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DDT Toxicity and Critical Body Residue in the Amphipod *Leptocheirus plumulosus* in Exposures to Spiked Sediment

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Abstract. The lethal and sublethal toxicity of dichlorodiphenyltrichloroethane (DDT) to the estuarine amphipod *Leptocheirus plumulosus* was determined using sediment spiked with ^{14}C-labeled compound. Juvenile amphipods were exposed to concentrations up to 9.9 nmol/g dry weight (3.5 μg/g). Acute effects on survival were determined in a 10-day experiment. Chronic effects on survival, growth, and reproduction were assessed in a 28-day experiment. The DDT in the sediments transformed to dichlorodiphenyldichloroethane (DDD), dichlorodiphenyldichloroethylene (DDE), and polar metabolites during the 14-day sediment storage prior to exposing the amphipods. The mixture of DDT and its breakdown products (tDDT) was comprised mostly of DDT at the beginning of the exposures. DDD was the prevalent compound at termination of the 28-day exposure. Complete mortality occurred at sediment concentrations of tDDT as low as 7 nmol/g (2.3 μg/g) in both acute and chronic experiments. Most of the mortality appeared to have occurred within the first 4 days of exposure. No sublethal reductions in growth or reproduction were observed in the 28-day experiment. In the 10-day experiment, where amphipods did not receive supplemental food, growth was significantly increased in DDT treatments where survival was not affected. The concentration of tDDT in amphipod tissues was determined at exposure termination. In the 10-day experiment, a mean body residue of 14 nmol/g wet weight was associated with significant mortality (30%). Lower critical body residues were observed in the 28-day experiment, where the median lethal tissue residue (LR_{50}) was 7.6 (6.8–8.4, 95% confidence interval) nmol/g wet weight. Based on previous studies, the lethal critical body residue for *L. plumulosus* is similar to those determined for freshwater amphipods and substantially lower than those determined for cladocerans and polychaetes.

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Dichlorodiphenyltrichloroethane (DDT), an organochlorine pesticide, was banned for most applications in the United States in 1972 and subsequently in many other nations, but it is still extensively used in some developing countries. DDT and its major metabolites have strong binding affinity to organic carbon and resist degradation in sediments (Kennish 1992). The toxicity of DDT and its major metabolites, dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE), to benthic organisms in water exposures has been extensively investigated using a wide number of taxonomic groups (US EPA 1980; Bonner and Wells 1987; Hoke *et al.* 1994, 1997; Lotufo *et al.* 2000a). Using water concentrations associated with mortality as an indicator of sensitivity, freshwater amphipods appear more sensitive to DDT than do other invertebrates (Phipps *et al.* 1995). Lethal body residues are also substantially lower for freshwater amphipods (Lotufo *et al.* 2000a) than for other invertebrates, such as cladocerans (Crosby and Tucker 1971) or polychaetes (Lotufo *et al.* 2000b).

Exposures to historically contaminated sediment containing high concentrations of DDT and its metabolites have been performed using marine amphipods (Swartz *et al.* 1994), freshwater amphipods (Hoke *et al.* 1994), and chironomids (Hoke *et al.* 1997). Exposures to sediments spiked with DDT and its major metabolites have been conducted using polychaetes (Murdoch *et al.* 1997; Lotufo *et al.* 2000b) and freshwater amphipods (Nebeker *et al.* 1989; Lotufo *et al.* 2001). From these studies, it is evident that freshwater amphipods are substantially more sensitive than chironomids and polychaetes to the lethal effects of sediment-associated DDT and its metabolites.

The estuarine amphipod *Leptocheirus plumulosus* has a wide distribution in the East Coast of the United States, occurring from Cape Cod, Massachusetts, to northern Florida (Bousfield 1973). *Leptocheirus plumulosus* is easily cultured in the laboratory and has been routinely used for assessing the toxicity of marine and estuarine sediments (US EPA 1994) and dredged material (US EPA and US ACE 1998). In this study, the toxicity of sediment-associated DDT to this amphipod was investigated. Using spiked sediments, acute effects were determined in a 10-day exposure, and chronic effects on survival, growth, and reproduction were assessed in a 28-day exposure.

Materials and Methods

Preparation of Dosing Solutions

Radiolabeled DDT (^{14}C-DDT, 24.95 Ci/mol, 98% radiopurity determined using thin-layer chromatography) was purchased from New England Nuclear Research Products (Boston, MA) and nonradiolabeled DDT (99% pure) was purchased from Sigma Chemical Co. (St. Louis, MO). The molecular mass of DDT is 354.48 g/mol. For spiking sediment, a separate 2-ml dosing solution was prepared for each concentration (Table 1). Dosing solutions consisted of a constant amount of [^{14}C]DDT (22.57 μCi, targeting a concentration of 57 × 10^3 dpm/g sediment dry weight) and the appropriate amount of nonradiolabeled DDT dissolved in acetone. Control dosing solutions consisted of 2 ml of acetone only.

