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FATE AND EFFECTS OF COMPOSITION B IN MULTISPECIES MARINE EXPOSURES

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Abstract—The vast majority of investigations into the bioavailability and toxicity of explosives to receptors in aquatic environments has focused on deriving toxicity metrics for discrete chemical exposures to single species using pure compounds at relatively high concentrations. This study assessed the environmental fate and potential for biological effects of a common military formulation, Composition B, under more realistic exposure scenarios (e.g., those that more closely simulate a breached artillery round or residual exposure following a low-order detonation). We used a novel approach incorporating multiple species and toxicity endpoints in sediment exposures over a 34-d exposure period. Composition B fragments exposed at the sediment surface rapidly released 2,4,6-trinitrotolune (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the overlying water column. In comparison, burial of fragments resulted in dramatically reduced exposure, bioconcentration, and toxicity. The addition of a conservative flow rate to the aquaria also reduced water and tissue concentrations by factors of two to three. Although the exposure system likely represented a worst-case scenario relative to most conditions found in coastal and estuarine environments, overlying water concentrations generally did not approach known toxicity thresholds, while porewater concentrations were sufficiently elevated above toxicity thresholds immediately adjacent to the fragments, limiting hazardous exposure only to very localized scales. Bioconcentration correlated closely with observed toxicity and was either not detectable (buried), or low (exposed), as is expected based on the low hydrophobicities of TNT and RDX. Environ. Toxicol. Chem. 2010;29:1330–1337. © 2010 SETAC

Keywords—Composition B TNT RDX Explosive Marine sediment

INTRODUCTION

Although the extent of such exposure in marine environments is largely unknown, the presence of unexploded ordnance (UXO) and dumped ammunition in aquatic environments have been reported ([1–4]; http://cradpdf.drdc.gc.ca/PDFS/unc57/p522640.pdf; [5]) and low concentrations of some explosive chemicals have been measured in marine sediments [1,6]. It is anticipated that commonly used explosive compounds such as 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) may leak from underwater corroded, or breached, UXO as well as from fragments of explosives formulations remaining following low-order (incomplete) detonations. It is possible that ecological receptors inhabiting the vicinity of these potential sources of contamination may suffer toxic effects if exposure concentrations of explosives compounds are high enough and uptake occurs.

To support the assessment of risk associated with the presence of explosives in coastal environments, laboratory-based toxicity data have recently been derived for a variety of marine species and endpoints in both aqueous [7–9] and sediment [5,10–13] exposures. Those studies have involved the contamination of water or sediment at unrealistically high concentrations in order to derive toxicity metrics such as median lethal concentrations (LC50s).

Realistic exposure of explosives compounds to the biota at coastal and estuarine sites is more likely to be localized adjacent to leaking projectiles or residues associated with low-order detonations, and at a low level further away, because of dilution into the surrounding water and transport by currents. In addition, explosives are subject to microbial degradation and binding to sediments [14,15].

The objective of the present study was to evaluate the fate and effects for fragments of Composition B (Comp B), a common military explosive formulation containing both TNT and RDX (in an ≈1:1.5 ratio by weight), simulating potential exposure following a low-order detonation. This study was performed in aquaria containing seawater and sediment under various exposure scenarios with multiple species and endpoints. One fish and four invertebrate species were included in the exposures to simulate real-world conditions more closely than previously conducted single-species studies, and to more accurately assess any differences in toxicity and bioaccumulative potential among species. The substrate for all treatments consisted of sandy, low organic carbon sediment to minimize sorption of compounds to sediment and hence maximize exposure to ecological receptors. Composition B fragments were either placed at the sediment surface or buried at a shallow depth; the overlying water was either maintained statically or slowly exchanged during the exposure.

MATERIALS AND METHODS

Chemicals

Except for the control treatments, each aquarium discussed below received two irregularly shaped Comp B fragments weighing a total of approximately 0.4 g (range = 0.39–0.41 g). Comp B fragments consist of 59.5% RDX, 39.5% TNT, and 1% paraffin wax by mass and were obtained from the Holston Army Ammunition Plant (Kingsport, TN, USA). Individual solubility limits of pure RDX and TNT at 25°C are reported at 60 and 101 mg/L [16], respectively. At 20°C, TNT is reported to have a solubility of 86 mg/L in full-strength sea-

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water [17]. Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) is an impurity associated with the manufacture of the military-grade RDX used in Comp B and was typically detected in water samples at a low level (≈10% by mass, of the RDX concentration), but was not detected in organism tissues. For simplicity, the HMX aqueous data are not shown or further discussed in this article.

Exposure apparatus

A 3 × 2 factorial experimental design was used to characterize effects of Comp B placement (Comp B exposed at the sediment surface or buried 1–2 cm below the sediment surface) and water exchange (static vs. flow) on the movement of explosives into the overlying water and pore water. The design was also used to evaluate the potential for uptake by and toxicity to marine fish and benthic and pelagic marine invertebrates.

Experimental units were modified 20-L glass aquaria (42 cm L × 21 cm W × 26.3 cm H), which were replicated three times for each treatment (Fig. 1). Previous studies have shown that TNT released into the marine environment is microbially transformed to aminated daughter products and becomes irreversibly bound to sediment [14,15]. Thus, each tank received a 3.5-cm thick layer of uncontaminated sandy sediment (Yaquina Bay, OR, USA; sand = 97.5%, silt/clay = 2.5%, total organic carbon [TOC] = <0.1%). Sandy sediment was selected to represent a worst-case scenario in terms of explosives compound partitioning into the overlying water and bioavailability.

