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USE OF A NOVEL SEDIMENT EXPOSURE TO DETERMINE THE EFFECTS OF TRICLOSAN ON ESTUARINE BENTHIC COMMUNITIES

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Abstract—Triclosan (5-chloro-2-[2,4-dichlorophenoxy]phenol) is a relatively new, commonly used antimicrobial compound found in many personal care products. Triclosan is toxic to marine organisms at the micrograms per liter level, can photodegrade to a dioxin, can accumulate in humans, and has been found to be stable in marine sediments for over 30 years. To determine the effects of triclosan on marine benthic communities, intact sediment cores were brought into the laboratory and held under flowing seawater conditions. A 2-cm layer of triclosan-spiked sediment was applied to the surface, and after a two-week exposure the meio- and macrofaunal communities were assessed for differences in composition relative to nonspiked cores. A high triclosan treatment (180 mg/kg dry wt) affected both the meio- and the macrobenthic communities. There were no discernible differences with a low-triclosan treatment (14 mg/kg dry wt). This exposure method is effective for testing the benthic community response to sediment contaminants, but improvements should be made with regard to the amount and method of applying the overlying sediment to prevent smothering of fragile benthic organisms. Environ. Toxicol. Chem. 2013;32:384–392. © 2012 SETAC

Keywords—Triclosan Sediment Benthic communities Estuarine Mesocosm

INTRODUCTION

Triclosan (5-chloro-2-[2,4-dichlorophenoxy]phenol) is a relatively new, commonly used antimicrobial compound in many personal care products (e.g., liquid hand soaps, detergents, toothpastes, mouthwash, and cosmetics) and is infused in many common consumer plastic products, such as kitchen utensils and children's toys (http://householdproducts.nlm.nih.gov/cgi-bin/household/brands?tbl=chem&id=1401; accessed February 2012). It is formulated in liquid soaps and disinfectants at levels up to 0.45% [1] and enters estuarine environments primarily via wastewater treatment plant effluent [2]. In a survey of published studies, triclosan was reported in over 50% of all surface waters with a median concentration of approximately 50 ng/L [3]. It has been found in sediment cores dating back to the mid-1960s at concentrations up to 800 μg/kg [4,5]. Concentrations of triclosan are generally higher toward core surfaces, indicating a recent increase in deposition [4,5]. In addition to its persistence in sediments, triclosan has been shown to degrade to a dioxin in sunlight [6–8]. The 96-h aqueous median lethal concentration (LC50) for *Americamysis bahia* (mysid) and *Ampelisca abdita* (amphipod) is 74 and 73 μg/L, respectively [9]. For *Palaemonetes pugio* (grass shrimp), the acute aqueous LC50s were 305 μg/L for adult shrimp, 154 μg/L for larvae, and 651 μg/L for embryos [10]. *Vibrio fischeri* (Microtox bacterium) was more sensitive, with a 15-min aqueous median inhibitory concentration (IC50) value of 53 μg/L [10]. The marine phytoplankton species *Dunaliella tertiolecta* in a standard 96-h static algal bioassay protocol was the most sensitive, with an LC50 of 3.55 μg/L [11].

Because of its accumulation and persistence in sediments, as well as the recognition of the potential risks associated with emerging contaminants, the effect of triclosan on intact marine benthic communities should be further evaluated. Meio- and macrobenthic communities form the basis of food chains in sediments in many estuarine ecosystems. These communities are at a higher risk than water-column organisms because of triclosan's propensity to accumulate in sediments. Determining the effects of triclosan on these communities allows us to understand better the risks of triclosan in estuaries.

To examine the effects of triclosan on these ecologically important communities, we adapted a novel exposure method that brings intact sediment cores into the laboratory and then exposes the existing benthic communities by addition of toxicant-spiked sediments to the core surface [12]. Traditionally, toxicity testing has been performed with single or some limited combination of culturable or collectable test organisms in a laboratory with spiked sediments [13]. Though this laboratory design allows us to control many experimental factors, it does not provide information on the many benthic organisms that make up a community, nor does it allow for organism interaction. Conversely, researchers have also conducted field

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experiments in outside mesocosms with natural organism assemblages or in field recolonization experiments [14,15]. Experimental field designs can give information on the sensitivity of many benthic organisms and allow for community interactions, but it is difficult to control the many variable factors in field exposures, and there are limitations on the type of toxicants that can be tested in field settings when there are exchanges with natural systems (e.g., many very toxic or persistent compounds would not be allowed). The novel hybrid system in our experiment brings intact benthic communities into the laboratory, yet controls many experimental factors and can be used with very toxic or persistent compounds. Our experiments are based on a design by Chandler et al. [12] in which cores were brought into the laboratory and spiked sediment places on the top. Chandler et al. added a population of harpacticoid benthic copepods to the cores, and the endpoints were copepod population measures and nematode counts. Our design differs from that of Chandler et al. in that we did not add copepods, our endpoints were both the meio- and macrofaunal benthic communities, and we added an additional DNA-free sediment layer to explore a genomic endpoint (Chariton et al., unpublished data). As in the Chandler et al. design, the addition of a 2-cm layer of treated surface sediment to the core sediment surface forces the benthic communities to move up into the oxidized layer of sediment to survive, exposing them to the toxicant. This exposure method allows the determination of changes in the entire meio- and macrobenthic sediment community, rather than testing one or two test species. The present study had two major objectives: (1) to examine the effect of triclosan-spiked sediments on estuarine meio- and macrobenthic communities, and (2) to determine whether this novel exposure method is effective for evaluating the toxicity of sediment contaminants of emerging concern.

