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Survival Time Analysis of Least Killifish (*Heterandria formosa*) and Mosquitofish (*Gambusia affinis*) in Acute Exposures to Endosulfan Sulfate

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Abstract Single-species flow-through toxicity tests were conducted to determine the times-to-death of two indigenous fish to South Florida—least killifish (*Heterandria formosa*) and mosquitofish (*Gambusia affinis*)—from acute exposure to endosulfan sulfate. Mortalities were recorded within 8-h periods from test initiation to termination at 96 h. The 96-h LC_{50}s for least killifish and mosquitofish estimated using the trimmed-Spearman–Karber method were 2.0 and 2.3 µg/l, respectively. An accelerated failure time model was used to estimate times to death at selected concentrations. Data were fit to log-normal, log-logistic, and Weibull distributions. Acute toxicity data fit to the Weibull distribution produced a better relative fit than log-normal or log-logistic distributions for both toxicity tests. The survival-time profiles and associated statistics illustrate the benefit of considering exposure duration as well as concentration when predicting acute risk to species' populations. Both toxicity tests had similar outcomes from exposure to endosulfan sulfate, with least killifish being slightly more likely to die at lower concentrations and shorter time periods than mosquitofish. From the models generated by the toxicity tests, times-to-death for least killifish and mosquitofish were estimated for environmentally relevant concentrations of total endosulfan at a site of concern in South Florida. When the results from the current toxicity tests were compared to environmental concentrations from previous screening-level ecological risk assessments, the durations necessary to potentially kill 10% or more of the populations of the two native south Florida fish species were estimated to be 77 and 96 h for least killifish and mosquitofish, respectively. However, the exposure values included the α and β isomers as well as endosulfan sulfate; therefore, an understanding of their toxicity might be important in understanding the survival dynamics of fish species in endosulfan sulfate-contaminated sites.

Technical endosulfan is a mixture of two stereoisomers (α and β endosulfan) at a ratio of 7:3. In South Florida, formulations containing technical endosulfan are applied to control target insects on crops (e.g., tomato, squash) with an annual usage of ~44,589 kg active ingredient endosulfan (FDOACS 2003). Through oxidation in freshwater and saltwater, including sediment, the main transformation product of the endosulfan isomers is endosulfan sulfate (Navarro et al. 2000; Shivaramaiah et al. 2005).

A probabilistic risk assessment of sediment monitoring data from 1990 to 2002 in South Florida freshwater canals identified total endosulfan ($\alpha + \beta +$ sulfate) as a chemical of potential ecological concern based on exceedence of sediment quality screening benchmarks (Carriger et al. 2006). At an agriculture site upstream of the Everglades National Park, designated as S-178, total endosulfan was found at levels that could produce potential risk to fish and arthropods (Carriger and Rand 2008). For the years 1999 and 2000, the 90th centile concentration estimate at this site was 0.2 µg/l, which is equivalent to chronic no-effect concentrations for fathead minnow (*Pimephales promelas*) survival, growth, and reproduction as reported by Macek et al. (1976). However, unlike arthropods, fish have a narrow range of sensitivities to technical endosulfan and

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exposure concentration increases within this range can potentially impact a wider range of fish species (Carriger and Rand 2008). This was observed in the steep slope of a log-logistic species sensitivity distribution when all available acute LC_{50} (lethal concentration estimated to kill 50% of the organisms) toxicity estimates for freshwater fish species were plotted (Carriger and Rand 2008). The high acute toxicity of technical endosulfan to fish species at environmentally relevant concentrations (Carriger and Rand 2008) and the limited acute toxicity data on endosulfan sulfate to native Florida fishes triggered a series of acute toxicity studies with endosulfan sulfate and small demersal fish (Carriger et al. unpublished data), which play a key trophodynamic role in the Everglades landscape because they are part of the forage base of many taxa, including wading birds (Lorenz and Serafy 2006). Acute toxicity tests with native fish and endosulfan sulfate indicated the 96-h LC_{50}s from measured concentrations were 2.3, 2.0, and 3.1 μg/l for mosquitofish (*Gambusia affinis*), least killifish (*Heterandria formosa*), and sailfin molly (*Poecilia latipinna*), respectively (Carriger et al. unpublished data). For fathead minnow (*Pimephales promelas*), an introduced species in South Florida, the 96-h LC_{50} was 2.8 μg/l (Carriger et al. unpublished data).

