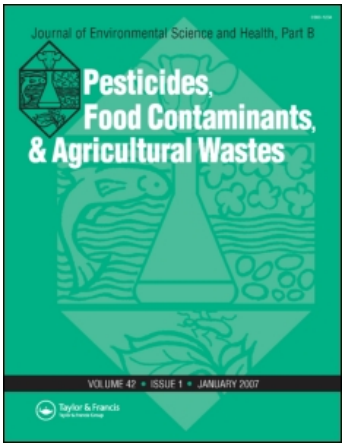

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Acute toxic effects of endosulfan sulfate on three life stages of grass shrimp, *Palaemonetes pugio*

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In this study, the toxicity of endosulfan sulfate, the primary degradation product of the insecticide endosulfan, was determined in three life stages of the grass shrimp (*Palaemonetes pugio*). After 96 h exposure to endosulfan sulfate, the grass shrimp adult LC50 was 0.86 μg/L (95% CI 0.56–1.31), the grass shrimp larvae LC50 was 1.64 μg/L (95% CI 1.09–2.47) and the grass shrimp embryo LC50 was 45.85 μg/L (95% CI 23.72–88.61 μg/L). This was compared to the previously published grass shrimp 96-h LC50s for endosulfan. The toxicity of the two compounds was similar for the grass shrimp life stages with adults more sensitive than larvae and embryos. The presence of sediment in 24h endosulfan sulfate–exposures raised LC50s for both adult and larval grass shrimp but not significantly. The USEPA expected environmental concentrations (EEC) for total endosulfan and endosulfan sulfate and the calculations of risk quotients (RQ) based on the more sensitive adult grass shrimp 96-h LC50 clearly show that environmental concentrations equal to acute EECs would prove detrimental to grass shrimp or other similarly sensitive aquatic organisms. These results indicate that given the persistence and toxicity of endosulfan sulfate, future risk assessments should consider the toxicity potential of the parent compound as well as this degradation product.

Keywords: Endosulfan sulfate; endosulfan; *Palaemonetes pugio*; insecticide.

Introduction

Cyclodiene insecticides, such as endosulfan, are used in commercial agriculture for food and non-food crops in many parts of the world. In the United State, an estimated 743,000 lbs of endosulfan active ingredient was applied between 2002 and 2006 to 17 different crops with the top three being cotton, tomato and apple.[1] The cyclodiene insecticides are neurotoxic and inhibit nervous system function by blocking the gamm-aminobutyric (GABA)-gated chloride channels.

Technical grade endosulfan is a racemic mixture of two isomers with 70% α and 30% ß. Both isomers degrade to the major degradation product endosulfan sulfate. This transformation is mainly by soil metabolism where endosulfan sulfate has a half-life of up to six years. Endosulfan sulfate is more persistent than parent isomers and represents 55% of total endosulfan (α+ ß + sulfate) measured in aquatic systems.[2] It has been well documented that endosulfan can migrate over long distances with detections found in many remote regions.[3] In areas of intensive agricultural activity such as southern Florida, USA, endosulfan levels have been found to exceed the USEPA freshwater and saltwater chronic criteria of 0.056 and 0.0087 μg/L, respectively.[4–5]

Endosulfan sulfate has also been detected in surface waters in various areas throughout the world. Maximum endosulfan sulfate levels have ranged from 0.038 μg/L in stream waters of Brazil[6] to 0.28 μg/L in Canadian farm ditches.[7] In the United States, endosulfan sulfate levels in surface waters have ranged up to 0.26 μg/L in the Chesapeake Bay region of Maryland.[8] In southern Florida, maximum endosulfan sulfate measurements have been reported to be 0.11 μg/L by Pfeuffer and Rand[9] in drainage canals monitored over a nine–year period. In this same region over a two–year sampling period, Harman-Fetcho et al.[10] detected endosulfan sulfate levels up to 0.00028 μg/L. In another study of South Florida drainage canals, DeLorenzo et al.[11] sampled 11 sites over a two–year period with maximum endosulfan sulfate levels ranging from 0.002 to 0.218 μg/L. In South Carolina, USA, in tidal creeks adjacent to agricultural fields, maximum endosulfan sulfate levels over a two–year period ranged from 0.119 to 0.147 μg/L. Over this two year period, endosulfan sulfate represented 43% of the total endosulfan measured.[12] Despite

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its frequent occurrence, no water quality criteria have been developed in the United States for endosulfan sulfate and there is a lack of toxicity data available for this degradation products.