Sediment Spiking

Uncontaminated sediment (Sequim Bay, Washington, USA) was presieved to <0.3 mm (total solids content = 34%; silt and clay content = 74%) and stored at 4°C until dosed. DDT was added to sediments via clean quartz sand. Each dosing solution was added to 50 g of dry sand contained in a 50-ml glass beaker under a fume hood. After all the solvent evaporated, DDT-coated sand was added to a 4-L beaker containing 2.5 kg of sediment. Sediment and sand were mixed for 16 h at 1,000 RPM using a laboratory impeller mixer (Lightnin, Avon, NY). The spiked sediment was homogenized and triplicate samples (0.05–0.1 g) were transferred to scintillation cocktail (3a70b, Research Product International, Mt. Prospect, IL) to verify the concentration and distribution of ^{14}C-label using liquid scintillation counting (LSC) on a Tri-Carb Liquid Scintillation Analyzer (Model 2500 TR, Packard Instrument, Meridien, CT). Sediment was also frozen for analysis of DDT breakdown products.

The spiked sediments were held at 4°C for 14 days and homogenized before use in the experiments. The recommended holding period for spiked sediments is 1 month (US EPA 1994). Because the bioavailability of sediment-spiked DDT is expected to decrease with aging (Harkey *et al.* 1994), storage periods longer than 14 days are likely necessary to allow equilibrium to develop between the bioavailable and the nonbioavailable fractions of DDT in the sediment. However, because rapid DDT breakdown in Sequim Bay sediment had been previously observed (Lotufo *et al.* 2000b), a short storage period was used in this study to ensure that DDT would be the prevalent compound at initiation of the sediment exposures.

Toxicity Experiments

Laboratory-cultured *L. plumulosus* (Emery *et al.* 1997) were exposed to DDT-dosed sediment in two concurrent experiments using the same batch of spiked sediments. An acute exposure was conducted according to a published 10-day toxicity test protocol for estuarine and marine amphipods (US EPA 1994). A chronic exposure was conducted according to the 28-day toxicity test protocol described in the US. Environmental Protection Agency draft document "Method for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-associated Contaminants with the Amphipod *Leptocheirus plumulosus*" (in review). The 28-day protocol incorporates the assessment of two sublethal endpoints, growth and reproduction, in addition to lethality. Sediments stored for 14 days were homogenized and added to experimental chambers (1-L beakers). While the sediment was being added to experimental chambers, triplicate samples (0.05–0.1 g) were taken to verify the concentration and distribution of ^{14}C-label using liquid scintillation counting LSC. Sediment was also frozen for analysis of DDT breakdown products. Each beaker received 200 g of ^{14}C-DDT-spiked sediment and 800 ml of 5 ppt artificial sea water and was placed in a water bath maintained at 25°C. Sea water was reconstituted using dry sea salts (40 Fathoms®, Marine Enterprise International, Baltimore, MD) and deionized water and was aged for at least 1 week prior to use. Each beaker received trickle flow aeration and was left undisturbed for 24 h. After this period, 20 experimental organisms were added to each beaker. Each treatment was composed of five replicates.

10-day Experiment: Experimental organisms were juvenile amphipods that passed through a 0.71-mm mesh and were retained on a 0.5-mm mesh (2–4 mm in length, average dry weight = 50 μg). Exposure beakers were maintained under fluorescent lights with constant illumination. Water quality parameters (temperature, pH, salinity, dissolved oxygen and ammonia concentrations) were measured in the overlying water at days 2 and 8 from all beakers. Beakers did not receive any external food source, and the overlying water was not renewed during the entire exposure. After 10 days, amphipods were recovered by sieving using a 0.5-mm mesh screen. All surviving animals from each replicate were enumerated, blotted dry on paper towels, and weighed wet. DDT-exposed amphipods were transferred to scintillation cocktail. After subsidence of chemiluminescence (24 h), the ^{14}C activity of each sample was quantified by LSC and used for calculating the concentration of DDT molar equivalents in the tissues. Control amphipods were assayed for total lipid content. Sediment (~ 3 g) was sampled from three beakers per treatment for each exposure prior to sieving. Sediment samples were used to measure moisture content, radioactivity, and the relative concentration of DDT parent compound and its breakdown products using thin-layer chromatography (TLC).

28-day Experiment: Experimental organisms were juvenile amphipods that passed through a 0.6-mm mesh and were retained on a 0.25-mm mesh (1.1–2 mm in length; average dry weight = 38 μg). Beakers were maintained under fluorescent lights on a 16:8 h light: dark regime to mimic a natural light cycle. Water quality parameters were measured at day 0 and weekly thereafter from three replicates per treatment. Amphipods were fed ground Tetramin® (Tetra Sales, Blacksburg, VA) three times weekly (1.5 mg/animal for the first 2 weeks, then 3 mg/animal for the last 2 weeks). The overlying water was renewed three times weekly (50% of volume). At the end of the 28-day exposure, the contents of each beaker were gently sieved (0.3-mm sieve) and surviving adults and offspring were enumerated. Three adult amphipods per replicate were assayed for radioactivity as described for the 10-day experiment. Control amphipods were analyzed for lipid content and DDT-exposed amphipods were analyzed for DDT biotransformation products.