In order to generate a dose–response model for predicting ecologically relevant population or community-level effects, a survival-time analysis was conducted on the acute toxicity data for endosulfan sulfate to mosquitofish (*G. affinis*) and least killifish (*H. formosa*), two important native demersal fish. Knowledge of the survival dynamics for these fish and endosulfan sulfate will assist regulators in decision-making on the movement of contaminated water from areas, like S-178, an endosulfan "hot spot" in the C-111 canal system (Carriger and Rand 2008; Rand et al. in press), which is a buffer area between agriculture and Everglades National Park. Dose–response models built using survival-time analysis can place time and concentration as covariates and thus model the temporal dynamics of effects (Zhao and Newman 2004).

Methods

Survival Analysis

Acute 96-h flow-through LC_{50} toxicity tests were conducted with endosulfan sulfate and mosquitofish and the least killifish at the Florida International University Ecotoxicology and Risk Assessment Laboratory following US Environmental Protection Agency (EPA) methodology (Carriger et al. unpublished data). Fish were juveniles purchased from reputable growers. Fish were acclimated to laboratory conditions prior to initiating the experiments. The temperature and photoperiod were kept at 24 ± 1°C and 16:8 h light:dark, respectively. Average hardness was 62 and 64 mg/l as $CaCO_3$ for least killifish and mosquitofish, respectively.

Tests consisted of exposures to a solvent control (dimethylformamide) and a geometric treatment series of five nominal test substance concentrations (0.625, 1.25, 2.5, 5.0, and 10 μg/l). Water samples were taken at the beginning and end of the test from each of two replicates for endosulfan sulfate analysis. All endosulfan sulfate analyses were based on average measured concentrations. Chemical analyses were done at the Southeast Environmental Research Center Environmental Analytical Research Laboratory. Residues were extracted three times using methylene chloride for a liquid:liquid extraction. Residues were then evaporated, concentrated, and analyzed using a gas chromatograph electron capture detector. Quantification was implemented in a Hewlett-Packard 5890 capillary gas chromatograph (Hewlett-Packard, Avondale, PA, USA) with an electron capture detector (Hewlett-Packard). Two samples from the nominal 0.625 μg/l treatment and one sample from the nominal 2.5 μg/l treatment in the least killifish test were lost during extraction and not included in the calculations used for analyses. The average recovery efficiency of surrogate DBOFB for the least killifish test was 96.3% (standard error: 2.8%; range: 68.7–120.6%). The average recovery efficiency of surrogate PCB103 for the least killifish test was 94.4% (standard error 2.6%, range 69.8–115.5%). The average recovery efficiency of surrogate PCB198 for the least killifish test was 95.2% (standard error 2.9%, range 67.6–113.7%). The average recovery efficiency of surrogate DBOFB for the mosquitofish test was 69.2% (standard error: 2.4%; range: 50.5–94.9%). The average recovery efficiency of surrogate PCB103 for the mosquitofish test was 70.5% (standard error: 2.5%; range: 50.2–101.1%). The average recovery efficiency of surrogate PCB198 for the mosquitofish test was 71.1% (standard error: 2.9%; range: 51.3–107.7%). One of the solvent control replicates for the mosquitofish test had a low recovery for all surrogates (40.4–44.5%) that was qualified and not included in the above calculations. The method detection limit for endosulfan sulfate was 0.0025 μg/l. All analytical work met National Environmental Laboratory Accreditation Program requirements.

Mortality was the end point used to evaluate the acute toxicity of endosulfan sulfate. Mortality measurements were taken three times a day, every 8 h until test termination at 96 h. Dead fish were immediately removed at each measurement interval. Conventional LC_{50}s were calculated using trimmed Spearman–Karber methods (Carriger et al. unpublished data). Survival-time data analysis was conducted following the methods described by

Newman (1995). The accelerated failure time model was chosen to estimate time-to-death at selected concentrations. Fish that survived beyond 96 h were censored. Data from both fish toxicity tests were fit to log-normal, log-logistic, and Weibull distributions using the LIFEREG Procedure in SAS 9.1.3. Natural logs were selected over common logs for the analysis (Zhao and Newman 2004). The accelerated failure time model $[\ln t = a + b \times \ln(\text{concentration}) + \varepsilon]$ estimated the times-to-death at selected concentrations. Error can be distributed in various ways. Here, we assumed that it would either fit a log-normal, log-logistic, or Weibull distribution. After a conversion, the previous equation becomes

$$\mathrm{TTD} = e^{\mu} e^{\beta(\ln \text{concentration})} e^{\sigma W}, \tag{1}$$

where TTD is time-to-death, μ is the intercept, β is a model parameter for the concentration, concentration is in units of micrograms per liter, σ is the scale parameter, and W is a response metameter for a specified distribution (*i.e.*, log-normal, log-logistic, Weibull) (Newman 1995; Zhao and Newman 2004). The response metameter for each distribution can be found in Table 7 in the Appendix of Newman (1995).