MATERIALS AND METHODS

Core collection

Sediment cores were collected from the John H. Chafee National Wildlife Refuge at Pettaquamscutt Cove (Narrow River), Narragansett, Rhode Island, USA (N 41°26.92′, W 71°27.51′) in June, 2010. This area is an estuarine wildlife refuge of over 300 acres of salt marsh and surrounding forest habitat (salinity range, 20–30 ppth), with no noted sources of contaminants. Cores were collected by carefully inserting a cylindrical clear Plexiglass tube (32 cm height, 15 cm i.d.) into the sediment approximately 15 cm deep. The Plexiglass tube was then sealed with plastic plugs, and the intact core was removed from the sediment bed with a winch. All cores were maintained at approximately 20°C and transported to the laboratory within 4 h.

Core maintenance and treatments

Twenty-four cores were maintained in the laboratory in a flow-through, filtered seawater system (average 6.4-h turnover rate, 30 ppt, 20°C, 16 h:8 h light:dark cycle) with aeration. Each core had a sediment depth of approximately 15 and 17 cm of overlying water (Fig. 1). This sediment core – overlying water system comprised our mesocosms. Mesocosms were randomly divided into the following four treatment groups, with six replicates each: (1) field control with no sediment added, (2) laboratory control with clean sediment added, (3) low-triclosan-amended sediment, and (4) high-triclosan-amended sediment. Two days postfield collection, 10-g sediment samples were collected from the top layer of three randomly selected mesocosms and stored at −4°C for 90 d until triclosan analyses were performed.

Three days post-collection, mesocosms were treated by the addition of a sediment slurry (∼60% treated or untreated Long Island Sound control sediment [LIS] and ∼40% seawater) to the core surface [12]. The control sediment, LIS, was a well-characterized reference sediment that has been used in our laboratory for many years [16]. Nominal concentrations for the spiked low- and high-triclosan treatments were 30 and 300 mg triclosan/kg dry LIS sediment, respectively. Prior to the treatment application, subsamples of each spiked sediment treatment were collected for chemical analysis. Each mesocosm (except for the field controls) received 610 ml of sediment slurry (∼2-cm-thick sediment layer), with the exception of the high-triclosan mesocosms, which received only 470 ml of treated slurry (slightly less than 2 cm). The sediment slurry was applied slowly to each mesocosms from a plastic pastry bag, submerged into the water near the sediment surface. Three days post-addition of the sediment slurry, aliquots of sediment were collected from the top layer of the low- and high-triclosan treatments (three random replicates/treatment) for chemical analysis.

A second DNA-free [17] layer of untreated sediment (∼60% DNA-free sediment and ∼40% seawater) was added to the top of all cores, except for the field controls, two weeks after application of the treated slurry. The DNA-free sediment was prepared by autoclaving LIS sediment at 121°C for 5 min. This sediment was prepared and used to facilitate the trial of a DNA-based molecular diversity technique, with the results of the present study provided in a companion paper (Chariton et al., unpublished data). The DNA-free sediment was necessary because the genomic endpoints cannot distinguish between dead and live DNA. The DNA-free sediment layer was applied in the same way as the treated slurry layer, except that it was added in two 1-cm increments (1–2 h between additions) to allow the sediment to settle and the organisms to migrate to the top layer (Fig. 1). After the addition of the DNA-free layer, the exposures continued for another week for migration into the oxic, clean layer. It is assumed that organisms that do not

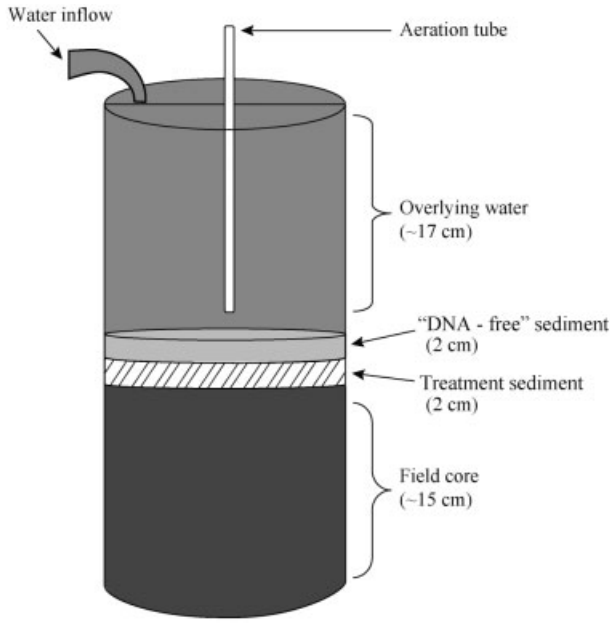

Fig. 1. Schematic of mesocosm core design.

migrate up into the upper 2 cm are not alive because they most likely will not survive in the anoxic layers below.

Live algae (*Isochrysis galbana*, *Dunaliella tertiolecta*, and *Tetraselmis suecica*; 2×10^7 cells/species/d for a total of 6×10^7 cells/d) were added to the mesocosms every 4 d [12] for the duration of the experiment, and physicochemical measurements (temperature, pH, dissolved oxygen, and salinity) were taken every 7 d throughout the experiment. Three weeks post-application of the first layer (and one week after the application of the DNA-free layer), final sediment samples were taken from all mesocosms. Sediment aliquots for triclosan analyses were taken from the top layer. Meiofaunal samples were taken by placing a 1.5-cm-diameter open-ended glass tube 2 cm deep into the mesocosm sediment, plugging the top to create a vacuum, and removing the sample (volume, 3.53 cm^3). The meiofaunal sample was rough sieved at 50 μm. For macrofaunal analysis, the remaining top 2 cm of sediment in each mesocosm was removed and rough sieved at 500 μm. All organism samples were preserved with buffered formalin and rose bengal, and stored at 4°C until analysis. With the exception of Nematodes, Kinorhyncha and Cnidaria, meiofauna were identified to Order, with the harpacticoid nauplii being enumerated separately from the adults. Macrofauna were identified to the lowest practicable level (generally to species).