Field-exposure chambers similar to those used in in situ deployments conducted by Burton et al. [18] were used to confine amphipods and polychaetes at a specified location in each tank. Briefly, these were cylindrical chambers constructed of cellulose acetate butyrate with an outer diameter of 7 cm and length of 12.7 cm. Two rectangular windows (4 × 8 cm each) were cut in each tube and covered with 300 μm Nitex mesh screens with aquarium-grade silicone glue. For each tank, two of these chambers were filled with 2 cm of sediment, capped with polyethylene lids on each end, and gently placed into the aquaria so that the sediment inside the chamber was flush with the surrounding sediment. The chambers were positioned so that they would be equidistant from the Comp B fragments (≈8 cm away). Test organisms were added through ports built into the chambers which were attached to 0.8 cm I.D. Tygon tubing that extended approximately 5 cm above the water surface.

After placement of the field chambers, 18 L of 30‰ reconstituted seawater (RSW, Instant Ocean) was added to each tank using a turbulence reducer. Three Comp B exposure treatments were used: an exposed fragment at the sediment surface; a buried fragment at a depth of approximately 2 cm; and a control consisting of no Comp B fragment. Each Comp B treatment type was performed in two separate aquaria under two different water exchange rates: static or flow. This resulted in treatments defined as: static control; flow control; static exposed (SE); flow exposed (FE); static buried (SB); and (FB) flow buried. The flow treatments resulted in renewal of each tank with clean seawater at a rate of 0.5 turnover (9 L) per day, which was supplied by a gravity-fed drip system, while tank water gradually exited the tanks through overflow ports.

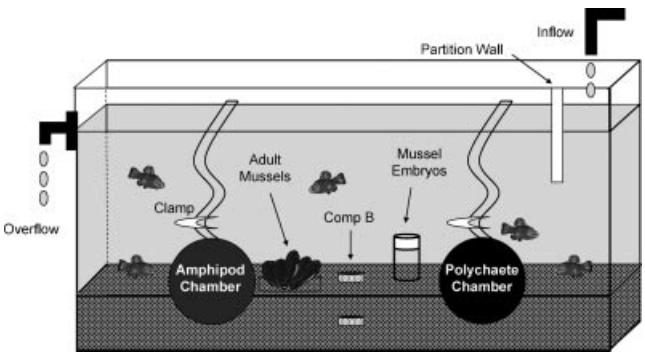

Fig. 1. Exposure system used to assess fate and effects of Composition B. Not drawn to scale.

On day zero, two Comp B fragments were placed immediately adjacent to one another, either both on (exposed) or in (buried) the sediment in the center of the tank. At day 24 all test organisms were introduced to the system for exposures of up to 10 days. Overlying water samples (1 ml from each tank) were removed every other day for a period of 34 d for chemical analysis. At exposure termination, following removal of test organisms and overlying water from the tanks, 1 to 2 ml pore water was sampled from the sediment immediately under and at 6 cm and 18 cm away from the Comp B fragments. Pore water was sampled using a fused-glass air stone attached to a 10 cc plastic syringe [19], then filtered (0.45 μm) and transferred to high-performance liquid chromatography (HPLC) sample vials for chemical analysis.

Experimental organisms

Survival and bioaccumulation were measured in five species: juvenile sheepshead minnows (*Cyprinodon variegatus*), two amphipod species (*Eohaustorius estuarius* and *Leptocheirus plumulosus*), the polychaete *Neanthes arenaceodentata*, and mussels (*Mytilus galloprovincialis*). Sublethal effects were investigated using embryo–larval development success of the same mussel species. The minnows were six-week-old juveniles (≈0.2 g wet weight each) obtained from cultures at Aquatic Biosystems. The amphipod *L. plumulosus* were 3- to 5-mm mixed age individuals obtained from cultures held at the U.S. Army Corps Engineer Research and Development Center (Vicksburg, MS, USA). The other amphipod species, *E. estuarius*, were 3- to 5-mm adults field-collected by Northwestern Aquatic Sciences from an uncontaminated site near Yaquina Bay, Oregon. *N. arenaceodentata* were six-week-old adults obtained from cultures from Dr. Don Reish (California State University, Long Beach, CA, USA). *M. galloprovincialis* small adults (≈1.5 cm, for survival and bioaccumulation) and large adults (≈5–7 cm, for obtaining gametes for embryogenesis exposures) were purchased from Carlsbad Aquafarm. All organisms were acclimated to the experimental conditions over a period of 5 to 7 d prior to addition to the aquaria, during which mortality was confirmed to be 5% or less.

Test organism exposure and analysis

Experimental procedures using the above-mentioned species were based on modifications of standard laboratory methods [20–22]. Amphipods and polychaetes, both deposit-feeding sediment burrowers, were added to the screened chambers through tubes that extended slightly above the surface of the water. This allowed the organisms to be added to the sediment without disruption of the surrounding media. Both species of amphipods, 20 individuals of each, were added to the same chamber following confirmation in a preliminary experiment that the two species could coexist, while five polychaetes were added to the other chamber. Five fish were then added and

allowed to swim freely throughout each tank. Finally, five small adult mussels were allowed to adhere to a 5-cm diameter watch glass, which was placed directly on the sediment surface, simulating a sediment–water interface exposure.

One in situ mussel chamber containing approximately 200 mussel embryos (<4 h old) was also placed at the sediment–water interface in each tank. Chambers were glass scintillation vials preconditioned with clean seawater. Embryos were contained in the vials with mesh-covered plastic screw caps, which were created by affixing a 20 μm Nitex screen with aquarium-grade silicone glue to the vial cap, with a 1.25-cm hole drilled through the center. This chamber allowed mixing with the surrounding seawater while preventing escape of the small (≈60 μm diameter) embryos. Mussel embryos were also exposed to grab samples collected from each of the tanks at the time of test organism addition, according to standard laboratory protocol describing static exposures [21].