From the Weibull, log-logistic, and log-normal models, log-likelihood statistics along with Akaike's information criterion (AIC) were calculated to determine the relative model fits. The AIC is equal to –2(log-likelihood statistics) + 2(number of parameters) (Atkinson 1980). Lower AIC values indicate greater information per model parameter.

When the Weibull model has an adequate fit, proportional hazards for the data can be assumed. With this assumption, one can state that y concentration produces risk x times greater than z concentration (Newman 1995). With log-transformed data, the relative risk from increasing concentrations is calculated using

$$e^{-\beta(\Delta \ln \mathrm{Conc})/\sigma}, \tag{2}$$

where β is a Weibull model parameter for the concentration, Δln Conc is the change in natural log concentration values, and σ is the scale parameter for the Weibull model (Newman 1995). Proportional hazards were used to calculate the risk that might occur to least killifish and mosquitofish from exposure to higher and lower actual or projected environmental concentrations for endosulfan sulfate.

Risk Predictions

From the survival time models developed, various concentrations of total endosulfan were used to predict the proportions of both fish species potentially affected by total endosulfan ($\alpha + \beta +$ sulfate) concentrations at a site of concern in South Florida (site S-178). S-178 is a structure used to regulate water flows on a canal in the Homestead Agricultural Area east of the Everglades National Park. The sampling site at S-178 is surrounded by agriculture. It is frequently monitored as part of the South Florida Water Management District's PEST program (four to six times annually for surface waters and semiannually for sediment) (Miles and Pfeuffer 1997). Consistent detections of endosulfan sulfate have been made in sediment and surface waters at S-178, with less frequent detections of the isomers (Miles and Pfeuffer 1997). Endosulfan sulfate has been found at some of the highest measured concentrations in South Florida at S-178 (Miles and Pfeuffer 1997). Due to its proximity to Everglades National Park, pesticide residues at S-178 have been a source of concern in the South Florida restoration effort (Carriger and Rand 2008; Miles and Pfeuffer, 1997).

Total endosulfan was used for exposure estimates because all three endosulfan products might be in water after agriculture applications (Rand et al. in press). Using total endosulfan allows greater conservativism in risk estimates than estimates of exposure from endosulfan sulfate alone. The 90th centile concentration estimate is used in probabilistic risk assessment as a conservative exposure benchmark (ECOFRAM 1999). The 90th centile concentration was selected because it is found at the upper end of a cumulative log distribution of exposure and represents a more extreme exposure event. For the current assessment, the estimated 90th centile concentration (0.2 µg/l) and the maximum concentration (1.35 µg/l) were selected at S-178 from the work of Carriger and Rand (2008). By applying these exposure concentrations to the experimentally derived failure time models, we estimated how long two native Florida fish species would have to be exposed to endosulfan at known environmentally relevant concentrations before mortalities occur. The toxicity tests were exclusively done with endosulfan sulfate, so all comparisons are made under the assumption that the α and β parent isomers have a similar toxicity to endosulfan sulfate (Rand et al. in press).

Results

Survival Analysis

Table 1 summarizes the results from both fish toxicity tests for each treatment. Concentrations are shown as average measured concentrations for two replicates per treatment on days 0 and 4. Number failed indicates the number of fish that died in the course of the test and the number censored indicates the number that survived (*i.e.*, right censored data). Both toxicity tests had similar outcomes from

Table 1 Summary of the number of censored (survival) and uncensored (mortality) values for the least killifish (LKF) and mosquitofish (MF) acute toxicity test

Species	Nominal concentration (μg/l)	Measured concentration (range) (μg/l)	Total	Failed (mortality)	Censored (survival)	Percent censored
LKF	0.625	0.394 (0.383–0.404)	20	0	20	100
	1.25	0.929 (0.823–1.021)	20	1	19	95
	2.50	1.767 (1.483–1.940)	20	5	15	75
	5.00	3.254 (3.109–3.464)	20	20	0	0
	10.0	6.843 (6.675–7.092)	20	20	0	0
MF	0.625	0.466 (0.447–0.477)	20	0	20	100
	1.25	0.937 (0.884–1.036)	20	0	20	100
	2.50	1.819 (1.544–2.012)	20	4	16	80
	5.00	3.510 (3.322–3.637)	20	19	1	5
	10.0	9.195 (8.209–10.099)	20	20	0	0

Note: Failed = died during the test

exposure to endosulfan sulfate, with least killifish being slightly more sensitive than mosquitofish.