This lack of data is especially important in coastal regions where, at least for endosulfan toxicity, saltwater organisms are generally considered more sensitive than freshwater organisms.[13] For this reason, the grass shrimp (*Palaemonetes pugio*), a common saltwater crustacean found along the Atlantic Coast of the United States, was chosen as the model test species for the pesticide exposures.[14] The purpose of this research was to determine endosulfan sulfate toxicity test values for grass shrimp and compare these values to previously reported values for the parent compound endosulfan to further define risk assessments of this degradation product.

Materials and methods

Collection and maintenance

Grass shrimp were collected from Leadenwah Creek (N 32°36′12″ ; W 80°07′00″), a relatively pristine tidal tributary of the North Edisto River Estuary, SC. Shrimp were acclimated in 76-L tanks at 25°C, 20 parts per thousand (ppt) salinity and 16-h light: 8-h dark cycle and fed a mixture of Tetramin® Fish Flakes and newly hatched *Artemia*. Gravid females were placed in brooding traps to allow larvae (zoea) to hatch and escape without interference. Larvae from at least 10 females were pooled for all tests. Embryos (Stage VI –oval eyespots, rapid heartbeat) for the embryo toxicity test were excised immediately prior to the test from at least 5 gravid females.

Chemical analysis

For the toxicity tests, technical grade endosulfan sulfate (99% purity) was obtained from Chem Service Inc. (West Chester, PA). The stock solution for dosing was made in 100% pesticide grade acetone which was used as a carrier in all tests. All treatments and the control received the same carrier concentration (0.1%). Analytical grade endosulfan sulfate was obtained from O2SI (Charleston, SC). D4-Endosulfan II was obtained from Cambridge Isotope Laboratories (Andover, MA) and was used as an internal standard.

Gas chromatography (GC) with tandem mass spectrometry (MS) detection was performed to confirm the nominal concentration of the endosulfan sulfate stock solution. Mass spectral data was acquired in the negative chemical ionization (GC/NCI/MS) mode using an Agilent 6890 GC (Agilent Technologies, Inc., Palo Alto, CA) coupled to an Agilent 5973 mass selective detector with a Gerstel PTV Large Volume Injector. A DB-5ms column was used for the separation of analytes in the GC. The quantitation and confirmation ions monitored for endosulfan sulfate were 385.80 and 387.80, respectively. The quantitation and confirmation ions monitored for the internal standard were 413.80 and 411.80, respectively. A ten-point calibration curve was used to verify the endosulfan sulfate concentration of the working stock solution; standards ranged in concentration from 0.05 ng/mL – 40 ng/mL. The stock solution was quantified to be 98.4% and 99.9% (n=2) of nominal for the 50 mg/L stock solution. All nominal treatment concentrations were made from this stock.

Adult toxicity tests

Two types of tests were conducted with adult grass shrimp. For the aqueous tests, adult grass shrimp were exposed in 4-L wide mouth glass jars containing 2-L of seawater at a salinity of 20 parts per thousand (ppt). Ten adult shrimp were randomly placed in each jar. The jars were incubated for 96 h with aeration on a 16-h light: 8-h dark cycle in a 25°C environmental chamber. Water quality parameters taken were temperature (°C), pH, salinity (ppt) and dissolved oxygen (mg/L). Every 24 hours dead shrimp were removed and the test solutions were renewed. Adult shrimp were not fed during the test. The nominal concentrations used for the 96-h endosulfan sulfate aqueous test were 0, 0.313, 0.625, 1.25, 2.50, and 5.00 μg/L.

For the 24-h endosulfan sulfate sediment test, 340 g of thoroughly mixed, unsieved sediment was added to each jar followed by 2 L of seawater. The sediment was allowed to settle 24 hours before dosing the water column. The nominal concentrations (in water) used for this test were 0, 0.625, 1.25, 2.5, 5.0, and 10.0 μg/L. Other test conditions were run as described previously.

Larval toxicity tests

Two types of tests with larval grass shrimp were conducted as with adults - a 24 h static test with sediment and a 96 h static-renewal test with no sediment. All tests were conducted as described above except 600 mL beakers were used. For the sediment tests, 68 g of sediment was used with 400 mL of seawater. There were 10 larvae per beaker with three replicates per treatment. Larvae were fed *Artemia* for the duration of the tests and tests were run as described for adults previously. The 96-h endosulfan sulfate seawater only concentrations were 0, 0.156, 0.313, 0.625, 1.25 and 2.5 μg/L. The 24-h endosulfan sulfate concentrations for the exposures with sediment were 0, 0.313, 0.625, 1.25, 2.50 and 5.00 μg/L.