The aquaria were maintained in a walk-in environmental chamber at 18°C with a 16:8 h light:dark photoperiod under gold fluorescent light bulbs (λ >500 nm) to prevent photodegradation of nitroaromatic compounds by UV radiation. Fish were fed approximately 100 newly hatched brine shrimp (*Artemia sp*.) nauplii daily during the experiment. The adult mussels were fed every other day by adding approximately 8×10^7 cells of a commercial phytoplankton mixture (Shellfish Diet 1800, Instant Algae®) to each aquarium. After 48 h, mussel larval chambers were removed from the tanks and preserved in buffered formalin for microscopic examination of the number of normally developed and alive D-shaped (prodissoconch I) larvae relative to control vials. After 10 d, amphipods, polychaetes, mussels, and fish were removed, assessed for survival, rinsed in deionized water, weighed, and frozen at 4°C for chemical analysis.

Water, tissue, and Comp B analysis

Overlying and porewater samples, as well as tissues, were analyzed using a modification of HPLC method 8330 [9]. Porewater samples were filtered (0.45 μm). Tissue samples underwent a solvent extraction with acetonitrile [11] prior to HPLC analysis.

Because TNT tends to undergo both microbial degradation prior to uptake and biotransformation within the organisms following uptake, TNT was expressed as the sum of TNT (sumTNT) and its major aminated transformation products, 2-aminodinitrotoluene (2-ADNT), 4-aminodinitrotoluene (4-ADNT), and diaminonitrotoluenes (DANT), in both water and tissue. The reporting limits for all analytes was 0.1 mg/L for water samples and 0.1 nmol/g for tissue samples.

Following removal from the relevant tank, each pair of Comp B fragments was rinsed and placed into glass scintillation vials, allowed to dry at room temperature for approximately one week, and weighed to the nearest 0.001 g calculation of the mass lost to the exposure system.

Data analysis

Statistical analyses were performed using SigmaStat 2.03. Differences among treatments were determined for RDX and sumTNT concentrations using two-way ANOVA ($\alpha = 0.05$) for overlying water prior to addition of test organisms, overlying water at the termination of exposure, and in pore waters at exposure termination. Treatment factors were flow condition and Comp B placement location. Therefore, three null hypotheses were tested: no effect of flow rate on water concentration; no effect of Comp B placement location on water concentration; and flow rate and Comp B placement location have no interactive effect on water concentration. Tukey's tests were performed a posteriori to determine pairwise differences. Porewater concentrations of RDX and sumTNT at different distances from the Comp B fragment in the exposure tank were analyzed using one-way analysis of variance (ANOVA) and a posteriori Tukey's tests for determining pairwise differences.

In addition, two-sample unequal variance *t* tests ($\alpha = 0.05$), with a Bonferroni's correction, were used for making comparisons between treatment effects for survival, embryo development, and bioaccumulation. Toxicity metrics (e.g., median effects concentrations) were determined using the Trimmed Spearman Karber method with the assistance of ToxCalc 5.0 (Tidepool Scientific).

RESULTS AND DISCUSSION

Overlying water concentration

The concentrations of RDX and sumTNT resulting from dissolution into the overlying water over time are shown in Figures 2 and 3, respectively. The concentration of sumTNT in the overlying water increased linearly in the SE treatment tanks during the first 10 d, and then remained at approximately steady state for the remainder of the study (Fig. 2). SumTNT concentration was highest in the SE treatment compared to any of the other three treatments, not surprisingly, because this was expected to represent a worst-case scenario. SumTNT approached a stable concentration range earlier in the FE treatment and remained approximately 60% lower than SE treatment ($p = 0.020$), which is explained by the loss of compounds through water outflow from the aquaria, coupled with dilution by the inflow of clean seawater. SumTNT consisted of 78% parent compound under FE, but only 34% parent TNT under SE conditions (Fig. 3), likely due to the removal of transformation products from the system under flow conditions, coupled with dissolution of parent TNT from the fragments.

The RDX behaved similarly to TNT in that overlying water concentrations in Exposed treatments increased linearly for the first 10 d followed by stabilization, while steady-state conditions occurred much more quickly (by day 3) under Flow conditions (Fig. 4). It is interesting to note that unlike TNT, RDX began to increase linearly again in the SE treatment following the addition of the test organisms (Fig. 4). The causes for the increase are unknown, but it could be associated with the onset of water circulation around the fragments by the various test organisms, as well as by the addition of gentle aeration to the system upon the addition of organisms.

Burial of the Comp B fragments resulted in negligible to no detectable sumTNT or RDX partitioning into the overlying water, regardless of whether conditions were static or flow-through (Figs. 2, 4). This finding is consistent with that of Ek et al. [23], in which cleaved artillery shells that were placed with the exposed TNT surface buried under 5 cm of sediment (TOC = 0.3%, 77% sand, 10% clay and silt, and 13% gravel) in laboratory exposures resulted in no detectable TNT in the overlying water above the buried shells over a 13-month evaluation period. In contrast, Ek et al. [23] reported substantial dissolution of TNT from the cleaved shells when they were placed in brackish water in the absence of sediment.

Both RDX and sumTNT overlying water concentrations were significantly affected by the presence and absence of