Chemical Analyses

Specific Activity Determination: A 50-μl aliquot of each dosing solution was diluted to approximately 50 ng/ml. Specific activities (dpm/μg) were calculated by determining the DDT concentration (μg/ml) and the total ^{14}C activity (dpm/ml) in the diluted dosing solutions. The DDT concentration was determined by gas chromatography (GC) on a Hewlett Packard (Avondale, PA) 6890 GC equipped with a ^{63}Ni electron-capture detector and 60 m × 0.32 mm capillary column (Supelco SPB-1; Bellefonte, PA). The ^{14}C activity was measured by LSC. Specific activities were used to convert ^{14}C activity measured in sediment and tissue samples using LSC to mass of DDT equivalents.

DDT Degradation Products Analysis: Sediments sampled prior to use in the experiments (following 14 days of storage after spiking) and at

Table 1. *Leptocheirus plumulosus* 10-day and 28-day experiments: mean (standard deviations) measured concentrations (nmol/g dry weight) of DDT equivalents in sediment samples taken at days 0 and at termination of the 10-day and 28-day experiments (numbers in brackets indicate concentrations expressed as μg/g dry weight)

Target	Initial (day 0)	Final	
		10-day Experiment	28-day Experiment
4.2 [1.5]	4.3 (0.6)	4.0 (0.1)	3.6 (0.1)
5.6 [2.0]	5.4 (0.2)	5.1 (0.4)	4.3 (0.6)*
7.1 [2.5]	6.6 (0.2)	5.9 (0.4)	6.1 (0.1)
8.5 [3.0]	8.6 (0.3)	8.2 (0.8)	8.3 (0.9)
9.9 [3.5]	11.3 (0.8)	10.2 (0.2)	9.4 (0.7)

* Significantly different from day 0 concentration.

Table 2. *Leptocheirus plumulosus* 10-day and 28-day experiments: specific growth constant rates

Sediment Treatment (nmol/g)	10-day Experiment	28-day Experiment
0	0.088 day^{-1}	0.124 day^{-1}
4.2	0.157 day^{-1}	0.125 day^{-1}
5.6	0.164 day^{-1}	0.125 day^{-1}
7.1	ND	ND
8.5	ND	ND
9.9	ND	0.131 day^{-1}

ND = not determined.

the end of the 10-day and 28-day exposures were extracted using an ASE 200 Accelerated Solvent Extractor (Dionex, Sunnyvale, CA) and analyzed for the relative concentration of DDT and its breakdown products using TLC. Wet sediment (3 g) was placed in aluminum pans and air-dried overnight at room temperature. Dry sediment was ground, weighed, mixed with approximately 20 g of anhydrous sodium sulfate, and transferred to an 11-ml extraction cell. The solvent system was acetone:hexane (1:1, v/v). For extraction from tissue samples, amphipods were sonicated for 20 s in 12 ml of ethylacetate:acetone (4:1, v/v). The extract was filtered through a sodium-sulfate column. The residual tissue was reextracted twice with 12 ml of cyclohexane. All extracts were combined. The volume of the sediment and tissue extracts was reduced under a stream of nitrogen in a Turbo Vap evaporator (Zymark, Hopkinton, MA). Concentrated extracts (0.3–0.5 ml) were spotted on precoated silica gel 60F-254EM TLC glass plates (Alltech Associates, Deerfield, IL) and developed in hexane:benzene (95:5, v/v). Developed plates were divided into 0.5-cm sections starting from the origin, the silica gel removed, and ^{14}C activity determined using LSC. The location on the plate of sections corresponding to DDT, DDD, and DDE standards was determined by visual observation under UV light. The relative fraction of DDT, DDD, DDE, or polar metabolites in the extracts was calculated by dividing the radioactivity of selected sections of the plate by the radioactivity of all sections combined.

Sediment Organic Carbon: Homogenized sediment was sampled before it was spiked and analyzed for organic carbon content on an Astro 2100 analyser (Zellweger Analytics, League City, TX) after acidification to remove carbonates.

Total Lipids: Control amphipods from the 10- and 28-day experiments were analyzed for total lipids using a colorimetric method (Lotufo *et al.* 2000b) modified from Van Handel (1985).

Calculations

Amphipod growth, or net gain in biomass, was estimated as the difference between the mean final and initial biomass (wet weight). Amphipod specific growth rate constants (see Table 2) were calculated using the natural log of the mean initial and final biomass and the exposure duration (Driscoll *et al.* 1998):

$$\text{growth rate} = \frac{\ln[\text{mean final wet weight}] - \ln[\text{mean initial wet weight}]}{\text{Exposure duration (days)}}$$

The molar concentrations of DDT parent compound and its metabolites in amphipods at termination of the 28-d experiment (Table 3) were estimated for each treatment by multiplying the mean concentration of DDT equivalents (measured using LSC) by the corresponding fraction of the total label comprised of each compound (determined using TLC).

