Bull Environ Contam Toxicol (2010) 84:545–549
DOI 10.1007/s00128-010-9978-z

Bioaccumulation of TNT and DDT in Sheepshead Minnows, *Cyprinodon variegatus* L., Following Feeding of Contaminated Invertebrates

G. R. Lotufo · W. M. Blackburn

Received: 31 August 2009 / Accepted: 6 April 2010 / Published online: 27 April 2010

Abstract The aim of this study was to determine the potential for dietary uptake by trophic transfer using the explosive 2,4,6-trinitrotoluene (TNT) and the substantially more hydrophobic dichlorodiphenyltrichloroethane (DDT) utilizing the amphipods *Leptocheirus plumulosus* as prey and the fish *Cyprinodon variegatus* as predator. Bioaccumulation did not change significantly over time for TNT but apparent steady-state was not reached for DDT at exposure termination after 7 days of dietary exposure. The bioaccumulation factor was 0.09 mg/mg for TNT and 0.34 mg/mg for DDT, confirming the low potential of TNT to bioaccumulate in fish.

Keywords TNT · DDT · Dietary uptake · Fish

Aquatic organisms can take up contaminants dissolved in water or present in food through ingestion. The importance of the dietary uptake route varies with species according to variables such as habitat, feeding mode, age, and sex. Net bioaccumulation will depend on chemical, animal, and environment-related parameters (Hellou et al. 2002). Bioconcentration, the non-dietary uptake of chemicals from water in aquatic animals, is expected to be relatively low for explosives because of their low hydrophobicity, as confirmed empirically (Lotufo et al. 2009). However, dietary exposure to organic contaminants results in significant bioaccumulation for a variety of compounds in predator organisms (e.g., Hellou et al. 2002). The dietary transfer of 2,4,6-trinitrotoluene (TNT) transformation products and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) from freshwater invertebrate to fish (Houston and Lotufo 2005; Belden et al. 2005a, b) and the dietary transfer of TNT transformation products from earthworms to salamanders (Johnson et al. 1999) has been reported. Those studies indicate that there is potential for dietary uptake of TNT and other explosives in fish. The purpose of this study was to provide environmentally realistic exposure estimates for the trophic transfer of TNT in estuarine and marine systems.

G. R. Lotufo (✉) · W. M. Blackburn
U.S. Army Engineer Research and Development Center, Waterways Experiment Station, EP-R, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199, USA
e-mail: guilherme.lotufo@usace.army.mil

Materials and Methods

To investigate the potential for dietary uptake of TNT, preexposed estuarine amphipods (*Leptocheirus plumulosus*) was used a prey and juvenile sheepshead minnows (*Cyprinodon variegatus*) were used a predator fish in laboratory experiments. The organochlorine pesticide dichlorodiphenyltrichloroethane (p,p′-DDT) was used as a comparative compound because it is substantially more hydrophobic, and hence more bioaccumulative, than TNT.

Laboratory cultured *L. plumulosus* (US Army Engineer Research and Development Center, Vicksburg, MS) and *C. variegatus* (Aquatic BioSystems, Fort Collins, CO) were used in the experiments. Non-labeled TNT was purchased from Sigma Chemical Co. (St. Louis, MO). Radiolabeled TNT ([^{14}C]TNT, 40 Ci/mol) was purchased from Chem Service (Westchester, PA). Radiolabeled DDT ([^{14}C]DDT, 25 Ci/mol) was purchased from Sigma Chemical Co. (St. Louis, MO). Manufacturer reported radiochemical purities and chemical purities were >98% for all compounds and were used as received.

