

TOXICITY AND BIOACCUMULATION OF DDT IN FRESHWATER AMPHIPODS IN EXPOSURES TO SPIKED SEDIMENTS

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Abstract—The amphipods *Hyaella azteca* and *Diporeia* spp. were exposed to sediments dosed with dichlorodiphenyltrichloroethane (DDT), and the toxicity and toxicokinetics were determined. The toxicity was evaluated with the equilibrium partitioning (EqP) and critical body residue approaches. The DDT in the sediments degraded during the equilibration period prior to organism exposure. Thus, the toxicity using EqP pore-water toxic units (TUs) was evaluated for DDT and its degradation product, dichlorodiphenyldichloroethane (DDD), as the ratio of the predicted interstitial water concentration divided by the water-only LC50 values. The sum of TUs (Σ TU) was assumed to best represent the toxicity of the mixture. For *H. azteca*, the 10-d LC50 was 0.98 and 0.33 Σ TU for two experiments. For *Diporeia* spp., no toxicity was found in the first experiment with up to 3 Σ TU predicted in the interstitial water. However, in the second experiment, the 28-d LC50 was 0.67 Σ TU. These data suggest that the EqP approach approximately predicts the toxicity for the combination of DDT and DDD in sediment, provided a toxic unit approach is employed. The critical body residue approach also used TUs because DDT is biotransformed by *H. azteca* and because of the dual exposure to DDT and DDD. Because biotransformation was only determined in the second experiment, the critical body residue approach could only be evaluated for that case. The TUs were calculated as the ratio of the concentration in the live amphipods divided by the respective LR50 (residue concentration required to produce 50% mortality) values. The LR50 was 1.1 Σ TU for *H. azteca* for the 10-d exposure and 0.53 for *Diporeia* spp. after a 28-d exposure. Thus, this approach was also quite successful in predicting the toxicity. The accumulation and loss rates for *H. azteca* were much greater than for *Diporeia* spp. Thus, 10-d exposures represent steady-state conditions for *H. azteca*, while even at 28-d, the *Diporeia* spp. are not at steady state.

Keywords—Dichlorodiphenyltrichloroethane Sediment Amphipods Equilibrium partitioning Critical body residue

INTRODUCTION

Highly hydrophobic chlorinated hydrocarbons persist in sediments long after their release into aquatic environments [1]. Dichlorodiphenyltrichloroethane (DDT), one of the most ubiquitous of such organochlorides, was banned for most applications in the United States in 1972, but the presence of historically discharged DDT may pose serious risk to the environment [2–4]. Concentrations of DDT metabolites in heavily contaminated sediments often exceed that of the parent compound [2,5,6].

The toxicity of DDT and its major metabolites has been determined for a wide variety of benthic invertebrates in water exposures [2,7–9] and in sediment exposures [2–4,7]. The relative toxicities of DDT, dichlorodiphenyldichloroethane (DDD), and dichlorodiphenyldichloroethylene (DDE) to benthic invertebrates differed greatly in water exposures. The DDT water concentrations that significantly affected survival of freshwater amphipods and chironomids were remarkably lower than DDD and DDE concentrations that promoted similar effects [2,7,9]. Similar comparative studies with sediment exposures have not been conducted. However, assuming equal bioavailability, toxicity differences observed in water exposures should be similar in sediment exposures. Equilibrium partitioning predicts that the bioavailability of nonionic organic compounds in sediment is proportional to the sediment organic carbon content [10]. Therefore, pore-water concentra-

tions of the freely dissolved compounds can be easily predicted for sediments with known organic carbon content using partition coefficients and the equilibrium partitioning (EqP) approach [10]. Pore-water concentrations may then be compared with toxicity data established using water-only exposures for quality assessment of contaminated sediments [10].

For most sediments tested, the effects of DDT and its major metabolites on the survival of the freshwater amphipod *Hyaella azteca* and of the midge *Chironomus tentans* were low, based on measured or predicted pore-water compound concentrations [2,7]. Low amphipod mortality at pore-water concentrations predicted to be toxic using EqP has been observed with fluoranthene [11–13] and dieldrin [14]. Burrowing avoidance was speculated to contribute to the lower-than-expected mortality by decreasing exposure to sediment-bound and pore-water contaminants [11,12,14]. Determination of tissue concentrations, in addition to sediment and pore-water concentrations, allowed more accurate assessments of sediment toxicity in studies that addressed the utility of the EqP approach. Even at exceedingly high fluoranthene sediment concentrations, mortality of the amphipods *H. azteca* and *Leptocheirus plumulosus* was only observed when tissue residues attained concentrations predicted as lethal in water-only exposures [11,12].

In this study, the effects of sediment-associated DDT on the survival of the freshwater amphipods *H. azteca* and *Diporeia* spp. were investigated in 28-d exposures. Tissue concentrations were measured throughout the exposure to allow

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calculation and comparison of toxicokinetic parameters in both species. Observed effects were related to pore-water concentrations predicted using EqP and were compared with water concentration previously determined to affect survival of these species [9]. The presence of DDT breakdown products in the sediment and in the tissues was determined and taken into consideration for a more accurate interpretation of the toxicity data.

MATERIALS AND METHODS

Experiment design

Toxicity and uptake kinetics of [^{14}C]DDT to *H. azteca* and *Diporeia* spp. were examined by exposing test organisms for 2, 4, 10, 17, and 28 d to sediment spiked at different concentrations. The DDT body residue was measured at the end of every exposure period. The DDT sediment concentrations were measured on days 0, 10, and 28. Two experiments were conducted with each species. The first *H. azteca* and *Diporeia* spp. experiments were initiated on October 7, 1996. Nominal concentrations were 2.8, 5.6, 11.3, 22.6, and 45.2 nmol/g dry wt (1, 2, 4, 8, and 16 $\mu\text{g/g}$ dry wt) for *H. azteca* and 4.2, 8.4, 16.8, 33.6, and 67.2 nmol/g dry wt (1.5, 3, 6, 12, and 24 $\mu\text{g/g}$ dry wt) for *Diporeia* spp. For each species, five replicate beakers from each concentration were analyzed at day 10 and triplicate beakers at other time points. A second experiment was initiated on May 27, 1997, with *H. azteca* and on April 24, 1998, with *Diporeia* spp. Nominal concentrations were 2, 3.4, 5.8, 9.9, and 16.8 nmol/g dry wt (0.7, 1.2, 2.1, 3.6, and 6 $\mu\text{g/g}$ dry wt) for *H. azteca* and 7, 14, 35, 88, and 220 nmol/g dry wt (2.5, 5, 12.5, 31.2, and 78 $\mu\text{g/g}$ dry wt) for *Diporeia* spp. Four replicate beakers were analyzed on days 4, 10, and 28; duplicate beakers were analyzed on days 2 and 17. One extra beaker per treatment was used to assay animals for DDT biotransformation products at day 10 in the second *H. azteca* experiment and at day 28 in the second *Diporeia* spp. experiment.

Sediment spiking

Sediment was collected by ponar grab from a 45-m-deep station (43.03°N, 86.37°W) in Lake Michigan, USA. The sediment was preserved to <1 mm and stored at 4°C until dosed. Radiolabeled [^{14}C]DDT (18.7 mCi/mmol, 98% radio pure, molecular weight = 354.48) and unlabeled DDT (99% pure) were purchased from Sigma Chemical (St. Louis, MO, USA). Separate 10-ml dosing solutions were prepared for each concentration. Dosing solutions consisted of a constant amount of [^{14}C]DDT (11.7 μCi , targeting a concentration of 13 disintegrations per min/mg dry sediment) and the appropriate amount of nonradiolabeled DDT dissolved in acetone. A small aliquot (up to 50 μl) of each stock solution was diluted to approximately 50 ng/ml for specific activity determination. For the first *H. azteca* and *Diporeia* spp. experiments, DDT was added to the sediment using the shell-coating procedure [15]. Dosing solutions (10 ml) were evaporated onto the inside of 3.8-L glass jars and sediment (2,800 g wet wt) was added. The jars were rolled for 1.5 h at 20°C, held overnight at 4°C, and rolled again at 20°C for 5 h. To allow for dissolution and partitioning of the spiked DDT, sediments were held to equilibrate at 4°C for 60 d prior to the initiation of the exposures. For the second *H. azteca* and *Diporeia* spp. experiments, quartz sand was coated with DDT and added to wet sediment. Each dosing solution and 30 g of dry sand were added to a 250-ml round-

bottomed flask. Acetone was evaporated using a rotor evaporator until the sand was dry and free flowing. The DDT-coated sand was transferred to a beaker. More sand (10 g) and 10 ml of acetone were added to each round-bottomed flask to remove the DDT encrusted to the glass. Acetone was evaporated under rotor evaporator until the sand was dry. All the DDT-coated sand (40 g) was added to a 3.8-L jar containing sediment (2,800 g wet wt). The sand was fully mixed with sediment with a metal spoon. All jars were rolled for 12 h at 22°C and stored overnight at 4°C. Jars were again rolled for 14 h followed by vigorous mixing of the sediment using a laboratory mixer for 20 to 30 min. After mixing, sediments were held to equilibrate at 4°C for 60 d prior to the initiation of the exposures.