Embryo toxicity tests

One toxicity test was run with grass shrimp embryos – a 96 h aqueous static-renewal test. Embryo exposure chambers were 24-well plates (sterile, polystyrene) with 2 mL test solution, one Stage VI embryo/well and one plate per control

and treatment. Plates were placed on an orbital shaker at 80 rpm in a 27°C environmental chamber with a 24-h dark cycle. The salinity of the control and treatment test solutions was 20 ppt. As with the adult and larval tests, every 24 h the test solution was renewed and any dead embryos removed. The nominal endosulfan sulfate concentrations were 0, 12.5, 25.0, 50.0, 100.0, 200.0 μg/L.

Statistics

The Median Lethal Concentration, $LC_{50,}$ was calculated with 95% confidence intervals using the Trimmed Spearman-Karber Method.[15] Differences between LC50 values were determined by an LC50 ratio test.[16] For the mortality data, analysis of variance (ANOVA) followed by Dunnett's procedure for comparison to the control response was performed to determine the Lowest Observed Effect Concentration (LOEC) and the No Observed Effect Concentration (NOEC).[17] For all statistical tests, alpha was set to 0.05 a priori.

Results

Average water quality parameters for the endosulfan sulfate toxicity tests are shown in Table 1. Dissolved oxygen levels ranged from 6.43 to 6.91 mg/L, pH ranged from 7.71 to 8.04, temperature ranged from 23.30 to 25.42 °C and salinity ranged from 19.62 to 20.40 ppt. These ranges were all within the acclimated conditions described previously and within natural grass shrimp field conditions.[14]

The results show that the 96-h endosulfan sulfate LC50s for embryos, larvae and adults were 45.85 μg/L (95% CI 23.72–88.61 μg/L), 1.64 μg/L (95% CI 1.09–8.87 μg/L) and 0.86 μg/L (95% CI 0.56–1.31 μg/L), respectively (Table 2). Embryos were significantly less sensitive to endosulfan sulfate than larvae and adults. Larval and adult grass shrimp sensitivities were statistically similar. However, LOEC and NOEC values for the aqueous–only exposures emphasized that adult grass shrimp were more sensitive than larvae and embryos (Table 2). The highest treatment in the embryo 96-h test, 200.0 μg/L, had 25% mortality within 72 hours. The highest treatment in the larval 96-h test, 2.5 μg/L, had 23.4% mortality within 72 hours. The highest treatment in the adult 96-h test, 5.0 μg/L, had 90% mortality within 48 hours.

In the 24-h exposures, the presence of sediment did not result in a significant decrease in toxicity for adults compared to the 24-h aqueous test. Since a 24-h aqueous LC50 was not obtained for larvae, comparisons to the 24-h exposure in the presence of sediment were not made (Table 2).

Table 1. Average water quality parameters with standard error for the embryo, larvae and adult grass shrimp endosulfan sulfate toxicity tests.

Lifestage Test	*Dissolved Oxygen (mg/L)*	*pH*	*Temperature (°C)*	*Salinity (ppt)*
Embryo	6.43 ± 0.13	8.04 ± 0.05	25.42 ± 0.79	20.16 ± 0.16
Larvae	6.91 ± 0.14	7.71 ± 0.18	24.22 ± 0.29	20.40 ± 0.40
Adult	6.92 ± 0.15	7.90 ± 0.08	23.30 ± 0.63	19.62 ± 0.32

Table 2. 96-h aqueous and 24-h sediment LC50 values (μg/L) with corresponding lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) values for grass shrimp embryos, larvae and adults exposed to endosulfan sulfate and endosulfan.

Test and Lifestage	*LC50 (95% confidence interval)*	*LOEC*	*NOEC*
	Endosulfan sulfate		
96-h Embryo (aqueous)	45.85 (23.72–88.61)	25.0	12.5
24-h Larvae (aqueous)	>2.50	–	–
96-h Larvae (aqueous)	1.64 (1.09–2.47)	1.25	0.625
24-h Adult (aqueous)	4.20 (1.99–8.87)	1.25	0.625
96-h Adult (aqueous)	0.858 (0.562–1.31)	0.313	<0.313
24-h Larvae (sediment)	2.96 (2.35–3.73)	0.625	0.313
24-h Adult (sediment)	6.63 (5.08–8.64)	0.625	<0.625
	Endosulfan		
96-h Embryo* (aqueous)	117.0 (0.73–18,810.0)	12.5	<12.5
96-h larvae* (aqueous)	2.56 (1.82–3.59)	1.25	0.63
96-h adult+ (aqueous)	1.01 (0.72–1.43)	0.1	0.01

*Key et al.[14]
+Scott et al.[25]

Discussion

The grass shrimp life stages showed a similar response to the parent compound, endosulfan,[18] as to the degradation product, endosulfan sulfate (Table 2). In comparing these results for 96-h exposures, toxicity was similar for both compounds in adult and larval grass shrimp. Due to the wide confidence intervals for endosulfan exposed embryos, there was no statistical difference with the endosulfan sulfate exposed embryos even though the 96-h LC50s were more than two times apart (Table 2). Lower embryo toxicity relative to adult and larval grass shrimp may be due to several physiological conditions as discussed by Key et al.[18]: (i) presence of an embryonic coat that protects the embryo until just before hatching; (ii) immaturity of the

Table 3. Estimated environmental concentrations (EEC) for total endosulfan (α+ß+sulfate) and endosulfan sulfate for three Southeast US crops along with corresponding risk quotients (RQ) for adult grass shrimp.