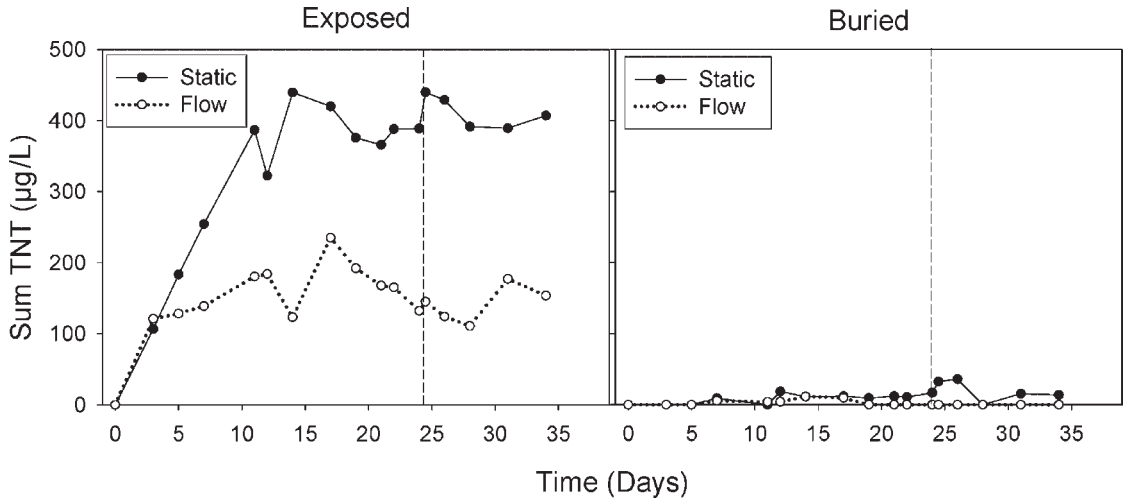

Fig. 2. Mean TNT concentration ($n = 3$) in the overlying water from exposures containing Composition B fragments, under both static and flow (0.5 turnover/day) conditions. TNT is expressed as the sum of the parent compound and the major transformation products, 4-aminodinitrotoluene, 2-aminodinitrotoluene, and diaminonitrotoluenes (sumTNT). Fragments were placed either at sediment surface (Exposed) or at a sediment depth of approximately 2 cm (Buried). Vertical dashed line represents when test organisms were added to the test systems (day 24). Concentrations higher than zero for the Buried treatment were below the reporting limit for the analytical method.

flow, and by Comp B placement location (buried or exposed). This was observed both prior to organism addition (day 24) and upon exposure termination (day 34) (Fig. 5). A significant interaction effect between the two factors was also observed. Whether prior to or after organism exposure, the SE treatment always resulted in significantly higher overlying water concentration, while the FE treatment was statistically higher than buried treatments only prior to organism addition. Increased variability associated with burrowing or feeding activities associated with individual organism replicates and transformation to nonidentified transformation products may explain the statistical trend change.

The ratio of explosive compound mass in the fragments and water volume in the tank would allow a maximum dissolved concentration of approximately 8.8 mg/L for sumTNT and 13.2 mg/L for RDX. However, final sumTNT and RDX concentrations in the overlying water (up to 0.4 and 1.3 mg/L, respectively) were substantially lower than these maximum attainable levels, and well below the solubility limits reported for those compounds [17,24,25]. This is consistent with the findings by Ek et al. [5], in which only minor loss of TNT from cleaved artillery shells was observed following observations more than 3 years after deployment in marine sediments. This might be expected based on reported slow dissolution of TNT and RDX from Comp B formulation [24,26] and the short exposure to water used in this study. In addition, military formulations such as Comp B have been reported to have lower dissolution rates than when compared to rates from pure crystal form in the absence of wax binders [24,27].

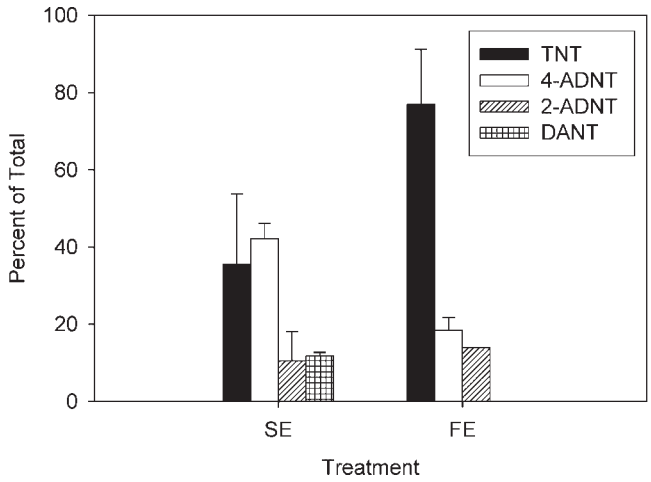

Fig. 3. Percentage of TNT measured as parent compound and its major transformation products, 4-aminodinitrotoluene (4-ADNT), 2-aminodinitrotoluene (2-ADNT), and diaminonitrotoluenes (DANT), measured in the overlying water at termination of experiments with Composition B fragments exposed under different scenarios. SE = static, exposed; FE = flow, exposed. Treatments in which fragments were buried had TNT concentrations at or close to detection limits and are not shown. $N = 3$.

Comp B contains 50% more RDX than TNT, which may explain why overlying water concentrations were higher for the former. The dissolution rate of RDX (0.00001 mg/s/cm^2), however, is reportedly as much as fivefold slower than TNT (0.00005 mg/s/cm^2; [16]). It is likely that volatilization, transformation to compounds not identifiable by HPLC Method 8330, and sorption of TNT to sediment contributed to the sumTNT loss following dissolution. The TNT tends to have a higher sorption affinity to soils [25,27] and marine sediments [28] relative to RDX. Based on these temporal trends, increasing concentrations of RDX, but stable concentrations of TNT for the SE treatment and stable concentrations of both compounds, would be predicted for the days or weeks following the 34-d exposure period used in this study.

Porewater concentration

Typically, sumTNT and RDX porewater concentrations were statistically higher directly under the Comp B fragments as compared to concentrations in the pore water at even short distances (as little as 6 cm) away from the fragments (Fig. 6). This difference was most dramatic for static treatments, whether or not the fragments were exposed or buried. Mean porewater sumTNT and RDX concentrations under the fragments were as much as one order of magnitude higher than the overlying water