The 12 mg/L TNT exposure water was prepared by adding a mixture of non-radiolabeled and [^{14}C]labeled TNT to reconstituted sea water (RSW, Crystal Sea®, Marine Enterprises International, Essex, MD) at 20 psu. This concentration is not lethal to *L. plumulosus* in exposures up to 12 h in preliminary exposures. The specific activity (dpm/μmol) of the isotopically diluted [^{14}C]TNT in the exposure water was determined by dividing the measured radioactivity (dpm per ml) by the TNT concentration (μg/mL). The 0.03 mg/L DDT exposure water was prepared by adding [^{14}C]DDT to RSW, and this concentration approximates the solubility limit for that compound. The radioactivity in the water was 95 dpm/μL for TNT and 4.8 dpm/μL for DDT. Three groups with approximately 200 juvenile *L. plumulosus* (0.5–1 mg) each were exposed to [^{14}C]TNT or [^{14}C]DDT for one hour in 300-mL tall beakers. A short exposure period was selected to maximize the fraction of the total radioactivity in the tissue corresponding to parent compound, as the concentration of transformation products increased with exposure duration in preliminary experiments. A subset of amphipods was analyzed for total radioactivity (both TNT and DDT exposures) and for the concentration of chemicals (TNT exposure only) in their tissues. Following exposure to TNT or DDT, remaining amphipods in each beaker were rinsed to remove surface-bound contaminants and fed live to each sheepshead minnow as described below.

Juvenile sheepshead minnows (100–250 mg) were placed in RSW at 20 psu and 23°C in 600 mL beakers containing one fish in each. Eighteen one-fish replicates each were fed TNT- or DDT-exposed amphipods twice daily (8 am and 5 pm) for up to 7 days. Ten additional one-fish replicates were used as controls and fed uncontaminated amphipods. Each fish was fed three contaminated live amphipods added to each exposure beaker. Three one-fish replicates were sampled on days 1, 2, 3, 4 and 7. Fish were then placed in beakers with uncontaminated water for 10 h to allow for evacuation of most of the gut contents before being analyzed for whole-body radioactivity. Fish were only assayed for radioactivity and not solvent-extracted for chemical analysis because preliminary experiments indicated that the TNT body burden in fish was below the detection limit.

For radioactivity analysis of TNT- and DDT-exposed amphipods, ten amphipods were placed in scintillation cocktail (3a70b, Research Product International, Mt. Prospect, IL) and then disrupted using a Branson Sonifier 450 high intensity probe-sonicator (Danbury, CT). After 24 h, the samples were assayed for radioactivity using liquid scintillation counting on a Tri-Carb liquid scintillation analyzer (Model 2500 TR, Packard Instrument, Meridien, CT). For amphipods and water samples, data were corrected for quenching using the external standards ratio method after subtracting the background. TNT-exposed amphipods were also extracted in acetonitrile as described in Houston and Lotufo (2005). Extracts were analyzed for TNT and its major transformation products with an Agilent 1100 Series HPLC (Palo Alto, CA) equipped with a Supelco RP-Amide C-16 column and a diode array detector. Sample injection volume was 100 μL with a flow rate of 1 mL per minute and column temperature of 45°C. An isocratic mobile phase consisting of 55% methanol and 45% water was utilized. Absorbance was measured at 230 and 254 nM. Peak identification was based on retention time with spectral analysis confirmation. The laboratory reporting limit for all analytes was 5 nmol/g. Recoveries ranged from 90 to 98%. At each sampling period, fish exposed to radiolabeled TNT or DDT were individually transferred to scintillation vials where each vial received 1.0 ml of tissue solubilizer (T2, Research Product International, Mt. Prospect, IL). Following complete tissue solubilization (overnight), each vial received 1.0 mL of 1.2 N hydrochloric acid, after which each was assayed for radioactivity in 15 mL of scintillation cocktail as described above.

For fish bioaccumulation data, randomized one-way analysis of variance (ANOVA) was used to determine differences between means of the various exposure periods at a 0.05 level of significance. Pairwise comparisons (Fisher LSD method) were used to determine significant differences between fish body burdens at different exposure periods. SigmaStat software (Systat Software, Inc. San Jose, CA) was use in all analyses.

Results and Discussion

For water, amphipod and fish samples, the sum concentrations of parent compound and all the degradation products for TNT and DDT were expressed as parent compound molar-equivalents. These were determined by dividing the radioactivity measurement (dpm/g) by the specific activity (dpm/μmol) measured for the exposure water (TNT) or provided by the manufacturer in the case of DDT. Parent compound and all of its degradation products are collectively referred to as SumTNT and SumDDT.