Biota-sediment accumulation factors (BSAFs) were calculated using tissue and sediment DDT-molar-equivalents concentrations measured at experiment termination as:

$$\text{BSAF} = \frac{(\text{mg DDT equivalents / g wet tissue}) / (\text{g lipids / g wet tissue})}{(\text{g DDT equivalents / g dry sediment}) / (\text{g organic carbon / g dry weight sediment})}$$

Statistical Analysis

All measurement values are expressed as mean ± 1 standard deviation. One-way analysis of variance (ANOVA) was used to analyze amphipod survival, growth, and reproduction data. The data conformed to

Table 3. *Leptocheirus plumulosus* 28-day experiment: mean DDT molar equivalents tissue concentrations; percent of total label in the tissue extracts represented by nonidentified polar metabolites (Polar), DDD, DDT, and DDE; and the estimated tissue concentrations for these compounds

Sediment Treatment (nmol/g)	DDT-Equivalents Tissue Concentration (nmol/g wet weight)	Compound	Percent of Total Radioactivity	Estimated Tissue Concentration (nmol/g wet weight)
		Polar	8.2	0.28
		DDD	76.3	2.59
4.2	3.44	DDT	6.0	0.20
		DDE	9.5	0.32
		Polar	7.6	0.40
		DDD	74.5	3.88
5.6	5.22	DDT	7.9	0.41
		DDE	10.0	1.29
		Polar	7.8	0.83
		DDD	71.8	7.61
9.9	10.56	DDT	8.2	0.87
		DDE	12.2	1.29

the assumption of homogeneity of variances. Pairwise comparisons of DDT treatments with the control treatment were conducted using the Williams test. The significance level (α) was set at 0.05. Mortality data were transformed by arcsine-square-root prior to analysis. Mean lethal concentration (LC_{50}) or mean lethal tissue residue (LR_{50}) values were calculated using the trimmed Spearman-Karber method (US EPA 1994). The LR_{50} values were calculated using body residue and survival from all replicates (Lotufo 1998; Lotufo *et al.* 2000a).

Results

Sediment

The fraction of total organic carbon in the spiked sediments was 1.78 ± 0.03% (n = 5). Mean measured concentrations of DDT molar equivalents in spiked sediments at day 0 were similar (< 15% difference) to target concentrations (Table 1). Low coefficient of variation for all treatments (3–13%) indicated that DDT was homogeneously distributed in the sedi ment. The sediment concentrations of DDT molar equivalents remained relatively constant during the 10-day and the 28-day exposures. Concentrations measured at experiment termination were not significantly different from day 0 concentrations, except for the 5.6 nmol/g treatment in the 28-day experiment (Table 1). Loss of radiolabeled compound during the exposure ranged from 3% to 20% of the initial amount. Most (61–78%) of the ^{14}C-labeled compound extracted from sediment sampled at the beginning of the experiment was DDT (Figure 1). The fractions corresponding to breakdown products ranged from 7% to 17% for DDD, 13% to 21% for nonidentified polar metabolites, and 1% to 4% for DDE (Figure 1). At the end of the 10-day exposure, the relative proportion of DDT had decreased considerably, ranging from 29% to 48%, whereas the relative proportions of DDD (24–37%) and nonpolar metabolites (24–31%) increased (Figure 1). At the end of the 28-day sediment exposures, most of the radioactivity in the sediment extract corresponded to DDD (44–57%), and the fraction corresponding to DDT ranged from 10% to 21% (Figure 1).

Exposure Concentrations and Toxicity

Mean percent survival in the control was 92% in the 10-day experiment and 91% in the 28-day experiment. Survival was high (>80%) in replicates from the 4.2 nmol/g treatment, and complete mortality was observed in all replicates from the 7.1 and 8.5 nmol/g treatments in both the 10-day and 28-day experiments (Figure 2). Survival in the 5.6 nmol/g treatment was significantly lower than in the control in the 10-day experiment but not in the 28-day experiment (Figure 2). In the highest treatment (9.9 nmol/g), complete mortality occurred in the 10-day experiment but some survival (up to 10%) was observed in the 28-day experiment. Most mortality appeared to have occurred early in the experiment, as daily visual inspection revealed that burrows disappeared from the sediment surface during the first 4 days of exposure in beakers where complete mortality was observed at experiment termination. LC_{50} values, calculated using mean initial sediment concentrations of DDT molar equivalents for each treatment, were 5.6 (5.5–5.7, 95% confidence interval [CI]) nmol/g dry weight for the 10-day experiment and 6.4 (6.3–6.5, 95% CI) nmol/g dry weight for the 28-day experiment. Expressed on a mass basis, LC_{50} values were 1.98 μg/g dry weight for the 10-day experiment and 2.27 μg/g dry weight for the 28-day experiment.