Organisms

Laboratory-reared *H. azteca* that passed through a 0.5-mm mesh and were retained in a 0.355-mm mesh (juvenile organisms approximately one to two weeks old; mean \pm standard deviation [SD] wet wt = 0.20 ± 0.04 mg for the first experiment and 0.42 ± 0.12 mg for the second experiment) were used in all experiments. Field-collected *Diporeia* spp. [9] that passed through a 2-mm mesh and were retained in a 1-mm mesh (juvenile organisms approximately 5–11 months old; mean \pm SD wet wt = 7.97 ± 0.98 mg for the first experiment and 3.01 ± 0.52 mg for the second experiment) were used.

Exposure

At the end of the 60-d equilibration period, the spiked sediments were homogenized. Five small sediment samples (approximately 0.2 g) were taken from each jar to verify the concentration and distribution of DDT in the sediment via liquid scintillation counting (LSC) and for total organic carbon content determination. Test beakers (300-ml-tall form) were filled with the appropriate sediment (100 g) and overlying water (150 ml) and were left to settle for 24 h. Ten test organisms were added to each beaker on day 0. Beakers were randomly placed into water renewal systems [16]. One third of the overlying water was renewed twice daily. Water quality parameters (hardness, alkalinity, pH, dissolved oxygen, and ammonia concentrations) were measured weekly during the experiment. Each beaker with *H. azteca* was fed once every day with 1 ml of yeast-cerophyl trout chow. *Diporeia* spp. were not fed for the duration of the experiment since they obtain sufficient nutrition from deposit feeding. Experiments with *Diporeia* spp. were conducted at 4°C and with *H. azteca* at room temperature (20–23°C).

At each sampling period, surface sediment was sieved through a 0.355-mm mesh (*H. azteca*) or a 1-mm mesh (*Diporeia* spp.), and dead or surviving amphipods were recovered and rinsed thoroughly. Samples of subsurface sediment were taken, excluding any organisms, for moisture content (0.5–1.5 g) and DDT concentration determination (0.1–0.2 g). A larger sample (5 g) was taken from one beaker per treatment at day 28 and analyzed for DDT breakdown products. Previous studies indicated that the concentration of contaminants in the subsurface material is a good representation of the exposure of the organisms [17]. Remaining sediment was sieved to recover additional amphipods. Live amphipods were enumerated and blotted dry on paper towels for wet weight and DDT concentration determination. Sample sizes consisted of all surviving *H. azteca* or five *Diporeia* spp. (fewer if survival <50%). Sediment and amphipod samples were transferred to a scintillation vial and 12 ml of scintillation cocktail (3a70b,

Research Product International, Mt. Prospect, IL, USA) was added. After 24 h, which allowed for subsidence of chemiluminescence and extraction of contaminants from the amphipods by the cocktail, ^{14}C activity was quantified by LSC on a tri-carb liquid scintillation analyzer (Model 2500 TR, Packard Instrument, Meriden, CT, USA). Samples were corrected for quench using the external standards ratio method after subtracting background. Specific activities were used to convert the radioactivity concentration to the concentration of DDT molar equivalents.

Growth rates of both species were calculated from the regression of the natural log of the wet weights versus exposure time. Lipid content (g lipids/g tissue dry wt) of *H. azteca* and *Diporeia* spp. was determined in five replicates at day 0 and at day 28 from control animals using a microgravimetric method [18] for both experiments. Mean measured dry-to-wet weight ratios of 0.275 and 0.269 for *H. azteca* and *Diporeia* spp. (unpublished data), respectively, were used for expressing lipid content as a fraction of the wet weight.

Chemical analyses

Specific activity determination. An aliquot of each dosing stock was diluted to a nominal concentration of 50 ng/ml. Specific activity was determined as disintegrations per minute per unit of mass (ng) of DDT in the dosing stock. An aliquot (1 ml) of each diluted stock was assayed for ^{14}C activity using LSC. Another aliquot from the same stock was analyzed for total DDT concentration by gas chromatography on a Hewlett Packard (Avondale, PA, USA) 5710 GC equipped with a ^{63}Ni electron-capture detector and 30-m DB-5 capillary column.

The DDT breakdown products. Sediment from all treatments was sampled at day 0 from the storage jars and from one beaker per treatment at day 28 for all experiments. Sediment samples (5 g) were dried with anhydrous sodium sulfate and transferred to Erlenmeyer flasks. Methylene chloride (50 ml) was added, and samples were sonicated (1 h at 30°C), incubated overnight (30°C), and sonicated again for 1 h. The volume of solvent was reduced to 0.5 ml under a stream of nitrogen. Amphipods from the extra beaker in the second *H. azteca* and *Diporeia* spp. experiments were sonicated for 20 s in 12 ml of ethylacetate:acetone (1:4, v:v). Each extract was filtered through a sodium-sulfate column. The residual tissue was reextracted twice with 12 ml of cyclohexane. The two extracts were combined and the volume reduced to 0.5 ml under a stream of nitrogen. Sediment or amphipod extracts were spotted on precoated silica gel 60F-254EM glass plates (Alltech Associates, Deerfield, IL, USA) and developed in hexane:benzene (95:5, v:v). The ^{14}C activities in the silica gel from the areas of the plate corresponding to DDT, DDE, DDD, and unidentified polar metabolites were determined using LSC.

Sediment organic carbon. Samples of the test sediments were analyzed for organic carbon content prior to dosing on a model 2400 CHN Elemental Analyzer (Perkin Elmer, Norwalk, CT, USA) after acidification to remove carbonates.

Calculation of predicted pore-water concentrations

Mean sediment concentrations for DDT and DDD in each sediment treatment at day 10 (*H. azteca*) or day 28 (*Diporeia* spp.) were estimated by multiplying the mean concentration of DDT equivalents in the sediment determined via LSC by the fraction of the total label comprised of each compound at day 0 determined via thin-layer chromatography. Predicted DDT and DDD pore-water concentrations were calculated using

Equation 1. Concentrations of DDT and DDD in the pore water were estimated using the equilibrium partitioning equation [10],

$$C_p = C_s / (f_{oc} K_{oc}) \quad (1)$$

where C_p is the estimated pore-water concentration (nmol/L), C_s is the estimated sediment concentration (nmol/g dry wt), f_{oc} is the fraction of organic carbon in the sediment, and K_{oc} is the organic carbon/water partitioning coefficient for the chemical of concern. The K_{oc} values for DDT and DDD were calculated using \log_{10} octanol/water partition coefficients ($\log_{10} K_{ow}$) values of 6.91 and 6.22 [19], respectively, and an equation for converting K_{ow} to K_{oc} [10] of

$$\log_{10} K_{oc} = 0.00028 + 0.983 \log_{10} K_{ow} \quad (2)$$

Statistics

All measurement values are expressed as mean \pm 1 SD. One-way analysis of variance was used to analyze amphipod survival data. Contaminant treatments were compared with the control treatment using Williams' test. Sediment concentrations at each treatment level for different exposure periods (0, 10, and 28 d) were compared using one-way analysis of variance. Tukey's honestly significant difference test was used for pairwise comparisons among all means. Significance level (α) was set at 0.05. Mortality data were transformed by arcsine-square-root prior to analysis. Mean lethal concentration (LC50) or mean lethal tissue residue (LR50) values were calculated using the trimmed Spearman-Kärber method.

Modeling

Accumulation data from the 28-d uptake experiment were fit to the two-compartment model [20]

$$\frac{dC_a}{dt} = k_u C_s - (k_e + k_g) C_a \quad (3)$$

where C_a is the concentration in the animal (nmol/g wet wt), k_u is the conditional uptake clearance rate coefficient (g sediment dry wt/g tissue wet wt/h), C_s is the concentration in the sediment (nmol/g dry wt), k_e is the conditional elimination rate constant (h^{-1}), k_g is the specific growth rate constant (h^{-1}), and t is time (h). Specific growth rate constants (k_g) for animals from each sediment concentration were calculated from the slope of the linear regression of the natural log of the wet weight per animal versus time (h) of exposure to the test sediment. Differences in growth rates between treatments were tested using the T method for unplanned comparisons among a set of regression coefficients [21]. Linear and nonlinear regression analyses were performed using Sigma Plot® (Release 4.0, SPSS, Chicago, IL, USA).

