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The role of various dissolved organic matter forms on chlorpyrifos bioavailability to the estuarine bivalve *Mercenaria mercenaria*

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Abstract

Dissolved organic matter (DOM) is comprised of a myriad of macromolecules with specific physical and chemical properties that may influence the bioavailability of hydrophobic pesticides to animals. This study was conducted to assess the role of various forms of DOM on the uptake and bioconcentration of the organophosphate insecticide chlorpyrifos (CHPY) to the bivalve *Mercenaria mercenaria*. Bivalves were exposed to DOM-free seawater (30‰) or to seawater containing a single form of DOM. DOM forms included two filtrate fractions of natural salt-marsh sediment DOM (DOM-($<0.45 \mu\text{m}$) and DOM-($<3 \text{ kDa}$)); natural purified humic (HA) and fulvic (FA) acids; and water soluble cyclic oligosaccharides alpha and beta cyclodextrins (CD- α and CD- β).

In ^{14}C -CHPY uptake and elimination experiments, juvenile bivalves were exposed to uniformly-labeled ^{14}C -CHPY and collected at time intervals during 48 h. The remaining bivalves were transferred to ^{14}C -CHPY-free elimination chambers with bivalve collection at time intervals over 144 h. Total uptake of ^{14}C -CHPY by bivalves in DOM-free seawater was $>40\%$ greater than in bivalves exposed to ^{14}C -CHPY in the presence of most DOM forms. These results are consistent with much faster ^{14}C -CHPY uptake rates estimated using a simple two parameter model. After the elimination period, bivalves exposed to DOM-free seawater had ^{14}C -CHPY body residue concentrations between 25% and 86% greater than

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bivalves in the presence of DOM forms. Experiments with larger bivalves showed that pulse-chase exposures with a 1.5 h exposure period to ^{14}C -CHPY was not long enough to detect differences in ^{14}C -CHPY tissue accumulation efficiencies across treatments. Our findings suggest that natural forms of DOM, at environmentally realistic organic carbon concentrations, reduced pesticide uptake and bioconcentration, consistent with much lower uptake rates relative to bivalves exposed to ^{14}C -CHPY in the absence of DOM. Interestingly, at the tested organic carbon concentrations CD- α and CD- β did not reduce ^{14}C -CHPY bioconcentration in *M. mercenaria*.

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1. Introduction

Dissolved organic matter (DOM) represents a large and chemically diverse organic carbon pool in coastal marine and estuarine environments (Santschi, Lenhart, & Honeyman, 1997). DOM consists of a variety of macromolecules that may provide a hydrophobic microenvironment that facilitates the binding of highly hydrophobic organic contaminants. DOM properties such as organic carbon content, abundance of hydrophobic and polar macromolecules, organic matter composition and molecular size have been recognized to be essential in the sorption of hydrophobic compounds (Stangroom, Lester, & Collins, 2000). Furthermore, due to its complexation capacity (Haitzer, Höss, Traunspurger, & Steinberg, 1998; Schwarzenbach, Gschwend, & Imboden, 1993) DOM may influence the fate and transport of many contaminants potentially reducing their bioavailability and toxicity to marine and estuarine fauna. In fact, studies have shown that DOM reduces the bioavailability to aquatic organisms of various metals (Guo, Santschi, & Ray, 2002; Kim, Ma, Allen, & Cha, 1999) and hydrophobic contaminants (Akkanen & Kukkonen, 2003; Haitzer, Burnison, Höss, Traunspurger, & Steinberg, 1999). Metals (Co, Hg, Ag, Fe and Zn) complexed to high molecular weight colloids (HMW; 1 kDa–0.2 μm and DOC concentration of 208 μM) were less bioavailable to the American oyster (*Crassostrea virginica*) compared to metals complexed to a low molecular weight permeate (<1 kDa and DOC concentration of 102 μM ; Guo et al., 2002). The presence of HMW colloids reduced metal uptake rate constant resulting in low soft tissue–metal concentrations. Similarly, the presence of DOM from different sources (Akkanen, Penttinen, Haitzer, & Kukkonen, 2001; Kukkonen & Oikari, 1991) and organic carbon contents (Akkanen & Kukkonen, 2003; Haitzer et al., 1999) greatly influenced the bioavailability of polycyclic aromatic hydrocarbons (PAHs) to aquatic invertebrates. For instance, Akkanen et al. (2001) found a reduction in bioavailability and bioconcentration of benzo[a]pyrene (B[a]P) to *Daphnia magna* related

to increasing DOM quantity (i.e., 0–20 mg/L) and quality (i.e., aromaticity and hydrophobicity).