Statistical results from the AIC tests are presented in Table 2. A lower AIC value indicates a better model fit to the data. The Weibull distribution had the best relative fit for both fish species based on lower AIC values. The shape parameter from the Weibull distribution was not used in the accelerated failure time model, but the scale parameter from the distribution was required (see Eq. 1 for the accelerated failure time model requirements).

Table 3 presents the LC_{50}s estimated at 24-h periods using the Weibull, log-normal, and log-logistic accelerated failure time models and trimmed Spearman–Karber calculations. Among the parametric models, LC_{50}s generally did not diverge more than ±0.2 μg/l. For mosquitofish at 24 hs, there was a 0.4 μg/l difference between the Weibull and log-normal models. The Weibull-generated LC_{50}s were generally closer to the log-logistic LC_{50}s than the log-normal ones. The trimmed Spearman–Karber LC50 s were closer to the accelerated failure time models for mosquitofish than for least killifish. Estimated LC_{50}s from the accelerated failure time models were more similar to the trimmed Spearman–Karber LC_{50}s at longer durations (*i.e.*, 72 and 96 h).

Table 4 presents the statistics for the best-fit Weibull and second-best-fit log-logistic distributions for least killifish and mosquitofish. Some of the implications that can be extracted from each of the parameters in Table 4 will be explained. The concentration chi-square values indicate a significant effect on time-to-death from exposure to endosulfan sulfate, with greater concentrations producing deaths more quickly (Newman 1995). The negative values for all βconcentration parameters indicate that survival time decreases with increasing concentration, as expected (Schlueter et al. 2000). When the scale parameters for the Weibull distributions in Table 4 equal 1, then an exponential distribution can be assumed. For both fish, the scale parameters were 0.3. The scale parameters indicate that hazard is not constant, and the greater flexibility of a Weibull distribution over an exponential distribution is required. A higher intercept indicates a longer duration for deaths to occur (Petraitis 1998). Thus, the Weibull model estimates the times to death at longer intervals for mosquitofish than least killifish. The shape parameters for the

Table 2 Log-likelihood statistics and AIC for all candidate distributions and best-fit Weibull accelerated failure time models for the endosulfan sulfate experiments

Species	Model	Log-likelihood	AIC	Accelerated failure time model[a]
LKF[b]	Log-normal	–39.08	84.2	ln T = 5.1939 – 1.1568 * ln (conc) + 0.4237 * W
LKF	Log-logistic	–38.36	82.7	ln T = 5.2251 – 1.1605 * ln (conc) + 0.2341 * W
LKF	Weibull	–34.86	75.7	ln T = 5.3638 – 1.1676 * ln (conc) + 0.2970 * W
MF[c]	Log-normal	–34.84	75.7	ln T = 5.4498 – 1.1496 * ln (conc) + 0.4277 * W
MF	Log-logistic	–33.95	73.9	ln T = 5.4450 – 1.1292 * ln (conc) + 0.2310 * W
MF	Weibull	–31.82	69.6	ln T = 5.6043 – 1.1378 * ln (conc) + 0.3110 * W

[a] See the text for an explanation

[b] LKF = least killifish

[c] MF = mosquitofish

Table 3 Estimated 24-h LC_{50}s from the log-normal, log-logistic, and Weibull accelerated failure time models and comparisons to trimmed Spearman–Karber LC_{50} estimates

Species	Distribution	Duration (h)	LC_{50} (µg/l)
LKF[a]	Log-normal	24	5.7
LKF	Log-logistic	24	5.8
LKF	Weibull	24	5.9
LKF	Trimmed Spearman–Karber	24	3.3
LKF	Log-normal	48	3.1
LKF	Log-logistic	48	3.2
LKF	Weibull	48	3.3
LKF	Trimmed Spearman–Karber	48	2.6
LKF	Log-normal	72	2.2
LKF	Log-logistic	72	2.3
LKF	Weibull	72	2.3
LKF	Trimmed Spearman–Karber	72	2.1
LKF	Log-normal	96	1.7
LKF	Log-logistic	96	1.8
LKF	Weibull	96	1.8
LKF	Trimmed Spearman–Karber	96	2.0
MF[b]	Log-normal	24	7.2
MF	Log-logistic	24	7.4
MF	Weibull	24	7.6
MF	Trimmed Spearman–Karber	24	X[c]
MF	Log-normal	48	3.9
MF	Log-logistic	48	4.0
MF	Weibull	48	4.1
MF	Trimmed Spearman–Karber	48	3.7
MF	Log-normal	72	2.8
MF	Log-logistic	72	2.8
MF	Weibull	72	2.9
MF	Trimmed Spearman–Karber	72	2.7
MF	Log-normal	96	2.2
MF	Log-logistic	96	2.2
MF	Weibull	96	2.3
MF	Trimmed Spearman–Karber	96	2.3

[a] LKF: least killifish

[b] MF: mosquitofish

[c] X: Data were not sufficient for estimating an LC_{50} value

Weibull distributions in Table 4 indicates an increasing rate of mortality ($\gamma > 1$) over time for the two fish species and endosulfan sulfate (Lee and Go 1997).