The concentration of SumTNT and SumDDT in water and in amphipods (Table 1) were similar for the various exposures. Variability was highest for amphipod body burdens and lowest for water contaminant concentrations. Most of the compounds accumulated in TNT-exposed amphipods were non-extractable in acetonitrile (78.5%); smaller fractions were not identified (19.2%) or identified as ADNTs (2.3%) or TNT (0.03%). Unextractable radioactivity detected in animal tissue likely corresponded to covalently bound products associated with organic

Table 1 SumTNT and SumDDT molar equivalents in the water, prey amphipods and fish (day 7) and transfer factors for water-to-amphipod and amphipod-to-fish

	Concentration*			Transfer factor	
	Water (nmol/mL)	Prey (nmol/g)	Fish (nmol/g)	Water-to-prey (mL/g)	Prey-to-fish (g/g)
SumTNT	52.1 ± 7.1 (14)	300 ± 138 (46)	20.5 ± 7.1 (35)	6.0 ± 2.9 (48)	0.068
SumDDT	0.088 ± 0.022 (25)	10.0 ± 6.4 (64)	3.9 ± 0.7 (18)	122 ± 77 (63)	0.388

* Values are presented as mean ± 1 standard deviation and the coefficient of variation, expressed as %, is in parentheses

molecules. Such strong binding has been reported previously for TNT in other invertebrates, including amphipods (Belden et al. 2005b; Rosen and Lotufo 2005; Houston and Lotufo 2005).

The concentration of DDT and its transformation products in prey amphipods was not determined. A previous study (Lotufo, unpublished) revealed most of the radioactivity in the tissues of *L. plumulosus* exposed to [^{14}C]DDT for 48-h corresponded to DDT parent compound (83.6%), and only smaller fractions corresponded to DDE (9.7%) and non-identified polar metabolites (6.7%). Therefore, in contrast to TNT, most of the SumDDT fed to fish in this study was likely present as DDT rather than its transformation products.

Upon termination of the 1-h exposure, SumDDT tissue concentration was higher in amphipods than in the water by a much higher factor than that for SumTNT, resulting in a higher water-to-amphipod transfer factor for DDT (Table 1). Higher bioaccumulation of SumDDT in *L. plumulosus* was expected based on the significant differences in the octanol-water partition coefficient (K_{ow}) between TNT (log K_{ow} = 1.6) and DDT (log K_{ow} = 6.2) (Houston and Lotufo 2005) and the positive relationship between log BCF and log K_{ow} (Meylan et al. 1999). Within 1 h or less of exposure, SumTNT approached steady state in adult mussels *Mytillus galloprovincialis* (Rosen and Lotufo 2007). Therefore, tissue concentrations approaching steady state were expected in *L. plumulosus* at termination of the 1-h exposure in this study, and the water-to-amphipod transfer factor is likely similar to the bioconcentration factor for TNT in that species. Bioconcentration factors for TNT ranged from 1.6 to 4 mL/mg for aquatic invertebrates in previous studies (Lotufo et al. 2009).

The concentration of SumTNT in fish fed SumTNT-laden amphipods remained relatively unchanged for the duration of the experiment and did not follow a consistent temporal pattern (Fig. 1). Only a small, non-significant increase in the mean fish burden occurred between days 2 and 7 of the dietary exposure. For SumDDT, the mean fish body burden increased steadily from day 1 to day 7 (Fig. 1). The concentrations measured after 4 and 7 days of dietary exposure were significantly higher than those measured after 1 and 3 days (Fig. 1). The amphipod-to-fish

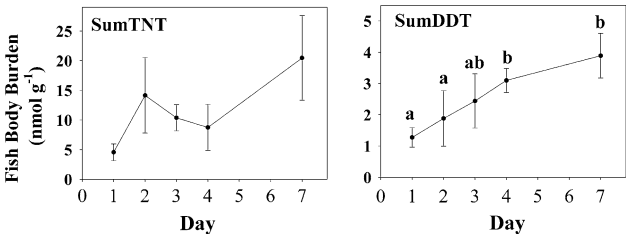

Fig. 1 Concentration of TNT or DDT molar equivalents determined using total radioactivity in sheepshead minnows fed TNT- or DDT-exposed amphipods. *Error bars* indicate ± 1 standard deviation (n = 3). For each sampling day, *bars* with the *same letter* indicate a significant difference ($p < 0.05$)

transfer factors, or dietary bioaccumulation factors, were calculated by dividing the concentration of SumTNT or SumDDT in the fish at termination of the 7-day exposure period by the mean concentration of SumTNT or SumDDT in amphipods fed to fish throughout the experiment. The bioaccumulation factor for SumDDT was substantially higher (5.7 times) than that for SumTNT (Table 1).