RESULTS

Sediment

The concentration of DDT molar equivalents in sediments at day 0 (Table 1) had coefficients of variation for replicates within a treatment less than 10%, suggesting the labeled compound was homogeneously distributed. The concentration of DDT molar equivalents in the sediment remained relatively constant during the 28-d exposure (Table 1). The sediment organic carbon content was $0.41 \pm 0.04\%$ for the first *H. azteca* and *Diporeia* spp. experiments, $0.60 \pm 0.07\%$ for the second *H. azteca* experiment, and $0.45 \pm 0.02\%$ for the second *Diporeia* spp. experiment.

Table 2. *Hyalella azteca* experiments; mean day 0 sediment concentrations; mean (standard deviation) days 2, 4, 10, and 28 percent survival; best estimate from nonlinear regression (standard error) for uptake clearance rate (k_u); organic carbon and lipid normalized k_u ($k_{u(OC-LIP)}$); elimination rate (k_e); elimination half-life (t_{95} , $0.693/k_e$); time to achieve 95% steady state (TSS₉₅, $2.99/k_e$); steady-state biota-sediment accumulation factor (BSAF_{SS}, $k_{u(OC-LIP)}/k_e$); and measured day-28 BSAF

Sediment concn. (nmol DDT equivalents/g dry wt)	% Survival				k_u (g dry sediment/g wet tissue/h) (SE)	$k_{u(OC-LIP)}$ (g organic carbon/g lipids/h)	k_e (h ⁻¹)	t_{95} (h)	TSS ₉₅ (h)	BSAF _{SS} $k_{u(OC-LIP)}/k_e$	Day-28 BSAF
	Day 2	Day 4	Day 10	Day 28							
First experiment											
0	93.3 (5.8)	80.0 (10.0)	80.0 (8.4)	90.0 (5.8)	ND ^a	ND	ND	ND	ND	ND	ND
2.1	80.0 (5.8)	83.3 (10.7)	82.0 (12.3)	83.3 (15.0)	0.0461 (0.0149)	0.0145 (0.0047)	0.0179 (0.0077)	32	167	0.81	0.44
5.6	80.0 (5.8)	90.0 (5.8)	86.0 (11.2)	86.7 (5.1)	0.0439 (0.0101)	0.0138 (0.0032)	0.0155 (0.0052)	37	193	0.89	0.57
9.1	80.0 (5.8)	55.0 (16.6)	28.0 (13.0) ^b	54.0 (16.7)	0.0366 (0.0083)	0.0115 (0.0026)	0.0151 (0.0050)	35	198	0.76	0.71
17.3	3.3 (5.1) ^b	0.0 ^b	0.0 ^b	0.0 ^b	ND	ND	ND	ND	ND	ND	ND
50.8	10.0 (5.8) ^b	0.0 ^b	0.0 ^b	0.0 ^b	ND	ND	ND	ND	ND	ND	ND
Second experiment											
0	90.0 (5.8)	96.3 (4.7)	95.0 (2.5)	92.5 (5.2)	ND	ND	ND	ND	ND	ND	ND
1.6	90.0 (0.0)	92.5 (6.3)	88.8 (6.2)	85.0 (4.8)	0.0525 (0.0389) ^c	0.0263 (0.0195) ^c	0.0322 (0.0258) ^c	21	93	0.82	1.07
3.0	87.5 (5.3)	92.5 (7.2)	67.5 (10.3) ^b	48.8 (6.2) ^b	0.0204 (0.0022)	0.0102 (0.0011)	0.0048 (0.0021)	144	623	2.13	2.08
5.3	52.5 (12.4) ^b	31.3 (6.2) ^b	21.3 (7.1) ^b	22.5 (6.6) ^b	0.0630 ^c (0.0361)	0.0315 (0.0015) ^c	0.0272 (0.0167) ^c	25	110	1.16	0.93
7.5	35.0 (10.6) ^b	15.0 (8.7) ^b	0.0 ^b	0.0 ^b	ND	ND	ND	ND	ND	ND	ND
13.2	7.5 (1.8) ^b	0.0 ^b	0.0 ^b	0.0 ^b	ND	ND	ND	ND	ND	ND	ND

^a ND = not determined.

^b Significantly different from control survival.

^c p value for the estimate >0.1.

Table 3. *Hyalella azteca* and *Diporeia* spp. experiments; median lethal concentrations (LC50s) and 95% confidence intervals (nmol DDT equivalents/g dry wt) calculated for different exposure periods

Day	<i>Hyalella azteca</i>		<i>Diporeia</i> spp.
	First experiment	Second experiment	Second experiment
2	10.7 (10.2–11.0)	5.4 (5.1–5.6)	64.0 (60.1–68.3)
4	9.9 (9.6–10.2)	4.2 (3.9–4.3)	50.5 (48.5–52.2)
10	8.2 (7.9–8.5)	3.1 (2.5–3.2)	34.7 (32.7–36.7)
17	7.3 (7.1–7.6)	3.1 (2.5–3.1)	24.3 (22.3–26.5)
28	9.9 (9.6–10.2)	2.8 (2.5–2.9)	8.2 (7.6–8.7)

second *H. azteca* experiment and in both *Diporeia* spp. experiments, the predicted DDT pore-water concentrations were typically higher than the predicted DDD pore-water concentrations. The predicted TUs for DDT far exceeded those for DDD. For both *H. azteca* experiments and in the second *Diporeia* spp. experiment, increases in the total pore-water TUs (Σ TU) DDT TUs + DDD TUs were accompanied by increases in mortality (Tables 5 and 6; Fig. 2). In the first *Diporeia* spp. experiment, increases in Σ TU to up to three were not accompanied by any significant increase in mortality (Table 5; Fig. 2B). Using the predicted Σ TUs for each sediment treatment and mortality data from individual replicates, 10-d LC50 values were 0.98 (0.95–1.00, 95% confidence interval [CI]) TU and 0.33 (0.32–0.34 CI) TU for the first and second *H. azteca* experiment, respectively. For the second *Diporeia* spp. experiment, the 28-d LC50 was 0.67 (0.64–0.69, CI) TU.

Biotransformation

Hyalella azteca and *Diporeia* spp. from the second experiment were analyzed for DDT biotransformation products. For day 28 *H. azteca*, most of the 14 C activity in the tissues was present as DDE, followed by DDT, DDD, and nonidentified polar metabolites (Table 7). For day 28 *Diporeia* spp., most of the 14 C activity corresponded to DDT, followed by DDD and nonidentified polar metabolites (Table 7). The metabolite DDE was not detected in the tissues of *Diporeia* spp.

Tissue concentrations and toxicity

Hyalella azteca. In the first experiment, decreased survival (<60%) at day 2 was associated with body residues ranging from 11 to 22 nmol DDT equivalents/g wet tissue (Fig. 3). At days 10 and 28, survival correlated poorly with body residue (Fig. 3). At day 10, body residues measured in amphipods from the 5.4 nmol DDT equivalents/g treatment, where mean survival was 87%, were very similar to body residues measured in the 9.1 nmol DDT equivalents/g treatment, where mean survival was much lower (28%). At day 28, body residues were measured only in treatments where survival was not significantly lower than in the control because of complete mortality in the two highest treatments. In the second experiment, decreased survival (<60%) at days 2, 10, and 28 occurred in association with body residues ranging from 6 to 19 nmol DDT equivalents/g wet tissue wet wt (Fig. 3). The LR50 values were calculated using survival and body residues measured as DDT molar equivalents for each replicate. Overall, the LR50 values calculated for different exposure periods were very similar in both experiments (Table 8). The LR50 values for the first experiment were higher than those for the second experiment by a factor of approximately two.

Diporeia spp. In the first experiment, body residues as high as 108 nmol/g wet wt were associated with high survival (>70%) for all exposure periods (Fig. 3), and consequently, an LR50 value could not be calculated. In the second experiment, survival decreased with increasing tissue concentrations. Low survival (<50%) was associated with body residues ranging from 20 to 110 nmol DDT equivalents/g wet wt (Fig. 3). The LR50 values were calculated using survival and body residues measured as DDT molar equivalents for each replicate. The LR50 values calculated for days 2 and 28 were very similar (Table 8). Insufficient partial mortality prevented the calculation of LR50 values for the 4-, 10-, and 17-d exposure periods. The LR50 values for *Diporeia* spp. were approximately 20% higher than those calculated for the first *H. azteca* experiment and two times higher than those for the second *H. azteca* experiment.