Due to its ability to describe proportional hazards, a Weibull distribution was chosen for analysis of the survival of mosquitofish and least killifish exposed to endosulfan sulfate. However, a graphic check of the fit of the proportionality of the data was first made. Figures 1 and 2 present the linearization of the toxicity test data for the Weibull model. In order to construct the Weibull regressions for treatments with complete mortalities, cumulative mortalities were only calculated up to the 19th fish out of 20 total per treatment. Parallel lines between treatments indicate a good fit to the Weibull distribution and potential proportional hazards. For each test, at least one of the concentrations did not produce lines that were exactly parallel. In particular, the 3.3-µg/l treatment in least killifish and the 1.8-µg/l treatment in mosquitofish were the least parallel of the three regressions. However, the relatively good fit from AIC statistics indicates that the Weibull model might be suitable for use in the current application.

From the Weibull model, Figure 3 presents the survival response curves for endosulfan sulfate and both fish species. The greater sensitivity of least killifish is observable in Figure 3a over mosquitofish in Figure 3b. The curves for least killifish are closer to each other, indicating that the least killifish are generally more sensitive to endosulfan sulfate than mosquitofish. From the survival–response curves, the percentage of species affected based on estimated concentrations or time of exposure can be predicted for an ecological risk assessment. At 60 h, the LC_{50} is predicted to be higher than the 96-h LC_{50} frequently used in water quality and level of concern setting for fish species. Figure 3 also includes the trimmed Spearman–Karber estimated LC_{50}s and 95% confidence intervals. The LC50 at 96 h estimated from the Weibull accelerated failure time model was closer to the trimmed Spearman–Karber LC_{50} for mosquitofish than for least killifish. However, the Weibull models for both fish species estimated LC_{50}s within the 95% confidence interval from the trimmed Spearman–Karber model.

Risk Predictions

Figures 4 and 5 display the results of the time-to-death using the two exposure benchmarks (the 90th centile, 0.2 µg/l, and the maximum concentration, 1.35 µg/l) for site S-178 in South Florida . It can be seen in Figures 4 and 5 that significant percentages (≥20%) of both fish species are predicted to die from the above two concentrations outside of the durations used in the test. In Figure 4, the models derived from the tests had to be extrapolated to accommodate the estimated 90th centile exposure concentration and the durations to death of ≥5% of the species. The maximum concentration (1.35 µg/l) detected at S-178 potentially produces mortalities in excess of 10% when exposed to this concentration for more than 77 h for least killifish and 96 h for mosquitofish.

From the Weibull proportional hazards model, an increase in concentration from the 90th centile concentration estimate (0.2 µg/l) to the maximum detected concentration at S-178 (1.35 µg/l) would produce relative risks of dying that are 1081 times greater for mosquitofish and

Table 4 Weibull and log-logistic accelerated failure time model statistics for least killifish and mosquitofish

Model	Species	Variable	df	Estimate (SE)	χ^2	$p > \chi^2$
Weibull	Least killifish	Intercept, μ	1	5.3638 (0.12)	2019.2	<0.0001
		[Endosulfan sulfate], β	1	–1.1676 (0.08)	218.0	<0.0001
		Scale, σ	1	0.2970 (0.04)		
		Shape, γ	1	3.3668 (0.40)		
Weibull	Mosquitofish	Intercept, μ	1	5.6043 (0.14)	1605.9	<0.0001
		[Endosulfan sulfate], β	1	–1.1378 (0.09)	197.9	<0.0001
		Scale, σ	1	0.3110 (0.04)		
		Shape, γ	1	3.2154 (0.37)		
Log-logistic	Least killifish	Intercept, μ	1	5.2251 (0.13)	1718.1	<0.0001
		[Endosulfan sulfate], β	1	–1.1605 (0.09)	168.0	<0.0001
		Scale, σ	1	0.2341 (0.03)		
Log-logistic	Mosquitofish	Intercept, μ	1	5.4450 (0.14)	1545.5	<0.0001
		[Endosulfan sulfate], β	1	–1.1292 (0.09)	175.0	<0.0001
		Scale, σ	1	0.2310 (0.03)		

