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Acute Toxicity of Diazinon to the Amphipod, *Gammarus pseudolimnaeus*: Implications for Water Quality Criteria Development

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Both the acute and chronic criteria for the organophosphate insecticide diazinon (100 ng/L) are presented in the U. S. Environmental Protection Agency's (USEPA) recently released water quality criteria document (USEPA 2000). The acute criterion is driven by the four lowest genus mean acute LC50 values in the diazinon data. These values are: *Gammarus fasciatus* = 200 ng/L; *Ceriodaphnia dubia* = 377 ng/L; *Daphnia magna* = 902 ng/L; and *Simocephalus serrulatus* = 1,587 ng/L. The second, third and fourth most sensitive species on this list are cladocerans, and the low toxicity values for each are logical and well documented by Giddings et al. (2000). However, the acute toxicity value for the amphipod, *G. fasciatus*, identified as the most sensitive species, is not logical since acute toxicity values for other amphipod species are much higher (2,000 to 184,000 ng/L as reported in Giddings et al. 2000). Also, the study reporting the low acute value for *G. fasciatus* was conducted in 1966 with unmeasured concentrations of 89% technical grade diazinon (i.e., possible toxic impurities) using one replicate per concentration (Johnson and Finley 1980; Mayer and Ellersieck 1986). Careful review of the raw data sheets for the 1966 study also revealed a "units problem" in the calculation of the LC50 values as it appears that the 96 h LC50 should be 2,000 ng/L and not 200 ng/L.

The objective of this study was to determine the acute (96h) toxicity of diazinon to the amphipod, *Gammarus pseudolimnaeus* and discuss regulatory implications. *G. pseudolimnaeus* is a closely related species in the same genus as *G. fasciatus,* and was tested as a surrogate for *G. fasciatus* because *G. fasciatus* is not commercially available. This approach is well grounded as Suter et al (1993) have documented that closely related species (i.e., species within the same genus) are expected to demonstrate similar sensitivity to chemical stressors. The Stephen et al. (1985) guidelines for developing water quality criteria also recognize that closely related species exhibit similar sensitivity to chemical stressors by using "genus mean acute values" (a geometric mean of all species within the same genus) in the calculation of a final acute value.

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MATERIALS AND METHODS

The test methods used for these experiments are modified from an EPA testing guideline for amphipods (USEPA 1986) and described in detail in Hall and Anderson (2004). Two static-renewal 96-h range-finding experiments and one 96-h definitive toxicity test were conducted with the amphipod *G. pseudolimnaeus.* Two range-finding tests were needed to accurately determine the range of concentrations needed for the definitive test. Well water from the University of Maryland's Aquatic Toxicology was used for testing mature amphipods obtained from Environmental Consulting and Testing in Superior, Wisconsin. Four replicates of 10 organisms each were tested in one liter glass beakers. Concentrations of diazinon were measured in the test containers for all three tests. Analytical grade diazinon (100% pure) used for these experiments was obtained from AccuStandard Inc., New Haven, Connecticut (CAS # 333-41-5, lot #12838). Wildlife International Ltd. in Easton, Maryland conducted the diazinon analysis using a GC/MS method with a detection limit of 50 ng/L described in detail in Hall and Anderson (2004). LC50 values with 95% confidence limits were calculated using the Trimmed Spearman Karber method.

RESULTS AND DISCUSSION

Water quality conditions measured during the two range-finding tests and the one definitive test are presented in Table 1. The four-day average temperature, pH, dissolved oxygen, and conductivity measurements were similar among the test conditions within each of the three experiments. Hardnesss (62.5 mg/L), alkalinity (100 mg/L), total organic carbon (0.28 mg/L), total nitrogen (27.96 μM), and total phosphorous (0.70 μM) were also reported for the laboratory well water used for the toxicity tests.

Nominal and measured diazinon concentrations were reported for the two range-finding experiments and one definitive test in Table 2. Generally, the nominal concentrations and measured concentrations for the range-finding tests were within 14% or less of each other when considering only the mean values for each experiment. For the definitive test, the test mean recovery was 105% (only a 5% difference between the nominal and measured test concentration). Results from the diazinon analytical measurements are presented in detail in Hall and Anderson (2004).

