1	28
Potassium (mg/l)	0.8	0.2	27

and 20.1 g/l. These concentrations were used in the time-to-death model and 96-h LC_{50} calculations.

Estimation of 96-h LC_{50}

The trimmed Spearman-Karber method (Hamilton et al., 1977) as implemented by the CT-TOX software (CT Dept. of Environ. Protection, 1990) was used to estimate the 96-h LC_{50} and associated 95% confidence interval. Mortalities at 96 h in the < 1.0, 10.3, 10.8, 11.6, 13.2, 15.8, and 20.1 g/l tanks were 0, 21, 28, 52, 91, 100 and 100%, respectively. With a 21.05% trim, the 96-h LC_{50}, was estimated to be 11.54 g/l (lower 95% confidence limit: 11.29 g/l; upper confidence limit: 11.80 g/l) using this modelfree method.

Selection of distribution type

There was no control mortality during the exposure. The highest concentration had complete mortality by the first sampling (8 h). Consequently, associated mortality data for the five intermediate concentrations were used to examine model fit graphically. Generally straight, parallel lines were obtained for the Weibull distribution when cumulative survival data were transformed as described above (Fig. 1). Further, the log likelihood for the model assuming a Weibull distribution (−193.86) was larger than those of the other distributions (log logistic: −197.90, log normal: −201.79, exponential: −385.67). When a gamma distribution was assumed, a slightly larger value was obtained (−192.68); however, the minimal improvement of fit was judged to be insignificant relative to the advantages of assuming a Weibull distribution.

The Weibull and gamma distributions, both generalized exponential distributions

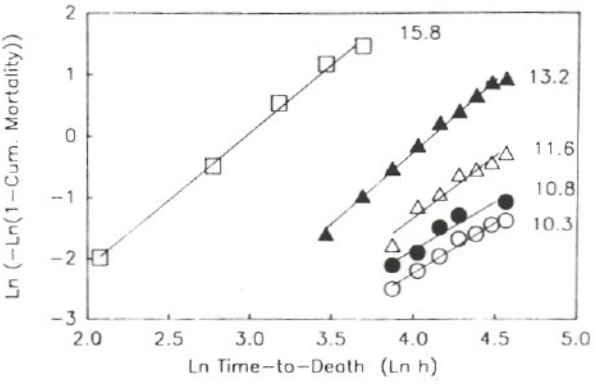

Fig. 1. Plot of transformed time-to-death data for mosquitofish exposed to 10.3 to 15.8 g NaCl/l demonstrating acceptable fit of the Weibull distribution. Two points from the 11.6 g NaCl/l and one point each from the 10.8 and 13.2 g NaCl/l treatments were omitted. These points which had values below −3 for ln[−ln(1−cumulative mortality)] were associated with spurious mortality at the beginning of the exposure period.

with very similar fits to the data, differ in one important aspect. The Weibull is the only candidate model that is a proportional hazard model. The Weibull has the same form for accelerated time (a covariate accelerates time-to-death) and proportional

TABLE 2

Sodium and chloride concentrations in exposure tanks. Each variable was measured in each of the tanks at the onset and end of the exposure period. See Materials and Methods for more detail.

Tanks	Mean	Standard deviation	*N*
	Na (g/l)		
11,12	6.522	0.195	4
1,2	5.219	0.287	3
3,4	4.329	0.088	4
5,6	3.810	0.120	4
7,8	3.584	0.146	4
9,10	3.445	0.109	4
13,14 (controls)	0.0072	0.0013	2
	Cl (g/l)		
11,12	13.575	0.222	4
1,2	10.545	0.088	3
3,4	8.870	0.037	4
5,6	7.742	0.102	4
7,8	7.172	0.171	4
9,10	6.522	0.195	4
13,14 (controls)	0.0087	0.0023	2

hazard (the effect of a covariate on the hazard is to multiply some base hazard by a constant) models (Dixon and Newman, 1991). If a proportional hazard model is appropriate then the hazard of one group of exposed individuals is proportional to that of another regardless of exposure duration. For example, the hazard of fish held at one salt concentration is proportional to that of fish held at another. Also, the hazard of a 0.100 g fish is proportional to that of a 1.000 g fish. As described by Dixon and Newman (1991), this allows estimation of relative risks that are constant over time for various classes of individuals.