In the 10-day experiment, growth was significantly higher (~2×) in the 4.2 and 5.6 nmol/g treatments than in the control (Figure 2). Growth was not significantly affected by exposure to DDT in the 28-day experiment (Figure 2). Growth rates for control amphipods were higher in the 28-day experiment than in the 10-day experiment (Table 2). For DDT-exposed animals, however, growth rates were higher in the 10-day experiment (Table 2). Offspring production in the 28-day experiment was

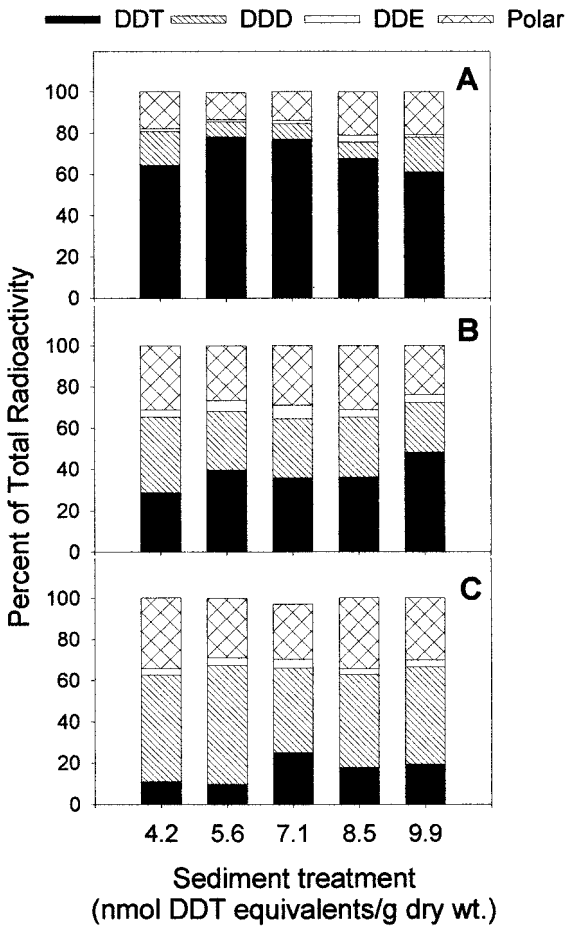

Fig. 1. *Leptocheirus plumulosus* 10-day and 28-day experiments: percent of the total radioactivity comprised of DDT, DDD, DDE, and nonidentified polar metabolites (Polar) in the sediment at day 0 (A) and at termination of the 10-day (B) and 28-day (C) experiments

significantly lower than in the control only in the 9.9 nmol/g treatment (Figure 2).

Bioaccumulation

Concentration of DDT equivalents in the tissues increased with increasing concentration of DDT equivalents in the sediment in both the 10-day and 28-day experiments (Figure 2). The amount of tDDT in the tissues was proportional to the amount of tDDT in the sediments, as tissue concentrations increased linearly with increasing sediment concentrations at day 28 (tissue concentration = 0.21 sediment concentration, $r^2 = 0.69$, regression not shown). In the two lowest treatments (4.2 and 5.6 nmol/g), tissue concentrations of DDT equivalents were significantly higher (~3×) in the 10-day experiment than in the 28-day experiment (Figure 2). Tissue concentration in control amphipods was assumed negligible because pre-spiking concentrations of DDT in the sediment were <0.005 μg/g.

Analyses of tissue extracts from adult amphipods exposed for 28 days revealed that the ^{14}C-labeled compounds bioaccumulated were DDD (75.2 ± 3.2% of the total molar equivalents), DDE (10.1 ± 1.6%), polar metabolites (8.0 ± 1.2%), and DDT (6.8 ± 1.5%) (Figure 3). The mean relative tissue concentration of DDT, DDD, DDE, and polar metabolites was compared to the mean relative concentration of DDT, DDD, and DDE in the sediment at day 28 (Figure 3). Polar metabolites in the sediment were excluded from the relationship because they are not expected to bioaccumulate. The relative