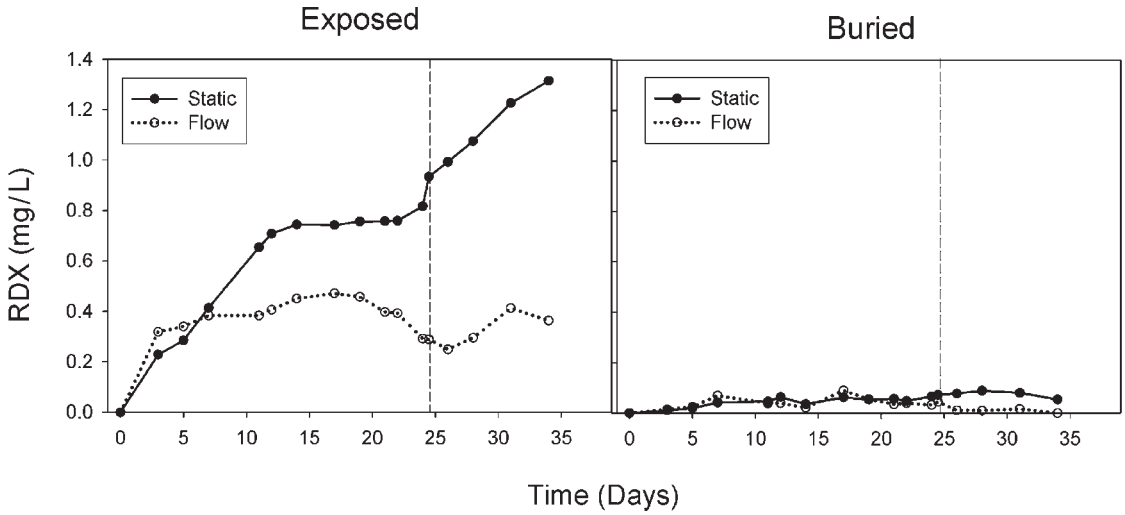

Fig. 4. Mean RDX concentration ($n = 3$) in the overlying water from exposures containing Composition B fragments, under both Static and Flow (0.5 turnover/day) conditions. Fragments were either placed at sediment surface (Exposed) or at a sediment depth of approximately 2 cm (Buried). Vertical dashed line represents when test organisms were added to the test systems (day 24). Concentrations higher than zero for the Buried treatment were below the reporting limit for the analytical method.

concentrations in SE treatment, and more than two orders of magnitude higher than the overlying water in the SB treatment. The porewater concentrations, however, were still substantially below the solubility limits for these compounds. Although porewater concentrations immediately adjacent to the fragments would be acutely toxic to the test organisms used in this study, the potential for effects would be limited to organisms continuously inhabiting only the immediate area of the fragments, as concentrations approached nontoxic concentrations just a few centimeters from the fragments (Fig. 6). Gentle flow conditions (FE and FB treatments) in the tanks resulted in substantially lower (by factors of two to seven) mean porewater concentrations immediately below the fragments, indicating that most marine organisms would not encounter lethal or sublethal porewater concentrations of explosives beyond distances of only a few centimeters from dissolving Comp B fragments.

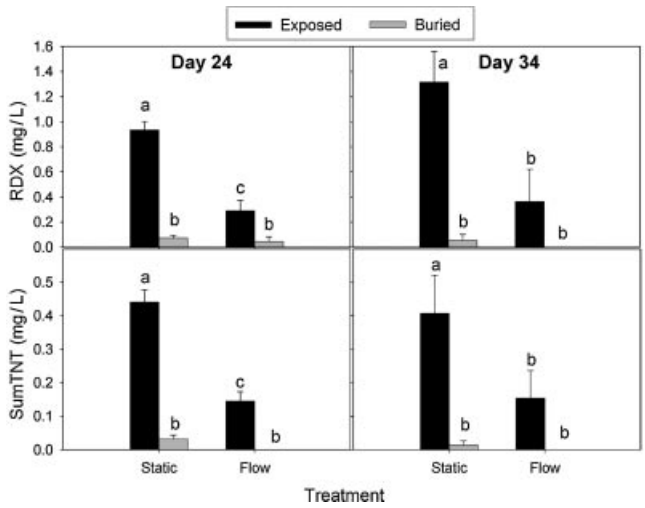

Fig. 5. Mean (±1 SD) TNT (lower) and RDX (upper) overlying water concentrations after 24 and 34 d of exposures with Composition B fragments. Organisms were added to the aquaria on day 24. $N = 3$. Letters indicated significant differences based on pairwise comparisons (Tukey's test, $\alpha = 0.05$) following two-way analysis of variance (ANOVA). TNT is expressed as the sum of the parent compound and its major transformation products: 4-aminodinitrotoluene, 2-aminodinitrotoluene, and diaminonitrotoluenes (SumTNT).

Fragment mass change

At termination of the 34-d exposure, approximately 16% of the initial Comp B fragment mass had been lost from exposed (SE and FE) fragments, while less than 2% loss was observed in treatments with buried (SB and FB) fragments (Fig. 7). Mean mass loss in both FE and FB treatments was about 10% greater than in SE and SB treatments, but this difference was not statistically significant. The fragment mass change is simply another means of illustrating the dissolution of Comp B under the different treatments.

Lethal toxicity

No statistically significant reduction in survival was observed for any test species relative to control (tanks without Comp B fragments), as shown in Table 1. High survival is expected, because observed water concentrations did not achieve previously determined survival thresholds for *E. estuarius* or *L. plumulosus* (TNT no-observed-effect concentration [NOEC] = 3.3 and 2.2 mg/L, respectively; RDX NOEC = 38.8 mg/L for both species; unpubl. data); nor *C. variegatus* (TNT NOEC = 1.5 mg/L, RDX NOEC = 7.4 mg/L; [8]) or adult *M. galloprovincialis* (TNT NOEC = 8.6 mg/L, RDX NOEC = 28.4 mg/L; [9]). Reduced survival of *L. plumulosus* and *N. arenaceodentata* (Table 1) were likely caused by factors unrelated to Comp B exposure, because control survival was below established toxicity test performance criteria [22]. Higher survival of *E. estuarius* indicates that this species may be particularly amenable to caging, in accordance with successful deployment in field studies [29]. However, recent in situ toxicity assessments incorporating use of *L. plumulosus* and *N. arenaceodentata* also resulted in high survival of those species after 4 d of exposure in surficial sediments at reference sites (Rosen et al., in prep.).