Equality of replicate tanks

The SAS procedure LIFETEST was used to test for equality between duplicate tanks. The two nonparametric methods (log-rank and Wilcoxon tests) indicated no statistically significant ($\alpha = 0.05$) deviation from the assumption of equality with one exception (13.2 g NaCl/l: P=0.035 for the Wilcoxon test Chi-square value of 4.45, df=1). The Wilcoxon test that puts more weight on earlier times-to-death than the Log-rank test (SAS, 1988a) was influenced by a few early deaths in one tank. The overall Chi-square value (10.96, df=5) for this method was not significant at α=0.05. The Log-rank test suggested no significant tank effect at this salt concentration (P=0.092, Chi-square value=2.83, df=1). Considering the number of comparisons made, the sensitivity of the Wilcoxon test to early times-to-death, and the lack of significance of the log-rank associated Chi-square value, all duplicates were judged to be similar enough to pool during model development.

Survival time model

Both fish size (g wet weight) and salt concentration (g NaCl/l) had significant effects on time-to-death (Table 3). The negative β estimated for the effect of salt concentration (−0.2953) indicates that time-to-death decreased as concentration increased. Conversely, the positive β for the influence of fish size (1.0602) indicates that smaller fish have shorter times-to-death than larger fish. The model predicting median time-to-death (MTTD) derived from these data was the following:

$$\text{MTTD} = e^{\mu} e^{\beta_{\text{w}} \cdot \text{wgt}} + \beta_{\text{s}} \cdot \text{salt conc.} e^{\sigma \cdot W}$$

or

$$\text{MTTD} = e^{7.8579} e^{(1.0602 \cdot \text{wgt}) + (-0.2953 \cdot \text{salt conc.})} e^{0.3046 \cdot W}$$

The σ is a scale parameter estimated by LIFEREG. The β_{w} and β_{s} are the estimated coefficients for the fish weight and salt concentration effects, respectively. W is the 50th percentile of the standardized distribution assumed for the error (SAS, 1988b). It is −0.3665 for the Weibull distribution (Dixon and Newman, 1991). With this equation, the predicted median time-to-death can be estimated for a Risher Pond fish

TABLE 3

Summary of Weibull model including effects of mosquitofish size (g wet weight) and exposure salt concentration (g NaCl/l).

Variable	df	Estimate (S.E.)	Chi square	P>Chi square
Intercept (μ)	1	7.8579 (0.0853)	8488.1	<0.0001
Salt Concentration (β_s)	1	-0.2953 (0.0052)	3257.8	<0.0001
Wet Wgt (β_w)	1	1.0602 (0.2566)	17.1	0.0001
Scale (σ)	1	0.3046 (0.0137)		

of a specified size exposed to any salt concentration provided concentration and size are within the ranges used in the exposure. If requested, estimates of median time-to-death and associated standard errors can be generated using the SAS procedure LIFEREG. Use of W's for other percentiles would provide time-to-death predictions for other levels of mortality, e.g., 1%, 5%, 90% of the exposed fish.

DISCUSSION

The predicted median times-to-death for an average-sized fish (0.136 g) at the various salt concentrations and associated standard errors are shown for the Weibull, log normal and log logistic models in Fig. 2. The log normal and log logistic models generated very similar results different from that of the trimmed, Spearman-Karber estimate. The predicted 96-h LC_{50} using the trimmed, Spearman-Karber method was consistent with predictions from the best model, the Weibull model. A 96-h LC_{50} derived from the Weibull model can be estimated using the average fish weight in the equation,

$$96\text{-h } LC_{50} = (\ln 96 - \mu - \beta_w \mathrm{Wgt} - \sigma W)/\beta_s$$

where μ = model intercept (7.8579); $\beta_w = \beta$ for the effect of wet weight (1.0602); $\beta_s = \beta$ for the effect of salt concentration (−0.2953); σ = model scale (0.3046); $W = -0.3665$ as described previously; and Wgt = fish wet weight (0.136 g).