The data obtained in this study are consistent with experimental results obtained when juvenile fathead minnows (*Pimephales promelas*) were fed the oligochaete *Lumbriculus variegatus* pre-exposed to [^{14}C]TNT, [^{14}C]RDX or [^{14}C]DDT (Houston and Lotufo 2005). In that study, the body burden in the *P. promelas* remained relatively unchanged over time for SumTNT or SumRDX, but [^{14}C]DDT increased over time and did not approach steady state in the tissue during the 14-day exposure period. Houston and Lotufo (2005) reported mean bioaccumulation factors, determined using total radioactivity in worms and fish, of 0.018, 0.010, and 0.422 g/g for SumTNT, SumRDX and SumDDT, respectively. When juvenile channel catfish (*Ictalurus punctatus)* were force-fed [^{14}C]TNT-spiked food pellets daily for up to 10 days, they bioaccumulated the ingested compound primarily as unknown transformation products, both extractable and non-extractable (Belden et al. 2005b). The bioacumulation factors for TNT (0.000024 g/g), sum of extractable compounds (0.00048 g/g), and SumTNT (0.00091 g/g) reported by Belden et al. (2005b) were exceedingly low relative to those determined in this study and for fathead minnows in another study (Houston and Lotufo 2005), likely because of the efficient

ability of catfish to metabolize and eliminate TNT (Ownby et al. 2005). The higher potential for trophic transfer of strongly hydrophobic organochlorine compounds such as DDT compared to compounds relatively less hydrophobic and more readily metabolized such as PAHs has been well documented (e.g., Fisk et al. 1998; Hellou et al. 2002).

Our finding of much greater trophic transfer of DDT compared to TNT in an aquatic prey-predator system corroborates results from an earlier similar investigation. Using earthworms exposed to TNT or PCBs (Aroclor 1260) as prey and salamanders as predator, Johnson et al. (1999) reported much greater dietary bioaccumulation for PCBs than for TNT and its metabolites. The bioaccumulation factor for DDT obtained in this study (0.207 g/g) was lower than similar factors (0.89–2.80 g/g) reported for hydrophobic organochlorine compounds in rainbow trout (Fisk et al. 1998), likely due to the short exposure duration used in the present study.

In this study, fish were held for 10-h in clean water to allow for the digestion of their last meal and also the egestion of undigested prey from their guts. However, this period may not have been sufficient for complete gut clearance resulting in potential for radioactivity associated with undigested prey in the fish gut to contribute at least a fraction of the radioactivity determined in whole fish. For SumTNT, the radioactivity associated with a meal (three amphipods), approximately 1,500 dpm, corresponded to a substantial portion (38%) of the mean radioactivity in fish at exposure termination (approximately 4,000 dpm). It is possible undigested prey could have accounted for a fraction of the whole body burden of SumTNT measured in fish. This could also have contributed to the high variability observed among replicates as well as the observed overall temporal variability. For SumDDT, however, the mean radioactivity associated with a meal (approximately 1,900 dpm) corresponds to a relatively small portion (10%) of the mean concentration in the fish at exposure termination (approximately 21,000 dpm), indicating that most of the body burden in fish is associated with radioactivity present in fish tissues rather than with undigested prey. In a previous study (Houston and Lotufo 2005), the potential contribution of residual gut content to the SumTNT or SumRDX whole-body concentration in fish fed contaminated prey was substantially higher than that estimated in this study. Therefore, fish fed explosive-contaminated prey may effectively accumulate those compounds at levels even lower those reported in those studies.