A significant fraction of DOM is composed of humic substances produced during the decay of biomatter (i.e., lignin and polysaccharides). Humic substances are operationally subdivided into humic and fulvic acids, and contain functional groups (i.e., carboxyl and phenolic –OH groups) forming a non-polar microenvironment that binds hydrophobic compounds (Schwarzenbach et al., 1993). Humic acids are generally water soluble only at high pH, while fulvic acids are water soluble under both acidic and basic conditions (Schwarzenbach et al., 1993). These two forms of humic substances differ in their molecular weights, composition and abundance of functional groups, and extent of polymerization (Stevenson, 1982). Compared to fulvic acids, humic acids are of relatively larger size (MW < 5000 Da) and lower water solubility resulting from their high carbon and low oxygen contents (Averett, Leenheer, McKnight, & Thorn, 1989). Consequently, specific chemical properties such as molecular size and functional groups in DOM forms of similar nature (i.e., humic vs. fulvic acids) may greatly influence pesticide binding potentially influencing their bioavailability.

Other natural substances with high pesticide inclusion abilities (i.e., cyclodextrins, CDs; Szejtli, 1998) could also be used as a surrogate for hydrophobic DOM constituents. CDs are water soluble cyclic oligosaccharides of six to eight glucose units with α -(1,4)-linked D-(+)-glucopyranose units (Szejtli, 1998). The molecular arrangement of CDs facilitates the formation of a conical cylinder with hydrophilic exterior and a hydrophobic inner cavity that allows the inclusion of hydrophobic compounds such as pesticides (Ishiwata & Kamiya, 1999; Szejtli, 1998). Such hydrophobic inner cavity results from the presence of opposing carbon–hydrogen bonds, hydrogen-bonded hydroxyls and oxygen molecules connecting adjacent glucose units. Therefore, CDs could also be used to assess the role of DOM-like molecules on pesticide uptake and bioconcentration.

In previous work with *Mercenaria mercenaria* (Bejarano, Widenfalk, Decho, & Chandler, 2003) we suggested that specific forms of DOM having particular molecular properties may differentially influence pesticide uptake, elimination and soft tissue assimilation patterns. Consequently, the purpose of this research was to further evaluate the role of various DOM forms (i.e., natural DOM, humic and fulvic acids, and CDs) on the uptake, bioconcentration and elimination of the highly hydrophobic insecticide chlorpyrifos (hereafter, CHPY) to the common suspension-feeding bivalve *M. mercenaria*. This insecticide has been detected at low concentrations in coastal waters (<3 nmol/L; Pait, DeSouza, & Farrow, 1992), and although is highly toxic to crustaceans it poses a low toxicity to mollusk (USEPA, 1986, 2000). Studies, however have shown that under sublethal concentrations (3–57 μ mol/L) CHPY readily bioaccumulates in mollusk tissues (USEPA, 1986; Serrano, Hernandez, Lopez, & Pena, 1997). We hypothesized that various DOM forms at environmentally realistic concentrations reduce CHPY uptake and bioconcentration in *M. mercenaria*. Understanding the processes influencing bioconcentration will further elucidate the fate and transport of organic contaminants in the environment.

2. Methods

2.1. Chlorpyrifos

Chlorpyrifos (*O,O*-diethyl-*O*-[3,5,6-trichloro-2-pyridyl]-phosphorothioate) is an organophosphorus insecticide that prevents the catalysis of acetylcholine at nerve synapses, by inhibiting the enzyme acetylcholinesterase (AChE). This pesticide, currently being eliminated for household purposes (USEPA, 2000), is known to pose acute and chronic risks to many non-target aquatic wildlife (Odenkirchen & Eisler, 1988), including estuarine species. This pesticides has a high organic carbon partitioning coefficient ($\text{Log } K_{oc} = 3.4\text{--}4.2$), thus is ideal for assessing pesticide uptake, elimination and bioconcentration from various DOM forms.