Chemical analysis of triclosan

Chemical analysis was performed as outlined elsewhere [4]. Briefly, 1 g of dried, homogenized sediment was spiked with 50 ng ^{13}C-triclosan as an internal standard and extracted in an accelerated solvent extraction unit (ASE 200; Dionex). Agilent silica solid-phase extraction (SPE) columns were used for extract cleanup and extracts were reduced to 1 ml hexane. Aliquots (100 μl) of the final extracts were evaporated to dryness under a stream of nitrogen and derivatized (e.g., silylation reaction) at 20°C for 60 min with 50 μl *N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide. After derivatization, sample extracts were brought up to 100 μl with ethyl acetate and analyzed within 24 h of derivatization. Analysis was performed with an Agilent 7890 gas chromatograph equipped with a 5975 mass selective detector operating in select ion monitoring mode. A five-point calibration curve was developed using certified standards derivatized in the same manner as the samples and analyzed daily. Triclosan and ^{13}C-triclosan were quantified using ions m/z 347 and 357, respectively. To assess extraction performance during the study, reagent-grade triclosan was spiked at a concentration of 50 ng/g into ASE 200 cells. Recoveries of triclosan averaged 110%, with a standard deviation of 4.9% ($n = 2$).

Statistical analysis

Differences in community indices (e.g., total abundance, taxonomic richness, and diversity [H']) and the abundances of selected meio- and macrofauna taxa were analyzed using a balanced, single-factor analysis of variance (ANOVA) with a post hoc Student–Newman–Keuls (SNK) test. Calculations for community indices were modified to accommodate the taxonomic resolutions used in the present study. Residuals were assessed using D'Agostino's tests for skewness, kurtosis, and omnibus normality [18], with homogeneity of variances examined using a modified Levene equal variance test [19]. When these assumptions were violated, appropriate transformations were performed [20]. In cases in which the data remained heteroscedastic, the level of significance was set at $p < 0.01$. All ANOVAs were performed in NCSS v7.1.3 software.

Multivariate analyses were performed using the Primer 6+ statistical package (Plymouth Marine Laboratory). Ordination of both the meio- and the macrobenthic data was performed by nonmetric multidimensional scaling (nMDS). Data were double-square root transformed prior to computation, with distances between samples measured by Bray – Curtis similarity coefficients [21]. For both the meio- and the macrofaunal data, statistical differences among treatments were tested by permutational multivariate analysis of variance (PERMANOVA) on the transformed data using Bray – Curtis dissimilarities between samples [22]. Differences among treatments were identified by pairwise a posteriori tests based on 9,999 random permutations. The five taxa that contributed most to the differences between significantly different treatments were identified using the Primer's SIMPER routine.

RESULTS

Physical and chemical measurements

Weekly measurements of temperature, pH, salinity, and dissolved oxygen were relatively stable and within the accepted boundaries for the studied communities (Table 1). Flow rates indicated a turnover time of approximately 6.4 h. Chemical measurements of triclosan indicated background concentrations <100 μg/kg, and spiked sediment concentrations were between 63 and 68% of nominal for the low and high concentrations, respectively (Table 2). High standard deviations in sediment triclosan concentrations after 3 and 7 d of equilibration may be due to patchiness caused by biological mixing in the mesocosms. After 3 d of equilibration in the mesocosm, triclosan concentrations in the overlying treated sediment declined to 46 to 60% of nominal.

Meiofauna

In total 10,008 individual meiofaunal organisms were extracted from the four treatments, with nematodes representing >73% of the total abundance (Supplemental Data, Table S1). The univariate attributes of the meiofaunal assemblages are summarized in Table 3, with the ANOVA results, including post hoc comparisons of means, presented in Table 4. The field control treatments were more diverse (H') and more taxonomically rich (S) and contained more than twice the number of organisms (N) as the laboratory control and high- and low-triclosan treatments. This finding was also reflected in the abundances of nematodes, harpacticoids and their nauplii, cnidarians, and polychaetes (Fig. 2a and Tables 3 and 4). No differences in univariate metrics were detected between the laboratory control and the low-triclosan treatments. However, the univariate attributes of the high-triclosan treatments generally differed from those of the other treatments, with total abundance half of that observed in the low-triclosan and

Table 1. Summary of the physicochemical measurements from weekly mesocosm samples (overlying water)

	Mean	SD	Range
Temperature (°C)	20.3	0.55	19.5–21.0
pH	8.00	0.05	7.76–8.10
Dissolved oxygen (ppm)	8.24	0.64	6.2–8.8
Salinity (ppt)	30.3	0.43	30.0–31.0
Turnover rate[a] (h)	6.40	2.75	3.84–14.87

[a] Time required to replace the entire water column of the mesocosm.
SD = standard deviation.