Environmentally realistic experimental approaches of employing whole invertebrates as prey and juvenile fish as predators proved successful for investigating the potential for dietary uptake of contaminants in aquatic systems in the present study and also in previous investigations (e.g., Houston and Lotufo 2005; Belden et al. 2005a). The use of whole invertebrates a contaminant vectors to predator fish in standardized, controlled, and realistic exposures provides advantages (e.g., exposure to mixture of parent and transformation products, low release of chemical from the diet into the water column) relative to the more frequently use of spiked commercial foods.

Collectively, this study and others (Houston and Lotufo 2005; Belden et al. 2005a, b) empirically confirmed the expected low bioaccumulative potential for explosives through dietary pathways for fish. Invertebrates living in close proximity to UXOs or in sediment contaminated with explosives may accumulate explosives and their transformation products in their tissues. Because of the low potential for dietary uptake of explosives, fish feeding on exposed invertebrates are unlikely to serve as a transfer vector of those explosives from invertebrates to humans.

Acknowledgements The US Navy's Pollution Abatement Ashore Environmental Sustainability Development to Integration Program and the Environmental Quality Technology Research Program of the US Army supported this research. Permission to publish this study was granted by the Chief of Naval Operations (N456) and the Chief of Engineers.

References

Belden JB, Lotufo GR, Lydy MJ (2005a) Accumulation of hexahydro-1,3,5-trinitro-1,3,5-triazine in channel catfish (*Ictalurus punctatus*) and aquatic oligochaetes (*Lumbriculus variegatus*). Environ Toxicol Chem 24:1962–1967

Belden JB, Ownby DR, Lotufo GR, Lydy MJ (2005b) Accumulation of trinitrotoluene (TNT) in aquatic organisms: part 2—bioconcentration in aquatic invertebrates and potential for trophic transfer to channel catfish (*Ictalurus punctatus*). Chemosphere 58:1161–1168

Fisk AT, Norstrom RJ, Cymbalisty CD, Muir DG (1998) Dietary accumulation and depuration of hydrophobic organochlorines-bioaccumulation parameters and their relationship with the octanol/water partition coefficient. Environ Toxicol Chem 17:951–961

Hellou J, Leonard J, Anstey C (2002) Dietary exposure of finfish to aromatic contaminants and tissue distribution. Arch Environ Contam Toxicol 42:470–476

Houston JG, Lotufo GR (2005) Dietary exposure of fathead minnows to the explosives TNT and RDX and to the pesticide DDT using contaminated invertebrates. Int J Environ Res 2:286–292

Johnson MS, Franke LS, Lee RB, Holladay SD (1999) Bioaccumulation of 2,4,6-trinitrotoluene and polychlorinated biphenyls through two routes of exposure in a terrestrial amphibian: is the dermal route significant? Environ Toxicol Chem 18:873–878

Lotufo GR, Lydy MJ, Rorrer GL, Cruz-Uribe O, Cheney DP (2009) Bioconcentration, bioaccumulation and biotransformation of explosives and related compounds in aquatic organisms. In: Sunahara GI, Lotufo GR, Kuperman RG, Hawari J (eds) Ecotoxicology of explosives. CRC Press, Boca Raton, p 136

Meylan WM, Howard PH, Boethling RS, Aronson D, Printup H, Gouchie S (1999) Improved method for estimating bioconcentration/bioaccumulation factor from octanol/water partition coefficient. Environ Toxicol Chem 18:664–672

Ownby DR, Belden JB, Lotufo GR, Lydy MJ (2005) Accumulation of trinitrotoluene (TNT) in aquatic organisms: part 1—Bioconcentration and distribution in channel catfish (*Ictalurus punctatus*). Chemosphere 58:1153–1159

Rosen G, Lotufo GR (2005) Toxicity and fate of two munitions constituents in spiked sediment exposures with the marine amphipod *Eohaustorius estuarius*. Environ Toxicol Chem 24:2887–2897

Rosen G, Lotufo GR (2007) Bioaccumulation of explosive compounds in the marine mussel, *Mytilus galloprovicialis*. Ecotoxicol Environ Saf 68:237–245