Uniformly radiolabeled ^{14}C -CHPY [pyridine-2,6- ^{14}C] (99% purity; specific activity = 31.4 mCi/mmol) was purchased from American Radiolabeled Chemicals (ARC, St. Louis, MO, USA). The original stock solution was diluted (8.3 $\mu\text{Ci/ml}$, 265 μM) and stored in ultra-pure analyzed acetone (>99.4% purity; Sigma–Aldrich, St. Louis, MO, USA) at $-20\text{ }^{\circ}\text{C}$ until use.

2.2. Target species

Bivalves (*Mercenaria mercenaria* Linnaeus) were obtained from an aquaculture center (The Atlantic Farms, Inc) in Charleston, SC, USA. *M. mercenaria* were maintained in 800-mL weekly-replaced and aerated artificial seawater (30‰) at $20\text{ }^{\circ}\text{C}$, and fed to excess a 1:1 phytoplankton mixture of *Isochrysis galbana* and *Dunaliella tertiolecta* cultured in nutrient-enriched seawater (Gillard, 1972). Bivalves of different size-classes (Length = 1.71 ± 0.17 mm, Weight = 1.16 ± 0.27 mg/whole bivalve and $L = 7.44 \pm 0.48$ mm, $W = 108.3 \pm 23.32$ mg/whole bivalve) and cultured separately were randomly selected from each culture, cleaned and transferred to food-free seawater for two days prior to bioavailability experiments.

2.3. Dissolved organic matter preparation and radiolabeling

Several ^{14}C -CHPY spiked forms of dissolved organic matter (DOM) were used to assess their role on pesticide bioavailability to *M. mercenaria*. Natural DOM concentrations in this study were selected to mimic realistic environmental concentrations. Salt-marsh sediment derived DOM was prepared by mixing clean North Inlet, SC, washed and processed estuarine sediments with artificial seawater (30‰). Slurry was placed in refrigerator ($4\text{ }^{\circ}\text{C}$) for 2 h and overlaying water decanted without disturbing the sediments. Sediments were centrifuged for 5 min at 4700 RPM, the supernatant collected into a clean beaker, filtered through a $0.45\text{ }\mu\text{m}$ membrane and aerated overnight. Prior to use, salt-marsh sediment filtrate was passed through a $0.45\text{ }\mu\text{m}$ membrane and a 200-mL aliquot further filtered through a Centriprep[®] Centrifugal Filter Device equipped with a 3 kDa Nominal Molecular Weight Limit (NMWL)-cut off membrane (Centriprep YM-3, AMICON Bioseparations, Millipore, Billerica, MA, USA). This arbitrary molecular weight cut off was chosen to

exclude small organic matter particles and to ensure the inclusion of relatively large DOM constituents. The $<0.45 \mu\text{m}$ fraction of filtrate contains DOM and large colloids (hereafter referred as DOM- $(<0.45 \mu\text{m})$), while the $<3 \text{ kDa}$ MW-filtrate contains the truly DOM fraction (hereafter referred as DOM- $(<3 \text{ kDa})$). Water aliquots were analyzed in triplicates for total organic carbon (TOC) concentration by combustion method using a TOC analyzer (TOC-5000A; Shimadzu Scientific Instruments, Inc.; Norcross, GA, USA). Measured TOC concentration in these two salt-marsh sediment DOM fractions was $11.57 \pm 0.18 \text{ mg organic carbon/L}$ and $9.08 \pm 0.09 \text{ mg organic carbon/L}$, respectively, with little variation across extractions ($<5\%$).

Humic (HA) and fulvic (FA) acids from the Suwannee River, GA, USA (International Humic Substances Society, IHSS) were used without further processing. These HA and FA have distinct molecular weights (~ 1100 and $\sim 800 \text{ Da}$, respectively) and aromatic carbon abundance (42% and 28%, respectively), resulting in a higher hydrophobicity of HA over FA (Averett et al., 1989). Small amounts of HA and FA were dissolved in filtered ($0.45 \mu\text{m}$ and 3 kDa membranes) artificial-seawater (30‰) to a concentration of $7.83 \pm 0.02 \text{ mg organic carbon/L}$ protected from light and stirred for 1h at room temperature prior to ^{14}C -CHPY radiolabeling. This concentration of organic carbon in HA and FA treatments was chosen under the assumption that humic substances comprise a significant fraction (50–80%) of the organic carbon pool in salt-marsh sediment derived DOM.