Sublethal toxicity

The only toxicity endpoint associated with a statistically significant effect was mussel embryo–larval development in SE treatment, where both the in situ (aquarium exposed) and ex situ

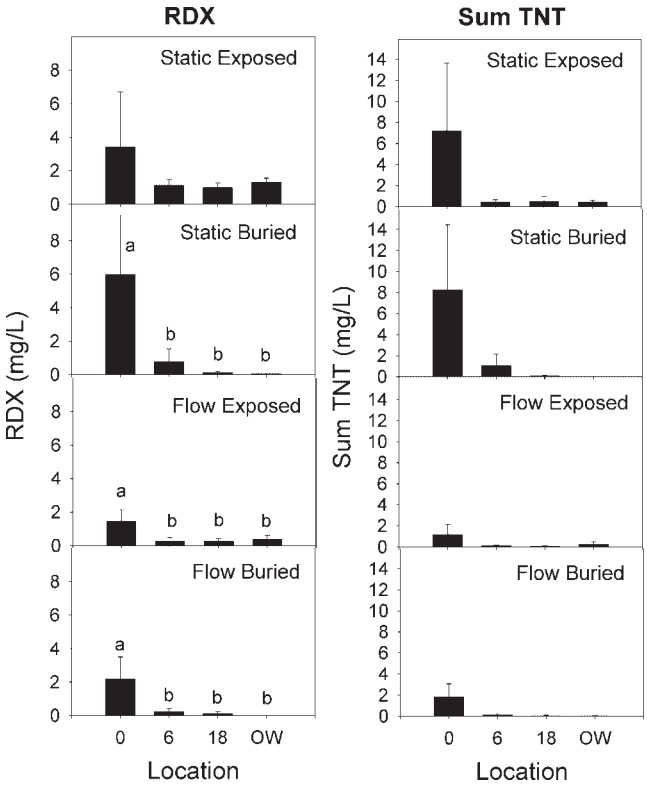

Fig. 6. Mean (±1 SD) TNT (left) and RDX (right) pore water concentrations at termination of exposures with Composition B fragments. Locations are distances (cm) from the fragments, with 0 representing pore water directly below or adjacent to the fragments. OW = overlying water, for comparison purposes. $N = 3$ for all treatments. Letters above bars indicate statistical differences from Tukey's multiple comparison tests. TNT is expressed as the sum of the parent compound and its major transformation products: 4-aminodinitrotoluene, 2-aminodinitrotoluene, and diaminonitrotoluenes (SumTNT).

(grab sample) exposures resulted in developmental abnormalities in all larvae (Table 1). The impacts to mussel larval development in the SE treatment are explained by relatively high overlying water TNT concentrations (0.43 mg/L) measured on the day that the mussel embryo exposures were initiated. This approached effects thresholds for TNT-spiked water exposure determined concurrently to the multispecies

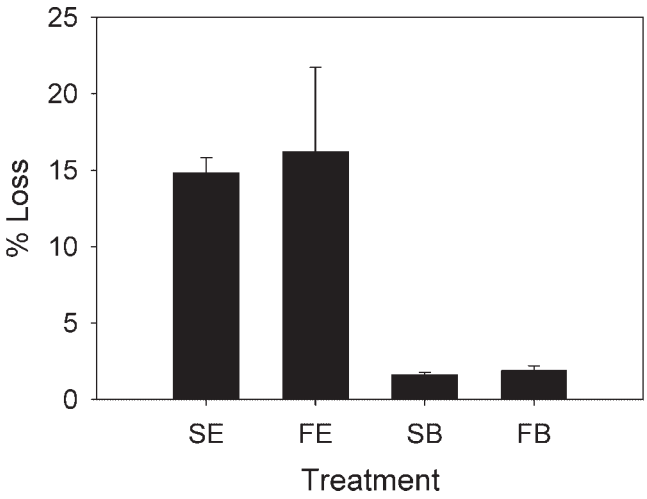

Fig. 7. Percent of initial mass of Composition B fragments lost to the exposure media following the experiments. SE = static exposed; FE = flow exposed; SB = static buried; FB = flow buried. $N = 3$.

exposure (TNT EC50 = 0.86 mg/L) and reported previously (TNT EC50 = 0.75 mg/L; [9]). The TNT concentration achieved in the overlying water samples were lower than the EC50s, but were within a factor of two of the reported values. Increased abnormal larval development could be due to the presence of possibly more toxic transformation products of TNT (transformation was not observed in aqueous exposures conducted by Rosen and Lotufo [9]), and synergistic effects associated with the presence of both subeffect concentrations of TNT and RDX.

The consistency between SE treatment response from grab samples collected from the tanks upon initiation of the embryogenesis exposures and response when deployed in the aquaria (Table 1) indicates that potential chemical concentrations inside the vials were similar to those outside the vials. This was validated in a subsequent experiment, in which concentrations between the contents of the vial and the surrounding environment were within 90% of steady state in less than 6 h, using salinity as a surrogate for a contaminant. Rapid equilibration of the exposure vials indicates that in situ monitoring using *M. galloprovincialis* embryos might be useful at sites where TNT is a potential contaminant of concern. Others have reported use of embryo–larval development tests in situ using larger chambers [30–32], but the use of scintillation vials for exposure chambers as described here may result in more accurate, simpler assessments. This is because the larvae can be counted directly in the vials on an inverted microscope, and there is low risk for loss of larvae during transfer steps to other vessels for counting.

Bioaccumulation

Whole-body residues for RDX and sumTNT are presented in Table 2. Body residues in *C. variegatus* and *M. galloprovincialis* tissues were similar and tracked the overlying water concentrations in general, with Static Exposed (worst case) treatments having the highest body burdens. The highest mean body residue in *M. galloprovincialis* was lower than the no-observed-effect residues (NOER) reported for that species (45.3 and 86.2 nmol/g, for TNT and RDX, respectively; [9]). The highest mean body residues in *C. variegatus* (Table 2) were 28.2 nmol/g sumTNT (the sum of 25.1 nmol/g ADNTs and 3.1 nmol/g TNT) and 35.3 nmol/g RDX. Those residues are lower by approximately 6-fold relative to the 5-d median lethal residue determined for TNT (19.1 nmol/g), 2-ADNT (275 nmol/g), and the RDX 10-d NOER (135 nmol/g) (unpublished data). Because of the large difference in the toxicity of TNT and ADNTs, differences in the composition of the TNT and transformation products mixture in the exposure water are critical, particularly for appropriate interpretation of body residues.