Table 2. Chemical measurements of triclosan in mesocosm sediments

Sample	Nominal triclosan concentration (mg/kg dry)	Average measured triclosan concentration (mg/kg dry; ± SD, where applicable; $n = 3$ except where noted)	Percentage of nominal
Top layer of field control (Narrow river sediment)		0.06 ± 0.04	
LIS sediment		0.0014 ± 0.001	
Initial treated sediment (LIS sediment)			
Low triclosan	30	20.3 ± 3.96[a]	67.7
High triclosan	300	187.7 ± 20.8	62.6
Top layer of cores 3 d after triclosan-treated sediment added			
Low triclosan	30	13.9 ± 4.31	46.4
High triclosan	300	181 ± 125	60.3
Top layer of cores 7 days after "DNA-free" LIS added			
Low triclosan	NA	1.19 ± 0.20	
High triclosan	NA	1.43 ± 0.81	

[a] $n = 2$.
LIS = Long Island Sound; NA = not applicable.

laboratory control treatments and one-fifth of the field control abundance. Furthermore, the high-triclosan assemblages were less rich and contained significantly lower abundances of nematodes, harpacticoids (including their nauplii), bivalves, and kinorhynchs than did the other treatments (Fig. 2a and Tables 3 and 4).

As indicated by the proximity of samples, the nMDS ordination plot derived from the meiofauna suggests that the field control and high-triclosan treatments contained the most dissimilar communities (Fig. 3a), with both treatments differing, but to a lesser extent, from the laboratory control and low-triclosan treatments. In contrast, assemblages from the laboratory control and low-triclosan treatments appeared to contain similar meiofaunal assemblages. The PERMANOVA detected a significant difference among the treatments (PERMANOVA: $F = 14.6$, $p = 0.001$). Post hoc comparisons supported the nMDS analysis, with significant differences detected among all treatments, with the exception of the laboratory control and low-triclosan treatments (Table 5 and Supplemental Data, Table S1).

Table 3. Summary of the univariate attributes of the meiofaunal assemblages collected at the conclusion of the exposures (means ± 1 standard error)

	Treatment			
Variable	Field control	Laboratory control	Low triclosan	High triclosan
Total abundance (N)	769 ± 72	382 ± 18	367 ± 31	149 ± 25
Number of taxa (S)	9.2 ± 0.4	7.7 ± 0.2	7.7 ± 0.3	5.7 ± 0.2
Diversity (H′)	1.02 ± 0.04	0.87 ± 0.04	0.84 ± 0.04	0.80 ± 0.05
No. of nematodes	538 ± 63.7	291 ± 19.0	283 ± 20.3	117 ± 20.1
No. of harpacticoids	103 ± 7.36	41.0 ± 2.42	35.8 ± 7.03	8.50 ± 2.43
No. of harpacticoid nauplii	62 ± 4.4	13 ± 1.3	22 ± 7.0	3.0 ± 0.67
No. of ostracods	37.2 ± 10.9	28.0 ± 1.7	18.0 ± 2.8	16.3 ± 3.5
No. of cnidarians	21 ± 3.1	4.8 ± 1.2	3.2 ± 0.5	3.7 ± 0.7
No. of bivalves	2.5 ± 0.5	0.8 ± 0.4	2.2 ± 0.9	0.2 ± 0.2
No. of kinorhynchs	2.3 ± 0.49	1.7 ± 0.33	2.0 ± 0.63	0.33 ± 0.21
No. of polychaetes	2.16 ± 1.3	0.33 ± 0.52	0.83 ± 1.2	0.17 ± 0.41
No. of amphipods	1.33 ± 0.82	0.67 ± 0.82	1.0 ± 1.3	0.0 ± 0.0

Table 4. Summary analysis of variance table for univariate meiofaunal community metrics and the abundances of selected taxa

Variable	SS	MS	F	p	Student–Newman–Keuls
Total abundance (N)[a]	1.64	0.545	40.9	<0.001	High < (lab = low) < field
Number of taxa (S)	31.1	12.4	22.9	<0.001	High < (lab = low) < field
Diversity (H′)	0.167	0.056	4.74	<0.05	(High = low = lab) < field
No. of nematodes[a]	1.44	0.479	30.6	<0.001	High < (lab = low) < field
No. of harpacticoids	3.88	1.29	32.3	<0.001	High < (lab = low) < field
No. of harpacticoid nauplii[b]	5.18	1.73	48.8	<0.001	High < (lab = low) < field
No. of ostrocods[b]	0.326	0.108	1.97	0.151	Not applicable
No. of cnidarians[a]	1.96	0.655	19.4	<0.001	(High = low = lab) < field
No. of bivalves[a,c]	0.778	0.260	0.259	<0.01	High < low and field
No. of kinorhynchs[a]	0.552	0.184	5.32	<0.001	High < (lab = low = field)
No. of polychaetes[a]	0.624	0.208	6.19	<0.01	(High = low = lab) < field
No. of amphipods	1.33	0.667	0.68	0.521	Not applicable

[a] Data log10 transformed.
[b] Log10 transformed with $p < 0.01$ because of heteroscedasticity.
[c] Comparisons were made only among field and low and high treatments because no individuals were present in the laboratory treatment group.
SS = sum of squares; MS = mean square.