Other forms of DOM consisted of water soluble cyclic oligosaccharides alpha (α) and beta (β) cyclodextrins (CDs), referred throughout as CD- α and CD- β , respectively. The 6 glucose unit, CD- α , has a molecular weight of 972; while the 7 glucose unit, CD- β , has a molecular weight of 1135. Molar quantities of CD- α and CD- β (98% purity, Sigma, St Louise, MO, USA) were dissolved in filtered artificial-seawater (Centriprep YM-3; 30‰) and stirred for 1h at room temperature prior to pesticide addition. The concentration of CDs in solution was higher ($10^{-4} \text{ M CD-}\alpha$ and CD- β) than pesticide concentration (10^{-8} M CHPY) to ensure pesticide complexation to CDs. Organic carbon content in CD- α and CD- β solutions were $1.4 \pm 0.1 \text{ mg organic carbon/L}$ and $2.28 \pm 0.1 \text{ mg organic carbon/L}$, respectively.

Fourier-Transform Infrared (FTIR) Spectroscopy was used to confirm pesticide complexation by verifying the shift in the absorbance spectra between pesticide free-CDs and pesticide bound-CDs. Spectra were obtained by loading $10 \mu\text{l}$ of liquid over a 2 cm^{-2} KGe attenuated total reflectance (ATR) crystal (Nicolet Nexus 670 FTIR; Nicolet Instrument Corp., Madison, WI, USA). FTIR spectra were acquired at 4 cm^{-1} resolution by co-adding 64 signal-averaged scans in the $4000\text{--}675 \text{ cm}^{-1}$ region. FTIR spectral analyses were performed following Sabapathy, Bhattacharyya, Cleland, & Hussey (1998).

In all experiments, the same source of filtered (i.e., $0.45 \mu\text{m}$ and 3 kDa membranes) seawater (30‰) utilized to dissolve CDs, HA and FA, was used as a control to assess for pesticide bioavailability from DOM-free seawater. Each DOM treatment solution and control was spiked with μL amounts of a ^{14}C -CHPY stock solution ($8.3 \mu\text{Ci/ml}$ in Ultra-Resi Analyzed Acetone®, Fisher, Pittsburgh, PA, USA). Solutions were labeled for 1 h under constant stirring at room temperature before exposing the bivalves. After incubations, $200 \mu\text{L}$ aliquots per treatment (3 each) were

withdrawn and placed into 20-mL glass scintillation vials. Water aliquots received 10 mL of liquid scintillation cocktail (ScintiSafe, ECONO1 Cocktail, Fisher Scientific, Pittsburgh, PA, USA), and were analyzed for initial ^{14}C -CHPY quantities using a liquid scintillation counter (Tri-Carb 2300 TR, LSC, Packard BioScience, Meriden, CT, USA) at 12–156 keV. All samples were counted for 55 min ($\pm 5\%$ error) and corrected for detector counting efficiency.

2.4. Bioavailability experiments

2.4.1. Uptake and elimination exposures

Several experiments were conducted to assess pesticide bioavailability from DOM sources to *M. mercenaria*. To determine patterns of ^{14}C -CHPY accumulation and elimination, juvenile bivalves ($L = 1.71 \pm 0.17$ mm, $W = 1.16 \pm 0.27$ mg/whole bivalve) were placed in crystallizing dishes and randomly allocated to each of the treatments ($n = 50$ per treatment). Bivalves were continually exposed to hot-radiolabeled treatments for 48 h, and water constantly withdrawn to monitor ^{14}C -CHPY changes in the test solution. To monitor pesticide accumulation, six bivalves per treatment were collected at time intervals (2, 4, 6, 12, 24 and 48 h) during the uptake period, placed in scintillation vials and digested for 24 h in 500 μL tissue solubilizer (SOLV-ABLE™, Packard Bioscience, Meriden CT, USA). Due to the relatively small size of bivalves, soft tissue separation from shells was not possible, thus whole bivalve pesticide concentration (i.e., pmol/bivalve) are reported for these experiments. After the 48 h uptake period, surviving bivalves were transferred to clean Teflon chambers containing filtered and aerated seawater, and left in chambers for a 144 h elimination period. Six bivalves per treatment were collected at 48, 96 and 144 h of elimination, placed in scintillation vials and digested as above. To account for any passive binding to bivalve surfaces (shell and soft tissues), fresh formalin-killed bivalves ($n = 5$ per treatment) were exposed to each of the treatment solutions for the length of the uptake period and treated in identical fashion as above.