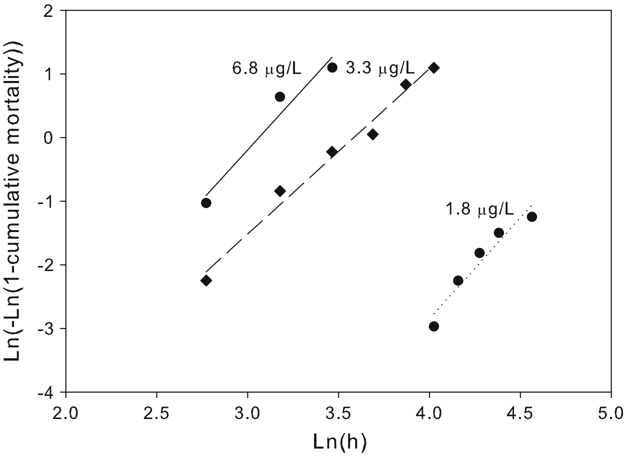

Fig. 1 Weibull linearization of least killifish survival time data

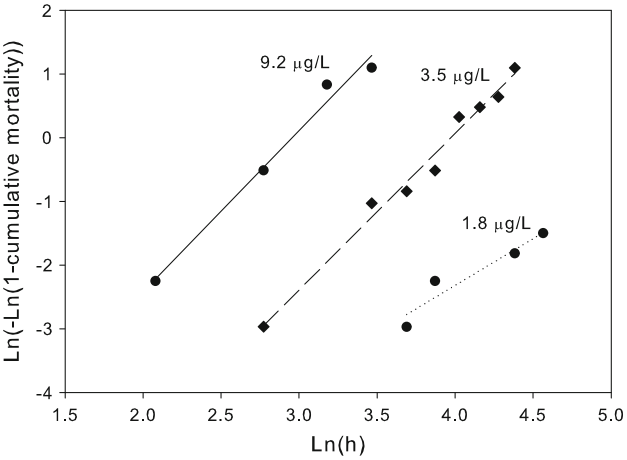

Fig. 2 Weibull linearization of mosquitofish survival time data

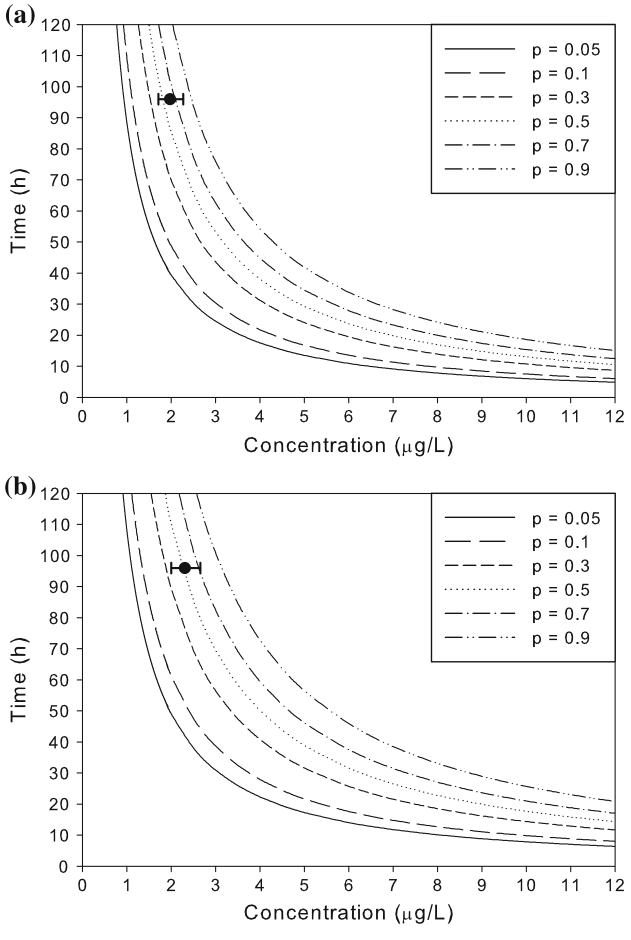

Fig. 3 Survival profiles for least killifish (**a**) and mosquitofish (**b**) exposed to endosulfan sulfate plus 96-h trimmed Spearman–Karber estimated LC_{50} and 95% confidence interval (error bars). p = proportion of dead fish