For the second *H. azteca* and *Diporeia* spp. experiments, mean tissue concentrations for DDT and DDD in each sediment treatment at day 10 (*H. azteca*) or day 28 (*Diporeia* spp.) were estimated by multiplying the tissue concentration of DDT equivalents by the fraction of the total label comprised of each compound (Table 7). To correlate the estimated body residue with critical body residues derived from water exposures, TUs were calculated by dividing the estimated tissue concentrations of DDT and DDD by the previously determined LR50s for those compounds [9] (Table 7). For *H. azteca*, estimated DDE tissue concentrations were higher than estimated DDT tissue concentration. However, because the LR50 for DDE was much higher, TUs for DDT far exceeded those for DDE. For *Diporeia* spp., estimated DDT tissue concentrations were higher than estimated DDD tissue concentrations, and because the LR50 for DDT was much smaller than for DDD, TUs were considerably higher for DDT. Those higher body residue TUs for DDT suggest that this compound should be contributing relatively more to the observed toxicity than DDE or DDD. For both *H. azteca* and *Diporeia* spp., increases in the Σ TUs were accompanied by increases in mortality (Table 7, Fig. 4). Using the estimated body residue Σ TUs for each sediment treatment and mortality data from individual replicates, 10-d LR50 TU values were 1.10 (0.99–1.24, CI) for the second *H. azteca* experiment and TU values for the 28-d LR50 were 0.53 (0.51–0.55, CI) for the second *Diporeia* spp. experiment.

Growth and lipid content

In the first experiment, *H. azteca* exhibited significant growth in all DDT treatments with surviving amphipods at day 28. Mean biomass at day 28 was 7.9 times higher than at day 0. Growth rates were similar and not significantly different across treatments, ranging from 0.0033 to 0.0048/h and averaging 0.0039/h (0.093/d). No significant growth was observed in the second *H. azteca* experiment except in the 5.4 nmol DDT equivalents/g treatment, where the estimated growth rate was much lower than those calculated for the first experiment (0.0008/h, or 0.013/d). *Diporeia* spp. showed no significant growth over the course of experiments 1 or 2.

Percent lipid content (g lipids/g wet wt) in control *H. azteca* was $0.88 \pm 0.04\%$ at day 0 and $1.79 \pm 0.41\%$ at day 28 for the first experiment and 0.66 ± 0.03 at day 0 and $1.73 \pm 0.25\%$ at day 28 for the second experiment. Percent lipid content in control *Diporeia* spp. was $11.59 \pm 1.18\%$ at day 0 and $10.85 \pm 0.62\%$ at day 28 for the first experiment and $5.97 \pm 0.75\%$ at day 0 and $6.27 \pm 1.21\%$ at day 28 for the second experiment. Although not measured, lipid content in DDT-

Table 4. *Diporeia* spp. experiments; mean day 0 sediment concentrations; mean (standard deviation) days 2, 4, 10, and 28 percent survival; best estimate from nonlinear regression (standard error) for uptake clearance rate (k_u); organic carbon and lipid normalized k_u ($k_{u(OC-LIP)}$); elimination rate (k_e); elimination half-life ($t_{1/2}$, $0.693/k_e$); time to achieve 95% steady state (TSS₉₅, $2.99/k_e$); steady-state biota-sediment accumulation factor (BSAF_{SS}, $k_{u(OC-LIP)}/k_e$); and measured day-28 BSAF

Sediment concn. (nmol DDT equivalents/g dry wt)	% Survival				k_u (g dry sediment/g wet tissue/h) (SE)	$k_{u(OC-LIP)}$ (g organic carbon/g lipids/h)	k_e (h ⁻¹)	$t_{1/2}$ (h)	TSS ₉₅ (h)	BSAF _{SS} $k_{u(OC-LIP)}/k_e$	Day-28 BSAF
	Day 2	Day 4	Day 10	Day 28							
First experiment											
0	100 (0.0)	100 (0.0)	100 (0.0)	96.7 (1.9)	NA ^a	NA	NA	NA	NA	NA	NA
3.7	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	86.7 (3.8)	0.042 (0.0005)	0.00017 (0.00020)	0.0003 (0.0004) ^b	2310	9967	0.56	0.10
7.8	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	83.3 (10.2)	0.033 (0.0007)	0.00013 (0.00003)	0 (0.0007) ^b	NA	NA	NA	0.11
25.5	100.0 (0.0)	100.0 (0.0)	92.5 (1.3)	86.7 (10.2)	0.0055 (0.0007)	0.00022 (0.00003)	0 (0.0004) ^b	NA	NA	NA	0.16
43.3	100.0 (0.0)	96.7 (5.1)	94.0 (8.3)	83.3 (6.9)	0.0016 (0.0001)	0.00006 (0.00001)	0.0009 (0.0003)	770	3322	0.07	0.03
58.8	100.0 (0.0)	100.0 (0.0)	88.0 (10.9)	80.0 (10.0)	0.0014 (0.0002)	0.00006 (0.00001)	0 (0.0004) ^b	NA	NA	NA	0.05
Second experiment											
0	100 (0)	95.0 (10.0)	92.5 (9.6)	97.5 (5.0)	NA	NA	NA	NA	NA	NA	NA
8.3	95.0 (7.1)	97.5 (5.0)	90.0 (11.5)	40.0 (14.1) ^c	0.0119 (0.0027)	0.00080 (0.00018)	0.0027 (0.0008)	257	1107	0.30	0.31
14.0	95.0 (7.1)	85.0 (17.3)	77.5 (20.6)	22.5 (12.6) ^c	0.0081 (0.0021)	0.00054 (0.00014)	0.0020 (0.0009)	347	1495	0.27	0.30
29.2	95.0 (7.1)	90.0 (14.1)	55.0 (5.8) ^c	7.5 (5.0) ^c	0.0046 (0.0023)	0.00031 (0.00015)	0.0023 (0.0008)	301	1300	0.13	0.11
87.7	15.0 (7.1) ^c	0.0 ^a	0.0 ^c	0.0 ^c	NA	NA	NA	NA	NA	NA	NA
239.0	10.0 (14.1) ^c	0.0 ^a	0.0 ^c	0.0 ^c	NA	NA	NA	NA	NA	NA	NA

^a NA = not applicable.

^b *p* value for the estimate >0.1.

^c Significantly different from control survival.

Table 5. *Hyalella azteca* experiments; sediment concentrations of DDT molar equivalents and DDT and dichlorodiphenyldichloroethane (DDD) percent of total label in sediment extracts, estimated sediment concentrations, predicted pore-water (PW) concentrations, compound toxic units (TUs), sum toxic units (Σ TU), and day-10 mean percent mortality

DDT equivalents sediment concn. (nmol/g)	Compound	% Total label	Estimated sediment concn. (nmol/g)	Predicted PW concn. (nmol/L)	TU ^a	Σ TU	Mean percent mortality
First experiment							
2.1	DDT	53.6	1.1	0.04	0.16	0.22	18
	DDD	33.83	0.7	0.13	0.06		
5.6	DDT	59.5	3.3	0.13	0.46	0.61	14
	DDD	30.3	1.7	0.32	0.15		
9.1	DDT	64.2	5.8	0.23	0.81	1.03	72
	DDD	27.17	2.5	0.46	0.21		
17.3	DDT	73.6	12.7	0.50	1.77	2.08	100
	DDD	20.5	3.5	0.66	0.31		
50.8	DDT	88.8	45.1	1.77	6.28	6.65	100
	DDD	8.4	4.3	0.80	0.37		
Second experiment							
1.6	DDT	90	1.4	0.04	0.14	0.14	11
	DDD	5	0.1	0.01	0.00		
3.0	DDT	82	2.5	0.07	0.23	0.25	32
	DDD	11	0.3	0.04	0.02		
5.3	DDT	85	4.5	0.12	0.43	0.46	79
	DDD	10	0.5	0.07	0.03		
7.5	DDT	83	6.2	0.17	0.59	0.64	100
	DDD	10	0.8	0.10	0.04		
13.2	DDT	86	11.4	0.30	1.08	1.15	100
	DDD	9	1.2	0.15	0.07		

^a Calculated using 10-d LC50 values of 0.28 and 2.41 nmol/L for DDT and DDD, respectively [9].

Table 6. *Diporeia* spp. experiments; sediment concentrations of DDT molar equivalents and DDT and dichlorodiphenyldichloroethane (DDD) percent of total label in sediment extracts, estimated sediment concentrations, predicted pore-water (PW) concentrations, compound toxic units (TUs), sum toxic units (Σ TU), and day-10 mean percent mortality

DDT equivalents sediment concn. (nmol/g)	Compound	% Total label	Estimated sediment concn. (nmol/g)	Predicted PW concn. (nmol/L)	TU ^a	Σ TU	Mean percent mortality
First experiment							
3.7	DDT	76	2.8	0.12	0.15	0.17	13
	DDD	18	0.7	0.12	0.02		
7.8	DDT	81	6.3	0.25	0.34	0.38	17
	DDD	14	1.1	0.20	0.04		
25.5	DDT	75	19.1	0.75	1.02	1.19	13
	DDD	19	4.8	0.91	0.16		
43.3	DDT	93	40.3	1.58	2.16	2.23	17
	DDD	5	2.2	0.41	0.07		
58.8	DDT	94	55.3	2.17	2.96	3.04	20
	DDD	4	2.4	0.44	0.08		
Second experiment							
8.3	DDT	74	6.1	0.22	0.30	0.33	60
	DDD	12	1.0	0.17	0.03		
14	DDT	91	12.7	0.46	0.62	0.65	77
	DDD	6	0.8	0.14	0.03		
29.2	DDT	95	27.7	0.99	1.35	1.38	92.5
	DDD	3	0.9	0.15	0.03		
87.7	DDT	93	81.6	2.92	3.98	4.12	100
	DDD	5	4.4	0.75	0.14		
239	DDT	94	224.7	8.04	10.97	11.19	100
	DDD	3	7.2	1.22	0.22		

^a Calculated using 28-d LC50 values of 0.73 and 6.1 nmol/L for DDT and DDD, respectively [9].