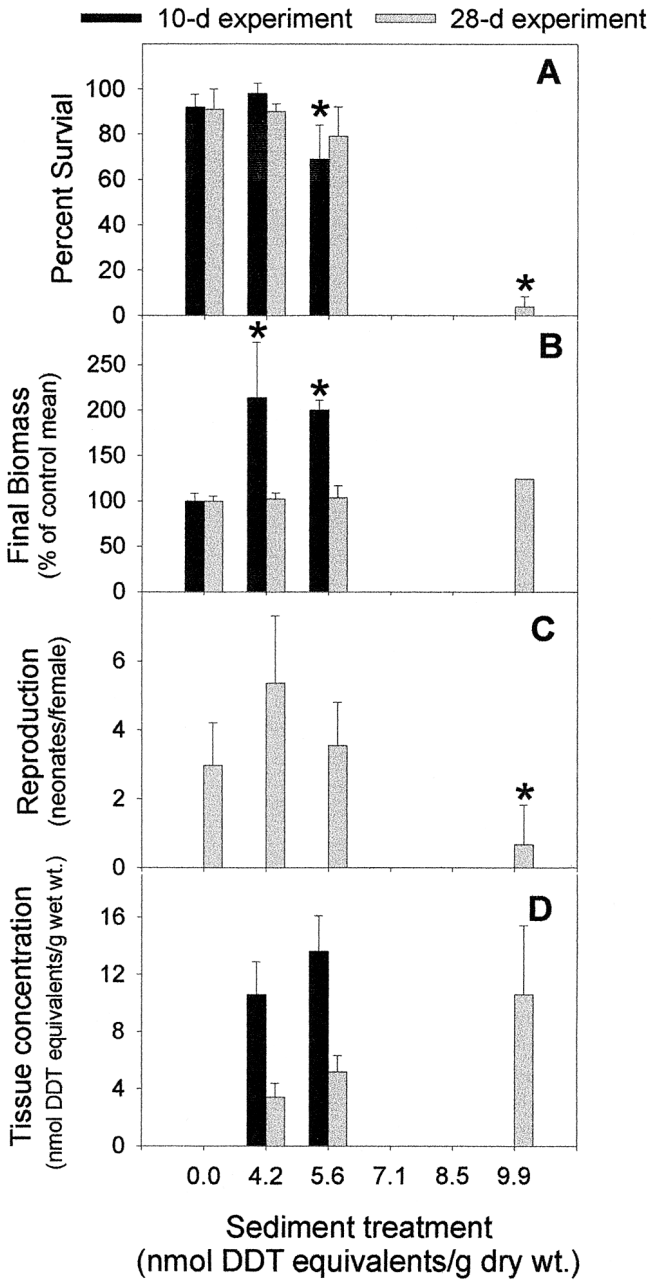

Fig. 2. *Leptocheirus plumulosus* 10-day and 28-day experiments: percent survival (A), mean growth (B), and mean percent reproductive output (neonates/surviving adult) (C) expressed as a percent of the control mean (B and C), and mean concentration of DDT molar equivalents in the tissues (D). Error bars represent 1 standard deviation. * Represents significant difference from control mean

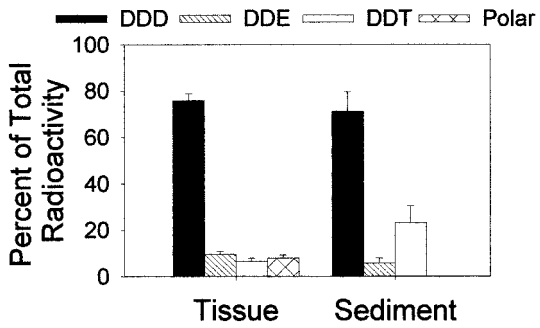

Fig. 3. *Leptocheirus plumulosus* 28-day experiments: relative concentration of DDD, DDE, DDT, and polar metabolites in amphipod tissues and of DDD, DDE, and DDT in sediments at day 28. Error bars represent 1 standard deviation

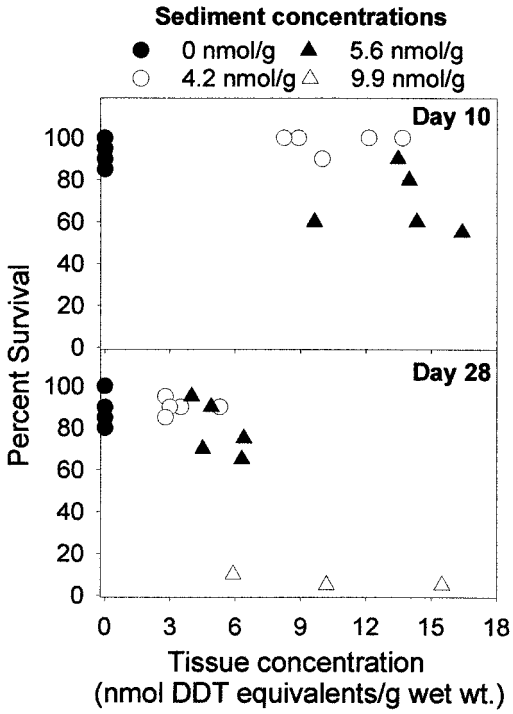

Fig. 4. *Leptocheirus plumulosus* 10-day (top) and 28-day (bottom) experiments: percent survival (y) as a function of tissue concentration of DDT molar equivalents at experiment termination (x). Symbols represent different sediment treatments

fraction of DDD was similar in the tissues and in the sediment, the fraction of DDE was higher in the tissues, and the fraction of DDT was considerably lower in the tissues (Figure 3).

Percent lipid content (g lipids/g wet weight × 100) in control amphipods at exposure termination in the 10-day experiment (1.92 ± 0.21%, n = 5) was significantly higher than in the 28-day experiment (1.65 ± 0.42%, n = 5). The BSAFs were calculated using control lipid content as a surrogate for the lipid content in DDT treatments. In the 10-day experiment, BSAFs were 2.51 ± 0.54 and 2.52 ± 0.46 for the 4.2 and 5.6 nmol/g treatments, respectively. In the 28-day experiment, BSAFs were 1.04 ± 0.32 and 1.32 ± 0.27 for the 4.2 and 5.6 nmol/g treatments, respectively.

Tissue Concentrations and Toxicity

In the 5.6 nmol/g treatment of the 10-day experiment, mortality (mean = 30%) occurred when body residues in surviving amphipods ranged from 10 to 17 nmol DDT equivalents/g wet weight (mean = 14 nmol/g) (Figure 4). The 10-day LR_{50} value could not be calculated because tissue concentrations were not measured in treatments where survival exceeded 50% (7.1, 8.5, and 9.9 nmol/g) due to complete mortality. Therefore, the 10-day LR_{50} can be assumed as higher than 14 nmol/g. In the 28-day experiment, mortality was high (>90%) in the highest treatment, where body residues ranged from 6 to 16 nmol DDT equivalents/g wet weight (Figure 4). An LR_{50} value of 7.6 (6.8–8.4, 95% CI) nmol/g wet weight was calculated for the 28-day experiment using DDT molar equivalents tissue concentration and mortality data for each replicate beaker.