In sediment exposures, neither RDX nor TNT or its major transformation products were detectable in *L. plumulosus* or *N. arenaceodentata* tissues, and were only detected in the FE *E. estuarius* tissues, for which the mean body residues were lower than the 4-d NOER for that species (162 and 268 nmol/g, for TNT and RDX, respectively, Lotufo and Rosen, unpublished data).

CONCLUSIONS

Although toxicity metrics for benthic and pelagic marine invertebrates have been previously derived using pure forms of common explosives such as TNT and RDX, few studies have reported data with direct relevance to expected exposure scenarios in the field. The results of this study suggest that exposure

Table 1. Summary (mean ± one standard deviation) of test organism survival (*C. variegatus, M. galloprovincialis*-adult, *E. estuarius, L. plumulosus, N. arenaceodentata*) following 10-d exposure and embryo-larval development success following 48-h exposure (*M. galloprovincialis* embryo-grab, in situ) in experiments with Composition B fragments

	Static	Flow	Static	Flow	Static	Flow
Test organism	Control	Control	Exposed	Exposed	Buried	Buried
Cyprinodon variegatus	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)
Mytilus galloprovincialis (adult)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)
Eohaustorius estuarius	100 (0)	90 (10)	90 (7.1)	90 (10)	90 (5)	95 (5)
Leptocheirus plumulosus	62 (2.9)	80 (7.1)	75 (21)	63 (13)	73 (20)	77 (32)
Neanthes arenaceodentata	87 (11)	80 (0)	70 (14.1)	80 (20)	87 (12)	93 (12)
M. galloprovincialis (embryo- grab)	92 (13)	85 (5.6)	0 (0)*	91 (9.4)	89 (3.7)	89 (0.9)
M. galloprovincialis (embryo- in situ)	70 (16)	74 (16)	0 (0)*	85 (13)	73 (22)	85 (13)

Asterisks indicate statistical difference from relevant control ($\alpha = 0.05$).

Table 2. Body burdens, expressed as means (± one standard deviation) from three replicate aquaria for each of four treatment types, determined on tissues from three test species exposed in test aquaria containing Composition B fragments

		Body Burden (nmol/g)		%
Species	Treatment	RDX	SumTNT	ADNTs
Cyprinodon variegatus	Static Exposed	35.3 (29.9)	28.2 (12.6)	89 (6.6)
	Static Buried	0.4 (0.7)	BDL	BDL
	Flow Exposed	10.8 (4.4)	13.7 (4.4)	79 (4.4)
	Flow Buried	BDL	BDL	BDL
Mytilus galloprovincialis	Static Exposed	46.4 (9.1)	31.1 (8.2)	82 (8.4)
	Static Buried	BDL	BDL	BDL
	Flow Exposed	31.5 (7.5)	18.7 (8.5)	34 (5.1)
	Flow Buried	BDL	BDL	BDL
Eohaustorius estuariusa	Static Exposed	108	74.4	87

BDL = below detection limit.
Replicates were composited due to insufficient tissue mass for Static Exposed treatment.
All other treatments resulted in tissue concentrations that were below method detection limits.

of TNT and RDX from Comp B fragments in laboratory-based simulated real-world exposure scenarios is unlikely to result in biological effects to most ecological receptors. Factors such as flow and burial of Comp B fragments also reduce the risk for exposure. It is unlikely that breached or corroded unexploded ordnance or Comp B fragments associated with low-order detonations are of significant risk to ecological receptors in the marine environment, except perhaps on very localized scales (e.g., sediment directly under exposed fragments of explosive formulations). Verification of this conclusion, however, should be pursued by determining water, sediment, and tissue residue concentrations, as well as toxicity to surrogate species, under true in situ exposures at field sites with underwater explosives present.

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REFERENCES

1. Darrach MR, Chutjian A, Plett GA. 1998. Trace explosives signatures from World War II unexploded undersea ordnance. *Environ Sci Technol* 32:1354–1358.
2. Dave G. 2003. Field test of ammunition (TNT) dumping in the ocean. In Munawar M, ed, *Quality Assessment and Management: Insight and Progress*. Aquatic Ecosystem Health and Management Society, Washington, DC, USA, pp 213–220.
3. Cusson B, Latreille A, Thiboutot S, Ampleman G. 2003. A great challenge: Characterization of sediments from the Lake St. Pierre Firing Range, St. Lawrence River, Quebec, Canada. *Proceedings*, 2nd International Symposium on Contaminated Sediments: Characterization, Evaluation, Mitigation/Restoration, Management Strategy Performance. Québec City, Canada, May 26–28, p 6.
4. Ampleman G, Thiboutot S, Lewis J, Marois A, Gagnon A, Bouchard M, Jenkins T, Ranney T, Pennington J. 2004. Evaluation of the contamination by explosives and metals in soils, vegetation, surface water and sediment at Cold Lake Air Weapons Range (CLAWR), Alberta, Phase II Final Report. Defence Research and Development Canada—Valcartier. Technical Report TR- 2004-204. Quebec, Canada.
5. Ek H, Dave G, Nilsson E, Sturve J, Birgersson G. 2006. Fate and effects of 2,4,6-trinitrotoluene (TNT) from dumped ammunition in a field study with fish and invertebrates. *Arch Environ Contam Toxicol* 51:244–252.
6. EA Engineering, Science, and Technology. 1996. Remedial investigation-Jackson Park Housing Complex, Naval Hospital Bremerton, Contract N44255-94-D-7309. Bellevue, WA, USA.
7. Nipper M, Carr RS, Biedenbach JM, Hooten RL, Miller K, Saepoff S. 2001. Development of marine toxicity data for ordnance compounds. *Arch Environ Contam Toxicol* 41:308–318.
8. Lotufo GR, Lydy MJ. 2005. Comparative toxicokinetics of explosive compounds in sheepshead minnows. *Arch Environ Contam Toxicol* 49:206–214.
9. Rosen G, Lotufo GR. 2007. Toxicity of explosive compounds in the marine mussel, *Mytilus galloprovincialis*. *Ecotoxicol Environ Saf* 68:228–236.
10. Green AS, Moore DW, Farrar D. 1999. Chronic toxicity of 2,4,6-trinitrotoluene to a marine polychaete and an estuarine amphipod. *Environ Toxicol Chem* 18:1783–1790.
11. Lotufo GR, Farrar JD, Inouye LS, Bridges TS, Ringelberg DB. 2001. Toxicity of sediment-associated nitroaromatic and cyclonitramine compounds to benthic invertebrates. *Environ Toxicol Chem* 20:1762–1771.