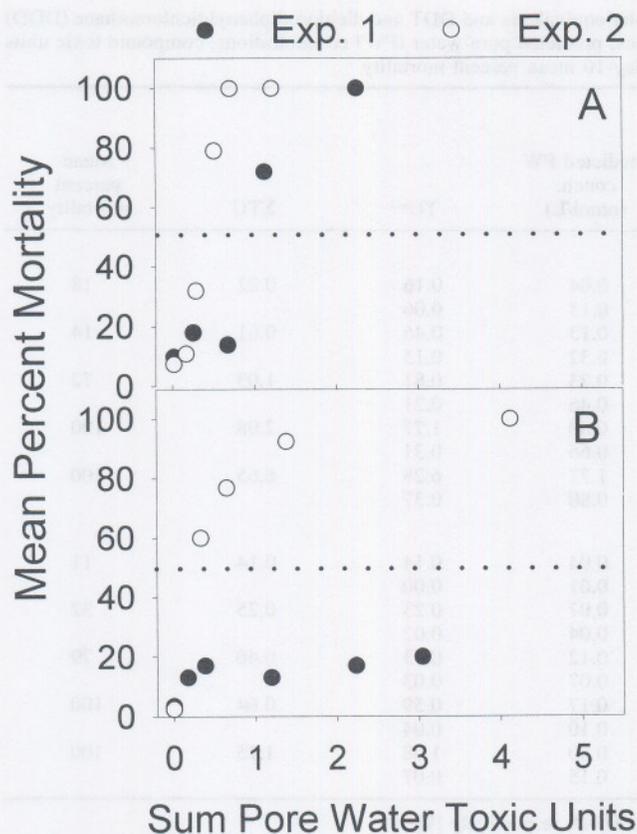


Fig. 2. Observed 28-d mortality versus predicted pore-water sum toxic units in the first (Exp 1) and second (Exp 2) *Hyalella azteca* (A) and *Diporeia* spp. (B) experiments.

exposed *H. azteca* may have been lower than that in the control animals, as suggested by water-only exposures [9]. Exposure to DDT is not expected to influence lipid content in *Diporeia* spp. over 28 d [9].

Uptake kinetics

For *H. azteca*, uptake data were collected over a period of 28 d only for the three lowest concentrations in each experiment because of complete mortality before day 10 in the two highest treatments (Table 2). In the first experiment, uptake clearance rate constants (k_u) were similar across sediment concentrations. The k_u estimates for the first experiment averaged 0.0422 g dry sediment/g wet tissue/h. Sediment organic carbon and lipid-normalized uptake rate constants ($k_{u(OC-LIP)}$) were estimated by dividing k_u by the mean total organic carbon in the sediment and by the overall mean total lipid content in control amphipods (days 0 and 28). The $k_{u(OC-LIP)}$ estimates for the first experiment averaged 0.0132 g organic carbon/g lipids/h. In the second experiment, the only reliable estimate for k_u ($p < 0.05$), obtained for the 3.0 nmol DDT equivalents/g treatment, was lower than k_u values calculated for the first experiment (Table 2). For *Diporeia* spp., uptake data were collected over a period of 28 d for all treatments except the two highest concentrations in the second experiment because of complete mortality before day 4. Overall, k_u tended to decrease with increasing compound concentration in the sediment (Table 4). In the second experiment, this decrease was more apparent and was accompanied by a decrease in survival (Table 4). For similar sediment concentrations, $k_{u(OC-LIP)}$ values for *Diporeia* spp. were consistently higher in the second experiment than

in the first experiment and were considerably lower than values estimated for *H. azteca*.

For *H. azteca*, elimination rate constants (k_e) in the first experiment were within a close range and averaged 0.0162/h for k_e . Elimination half-lives ($t_{1/2}$) averaged 34.8 h. In the second experiment, the only reliable estimate for k_e ($p < 0.05$), obtained for the 3.0 nmol DDT equivalents/g treatment, was approximately three times lower than the mean k_e obtained for the first experiment (Table 4). For *Diporeia* spp., reliable estimates for k_e in the first experiment were only obtained for the 43.3 nmol DDT equivalents/g treatment (Table 4). Exceedingly low k_e estimates can be explained by the linear nature of the uptake curves (Fig. 5). In the second experiment, estimates for k_e (Table 4) were lower than the mean calculated for the first *H. azteca* experiment by approximately a factor of seven.

Steady state and biota-sediment accumulation factors

Visual inspection of uptake curves indicates that apparent steady state was reached in less than 10 d for *H. azteca* in both experiments (Fig. 5). By day 28, *Diporeia* spp. was far from approaching steady state in the first experiment and the uptake curve was approaching an asymptote in the second experiment (Fig. 5). Time to achieve 95% steady-state residues, calculated as $2.99/k_e$ (Tables 2 and 4), confirms that *H. azteca* was approaching steady state by day 8 and indicates that *Diporeia* spp. would take periods much longer than the experiment duration (>45 d) to approach steady state. For *H. azteca*, steady-state biota-sediment accumulation factors ($BSAF_{ss}$), estimated as $k_{u(OC-LIP)}/k_e$ [20], were higher than for *Diporeia* spp., with the mean value for *H. azteca* (0.26) approximately five times higher than the mean value for *Diporeia* spp. (0.05). For both *H. azteca* experiments (Table 2) and in the second *Diporeia* spp. experiment (Table 4), $BSAF_{ss}$ was similar to 28-d BSAFs. For the *Diporeia* spp. first experiment, 28-d BSAFs were considerably lower than $BSAF_{ss}$ (Table 4).

DISCUSSION

Pore-water toxic units

Although the exposure sediment was spiked only with DDT, transformation of DDT during the storage resulted in the formation of DDD and, to a minor extent, nonidentified polar metabolites. Transformation of DDT to DDD in spiked sediments has been previously observed [22–24]. The relative proportion of DDT and its metabolites in the sediment differed among treatments both within and among experiments. The extent of DDT degradation in the sediment tended to decrease with increasing concentration of DDT molar equivalents in the sediment, especially in the first *H. azteca* and *Diporeia* spp. experiments. The apparent negative relationship between the DDT concentration in the sediment and the relative fraction transformed suggests that the transformation of DDT was mediated by microbes. Low relative DDT degradation at high sediment concentrations may have occurred because of saturation of the microbial enzymatic systems or because of lower activity of the microbial community resulting from exposure to DDT.

Differences in the relative proportion of DDT and DDD among the exposure sediments were significant, with the DDT:DDD concentration ratio ranging from 18.6 to 1.6 at the initiation of organism exposures. Although the relative lethality of DDT and its major metabolites (DDD and DDE)

Table 7. *Hyalella azteca* and *Diporeia* spp. second experiments; sediment treatment, 28-d DDT molar equivalents tissue concentrations; percent of total label in 28-d tissue extracts represented by nonidentified polar metabolites, DDT, dichlorodiphenyldichloroethane (DDD), and dichlorodiphenyldichloroethylene (DDE); estimated tissue concentrations for these compounds; median lethal residue values determined in water exposures (water LR50, [9]); compound toxic units (TUs); sum toxic units (Σ TU); and 28-d mean percent mortality

Sediment concn.	DDT equivalents (nmol/g)		Compound	% Total label	Estimated tissue concn. (nmol/g wet wt)	Water LR50 (nmol/g wet wt)	TU	Σ TU	Mean percent mortality
	Tissue concn. (wet wt)								
<i>Hyalella azteca</i>									
1.6	1.4		Polar	4	0.1	NA ^a	NA	0.24	15
			DDT	34	0.5	2	0.24		
			DDD	6	0.1	47	0.00		
			DDE	56	0.8	389	0.00		
3.0	6.7		Polar	6	0.4	NA	NA	0.76	51
			DDT	22	1.5	2	0.74		
			DDD	11	0.7	47	0.02		
			DDE	61	4.1	389	0.01		
5.3	12.4		Polar	4	0.5	NA	NA	1.86	81.5
			DDT	29	3.6	2	1.80		
			DDD	16	2.0	47	0.04		
			DDE	51	6.3	389	0.02		
<i>Diporeia</i> spp.									
8.3	25.6		Polar	4	1.0	NA	NA	0.35	60
			DDT	53	13.6	44	0.31		
			DDD	43	11.0	263	0.04		
14	56.3		Polar	5	2.8	NA	NA	0.89	77
			DDT	64	36.0	44	0.82		
			DDD	31	17.5	263	0.07		
29.2	74.9		Polar	5	3.7	NA	NA	1.21	92
			DDT	66	49.4	44	1.12		
			DDD	29	21.7	263	0.08		

^a NA = not applicable.