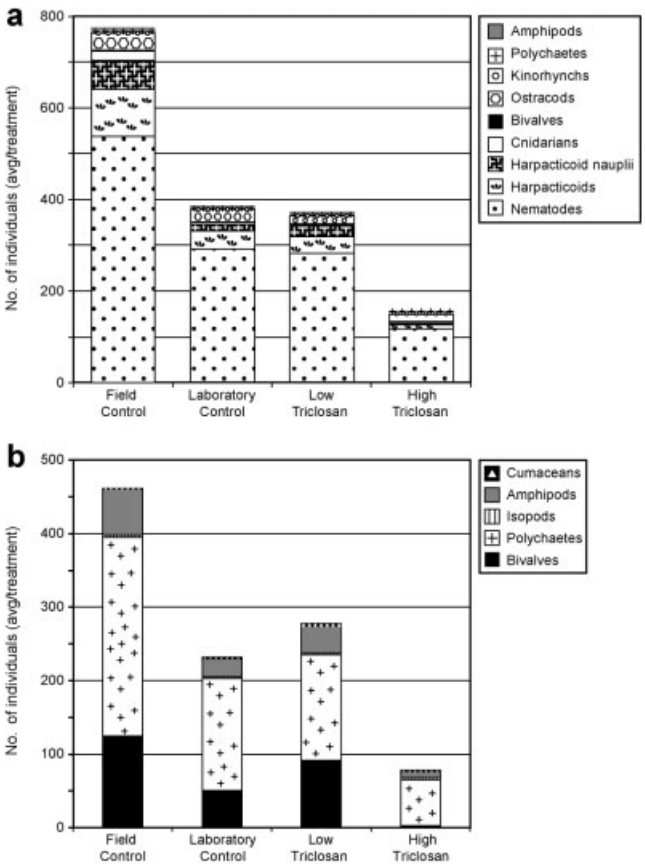

Fig. 2. Benthic community abundance with respect to treatment. **(a)** Meiofaunal abundance. **(b)** Macrofaunal abundance.

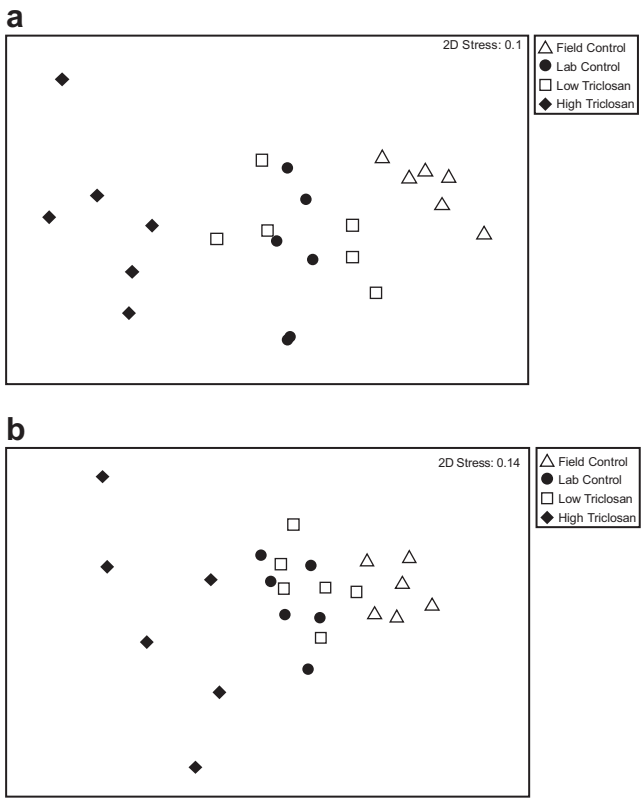

Fig. 3. Nonmetric multidimensional scaling ordination plot for benthic communities sampled from the four treatments. **(a)** Meiofaunal communities. **(b)** Macrofaunal communities.

Similarity percentage (SIMPER) analysis confirmed that the field control and high-triclosan treatments contained the most dissimilar meiofaunal assemblages (Table 6). These differences were driven primarily by pronounced reductions in the representations of harpacticoids and their nauplii, nematodes, bivalves, and polychaetes in the high-triclosan treatment. Compared with both the laboratory control and the low-triclosan treatments, the composition of the high-triclosan treatments contained lower abundances of bivalves, nematodes, kinorhynchs, and harpacticoids and their nauplii. Amphipods were

Table 5. Multivariate pairwise comparisons among treatments performed following permutational multivariate analysis of variance (PERMANOVA) derived from the meiofaunal data

Treatment	T	*p* (PERMANOVA)	Dissimilarity (%)
Field control, high triclosan	6.68	0.0036	33.3
Low triclosan, high triclosan	3.37	0.001	22.1
Laboratory control, high triclosan	3.60	0.003	21.9
Field control, laboratory control	3.63	0.003	17.5
Field control, low triclosan	2.97	0.005	16.8
Laboratory control, low triclosan	1.01	0.429	12.3

Table 6. Summary of SIMPER analyses of meiofaunal assemblages contributing to significant differences between treatments (permutational multivariate analysis of variance [PERMANOVA]: $p < 0.05$)

Seven major taxa contributing to dissimilarities	Field control and high triclosan	Low triclosan and high triclosan	Laboratory control and high triclosan	Field control and laboratory control	Field control and low triclosan
Nematodes	×	×	×	×	×
Harpacticoids	×	×	×		×
Harpacticoid nauplii	×	×		×	×
Cnidarians				×	×
Bivalves	×	×	×	×	
Kinorhynchs		×	×		
Polychaetes	×			×	×

Table 7. Summary of the univariate attributes of the macrofaunal assemblages (mean ± 1 standard error)

Variable	Field control	Laboratory control	Low triclosan	High triclosan
Total abundance (N)	467 ± 32	231 ± 21	279 ± 38	79 ± 14
Number of taxa (S)	19 ± 1.0	12. ± 1.0	15 ± 1.0	10 ± 1.0
Diversity (H′)	2.0 ± 0.03	1.8 ± 0.07	1.8 ± 0.06	1.2 ± 0.13
No. of polychaetes	270 ± 23.3	152 ± 30.9	145 ± 29.2	62.7 ± 13.0
No. of bivalves	125 ± 11.4	51.2 ± 6.06	91.2 ± 20.2	2.67 ± 0.803
No. of amphipods	61.8 ± 12.4	24.8 ± 11.9	37.3 ± 12.9	8.50 ± 4.85
No. of isopods	3.7 ± 1.3	1.7 ± 0.56	1.2 ± 0.31	2.5 ± 1.1
No. of cumaceans	0.67 ± 0.21	0.50 ± 0.34	3.2 ± 2.1	2.2 ± 1.3

relatively rare (only eight were found in the field control), with none found in the high-triclosan treatments. Differences between the field and laboratory controls were driven primarily by the relative abundances of harpacticoid nauplii, polychaetes, bivalves, nematodes, and cnidaria. In general, the relative abundances of the same groups drove the differences between the field control and low-triclosan treatments, although the contributions of the groups differed marginally (Table 6).