Water Quality

10-day Experiment: Water quality parameters (n = 30) in the overlying water ranged from 6.2 to 7.1 mg/L for dissolved oxygen, 24.3 to 24.8°C for temperature, 6 to 9‰ for salinity, 6.63 to 8.12 for pH, and 7 to 8 mg/L for ammonia.

28-day Experiment: Water quality parameters (n = 30) in the overlying water ranged from 6.4 to 8.1 mg/L for dissolved oxygen, 24.4 to 24.9°C for temperature, 6 to 8‰ for salinity, 7.55 to 8.19 for pH, and 6 to 8 mg/L for ammonia.

Discussion

Sediment

Although sediments were spiked with DDT only, amphipods were exposed to a mixture of DDT and its metabolites. During the 14-day storage period that followed spiking, DDT transformed to DDD, nonidentified polar metabolites and, to a minor extent, DDE. Further degradation of DDT occurred during the 10-day and 28-day experiments. Extensive transformation of DDT to DDD or DDE in spiked sediments has been previously observed (Albone *et al.* 1972; Van den Hoop *et al.* 1999; Lotufo *et al.* 2000b, 2001).

Although the concentration of DDT molar equivalents (hereafter referred to as tDDT) remained relatively unchanged throughout the sediment exposures, the relative concentration of DDT relative to the concentrations of its metabolites decreased considerably. Lotufo *et al.* (2000a) found that DDT was more lethally toxic to freshwater amphipods than DDD or DDE. Therefore, the toxicity of the DDT-spiked sediments to amphipods likely decreases as the relative concentration of DDT in the sediment decreases due to degradation.

Bioaccumulation

For the same sediment treatment, tDDT tissue concentrations were substantially lower in the 28-day experiment than in the 10-day experiment (Figure 2) and resulted in substantially lower BSAFs for the 28-day experiment. Differences in experimental conditions between the 10-day and 28-day experiments may have contributed to the observed differences in bioaccumulation. Lack of supplemental food in the 10-day experiment may have promoted higher ingestion of contaminated sediment and, consequently, higher compound bioaccumulation. The concentration of DDT in the water surrounding amphipods in their burrows may have been lower in the 28-day experiment, where overlying water renewals were frequent, than in the 10-day experiment, where water renewals were not performed. In addition, constant illumination in the 10-day experiment, which is intended to increase the likelihood that amphipods remain in the sediment, may have resulted in higher exposures in the 10-day experiments.

The relative concentration of DDT in the sediment was considerably higher in the sediment than in the tissues at termination of the 28-day experiment (Figure 3). We have demonstrated that *L. plumulosus* biotransforms DDT to DDE and polar metabolites but not to DDD (unpublished data). A fraction of the DDT entering the tissues was likely transformed to DDE and to polar metabolites. DDD and DDE likely remained untransformed, as previously observed with freshwater amphipods (Lotufo *et al.* 2000a).

In this study, BSAFs were calculated using control lipid content as a surrogate for the lipid content in DDT treatments because it was necessary to use all DDT-exposed amphipods in the TLC analysis of DDT biotransformation products. Lipid content in DDT-exposed amphipods may have been lower than in control amphipods, as observed with *Hyalella azteca* (Lotufo *et al.* 2000a). Therefore, actual BSAFs may have been higher than values calculated using control lipid content. The BSAF values calculated in the present study for tDDT using *L. plumulosus* were in the same range of values calculated for other invertebrates using sediment spiked with DDT (Mulsow and Landrum 1995; Lotufo *et al.* 2001).

Survival

The effects of tDDT on survival followed a sharp threshold pattern (Figure 1). This pattern is in contrast to the stepwise increase in mortality observed in freshwater amphipods exposed to increasing concentrations of sediment-associated tDDT (Lotufo *et al.* 2001). No burrows were observed on the sediment surface in beakers from the 7.1 and 8.5 nmol/g sediment treatments in the 10-day and 28-day experiments after day 4, indicating that complete amphipod mortality occurred very early in both experiments. Although younger amphipods were used in the 28-day experiment, DDT sediment concentrations associated with reduced survival (*e.g.*, LC_{50} values) were lower in the 10-day experiment than in the 28-day experiment.

Mortality appeared to have occurred when tDDT tissue concentrations exceeded approximately 14 nmol/g wet weight in the 10-day experiment and 6 nmol/g wet weight in the 28-day experiment. Lower lethal body burdens of tDDT with increasing exposure time is consistent with a previous study examining halobenzene critical body residues in fish (Yu *et al.* 1999). Higher lipid content in 10-day amphipods likely contributed to the higher lethal body residue observed in the 10-day experiment. In intra- and interspecific comparisons, organisms with higher lipid content have been found to be more tolerant to organic contaminants (Geyer *et al.* 1993; DeMaagd *et al.* 1997). Higher lipid content in 10-day amphipods was unexpected because they were not fed supplemental food, as were amphipods in the 28-day experiment.