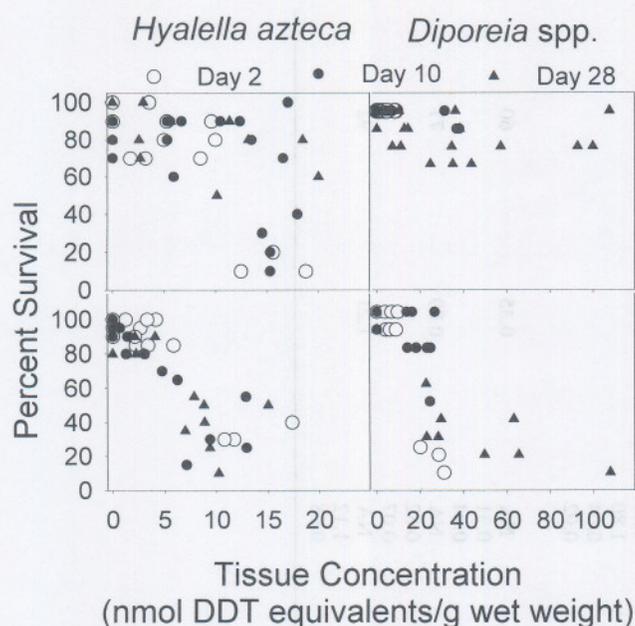


Fig. 3. The DDT equivalents body residue versus percent survival at days 0, 10, and 28 in the first (top graphs) and second (bottom graphs) *Hyalella azteca* and *Diporeia* spp. experiments.

to benthic invertebrates has been assumed to be similar [3,4,25], water-only experiments indicated that the lethal toxicities of DDT, DDD, and DDE are remarkably different for *H. azteca* [2,9] and *Diporeia* spp. [9]. Therefore, the effects of DDT-spiked sediments to *H. azteca* and *Diporeia* spp. in this study were investigated using the toxic units approach for contaminant mixtures, where the relative toxicological importance of each contaminant in the mixture can be evaluated. The toxicity of DDT and DDD to *H. azteca* and *Diporeia* spp. had previously been investigated in water-only, but not in sediment, exposures. Therefore, toxic units were calculated for pore-water concentrations of freely dissolved DDT and DDD that were predicted using the equilibrium-partitioning theory.

In the second *H. azteca* experiment and in both *Diporeia* spp. experiments, predicted pore-water concentrations in the sediments at day 0 were equal or higher for DDT than DDD. In the first *H. azteca* experiment, predicted concentrations in the pore water were higher for DDD than for DDT. Because

Table 8. *Hyalella azteca* and *Diporeia* spp. experiments; Median lethal residues (LR50s) and 95% confidence intervals (nmol DDT equivalents/g wet tissue) calculated for different exposure periods

Day	<i>Hyalella azteca</i>		<i>Diporeia</i> spp.
	First experiment	Second experiment	Second experiment
2	ND ^a	7.1 (6.6–7.7)	18.5 (17.2–19.9)
4	ND	8.1 (5.7–11.1)	ND
10	15.2 (15.0–15.4)	7.4 (4.7–11.6)	ND
17	14.1 (13.2–15.1)	5.2 (4.1–6.6)	ND
28	ND	8.0 (6.7–9.7)	16.8 (13.5–20.9)

^a ND = not determined because of insufficient partial mortality.

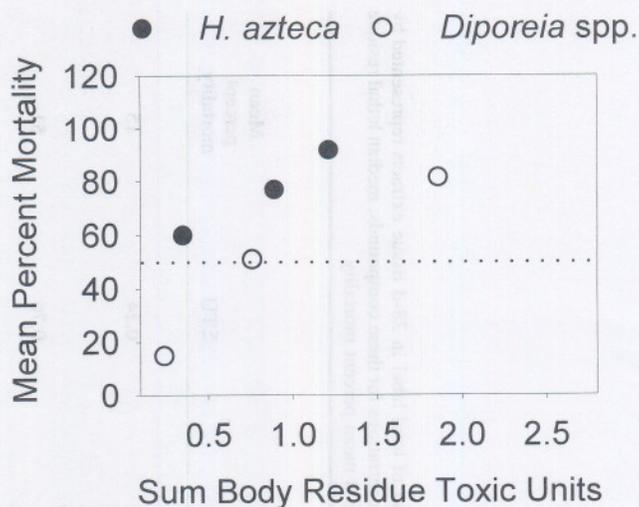


Fig. 4. Observed 28-d mean percent mortality versus predicted sum body residue toxic units in the second *Hyalella azteca* and *Diporeia* spp. experiments.

the previously determined water LC50 was much higher for DDD than for DDT [9], the number of pore-water TUs was much lower for DDD, even when the pore-water concentration for DDD was higher than for DDT. Therefore, the contribution of DDT to the observed mortality far exceeded the contribution from DDD in most treatments. The contribution of DDD to the total pore-water TUs never exceeded 33%, whereas the contribution of DDT exceeded 90% in most treatments. Assuming that the toxicity of compounds freely dissolved in the pore water or in solution in water-only exposure is similar and that the effects of DDT and DDD on survival are additive, 50% mortality in exposures to spiked sediments should occur when the sum of the pore-water toxic units for DDT and DDD (Σ TU) is one. In the *H. azteca* first experiment, mortality in exposures to DDT-spiked sediments occurred as predicted by the EqP- Σ TU model, as the Σ TU LC50 was 0.98 TU. Mortality was higher than expected by the Σ TU LC50 model in the second *H. azteca* and *Diporeia* spp. experiments, as the calculated Σ TU LC50 for those experiments (0.33 and 0.67 TUs, respectively) were lower than one. In the first *Diporeia* spp. experiment, however, significant mortality did not occur during

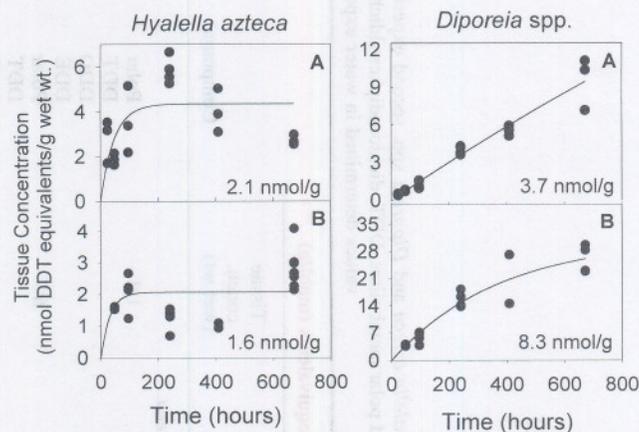


Fig. 5. *Hyalella azteca* and *Diporeia* spp. bioaccumulation of DDT molar equivalents in the course of 28-d exposures to various concentrations of DDT molar equivalents in the sediment in the first (A) and second (B) experiments.

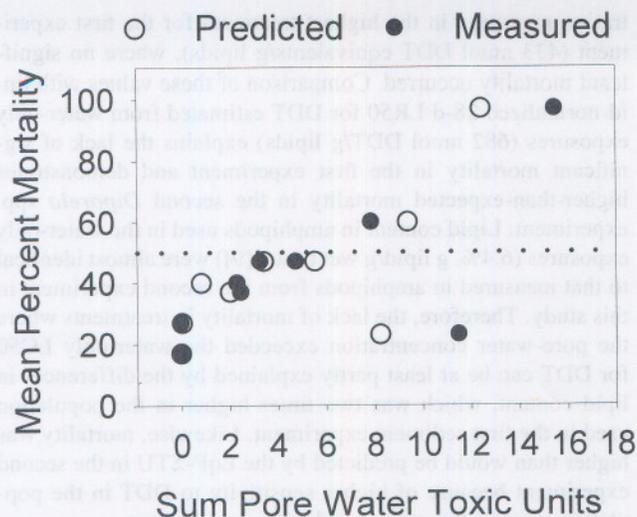


Fig. 6. Observed mortality versus sum pore-water toxic units in *Hyalella azteca* exposed to DDT-, dichlorodiphenyldichloroethane (DDD)-, and dichlorodiphenyldichloroethylene (DDE)-contaminated field-collected sediment in the study of Hoke et al. [2]. Pore-water concentrations were measured (solid circles) or predicted using equilibrium partitioning calculations (open circles).

the experiment, even in the highest sediment treatment where the pore-water Σ TU was 3.04. Because of their high lipid content, *Diporeia* spp. used in the first sediment experiment may have been more tolerant than *Diporeia* spp. used in the second experiment and for determining the LC50 in aqueous exposures [9].