Macrofauna

In total 6,332 macrofaunal organisms were collected from the experiment, with the bivalve *Gemma gemma* and the polychaetes *Prionospio heterobranchia* and *Lumbrineries hebes* making up 68% of the total abundance (Supplemental Data, Table S1). The univariate attributes of the macrofaunal assemblages are summarized in Table 7, with the ANOVA results, including post hoc comparisons of means, in Table 8. As in the case of the meiofauna, macrofaunal communities sampled from the field control contained a greater mean number of organisms and were more taxonomically rich than the other treatments (Fig. 2b and Table 7). The abundances of bivalves and polychaetes were also greater in the field control than in the other treatments, and no significant differences were detected in the measured univariate attributes between the laboratory control and the low-triclosan treatments (Table 8). The high-triclosan treatment had a pronounced effect on the univariate attributes of the macrofaunal assemblages. The mean abundance of macrofauna in the high-triclosan treatment was less than 17% of that in the field control and approximately one-third of that in the other two treatments (Table 7). The high-triclosan treatments were significantly less rich and diverse and contained a lower abundance of bivalves than the other treatments (Fig. 2b and Table 7). Though no statistical differences in the abundances of amphipods were detected among the high-triclosan, laboratory control, and low-triclosan treatments, mean amphipod abundance in the high-triclosan treatment was lower than that in the field control. Isopod and cumacean abundances were not negatively influenced by the triclosan treatments, and polychaete abundance was significantly greater only in the field control.

Examination of the nMDS ordination plot suggests that the macrofaunal assemblages sampled from the high-triclosan treatment were distinct from the other treatments, whereas the differences among the field control, laboratory control, and low-triclosan treatments were less evident (Fig. 3b). The PERMANOVA confirmed that there was a significant difference among the treatments (PERMANOVA: $F = 5.13$, $p = 0.001$). Post hoc analysis revealed patterns similar to those observed with the meiofauna, with all assemblages containing significantly different compositions, with the exceptions of the laboratory control and the low-triclosan treatments (Table 9). As in the case of the

Table 8. Summary analysis of variance table for univariate macrofaunal community metrics and the abundances of selected taxa

	SS	MS	F	*p*	Student–Newman–Keuls
Total abundance (N)	461,193	155,731	33.6	<0.001	High < (lab = low) < field
Number of taxa (S)	262	87.6	19.2	<0.001	High < low = lab < field
Diversity (H′)	1.90	0.633	15.4	<0.001	High < (lab = low = field)
No. of polychaetes	131,350	43,784	11.6	<0.001	(High = low = lab) < field
No. of bivalves[a]	9.42	3.14	77.5	<0.001	High < (lab = low) < field
No. of amphipods	9102	3034	4.16	<0.05	High < field
No. of isopods[a]	0.203	0.067	0.74	0.541	Not applicable
No. of cumaceans[a]	0.279	0.093	0.86	0.480	Not applicable

[a] Data were log10 transformed.
SS = sum of squares; MS = mean square.

Table 9. Multivariate pairwise comparisons among treatments performed following permutational multivariate analysis of variance (PERMANOVA) derived from the macrofaunal data

Treatment	T	*p* (PERMANOVA)	Dissimilarity (%)
Field control, high triclosan	3.08	0.003	54.7
Low triclosan, high triclosan	2.15	0.002	46.1
Laboratory control, high triclosan	1.96	0.002	44.0
Field control, laboratory control	2.47	0.003	36.6
Field control, low triclosan	2.15	0.002	34.4
Laboratory control, low triclosan	1.04	0.434	28.5

Table 10. Summary of SIMPER analyses of macrofaunal assemblages with significantly different treatments (permutational multivariate analysis of variance [PERMANOVA]: $p < 0.05$)

Four major taxa contributing to dissimilarities	Field control and high triclosan	Low triclosan and high triclosan	Laboratory control and high triclosan	Field control and laboratory control	Field control and low triclosan
Polychaetes					
Clymenella torquata[a]				×	×
Exogone verugera	×			×	×
Neanthes virens					×
Polydora cornuta	×	×	×		
Prionospio heterobranchia	×	×	×		
Bivalves					
Gemma gemma	×	×	×		
Amphipods					
Ampelisca abdita	×	×	×	×	
Microdeutopus gryllotalpa			×	×	×
Nemertea				×	×

[a] Taxon restricted to the field control and completely absent in all other treatments.

meiofauna, the most dissimilar assemblages were between the field control and high-triclosan treatments.

Differences between the high-triclosan treatment and the other three treatments were due predominantly to reductions in the relative abundances of the bivalve *Gemma gemma*, the polychaetes *P. heterobranchia* and *Polydora cornuta*, and the amphipod *A. abdita* (Table 10). The polychaete *Exogone verugera* also contributed to differences between the field control and the high-triclosan treatment. The relative abundance of *Microdeutopus gryllotalpa* was lower in the high-triclosan treatment compared with the laboratory control. Much of the difference between the field control compared with the laboratory control and low-triclosan assemblages was due to taxa present in much greater numbers in the field control assemblages. These included the polychaetes *E. verugera, Neanthes virens*, and *Clymenella torquata*. Reductions in the relative abundances of the amphipods *M. gryllotalpa and A. abdita* and an unidentified nemertean also contributed to these differences.