Crop	*Chemical*	*Peak EEC for maximum labeled use rate* (μg/L)*	*Risk Quotient** (EEC/96-h LC50)*
Cotton	Total Endosulfan	7.53	7.45
	Endosulfan Sulfate	4.14	4.83
Tobacco	Total Endosulfan	6.87	6.80
	Endosulfan Sulfate	3.78	4.41
Tomato	Total Endosulfan	19.1	18.91
	Endosulfan Sulfate	10.5	12.24

*USEPA.[2]
**USEPA.[24]

embryonic nervous system; (iii) metabolic rate differences from larvae and adults; and (iv) development of phase 1 monooxygenases.

Some saltwater aquatic organisms exposed to endosulfan sulfate for 96 h have shown lower sensitivity than grass shrimp but within the same order of magnitude. Tests with sheepshead minnow (*Cyprinodon variegatus*) and mysids (*Americamysis bahia*) revealed 96-h LC50s of 3.1 and 7.9 μg/L, respectively.[3] Research with freshwater organisms has shown mixed results in acute toxicity comparisons. Rainbow trout (*Oncorhynchus mykiss*) and the invertebrate *Daphnia magna* were significantly less sensitive to endosulfan sulfate than endosulfan. However, the invertebrate *Hyalella azteca* showed no difference in toxicity between the two compounds. The authors also studied effects on another salmonid fish and a freshwater crustacean and concluded that endosulfan sulfate was at least as toxic as endosulfan.[19]

The presence of sediment in this current study did not significantly raise LC50 values of endosulfan sulfate exposed shrimp. The only other reported estuarine sediment test with endosulfan sulfate was with the amphipod, *Leptocheirus plumulosus*, exposed for 10 days resulting in an EC50 (survival) of 74 μg/L based on pore water concentrations.[3] Sorption studies with α and ß endosulfan reported by the USEPA[2] have shown these compounds to have a high affinity for sediments. Endosulfan sulfate has not had a similar study but it is expected to be comparable to the parent compound.[2] Endosulfan sulfate is most probably formed by the metabolism of soil microbes. This degradation product can be formed directly in the contaminated water body from endosulfan spray drift or runoff, or it can be carried there in the degraded form via water or sediment runoff.[20] Associated research with total endosulfan (α+ ß + sulfate) has shown that bioconcentration occurs in estuarine fish and invertebrates including grass shrimp[21–22] since endosulfan is lipophilic and can easily diffuse into cells.[23]

The USEPA has determined expected environmental concentrations (EECs) for total endosulfan and endosulfan sulfate for several agricultural crops that are allowed to be treated with endosulfan products within southeastern US coastal counties.[2] Using these acute peak water concentrations allows for the calculations of risk quotients (RQs) based on the more sensitive adult grass shrimp life stage 96-h LC50. When these RQs are compared to a preset level of concern (LOC)[24] of 0.5, it is clearly seen that if these acute EECs occurred, grass shrimp populations or other similarly sensitive aquatic organisms would be at risk (Table 3). These EECs are a worst case scenario and actual published measurements of endosulfan sulfate in the United States have not been as high. As stated earlier, maximum published endosulfan sulfate levels measured in US surface waters have ranged from 0.218 to 0.26 μg/L.[8,11] While these measured values are all below grass shrimp LOECs for embryos, larvae and adults (Table 2), caution should still be taken since EECs show the potential for higher values to occur. Also, it must be remembered that measured endosulfan sulfate values represent only 43% to 55% of the total endosulfan in the water column[2,12] so that total endosulfan levels could exceed the LOECs presented in this research.

With the continued use of endosulfan, its degradation products will also continue to be a risk to aquatic organisms. Further research is needed on the effects of endosulfan sulfate on biological markers of exposure and the effects on saltwater invertebrates after chronic exposures. The results of this research and other research discussed indicate that, given the persistence and toxicity of endosulfan sulfate, future risk assessments should consider the toxicity potential of this degradation product in addition to its parent compound, endosulfan.

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