With the exception of the first *Diporeia* spp. experiment, mortality in exposures to DDT and DDE occurred at pore-water concentrations predicted by EqP to be toxic using water-only toxicity data, which indicates that organic carbon content strongly influenced the bioavailability of sediment-bound DDT to freshwater amphipods in our experiments. In a previous study [26], organic carbon normalization accounted for most of the variability in acute lethality when *H. azteca* were exposed to different sediments spiked with DDT. Sediment organic carbon content seems to influence the bioavailability of DDT and its metabolite compounds to freshwater invertebrates in historically contaminated sediments as well. A strong correlation between the measured pore-water concentration of DDT and its metabolites and mortality was observed when *H. azteca* [2] or *Chironomus tentans* [7] were exposed to sediments collected from a contaminated site near Huntsville, Alabama, USA.

The data presented in Hoke et al. [2] were used to determine the relationship between EqP-predicted pore-water concentrations and *H. azteca* mortality in historically contaminated sediments. The concentrations of *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE and the organic carbon contents of field-collected sediments provided in Table 2 from Hoke et al. [2] as well as K_{ow} values from De Bruijn et al. [19] were used in the calculation of predicted pore-water concentrations. Ten-day water-only LC50 values obtained by Hoke et al. [2] for *H. azteca* were used for estimating pore-water TUs for *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE. Mortality increased with increasing Σ TU, but was low (<50%) for TUs ranging from one to six and only approached 100% when Σ TU was over 12 (Fig. 6), demonstrating that mortality was underpredicted using the EqP- Σ TU model. This finding sharply contrasts with the relationship between Σ TU and mortality (Fig. 2) and the Σ TU LC50 values

of 0.33 and 0.98 obtained in this study for DDT-spiked sediments. Mortality was also lower than expected when pore-water TUs for *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE were calculated using measured concentrations (Fig. 6). These remarkable differences in expected mortality between this study and that by Hoke et al. [2] may have resulted from differences in the partitioning of DDT and its major metabolites into the pore water as freely dissolved compounds. Partition coefficients for these compounds may increase as the contact time with the sediment increases. Contact time was only 60 d in this study but was over 20 years in sediments used by Hoke et al. [2]. When *C. tentans* were exposed to historically contaminated sediments collected from the same sites as the samples used by Hoke et al. [2], mortality was also lower than expected based on EqP- Σ TU, as 50% occurred between 2 and 5 pore-water Σ TU [7]. Therefore, compound-specific toxicity data derived from laboratory exposures using spiked sediments should be used with caution for establishing guidelines for quality assessment field sediments since toxicity may be overestimated.

Evidence from this study suggests that the toxicity of sediment-spiked DDT and its major metabolites to benthic invertebrates can be predicted from effects determined in water-only exposures using the EqP model. In exposures to sediment-spiked fluoranthene (a polycyclic aromatic hydrocarbon), however, mortality of *H. azteca*, *Diporeia* spp. [11], or *Leptocheirus plumulosus*, an estuarine amphipod, was low at predicted pore-water concentrations similar to the water-only LC50 for those species. The differences in the predictions for the two classes of contaminants chlorinated hydrocarbons and polycyclic aromatic hydrocarbons are consistent with previous efforts. The bioavailability of sediment-associated chlorinated hydrocarbons is often greater than that of polycyclic aromatic hydrocarbons even from the same sediment [27–29]. This difference in bioavailability appears to result in part from differences in the partitioning of different contaminants to different sediment particles [28,30], the character of the organic matter that controls the binding and bioavailability [31], and the feeding selectivity of the organism [28].

Body residues toxic units

Hyalella azteca biotransforms DDT to DDE but not to DDD, whereas *Diporeia* spp. biotransforms DDT to DDD but not to DDE [9]. Therefore, DDT and DDD detected in the tissues of *H. azteca* in the second experiment were taken up from sediment, whereas DDE was a product of DDT biotransformation. For *Diporeia* spp., DDD in the tissues may have resulted from both direct uptake and from biotransformation of DDT. In aqueous exposures, the DDT, DDD, and DDE critical body residues for *H. azteca* and *Diporeia* spp., expressed as LR50, differed greatly [9] and DDT caused the greatest impact on survival. Therefore, the relative proportion of DDT and its metabolites in the tissue should be taken into account in residue-based assessments of DDT toxicity. Assuming that critical body residues are independent of the exposure route and that the effects of DDT, DDD, and DDE on survival are additive, 50% mortality in exposures to spiked sediments should occur when the sum of the toxic units for tissue concentrations of DDT, DDD, and DDE (Σ TU) is one. In the *H. azteca* second experiment, the Σ TU LR50 was 1.10 TUs, confirming the approach prediction. In the *Diporeia* spp. second experiment, however, mortality was somewhat higher than predicted by the Σ TU LR50 model since the Σ TU LR50

was 0.53 TU. The use of critical body residues estimated from water-only exposures appears to be a reliable method for predicting mortality in sediment exposures to DDT and its major metabolites. Similarly, mortality of freshwater amphipods in sediment exposures to fluoranthene occurred when the internal concentration of this compound attained critical body residues estimated from water-only exposures [11]. Thus, the body residue approach offers advantages for evaluating the hazard of contaminants to organisms based on the bioaccumulated residues and without the need to predict or interpret the bioavailability of the contaminant.

Comparative sensitivity

The DDT-spiked sediments exerted a greater impact on the survival of *H. azteca* in the second experiment, as evidenced by the organic carbon-normalized 10-d LC50 values of 2,000 and 517 μg DDT equivalents/g organic carbon for the first and second experiments, respectively. The 10-d ΣTU LC50 was three times higher for the first experiment, indicating that differences in the relative proportions of DDT and DDD in the spiked sediments may have only partially accounted for the difference in sensitivity between the two experiments. Substantial growth occurred in the first experiment, whereas no growth was observed in the second experiment. The reasons for the higher sensitivity and lack of significant growth of *H. azteca* in the second experiment are unknown; the age and lipid content of the test organisms and experimental conditions were comparable and adequate water quality was maintained in both experiments. The 10-d LC50 value for the second experiment was comparable to values calculated by Nebeker et al. [26] (272–473 $\mu\text{g}/\text{g}$ organic carbon) for toxicity tests where sediments with different organic carbon contents were spiked with DDT and two-month-old *H. azteca* were used.

In the second *H. azteca* experiment, survival correlated strongly with body residues and mortality increased with increasing body residues within the range of 5 to 20 nmol DDT molar equivalents/g wet tissue. In the first experiment, however, mortality correlated poorly with tissue concentrations of DDT equivalents when data from all exposure periods are considered. As observed in the second experiment, DDT and its metabolites, DDE and DDD, were likely present in the tissues of *H. azteca*. Most mortality in the first experiment occurred during the first 48 h of exposure. During this period, the extent of DDT biotransformation in the tissues was likely small and survival was dramatically decreased when tissue concentrations exceeded 10 nmol DDT molar equivalents/g wet tissue, as observed in the second experiment. For days 10 and 28, however, an unexpectedly wide range of mortality (10–90%) was observed for tissue residues ranging from 12 to 18 nmol DDT molar equivalents/g wet tissue. Although not measured, it is possible that the relative tissue concentrations of DDT, DDD, and DDE were highly variable in amphipods from the first experiment. Because the critical body residues for DDT and its metabolites differ greatly [9], high variability in the relative concentration of compounds in the tissues would result in high variability in survival within a narrow range of DDT-equivalents tissue concentrations.

For *Diporeia* spp., large differences in sensitivity to DDT were observed between the first and second experiments. The higher tolerance of organisms from the first experiment is likely explained by their higher lipid content. The lipid-normalized 28-d LR50 for the second experiment (268 nmol DDT equivalents/g lipids) was much lower than the mean tissue concen-

tration measured in the highest treatment for the first experiment (433 nmol DDT equivalents/g lipids), where no significant mortality occurred. Comparison of these values with lipid-normalized 28-d LR50 for DDT estimated from water-only exposures (682 nmol DDT/g lipids) explains the lack of significant mortality in the first experiment and demonstrates higher-than-expected mortality in the second *Diporeia* spp. experiment. Lipid content in amphipods used in the water-only exposures (6.4%, g lipid/g wet tissue [9]) were almost identical to that measured in amphipods from the second experiment in this study. Therefore, the lack of mortality in treatments where the pore-water concentration exceeded the water-only LC50 for DDT can be at least partly explained by the differences in lipid content, which was two times higher in the population used in the first sediment experiment. Likewise, mortality was higher than would be predicted by the EqP- ΣTU in the second experiment because of higher sensitivity to DDT in the population used in the second sediment experiment.