DISCUSSION

Overall changes in benthic communities

Overall, the meio- and macrofaunal communities followed the same trends in their responses to the high-triclosan treatment, with decreases both in total abundance and in number of taxa (Tables 3 and 7). Specifically, bivalve abundance decreased (e.g., the macrofaunal species *G. gemma* and *Tellina agilis*), and most polychaetes, regardless of family or feeding strategy, also declined. Even though we expected crustaceans to be sensitive, the results were variable. For example, harpacticoids, their nauplii, and the macrofaunal amphipods *Ampelisca* and *Microdeutopus* were adversely impacted by the high-triclosan treatment; however, the meiofaunal amphipods and other macrofaunal crustaceans (the cumacean *Leucon americanus,* the isopod *Edotea triloba*, and other ostracods) did not show a significant effect of triclosan (Supplemental Data, Table S1).

The field control had no sediment added, so it is not surprising that this group had the highest N, S, and H′ values (Tables 3 and 7). However, unexpectedly, the communities did not discriminate between the laboratory control (clean sediment) and the low-triclosan treatment (Table 4 and 8). This can be attributed to a detrimental effect on communities from the addition of two 2-cm layers of sediment and the ability or inability of different organisms to migrate twice.

For both macro- and meiobenthic communities, the ordination plots (Fig. 3a and b) indicate a greater variation in replication, or less clumping, for the high-triclosan treatment compared with the low-triclosan treatment and the field and laboratory controls. This varied community response might indicate that a different mechanism is responsible for the change in community function resulting from a chemical disturbance rather than a natural sedimentation event. The chemical disturbance may accentuate subtle differences existing in the individual mesocosms or promote differential competitive exclusion across replicates.

The observed changes in the benthos can be categorized into three separate responses. Those biota and community attributes that are more sensitive to the experimental artifact of sedimentation, (high = lab = low) < field; those that are more sensitive to triclosan concentrations, high < (lab = low = field); and those sensitive to both sedimentation and triclosan, high - < (lab = low) < field (Tables 4 and 8). It appeared that kinorhynchs and macrofaunal diversity were sensitive to triclosan exposure; cnidarians, polychaetes, and meiofaunal diversity were sensitive to sedimentation effects; and nematodes, harpacticoids and their nauplii, macrofaunal bivalves, total abundance, and number of taxa were sensitive to both sedimentation and chemical exposure. In the case of kinorhyncha, there is a small but growing body of evidence suggesting that this phylum is sensitive to a range of environmental contaminants [23–25].

Decline in laboratory control versus field control mesocosm

Changes in community structure were compared among different treatments. Results were not compared with the structure in the field because samples for time 0 community structure were not taken. We would expect some changes from loss of very sensitive organisms as the cores moved from the field to the laboratory. This loss of sensitive species is almost inevitable but is the trade-off for being able to control environmental conditions and factors that affect the mesososm.

There was a marked decrease in benthic community parameters between the field and laboratory control mesocosms for both the meio- and the macrobenthic communities as well as between the field mesocosms and all other mesocosms (Tables 3 and 7). This difference indicated that the benthic communities in the triclosan-treated and laboratory control mesocosms changed because of the sediment addition on top of the cores. The difference likely is due to smothering, because the layers of

added sediment were well aerated, so hydrogen sulfide or ammonia concentrations were probably not at toxic levels.

The addition of 2 cm of sediment was chosen because Chandler et al. [12] indicated that a 2-cm top application of clean sediment did not cause any meiofaunal mortality in their core exposure studies. Trannum et al. [26] indicate that up to 2.5 cm of clean sediment did not cause notable changes to benthic macrofaunal community structure. However, Miller et al. [27] performed studies indicating that, not surprisingly, different macrobenthic organisms respond differently to sedimentation. Some sessile, suspension-feeding and reef-building polychaetes, such as *Sabellaria vulgaris,* died after 2 cm of clean sediment had been placed on the surface, whereas others, such as the infaunal red-gilled mud worm, *Marenzelleria viridis*, and the epifaunal, motile mud snail, *Ilyanassa obsoleta*, survived applications of clean sediments up to 5 and 15 cm, respectively. Thrush et al. [28] reported that layers as small as 0.7 cm of terrestrial sediment on top of estuarine sediments could cause a major change in macrobenthic communities; however, terrestrial sediment might have adverse effects on estuarine fauna in addition to smothering.

Sandulli and De Nicola-Giudici [29] hypothesized that meiofaunal community decline as a result of smothering likely was caused by a reduction in the interstitial spaces from increasing the organic coating on sediment particles. The second layer of sediment applied to our mesocosms had been autoclaved to destroy any DNA that would interfere with the genetic analysis of meiofauna [17]. Autoclaving the sediment appeared to have broken down the sediment structure (i.e., it was a bit soupier), which possibly increased the silt component and decreased the interstitial spaces in the sediment. In hindsight, the organisms might have been able to tolerate the application of one 2-cm layer of clean sediment, but two 2-cm applications seemed to cause a notable decrease in benthic community diversity. This smothering effect made it very difficult to discern any benthic community changes at the low triclosan concentration.