This size-normalized, 96-h LC_{50} predicted from the Weibull model for a fish of average weight was 11.26 g NaCl/l, a value slightly lower than that derived using the trimmed Spearman-Karber method. The difference between the two results is reason-

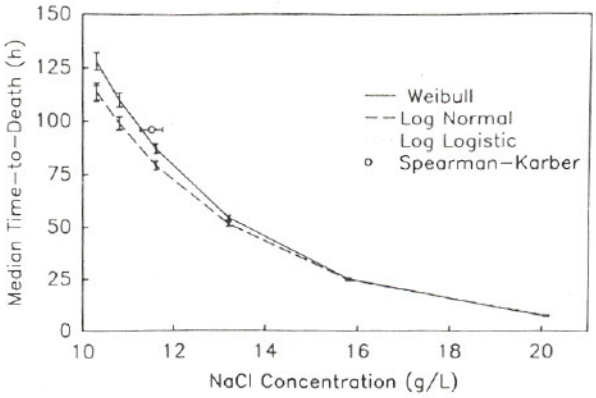

Fig. 2. Predicted median times-to-death (± standard error) for an average sized mosquitofish (0.136 g wet weight) over the range of 10 to 20 g NaCl/l. Predicted values for the log normal and log logistic models were so similar that the points and associated standard errors overlie each other. The Weibull model generated estimates most consistent with the 96-h LC_{50} (± 95% confidence interval) calculated with the trimmed Spearman-Karber method.

able as the traditional method takes only the final mortality data into consideration while the survival time model uses data from all time intervals. Also, the traditional method does not incorporate the nonlinear effect of size on mortality.

Covariates are not routinely incorporated into LC_{50} test protocol. Indeed, standard methods often minimize the effect of covariates by testing a sample of similar individuals held under similar conditions. This enhances the precision of LC_{50} estimation but limits the researcher's ability to predict effects to individuals within field populations. Fish size is a good example of such a compromise in predictive power. Frequently, the range in fish sizes is restricted in a test. Although Anderson and Weber (1975) modified the survival time techniques of Bliss (1936) to predict LC_{50} values across size classes, such an approach has no conceptual or statistical advantage over the techniques discussed herein. As demonstrated, the survival time techniques can readily incorporate fish size for normalization or predictive purposes. As illustrated in Fig. 3, the effect of size on time-to-death for a range of salt concentrations can be predicted.

Proportional hazard models such as that developed in this work could easily generate the concentration-duration-response curves recommended by Suter et al. (1987) for chronic toxic exposures. They suggested that functions predicting toxic impact of exposure at a specified duration and concentration should be developed for all life stages. Such information could be used to predict effect over a range of exposure concentrations and durations. Splines (Smith, 1979; Harrell et al., 1988) could be used to link relationships between life stages.

The effect of covariates such as size, toxicant concentration, and water quality can also be expressed in terms of relative risk. For example, the relative risk of a 0.100 g

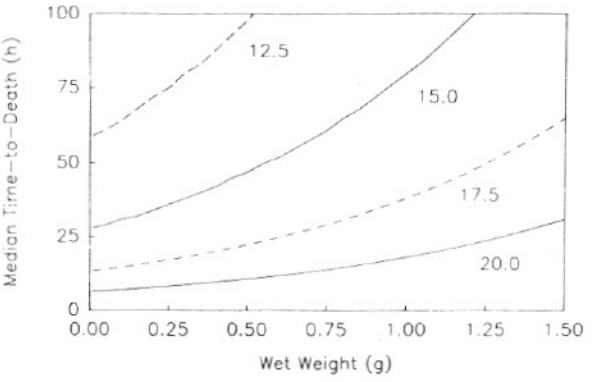

Fig. 3. Mosquitofish size (g wet weight) and salt concentration (g NaCl/l) effects on predicted median times-to-death.

fish to a 1.000 g fish is $e^{\beta\Delta Wgt}$ or $e^{1.0602 \cdot 0.9000}$ (Dixon and Newman, 1991). The smaller fish has a risk of death 2.6 times that of the larger fish. Similarly, a fish at 20 g NaCl/l has a risk 19.2 times higher than that of a fish at 10 g NaCl/l. Further, the small fish at the higher concentration has a risk 49.8 times higher than that of the larger fish at the lower concentration.