The *Gammarus* survival data for the two range-finding tests and the definitive test are presented in Table 3. The percent control survival (>80%) was acceptable for all three tests. For the first range-finding experiment, 75% survival was reported at the highest test concentration (9,970 ng/L) after 4 days. *Gammarus* survival after four days of exposure ranged from 0 to 2.5% at diazinon concentrations >

50,000 ng/L in the second range finding test. For the definitive test, the highest test concentration (32,000 ng/L) resulted in 12.5% survival after four days.

Table 1. Average water quality values from four-day diazinon acute toxicity tests with *Gammarus pseudolimnaeus* on February 16, March 1, and March 15, 2004.

Test	Nominal Concentration (ng/L)	Temperature (° C)	pH	Conductivity (µs/cm)	D. O. (mg/L)
Range-Finding 1	Control[a]	18.1	8.29	267.0	9.16
	100	18.4	8.23	262.7	8.88
	316	18.8	8.26	268.3	8.84
	998	18.6	8.31	269.8	8.70
	3,160	18.6	8.31	267.7	8.81
	9,970	18.9	8.28	270.6	8.56
	Test Mean	18.6	8.28	267.7	8.83
Range-Finding 2	Control	17.8	8.27	263.1	9.36
	5,000	17.8	8.24	256.9	9.15
	15,800	17.8	8.25	264.5	9.30
	50,000	17.9	8.31	264.0	9.72
	158,000	17.4	8.54	255.7	11.46
	500,000	17.4	8.54	255.7	11.46
	Test Mean	17.7	8.36	260.0	10.08
Definitive	Control	18.0	8.28	263.1	9.44
	2,000	18.1	8.26	262.0	9.18
	4,000	17.8	8.30	261.9	9.23
	8,000	17.9	8.26	261.3	8.95
	16,000	17.8	8.27	261.1	9.30
	32,000	18.0	8.31	262.2	9.39
	Test Mean	17.9	8.28	261.9	9.25

[a] The toxicity lab well water (Control water) was analyzed for the following parameters: Hardness = 62.5 mg/L, Alkalinity = 100 mg/L, Total Organic Carbon = 0.2762 mg/L, Total Nitrogen = 27.96 µM, Total Phosphorous = 0.70 µM.

The 48, 72, and 96 h LC50 values for the definitive *Gammarus* toxicity test are 27,290, 20,210, and 16,820 ng/L, respectively, based on measured concentrations (Table 4). The 24 hour LC50 value could not be calculated because mortality did not exceed 50% in any treatment.

The Stephen et al. (1985) guidelines for developing water quality criteria state that "acute values that appear to be questionable in comparison with other acute or chronic values for the same species and for other species in the same genus should not be used in the calculation of a species mean acute value". We believe that this statement applies to the *G. fasciatus* data point used for the calculation of the diazinon acute water quality criterion (USEPA, 2000).

Table 2. Nominal and measured concentrations of diazinon in the toxicity test solutions.

	Diazinon Concentration (ng/L)			
Test	Nominal Concentration	Day 0	Day 4	Mean
Range- Finding 1	100	85	132	108.5
(February 16, 2004)	998	798	768	783
	9,970	7,876	6,480	7,178
Range- Finding 2	5,000	4,460	4,450	4,455
(March 1, 2004)	50,000	44,000	43,000	43,500
	500,000	390,000	[a]	[a]
Definitive	2,000	2,440	2,040	2,240
(March 15, 2004)	4,000	4,760	3,920	4,340
	8,000	8,960	7,680	8,320
	16,000	16,320	14,720	15,520
	32,000	35,200	29,760	32,480

[a] Not measured as there were no surviving amphipods in the test treatment.

Table 3. *Gammarus pseudolimnaeus* survival during four day acute toxicity tests with diazinon (nominal concentrations) in February and March, 2004.