2.4.2. Pulse-chase exposures

Previous to pulse-chase exposures, an experiment with large bivalves was performed to confirm similar uptake and elimination patterns between bivalves of different size classes. In this experiment large bivalves ($L = 7.44 \pm 0.48$ mm, $W = 108.3 \pm 23.32$ mg/whole bivalve) were exposed to free-DOM seawater (DOM-free) and salt-marsh sediment DOM (DOM-(<3 kDa)) under identical conditions as previously described. Bivalves were collected after 6, 12 and 24 h of exposure to the radiolabeled treatments ($n = 7$ per treatment), and after 18 and 24 h of elimination in clean water. Fresh formalin-killed bivalves ($n = 4$ per treatment) were exposed to these two treatments for the length of the uptake period and treated in identical fashion as above. Soft tissues of sacrificed bivalves were separated from the shell, and digested for 24 h as above. Scintillation cocktail was added to all samples (i.e., shells and soft tissue) and counted for ^{14}C -CHPY.

Elimination rates and ^{14}C -CHPY soft tissue assimilation efficiencies from different DOM types, were determined by pulse chase exposures (Bejarano et al., 2003). Ten

bivalves were randomly allocated to each treatment with exposure to the radiolabeled material allowed for 1.5 h maximum. Only bivalves exhibiting an active behavior were used in the experiment. After exposure to the radiolabeled treatments, bivalves were carefully rinsed and individually transferred to Teflon chambers containing 2-mL ^{14}C -CHPY-free seawater. Bivalves were regularly transferred to clean Teflon chambers containing 2-mL of seawater. To account for ^{14}C -CHPY excretion, fecal elimination and desorption from bivalve surfaces, fecal material plus overlaying water (containing ^{14}C -CHPY desorbed from feces and tissues) were removed at time intervals (2, 4, 6, 12, 24 h) and transferred to scintillation vials. Upon assay termination, all bivalves were sacrificed, frozen ($-40\text{ }^{\circ}\text{C}$) to facilitate soft tissue separation from the shell, and digested as above. Scintillation cocktail was added to all samples (i.e., fecal pellets, shells and soft tissue) and counted for ^{14}C . To account for any passive binding to bivalve surfaces, fresh formalin-killed bivalves ($n = 3$ per treatment) were exposed to each of the solutions for the length of the pulse chase exposure period and treated in identical fashion as above. The portion of tissue assimilated ^{14}C -CHPY was calculated as $\text{Tissue AE}\% = (^{14}\text{C Tissues}/\text{Total } ^{14}\text{C ingested}) \times 100$. Where total ^{14}C ingested was estimated through a mass balance approach as follows: $\sum ^{14}\text{C Tissues} + ^{14}\text{C Excretion} + ^{14}\text{C Fecal elimination} + ^{14}\text{C Respiration}$ (Bejarano et al., 2003). Only data from living bivalves at the end of the experiment (70–100% survival) were included in the analysis.

Bivalves were not fed during the experiments to account mostly for pesticide bioconcentration from the exposure media via filtration through gills, although background DOM/colloids that were present may have been ingested. All data were corrected for passive binding by subtracting the amount of ^{14}C in formalin killed bivalves from total ^{14}C values. Similarly, data were corrected for respiration loss of ^{14}C -CHPY, estimated to range between 4% and 6% (A.C. Bejarano, unpublished).

2.5. Statistical Analyses

All variables were tested for normality using the Kolmogorov–Smirnov Test for Goodness of Fit and transformed accordingly (Sokal & Rohlf, 1981). Differences in ^{14}C -CHPY across treatments were determined by one-way analysis of variance (ANOVA; PROC GLM, SAS Institute Inc., Cary, NC, USA) using the Bonferroni adjustment for all possible a posteriori multiple comparisons. All tests for significance were performed using an alpha level of 0.05 ($\alpha = 0.05$).