The 28-day LR_{50} for *L. plumulosus* was determined using survival and tissue concentration data measured in each replicate. Only one or two amphipods from three replicates in the highest sediment concentration were available for sampling from treatments where significant mortality occurred because of complete mortality in the remaining replicates. Therefore, the limited data used to calculate the 28-day LR_{50} may have influenced its accuracy. The tDDT lethal body residue for *L. plumulosus* (28-day LR_{50} = 7.6 nmol/g wet weight) was similar to those previously determined for other amphipods. The tDDT 10-day LR_{50} for *H. azteca* was 10 nmol/g wet weight in a water-only exposure (Lotufo *et al.* 2000a) and was 7 and 15 nmol/g wet weight in a sediment exposure (Lotufo *et al.* 2001). The tDDT 28-day LR_{50} for the cold-water amphipods *Diporeia* spp. was 17 nmol/g wet weight in a sediment exposure (Lotufo *et al.* 2001). Other invertebrates differ greatly in their sensitivity to DDT. Nimmo *et al.* (1970) reported a very low DDT lethal body residue (0.4 nmol/g wet weight) for the pink shrimp *Penaeus duoduroum*, whereas much higher lethal body residues were reported for the cladoceran *Daphnia magna* (3,200 nmol/g wet weight) (Crosby and Tucker 1971) and for the polychaete *Neanthes arenaceodentata* in sediment exposures (no mortality at DDT tissue concentrations as high as 400 nmol/g wet weight) (Lotufo *et al.* 2000b). It is possible that the mode of action for DDT-induced mortality is not the same for all species of aquatic organisms. DDT has been described as a neurotoxin (Bloomquist 1996). By acting by a specific mode action, DDT is expected to promote mortality at tissue concentrations well below levels observed for organic compounds that act by general narcosis (McCarty and Mackay 1993), as was observed with amphipods. DDT may have a weak neurotoxic action in other invertebrates, such as *D. magna* and *N. arenaceodentata*, where it may produce mortality mostly by a nonspecific narcotic mechanism.

The toxicity of DDT and its major metabolites has been assumed to be equal and dose-additivity (ΣDDT approach) has been applied to predict effects from these compounds (*e.g.*, Swartz *et al.* 1994; Ferraro and Cole 1997). However, comparative studies using DDT, DDD, and DDE have demonstrated considerable differences in the relative toxicity of these compounds to benthic invertebrates. DDT was found to be more lethally toxic than DDD or DDE to two species of freshwater amphipods (Lotufo *et al.* 2000a) and planarians (Kouyoumjian and Uglow 1974), but DDD was more toxic than DDT to chironomids (Phipps *et al.* 1995; Hoke *et al.* 1997). Although compound-specific lethal body residues were not determined for *L. plumulosus* in the present study, the relative contribution of DDT to lethality was expected to be higher than the contribution of DDD or DDE, as observed with freshwater species. Differences in critical body residue for tDDT between the 10-day and the 28-day exposures may also

have been partly due to differences in the relative concentration of DDT and its metabolites in the tissues of both sets of amphipods. However, the existence of such difference cannot be verified because DDT biotransformation was not assessed in amphipods from the 10-day experiment.

Growth

The specific growth constants for control animals in the 10-day experiment (0.088 day^{-1}) and 28-day experiments (0.124 day^{-1}) were within the range of mean values observed in similar experiments performed in our laboratory (0.075–0.083 day^{-1} and 0.111–0.147 day^{-1}, for 10-day and 28-day exposures, respectively, unpublished data). Higher growth rates in the 28-day experiments are expected because amphipods are fed supplemental food in the 28-day experiments but not in 10-day experiments.

In the present 10-day experiment, exposure to sublethal concentrations of sediment-associated DDT significantly enhanced amphipod growth. The growth rates in the two lowest DDT treatments of the 10-day experiment were approximately twice as high as in the control for that experiment and 30% higher than in the control of 28-day control animals, where amphipods received supplemental food. Increased invertebrate growth in exposures to sublethal concentrations of contaminants has been previously reported (Jenkins and Sanders 1986; Green *et al.* 1999) and was attributed to hormesis (Green *et al.* 1999). No sublethal effects on reproduction were observed.

Conclusions

Differences in toxicity and bioaccumulation of DDT in *L. plumulosus* were observed between a 10-day and a 28-day exposure to spiked sediment. Constant illumination and lack of overlying water renewal and supplemental feeding in the 10-day protocol were likely responsible for the higher tDDT bioaccumulation in the 10-day exposure relative to the 28-day exposure. Most mortality apparently took place in the first few days of exposure in both experiments. Although the sediment LC_{50} value was lower for the 10-day experiment than for the 28-day experiment, lethal body residues were lower for amphipods from the 28-day experiment. Differences in lethal body residues between the two exposure periods are likely due to relative concentration of DDT and its breakdown products in the tissues. No sublethal reductions in growth or reproduction were observed in the 28-day experiment. The substantial increases in growth observed in DDT treatments in the 10-day experiment (but not in the 28-day experiment) were likely due to hormesis. Lethal sediment concentrations and critical body residues for DDT and its metabolites for *L. plumulosus* was similar to those previously determined for freshwater amphipods.

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