Extrinsic factors such as the influence of water quality on toxic effect may also be amenable to survival time analysis. For example, the power models used to adjust for the influence of hardness on metal LC_{50} have no advantage over survival time techniques described in this work. Indeed, methods used to derive power models can produce biased predictions (Newman, 1991). To our knowledge, survival time models such as those described here have never been applied in this manner despite frequent use of the time-to-death approach in a variety of studies predating technique standardization (see Bliss, 1936; Shepard, 1955; Costlow et al., 1960; Sprague, 1969; Swarts et al., 1978 as examples). Similar examples for which survival analysis may have greatly enhanced incorporation of intrinsic and extrinsic covariates that modify toxic effect are replete in the literature and standard textbooks such as Rand and Petrocelli (1985).

Sole reliance on endpoint techniques not only restricts covariate incorporation but also restricts the ability to predict toxic impact with time. An LC_{50} can be estimated at a series of times to compensate; however, each estimate uses only the percentage mortality data associated with that one time. The information associated with earlier or later times is ignored and, consequently, statistical power is wasted. In contrast, the survival time model can be incorporated easily into simulations for predicting toxic impact through time. Such simulations can incorporate temporal changes in toxicant concentration also. Shifts in fish size in the population as a consequence of growth or differential mortality could also be included. These enhancements would greatly im-

prove attempts to assess risk in situations such as that examined by Wang and Hanson (1985) in which exposure time and total residual chlorine varied.

In conclusion, noting time-to-death during testing does not detract from estimation of endpoint parameters such as 96-h LC_{50} and enhances data interpretation. Whether the additional effort required to note time-to-death is warranted depends on the particular objectives of the research. Serious consideration should be given to survival time models when increased statistical power, incorporation of covariates, prediction of impact through time, expression of toxic impact as relative risk (proportional hazard models), or linkage to life table characteristics is important. Survival time models can significantly enhance our ability to understand the impact of toxicants in aquatic systems. This is particularly important as methods are extended to estimating ecological risk.

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REFERENCES

Anderson, P.D. and L.J. Weber, 1975. Toxic response as a quantitative function of body size. Toxicol. Appl. Pharmacol. 33, 471–483.

Blackstone, E.H., 1986. Analysis of death (survival analysis) and other time-related events. In: Current status of clinical cardiology, edited by F.J. Macartney, MTP Press Limited, Cambridge, MA, pp. 55–101.

Bliss, C.I., 1936. The size factor in the action of arsenic upon silkworm. Exp. Biol. 13, 95–110.

Buikema Jr., A.L., B.R. Niederlehner and J. Cairns, 1982. Biological monitoring. Part IV: Toxicity testing. Water Res. 16, 239–262.

Chagnon, N.L. and S.I. Guttman, 1989a. Differential survivorship of allozyme genotypes in mosquitofish populations exposed to copper and cadmium. Environ. Toxicol. Chem. 8, 319–326

Chagnon, N.L. and S.I. Guttman, 1989b. Biochemical analysis of allozyme copper and cadmium tolerance in fish using starch gel electrophoresis. Environ. Toxicol. Chem. 8, 1141–1147.

Costlow Jr., J.D., C.G. Bookhout and R. Monroe, 1960. The effect of salinity and temperature on larval development of *Sesarma cinereum* (Bosc) reared in the laboratory. Biol. Bull. 118, 183–202.

Cox, D.R. and D. Oakes, 1984. Analysis of survival data. Chapman and Hall, New York, 201 pp.

CT Department of Environmental Protection, 1990. CT-TOX MULTI-METHOD PROGRAM. Bureau of Water Management, Water Toxics Laboratory, 122 Washington Street, Hartford, CT 06106.

Diamond, S.A., M.C. Newman, M. Mulvey, P.M. Dixon and D. Martinson, 1989. Allozyme genotype and time to death of mosquitofish, *Gambusia holbrooki* (Baird and Girard), during acute exposure to inorganic mercury. Environ. Toxicol. Chem. 8, 613–622.