Some of the declines in organism abundance can be explained by size and living strategy. For example, *Clymenella torquata*, the bamboo worm, is a relatively large, head-down deposit feeder that was present only in the field control. A larger organism might generally better survive the addition of sediment, but head-down feeders might become buried within their burrows before migrating upward. Other worms, such as the subsurface deposit feeder *Leitoscoloplos fragilis* and the carnivore/omnivore *Lumbrineris hebes*, showed no effect (Supplemental Data, Table S1). The amphipods *A. abdita* and *M. gryllotalpa* were also identified as organisms that contributed to the differences between field and laboratory controls (Table 10). Both of these amphipods are tube builders and might have been trapped in their tubes and unable to escape the sediment application. *Corophium*, in contrast, is a large and active surface-burrowing amphipod that was unaffected by sediment additions. The infaunal nemerteans (ribbonworms) were quite sensitive to sedimentation, which might have been due to the filling of the sediment interstices. Overall, mortality may be related to where the organisms were living when the sediment was applied and whether they were able to migrate upward in time to avoid being smothered.

The changes in benthic community structure that occurred after the addition of test sediment suggest that this experimental design does not evaluate the intact community that exists in the field; however, it does allow for testing of a number of different genera that are not normally found in laboratory toxicity tests. In addition, the mesocosm design tests those genera under more realistic field conditions than classic sediment toxicity tests, and allows community interactions not found in single- or even multiple-organism testing. Given the findings of the present study, we are experimenting with different methods of applying the treatment layers so that organisms are able to migrate to the aerobic layer of sediment.

Comparison with aqueous LC50s

The high-triclosan treatment resulted in changes in the composition of the meio- and macrobenthic communities relative to the other treatments and both of the controls. We used the following equilibrium partitioning theory equation [30] to calculate the concentration of triclosan in the interstitial water (C_w) of the upper 2 cm of the low- and high–triclosan-treated mesocosms.

$$K_{OC} = \frac{K_d}{f_{OC}} = \frac{C_{sed}/C_w}{f_{OC}}$$

$$C_w = \frac{C_{sed}}{K_{OC} \times f_{OC}}$$

where K_{OC} is the organic carbon normalized partition coefficient, K_d is the water partition coefficient, f_{OC} is the sediment organic carbon concentration (kg OC/kg sediment dry), C_{sed} is the sediment concentration of the chemical (μg/kg dry), and C_w is the water concentration of the chemical. We used a K_{OC} of 66,100 L/kg for triclosan in 30 ppt seawater (modified from Sabaliunas et al. [31]), and an f_{oc} of 0.02 kg/kg dry for the spiked LIS sediment. We calculated concentrations of 137 and 10 μg/L triclosan in the interstitial water of the high- and low-triclosan-treated mesocosms, respectively. Perron et al. [9] reported a 96-h water-only LC50 for the amphipod *A. abdita* of 73 μg/L. The low-triclosan-treated mesocosms had predicted interstitial water concentrations below *A. abdita's* LC50, and no difference was noted for amphipods or the benthic community between the low-triclosan and the laboratory control mesocosms. The high-triclosan mesocosms had an interstitial water concentration of 137 μg/L, which was within a twofold range of the *A. abdita* LC50 of 73 μg/L. In the mesocosms, *A. abdita* numbers decreased by 56% between the laboratory control and the high-triclosan mesocosms (Supplemental Data, Table S1). The comparability in the amphipod responses in our mesocosm and in the toxicity tests is encouraging and suggests that the toxicity test is somewhat predictive of the mesocosm response. Although we should have seen slightly higher toxicity to *A. abdita* in the high-triclosan mesocosm given the higher interstitial water concentrations, the exposure system in the mesocosm experiment was slightly different from that of the LC50 experiment. The mesocosm used flow-through overlying water with a turnover of approximately four times per day, whereas the design used to determine the LC50 was static, allowing higher and more constant concentrations of toxicants in the overlying water. The difference in the overlying water concentration would affect *A. abdita* exposure, because this amphipod is a benthic tube builder and irrigates its tube with overlying water. Therefore, in a flow-through system like our mesocosm, *A. abdita* would experience lower exposure concentrations.

We recognize that the tested concentration, particularly the high-triclosan treatment (181 mg/kg dry), is higher than many current field concentrations (0.8 mg/kg dry) and is higher than in biosolids (13 mg/kg dry) [32]. However, the use of triclosan has

rapidly increased over the last decade. Informal supermarket surveys indicate that currently approximately one-fourth of all on-the-shelf dishsoaps contain triclosan at the 0.1% concentration. The Centers for Disease Control recently released data showing that triclosan concentrations have increased in the United States population by up to 50% over the last 5 years (http://www.nrdc.org/media/2010/100805.asp). Because triclosan is persistent and accumulates in marine sediments, if current trends continue, triclosan concentrations could reach much higher levels that might adversely affect benthic communities.

SUMMARY

This research indicates that triclosan concentrations of 181 mg/kg dry weight in marine sediments adversely affect marine benthic communities. With an organic carbon fraction of 2%, this is equivalent to 137 μg/L triclosan in the interstitial water. Macrofaunal bivalves, as well as meiofaunal nematodes, harpacticoids, and kinorhynchs, were the most sensitive to triclosan. A lower concentration of 14 mg/kg triclosan dry weight in sediments (10 μg/L in interstitial water) had no discernible effect on marine benthic communities. The exposure system that we used to test intact benthic communities was sensitive enough for us to discern differences between the high triclosan and the laboratory control. However, there also was a difference between the field and the laboratory control, most likely as a result of smothering. This experimental design shows promise for being able to evaluate contaminants of emerging concern using intact communities under controlled field conditions. Modifications to the application of the stressor layers are being investigated to decrease the smothering effect of applying top layers of test sediments on some taxa.

SUPPLEMENTAL DATA

Table S1 Meio- and macrobenthic community counts.

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