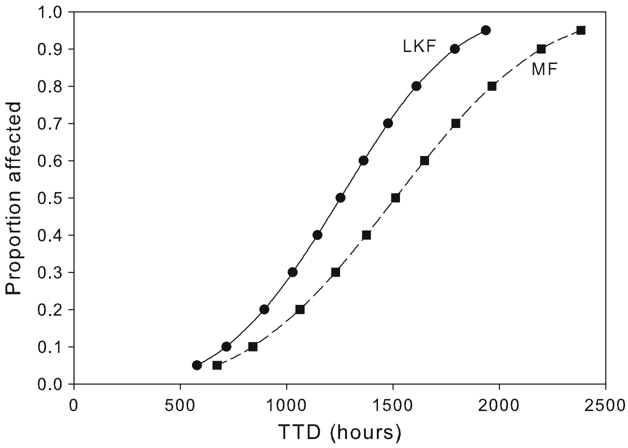

Fig. 4 Predicted times to death (TTDs) of least killifish (LKF) and mosquitofish (MF) based on endosulfan sulfate toxicity data for a potential exposure scenario to a calculated 90th centile concentration estimate for total endosulfan (0.2 μg/l) at S-178

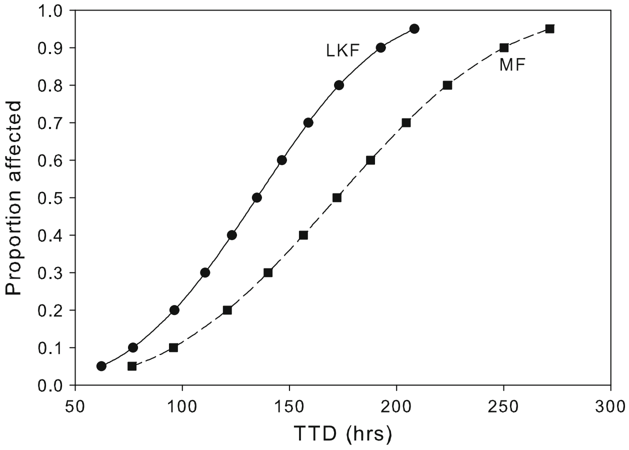

Fig. 5 Predicted times to death (TTDs) of least killifish (LKF) and mosquitofish (MF) based on endosulfan sulfate toxicity data for a potential exposure scenario to the maximum detected concentration for total endosulfan (1.35 μg/l) at S-178

1821 times greater for least killifish. Thus, least killifish that are in 1.35 μg/l endosulfan sulfate are 1821 times more likely to die than least killifish in 0.2 μg/l endosulfan sulfate.

Discussion

Results from the survival analysis models fit to the acute toxicity data for mosquitofish and least killfish produced LC$_{50}$s at 24-h intervals that were similar to what was calculated using trimmed Spearman–Karber estimates. The calculated 96-h LC$_{50}$s from the current tests are consistent with previous 96-h freshwater fish toxicity tests with endosulfan sulfate and rainbow trout (*Oncorhynchus mykiss*) at 1.4 μg/l nominal (Wan et al. 2005) and bluegill sunfish (*Lepomis macrochirus*) at 3.8 μg/l measured (US EPA 2007). The survival-time models for least killifish and mosquitofish confirmed their similar sensitivity to endosulfan sulfate.

Previous screening level ecological risk assessment studies using distributions of LC$_{50}$s for fish species and exposure concentrations at S-178 found exceedences of concern for total endosulfan (Carriger and Rand 2008, Rand et al. in press). When the results from the current toxicity tests were compared to environmental concentrations from these screening-level ecological risk assessments, the durations necessary to potentially impact 10% or more of the populations of native South Florida fish species for the maximum concentration (1.35 μg/l) was 96 h for mosquitofish. For least killifish, the predicted duration to impact 10% of the fish population was 77 h for the same concentration. The geographic scale of the results is focused on concentrations at a site surrounded by agriculture that has some of the highest historical surface water concentrations of endosulfan sulfate in all of South Florida. However, the survival analysis models reported in the current article can be applied to other sites and scenarios with endosulfan sulfate and small demersal fish. In addition, the exposure values used for risk predictions included the α and β isomers as well as the sulfate, so knowledge of the toxicity of the former might be important in understanding the survival dynamics of similar fish species potentially exposed to endosulfan sulfate.

In order to conduct an acute survival analysis experiment, measurements beyond every 24 h are required. The benefits of the additional information on potential risks more than offset the extra work gathering the data. Costs could potentially be higher for the additional work, but having survival analysis measurements allows regulators to see relative risk scenarios for different levels of a chemical stressor as well as the duration necessary for a fish kill or mass mortality from exposure to a chemical. As pointed out previously (Newman 1995; Zhao and Newman 2004), acute toxicity tests designed to produce conventional LC/EC$_{50}$s do not impart enough information for ecological decision-making or to determine the true sensitivity of species. However, additional considerations beyond the LC$_{50}$ at 96 h can be used (e.g., the slopes from dose–response curves) to circumvent the assumptions that an LC$_{50}$ is protective of a population or community of organisms (Hart 2001). A robust time-to-death model would allow analysts to take into account the slope at any single time period during a toxicity test, or beyond, if the baseline hazard function can be represented.