A portion of the difference in sensitivity between the two *Diporeia* experiments may be a consequence of size differences since smaller amphipods were field collected and used in the second experiment. *Diporeia* collected in April from a 30-m station tended to be smaller than those collected in October [32]. Smaller *Diporeia* exhibit higher feeding rates and higher biota-sediment accumulation factors for accumulation of nonpolar contaminants based on the toxicokinetics [33]. Thus, the more rapid accumulation could have led to greater exposure in a shorter time frame for the organisms in the second experiment. This is supported to some extent in the measured uptake coefficients between the two experiments, where the organisms in the second experiment had larger uptake coefficients. This should lead to greater exposure at the receptor site in a shorter time frame, which may lead to greater sensitivity.

Unlike *H. azteca*, *Diporeia* spp. are not amenable to culturing in the laboratory and therefore were field collected for use. Animals were collected in September of 1996 for the first experiment and in April of 1998 for the second experiment. The higher-than-expected response in *Diporeia* spp. may have resulted in part from additional environmental stressors that were affecting the animals collected for the second experiment. The *Diporeia* spp. population in southern Lake Michigan has experienced a rapid decline beginning in the early 1990s at the extreme southern portion of the lake [34]. This decline has spread rapidly. Between the two collection dates at 45-m depth off Muskegon, Michigan, USA, near our site of collection for *Diporeia* spp., the population declined approximately 40% and subsequently declined even further (approximately 60%) by the following year (T.F. Nalepa, unpublished data). Thus, the factors that resulted in the population decline likely produced a stressed population that yielded greater responses than expected to DDT exposures in the second experiment.

Toxicokinetics

The uptake clearance rate for DDT equivalents from sediment was higher in *H. azteca* than in *Diporeia* spp., even after normalization by sediment organic carbon and animal lipid content. Elimination rates were also much larger for *H. azteca* than for *Diporeia* spp. in sediment exposures. The uptake clearance and elimination rates for DDT, DDD, and DDE from water were also considerably higher for *H. azteca* than for *Diporeia* spp. [9]. Higher surface-to-volume ratios for *H. azteca*, as well as the higher temperature used in their exper-

iments, likely contributed to the higher uptake and elimination rates, as speculated for water-only exposures to DDT and its major metabolites [9]. Even after accounting for differences in lipid content, compound uptake from sediment in *Diporeia* spp. was much lower in the first experiment. The uptake clearance in the second experiment was similar to previously determined values for accumulation from sediment [27], while the values for the first experiment were much lower.

Diporeia spp. are intermittent feeders, with the smaller amphipods feeding more based on the gut fullness measured in field-collected organisms [35]. *Diporeia* spp. feeding rates have been confirmed to be substantially faster in smaller animals in laboratory studies (unpublished data). Further, smaller amphipods contain lower lipid content than larger organisms [33]. Thus, it may be that the organisms with lower lipid content in the second experiment had greater feeding rates than the higher lipid content organisms in the first experiment. In addition, because the *Diporeia* spp. for the second experiment may have been stressed due to environmental conditions, they may have fed at a greater rate. Because the rate of accumulation of contaminants depends on both the accumulation from the interstitial water and from ingestion of contaminated particles, changes in feeding rate can affect the accumulation of contaminants. As was stated above, the measured uptake coefficients tend to support a greater feeding rate and greater exposure in the second experiment.

Elimination rates were also lower in the first experiment. Elimination rate measured using the uptake data in the second *Diporeia* spp. experiment was much higher than previous experimentally measured elimination rates [9] despite similar lipid content in animals used in both studies. The rate of compound uptake may decrease as organisms become more intoxicated, as speculated when *H. azteca* and *Diporeia* spp. were exposed to fluoranthene [11]. Mortality increased during the second *Diporeia* spp. experiment in all treatments, reflecting increased intoxication. As the nonlinear model used to estimate k_e in the sediment exposure assumes that k_u is constant throughout the experiment, a decrease in k_u with time in the sediment exposure would result in a slower increase in tissue concentrations toward the end of the experiment that forces the uptake curve toward an asymptote, resulting in an inaccurately high estimate for k_e . Elimination rates determined for the first *Diporeia* spp. experiment, where no significant mortality was observed, were very similar to the experimentally measured elimination rates.

For *Diporeia* spp., uptake rate tended to decline with increasing DDT concentration in the sediment (this study) but not in the water [9]. Loss of swimming ability occurred at sublethal concentrations in water exposures [9]. Compound uptake in sediment may decrease with increasing immobilization because, as animals lose their locomotor ability, they may deplete the pool of bioavailable compound in their surrounding environment and thus avoid exposure to less depleted pools. Changes in toxicokinetics with increasing sediment concentrations leading to toxic responses have also been observed for *Diporeia* spp. exposed to pyrene [36]. However, the uptake rates increased with increasing dose and then declined again at concentrations that led to mortality. The changes in rates were speculated to be affected by changes in feeding rate as well as by other behaviors. Thus, while the feeding rate was likely somewhat greater for the April-collected organisms than for those collected in October, the decline in uptake coefficients with dose was similar between for the two studies. Thus, re-

ductions in feeding rates with increasing dose could also have played a substantial role in the decline in uptake rates with increasing DDT concentrations.

The strong correlation between pore-water toxic units and mortality in sediment exposures to DDT indicates that pore-water concentration reflects the bioavailability of DDT to *H. azteca* and *Diporeia* spp. Calculation of uptake clearance rates using pore water as the source compartment is complicated by the fact that DDD and DDT were present in the sediment and their relative concentrations were not measured at intermediate time points during the 28-d exposure.

For *Diporeia* spp., DDT metabolism was minimal (<5%) in the 29.9 nmol/g treatment of the second experiment. Using predicted pore-water concentrations estimated using sediment DDT molar equivalents concentrations and the K_{ow} for DDT [19], the uptake clearance rates for the 29.9 nmol/g treatment was 1,919 ml/g lipid/h. This value was similar to the uptake rate measured for DDT in water, approximately 1,100 ml/g lipid/h [9], which suggests that pore water may have been the major source of DDT uptake for *Diporeia* spp. in sediment exposures.

Highly hydrophobic organic compounds, such as DDT, are typically taken up and eliminated very slowly in benthic invertebrates in sediment exposures (e.g., [37,38]). Therefore, critical body residues for these compounds may not be attained in short-term exposures to low and moderately contaminated sediments due to nonattainment of steady-state concentrations. In this study, DDT tissue concentrations in *H. azteca* reached apparent steady state in approximately 8 d. Based on the rate of DDT uptake from sediment in this study, the 10-d duration of the U.S. Environmental Protection Agency recommended acute test with *H. azteca* [16] should be a long enough exposure for most highly hydrophobic organic compounds with environmental concern to reach steady state. For *Diporeia* spp., DDT tissue residues were far from reaching steady state after a 28-d exposure to spiked sediments. Similarly, time to achieve apparent steady-state body residues in *Diporeia* spp. was also exceedingly long for other chlorinated hydrocarbons and for polycyclic aromatic hydrocarbon congeners in sediment exposures [29]. Therefore, the use of *Diporeia* spp. in short-term toxicity testing for assessing the quality of contaminated sediments may not be appropriate.

In this study, two different procedures were used to spike DDT to sediment. Measured concentrations were similar to the target concentrations with both procedures, indicating that most DDT coating either glass jars or sand grains ended up bound to sediment particles during the mixing period. The bioavailability of DDT to amphipods was apparently not influenced by the spiking method, as indicated by similar BSAFs for each amphipod in both experiments.

SUMMARY AND CONCLUSIONS

The DDT spiked into sediments and held for equilibration degraded differentially depending on the DDT concentration, with greater degradation at lower sediment concentrations. This resulted in unequal exposures of organisms to mixtures of these contaminants for the different treatments. However, DDT is substantially more toxic than the degradation products; therefore, on a toxic units basis, DDT contributed the majority of the toxicity to the amphipods at all doses. The toxicity was evaluated using a toxic units approach, and both the equilibrium partitioning and the critical body residue approaches yielded reasonable and equally accurate interpretations of the

toxicity. This is substantially different from sediment exposures of these two amphipod species to polycyclic aromatic hydrocarbon congeners, where the equilibrium partitioning approach substantially overestimated the toxicity. The two amphipods appear to be similar in sensitivity to DDT and its degradation products if both are allowed to come to steady state. However, *Diporeia* spp. will require greater than 45 d to attain steady state, while *H. azteca* will be at steady state in less than 10 d when exposed to contaminated sediments as determined from the toxicokinetics. The absence of sensitivity of *Diporeia* spp. in the first experiment is thought to be attributed to very high lipid content, which would alter the toxicokinetics, reducing the exposure and increasing the storage capacity, limiting the amount of toxicant at the site of action. Further, the apparent enhancement of toxicity of the second experiment may have been due to additional environmental stressors that were contributing to population decline. Thus, accounting for differences in organism health is critical to proper evaluation and prediction of toxic responses for these amphipods. Field-collected organisms, such as *Diporeia* spp., can introduce unexpected complications into bioassay results that are not readily predictable.

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