12. Steevens JA, Duke BM, Lotufo GR, Bridges TS. 2002. Toxicity of the explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine in sediments to *Chironomus tentans* and *Hyalella azteca*: Low-dose hormesis and high-dose mortality. *Environ Toxicol Chem* 21:1475–1482.
13. 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.
14. Elovitz MS, Weber EJ. 1999. Sediment-mediated reduction of 2,4,6-trinitrotoluene and fate of the resulting aromatic (poly)amines. *Environ Sci Technol* 33:2617–2625.
15. Conder JM, La Point TW, Steevens JA, Lotufo GR. 2004. Recommendations for the assessment of TNT toxicity in sediment. *Environ Toxicol Chem* 23:141–149.
16. Brannon JM, Pennington JC. 2002. Environmental fate and transport process descriptors for explosives. ERDC/EL TR- 02-10. U.S. Army Engineer Research and Development Center, Vicksburg, MS.
17. Prak DJL, O'Sullivan DW. 2006. Solubility of 2,4-dinitrotoluene and 2,4,6-trinitrotoluene in seawater. *J Chem Eng Data* 51:448–450.
18. Burton GA Jr, Greenberg MS, Rowland CD, Irvine CA, Lavoie DR, Brooker JA, Moore L, Raymer DFN, McWilliam RA. 2005. In situ exposures using caged organisms: A multi-compartment approach to detect aquatic toxicity and bioaccumulation. *Environ Pollut* 134:133–144.
19. Winger PV, Lasier PJ. 1991. A vacuum-operated pore-water extractor for estuarine and freshwater sediments. *Arch Environ Contam Toxicol* 21:321–324.
20. U.S. Environmental Protection Agency. 1994. Methods for measuring the toxicity of sediment-associated contaminants with estuarine and marine amphipods. EPA-600/R-94/025. Narragansett, RI.
21. U.S. Environmental Protection Agency. 1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. EPA/600/R-95/136. Washington, DC.
22. U.S. Environmental Protection Agency and U.S., Army Corps of Engineers. 1998. Evaluation of Dredged Material Proposed for Discharge in Water of the U.S. — Testing Manual. EPA 823-B- 98-004. Office of Water and Department of the Army, US Army Corps of Engineers, Washington, DC.
23. Ek H, Nilsson E, Birgersson G, Dave G. 2007. TNT leakage through sediment to water and toxicity to *Nitocra spinipes*. *Ecotoxicol Environ Saf* 23:2617–2625.
24. Lynch JC, Brannon JM, Delfino JJ. 2002. Effects of component interactions on the aqueous solubilities and dissolution rates of the explosive formulations octol, composition B, and LX–14. *J Chem Eng Data* 47:542–549.
25. Larson SL, Martin WA, Escalon BL, Thompson M. 2008. Dissolution, sorption, and kinetics involved in systems containing explosives, water, and soil. *Environ Sci Technol* 42:786–792.
26. Brannon JM, Price CB, Yost SL, Hayes C, Porter B. 2005. Comparison of environmental fate and transport process descriptors of explosives in saline and freshwater systems. *Mar Pollut Bull* 50:247–251.
27. Dontsova KM, Yost SL, Simunek J, Pennington JC, Williford CW. 2006. Dissolution and transport of TNT, RDX, and Composition B in saturated soil columns. *J Environ Qual* 35:2043–2054.
28. Yost SL, Pennington JC, Brannon JM, Hayes CA. 2007. Environmental process descriptors for TNT, TNT-related compounds and picric acid in marine sediment slurries. *Mar Pollut Bull* 54:1262–1266.
29. Anderson BS, Hunt JW, Phillips BM, Nicely PA, Tjeerdema RS, Martin M. 2004. A comparison of *in situ* and laboratory toxicity tests with the estuarine amphipod *Eohaustorius estuarius*. *Arch Environ Contam Toxicol* 46:52–60.
30. Anderson BS, Phillips BM, Hunt JW. 1998. Marine Bioassay Project (Ninth Report): Investigations of receiving water toxicity in coastal waters. State Water Resources Control Board, California Environmental Protection Agency, Sacramento, CA, USA.
31. Beiras R, Vazquez E, Bellas J, Lorenzo JI, Fernandez G, Mach JC, Casas L. 2001. Sea-urchin embryo bioassay for *in situ* evaluation of the biological quality of coastal seawater. *Estuar Coastal Shelf Sci* 52:29–32.
32. Geffard O, His E, Budzinski H, Seaman M, Garrigues P. 2001. In situ monitoring of sea water quality with the embryo-larval bioassay of *Crasssostrea gigas* and *Mytilus galloprovincialis*. *Comptes rendus de l'Acadaemie des sciences, Saerie III, Sciences de la vie* 324:1149–1155.