By observing survival at intervals less than 24 h, we were able to fit models to our toxicity test data that provided significant implications about the effects of endosulfan sulfate on both fish species. In addition to deriving conventional LC_{50}s, we predicted time-to-death, relative risks, and hazard increases to least killifish and mosquitofish populations from endosulfan sulfate exposures. The survival response curves will allow future risk assessments to consider the duration as well as the concentration necessary for adversely affecting proportions of species' populations.

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References

Atkinson AC (1980) A note on the generalized information criterion for choice of a model. Biometrika 67:413–418

Carriger JF, Rand GM (2008) Aquatic risk assessment of pesticides in surface waters in and adjacent to the Everglades and Biscayne National Parks: II. Probabilistic analyses. Ecotoxicology 17:680–696

Carriger JF, Rand GM, Gardinali PR, Perry WB, Tompkins MS, Fernandez AM (2006) Pesticides of potential ecological concern in sediment from south Florida canals: an ecological risk prioritization for aquatic arthropods. Soil Sed Contam 15:21–45

ECOFRAM (1999) ECOFRAM aquatic report. Ecological Committee on FIFRA Risk Assessment Methods, report from the Aquatic Workgroup to the United States Environmental Protection Agency Office of Pesticide Programs, Washington DC

FDOACS (2003) Summary of agricultural pesticide usage in Florida: 1999–2002. Florida Department of Agriculture and Consumer Services, Division of Agricultural Environmental Services. Bureau of Pesticides, Tallahassee, FL

Hart A (2001) Probabilistic risk assessment for pesticides in Europe: implementation & research needs. A report from the European Workshop on Probabilistic Risk Assessment for the Environmental Impacts of Plant Protection Products, The Netherlands

Lee ET, Go OT (1997) Survival analysis in public health research. Annu Rev Public Health 18:105–134

Lorenz JJ, Serafy JE (2006) Subtropical wetland fish assemblages and changing salinity regimes: implications for Everglades restoration. Hydrobiology 569:401–422

Macek KJ, Lindberg MA, Sauter S, Buxton KS, Costa PA (1976) Toxicity of four pesticides to water fleas and fathead minnows. US Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN

Miles CJ, Pfeuffer RJ (1997) Pesticides in canals of South Florida. Arch Environ Contam Toxicol 32:337–345

Navarro S, Barba A, Segura JC, Oliva J (2000) Disappearance of endosulfan residues from seawater and sediment under laboratory conditions. Pestic Manage Sci 56:849–854

Newman MC (1995) Quantitative methods in aquatic ecotoxicology. Lewis Publishers, Boca Raton, FL

Petraitis PS (1998) Timing of mussel mortality and predator activity in sheltered bays of the Gulf of Maine, USA. J Exp Marine Biol Ecol 231:47–62

Rand GM, Carriger JF, Gardinali PR, Castro J (in press) Endosulfan and its metabolite, endosulfan sulfate, in freshwater ecosystems of South Florida: a probabilistic aquatic ecological risk assessment. Ecotoxicology

Schlueter MA, Guttman SI, Duan Y, Oris JT, Huang X, Burton GA (2000) Effects of acute exposure to fluoranthene-contaminated sediment on the survival and genetic variability of fathead minnows (*Pimephales promelas*). Environ Toxicol Chem 19:1011–1018

Shivaramaiah HM, Sanchez-Bayo F, Al-Rifai J, Kennedy IR (2005) The fate of endosulfan in water. J Environ Sci Health B 40: 711–720

US EPA (2007) Appendix 1 to 2007 addendum: environmental fate and ecological risk assessment of endosulfan. US Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Washington DC

Wan MT, Kuo J-N, Buday C, Schroeder G, Van Aggelen G, Pasternak J (2005) Toxicity of α-, β-, $(\alpha + \beta)$-endosulfan and their formulated and degradation products to *Daphnia magna*, *Hyalella azteca*, *Oncorhynchus mykiss*, *Oncorhynchus kisutch*, and biological implications in streams. Environ Toxicol Chem 24:1146–1154

Zhao Y, Newman MC (2004) Shortcomings of the laboratory-derived median lethal concentration for predicting mortality in field populations: eExposure duration and latent mortality. Environ Toxicol Chem 23:2147–2153