Diamond, S.A., M.C. Newman, M. Mulvey and S.I. Guttman, 1991. Allozyme genotype and time to death

of mosquitofish, *Gambusia holbrooki*, during acute inorganic mercury exposure: a comparison of populations. Aquat. Toxicol. 21, 119–134.

Dixon, P.M. and M.C. Newman, 1991. Analyzing toxicity data using statistical models for time-to-death: an introduction. In: Metal ecotoxicology. Concepts and applications, edited by M.C. Newman and A.W. McIntosh, Lewis Publishers, Chelsea, Michigan, pp. 207–242.

Finney, D.J. 1964. Statistical method in biological assay. Hafner, New York, 668 pp.

Gaddum, J.H., 1953. Bioassays and mathematics. Pharmacol. Rev. 5, 87–134.

Hamilton, M.A., R.C. Russo and R.V. Thurston, 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11, 714–719.

Harrell Jr., F.E., K.L. Lee and B.G. Pollock, 1988. Regression models in clinical studies: determining relationships between predictors and response. J. Natl. Cancer Inst. 80, 9–12.

Miller, R.G., 1981. Survival analysis. John Wiley & Sons, New York, 185 pp.

Newman, M.C., 1991. A statistical bias in the derivation of hardness-dependent metals criteria. Environ. Toxicol. Chem. 10, 1295–1297.

Newman, M.C., S.A. Diamond, M. Mulvey and P. Dixon, 1989. Allozyme genotype and time to death of mosquitofish, *Gambusia affinis* (Baird and Girard) during acute toxicant exposure: a comparison of arsenate and inorganic mercury. Aquat. Toxicol. 15, 141–156.

Newman, M.C. and M.G. Heagler, 1991. Allometry of metal bioaccumulation and toxicity. In: Metal ecotoxicology. Concepts and applications, edited by M.C. Newman and A.W. McIntosh, Lewis Publishers, Chelsea, Michigan, pp. 91–130.

Rand, G.M. and S.R. Petrocelli, 1985. Fundamentals of Aquatic Toxicology, Hemisphere Publishing Corp., New York, pp. 666.

SAS Institute, 1985. SAS Procedures guide for personal computers. SAS Institute, Inc., Cary, North Carolina, 373 pp.

SAS Institute, 1988a. SAS Technical Report: P-179, Release 6.03. SAS Institute, Inc., Cary, North Carolina, 255 pp.

SAS Institute, 1988b. SAS/STAT User's Guide, Release 6.03. SAS Institute, Inc., Cary, North Carolina, 1028 pp.

Shepard, M.P., 1955. Resistance and tolerance of young speckled trout (*Salvelinus fontinalis*) to oxygen lack, with special reference to low oxygen acclimation. J. Fish. Res. Board Can. 12, 387–446.

Smith, P.L., 1979. Splines as a useful tool and convenient statistical tool. Am. Statist. 33, 57–62.

Sprague, J.B., 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3, 793–821.

Stephan, C.E., 1977. Methods for calculating an LC_{50}. In: Aquatic toxicology and hazard evaluation, ASTM STP 634, edited by F.L. Mayer and J.L. Hamelink, American Society for Testing and Materials, Philadelphia, Pennsylvania, pp. 65-84.

Suter, G.W., II, A.E. Rosen, E. Linder and D.F. Parkhurst, 1987. Endpoints for responses of fish to chronic toxic exposures. Environ. Toxicol. Chem. 6, 793–809.

Swarts, F.A., W.A. Bunson and J.E. Wright, 1978. Genetic and environmental factors involved in increased resistance of brook trout to sulfuric acid solutions and mine polluted waters. Trans. Am. Fish. Soc. 107, 651–677.

Wang, M.P. and S.A. Hanson, 1985. The acute toxicity of chlorine on freshwater organisms: time-concentration relationships with constant and intermittent exposures. In: Aquatic toxicology and hazard evaluation, ASTM STP 891, edited by R.C. Bahner and D.J. Hansen, American Society for Testing and Materials, Philadelphia, Pennsylvania, pp. 213–232.