Range-Finding Test 1 Percent Survival (February 16, 2004)						
Day of Test	Control	100 (ng/L)	316 (ng/L)	998 (ng/L)	3,160 (ng/L)	9,970 (ng/L)
Day-1	97.5	100	100	92.5	95.0	92.5
Day-2	97.5	92.5	95.0	90.0	95.0	82.5
Day-3	95.0	80.0	95.0	87.5	85.0	80.0
Day-4	95.0	72.5	95.0	82.5	82.5	75.0
Range-Finding Test 2 Percent Survival (March 1, 2004)						
Day of Test	Control	5,000 (ng/L)	15,800 (ng/L)	50,000 (ng/L)	158,000 (ng/L)	500,000 (ng/L)
Day-1	100	87.5	82.5	40.0	0.0	0.0
Day-2	90.0	85.0	50.0	10.0	0.0	0.0
Day-3	85.0	70.0	35.0	5.0	0.0	0.0
Day-4	80.0	55.0	30.0	2.5	0.0	0.0
Definitive Test Percent Survival (March 15, 2004)						
Day of Test	Control	2,000 (ng/L)	4,000 (ng/L)	8,000 (ng/L)	16,000 (ng/L)	32,000 (ng/L)
Day-1	97.5	100	90.0n	92.5	87.5	75.0
Day-2	92.5	85.0	80.0	82.5	72.5	35.0
Day-3	90.0	72.5	70.0	77.5	55.0	22.5
Day-4	82.5	67.5	62.5	70.0	45.0	12.5

The *G. pseudolimnaeus* 96-h LC50 value of 16,820 ng/L reported in this study is almost two orders of magnitude higher than the LC50 of 200 ng/L for a closely related gammarid in the same genus (*Gammarus fasciatus*). Our reported 96-h LC50 is much more consistent with other acute toxicity values reported for other amphipods (2,000 to 184,000 ng/L) as reported in Giddings et al. 2000. Our 96 h LC50 is also comparable, but lower, than a recently reported 96h diazinon LC50 of ~ 60,000 ng/L for *G. pseudolimnaeus* (Marines et al. 2004). Further, our experiments are based on measured diazinon concentrations, while the previous *G. fasciatus* study (Johnson and Finley 1980; Mayer and Ellersieck 1986) was not. Measuring concentrations of chemicals in test containers during toxicity tests is preferred over the use of nominal concentrations in the development of water quality criteria (Stephen et al. 1985). In addition, two range-finding experiments with measured diazinon concentrations in tests containers were also used in our study to establish a precise range of concentrations needed for the definitive test. This level of preliminary testing (two range-finding experiments) is beyond standard procedures used for most aquatic toxicity testing with chemicals.

Table 4. Diazinon LC50 values (calculated using measured concentrations) from the four-day definitive acute toxicity test with *Gammarus pseudolimnaeus* during March, 2004.

Test Start Date	Time Period (hours)	LC50 Value (ng/L)	95% Lower Conf. Limit	95% Upper Conf. Limit
3/15/04	24[a]	--	--	--
	48	27,290	22,450	33,180
	72	20,210	15,790	25,870
	96	16,820	12,820	22,080

[a] LC50 value was not calculated because mortality did not exceed 50% in any treatment.

Since only the four lowest genus mean acute values are used to derive the final acute value, and the LC50 for *G. fasciatus* (200 ng/L) is currently the lowest acute value in the data set used to derive the final acute toxicity value for diazinon (USEPA 2000), replacing the *G. fasciatus* LC50 value of 200 ng/L with the *G. pseudolimnaeus* 96-h LC50 of 16,820 ng/L shifts the four lowest genus mean acute values in the acute diazinon data set to: 370 ng/L for *Ceriodaphnia dubia*; 902 ng/L for *Daphnia magna*; 1,600 ng/L for *Simocephalus serrulatus* and 6,500 ng/L for *Hyalella azteca* (see Table 3 in USEPA 2000). The final acute value for diazinon is therefore 330/2 or 165 ng/L. This new value is higher than the currently proposed value of 100 ng/L.

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