Several nonlinear models were evaluated to describe simultaneous uptake and elimination of ^{14}C -CHPY in *M. mercenaria* (Bahner & Oglesby, 1979; Newman, 1995; Wen, Kalf, & Peters, 1999). However, none of these models provided a good fit to our data. We employed a simple two-parameter model described by ^{14}C -CHPY_(clam) = $K_u * t * e^{(-K_e * t)}$ where K_u and K_e are estimates of ^{14}C -CHPY uptake and elimination, respectively. A nonlinear regression model using the Marquardt–Levenberg iterative fitting method (NLIN; PROC NLIN, SAS) was used to determine parameter estimates of K_u and K_e for each of the treatments per experiment. The Runs test was performed to determine deviation of the data from the proposed

model (GraphPad Prism 4.00, GraphPad Software, San Diego California, USA; $\alpha = 0.01$). Simultaneously, an F test for equality of curves ($\alpha = 0.05$) was performed to test for differences between treatment curves within experiments. This test compares the nonlinear fitting model of each treatment per experiment to a pooled-single model of all treatments (GraphPad Prism 4.00). Differences in uptake and elimination parameter estimates (K_u and K_e) across treatments were determined by Bonferroni test for simultaneous comparisons ($\alpha = 0.05$).

Elimination rates (K_e) from pulse-chase exposures were obtained with a simple one-phase exponential decay model described by $^{14}\text{C-CHPY}_{(\text{bivalve})} = \text{Total } ^{14}\text{C-CHPY Uptake} + a * e^{(-K_e * t)}$. Differences in rates across treatments within experiments were determined via Bonferroni test for simultaneous comparisons ($\alpha = 0.05$).

3. Results

3.1. Bioavailability experiments

3.1.1. Uptake and elimination exposures

Three independent experiments were conducted to assess $^{14}\text{C-CHPY}$ uptake, elimination and total residue in small bivalves following exposure to seawater (30‰) or seawater containing various (DOM) forms. Throughout the duration of each experiment, salinity and pH were constant (30 ± 1.5 and 8.02 ± 0.10 , respectively) and did not differ across treatments. All treatments within experiments were radiolabeled with similar amounts of $^{14}\text{C-CHPY}$, with constant pesticide concentration (>80% of initial measured $^{14}\text{C-CHPY}$) throughout the 48 h exposure.

In the first experiment bivalves were exposed to $^{14}\text{C-CHPY}$ in DOM-free seawater and in the presence of salt-marsh sediment DOM filtered through a $0.45 \mu\text{m}$ (DOM-(< $0.45 \mu\text{m}$)) and 3 kDa membrane (DOM-(< 3 kDa)). Mean $^{14}\text{C-CHPY}$ exposure concentration across treatments was on average $51.15 \pm 3 \text{ nmol/L}$. Total $^{14}\text{C-CHPY}$ uptake in bivalves exposed to DOM-(< 3 kDa) was reduced by 68.45% ($2.36 \pm 1.36 \text{ pmol/bivalve}$) compared to $^{14}\text{C-CHPY}$ in bivalves exposed to the DOM-free seawater treatment ($7.48 \pm 1.05 \text{ pmol/bivalve}$; $p < 0.0001$; Fig. 1(a)). However, $^{14}\text{C-CHPY}$ concentration in bivalves exposed to DOM-(< $0.45 \mu\text{m}$) ($5.07 \pm 2.26 \text{ pmol/bivalve}$) was not statistically different from the concentration in DOM-free seawater and DOM-(< 3 kDa) bivalves. Furthermore, the remaining amount of $^{14}\text{C-CHPY}$ after the 144 h elimination period in whole bivalves was reduced by 72% ($0.28 \pm 0.21 \text{ pmol/bivalve}$) and 86% ($0.14 \pm 0.06 \text{ pmol/bivalve}$) in the DOM-(< $0.45 \mu\text{m}$) and DOM-(< 3 kDa) treatments respectively, compared to DOM-free seawater bivalves ($1 \pm 0.8 \text{ pmol/bivalve}$; $p < 0.05$). Total $^{14}\text{C-CHPY}$ uptake and final pesticide residue were not statistically different between DOM-(< 3 kDa) and DOM-(< $0.45 \mu\text{m}$) bivalves ($p > 0.05$).

The simple two-parameter model showed an overall good fit between the predicted and observed data points (Table 1; Fig. 2(a)). However, mean predicted total $^{14}\text{C-CHPY}$ uptake after the 48 h uptake period was slightly lower than measured concentrations. Based on this model estimated $^{14}\text{C-CHPY}$ uptake rate (K_u) in bival-

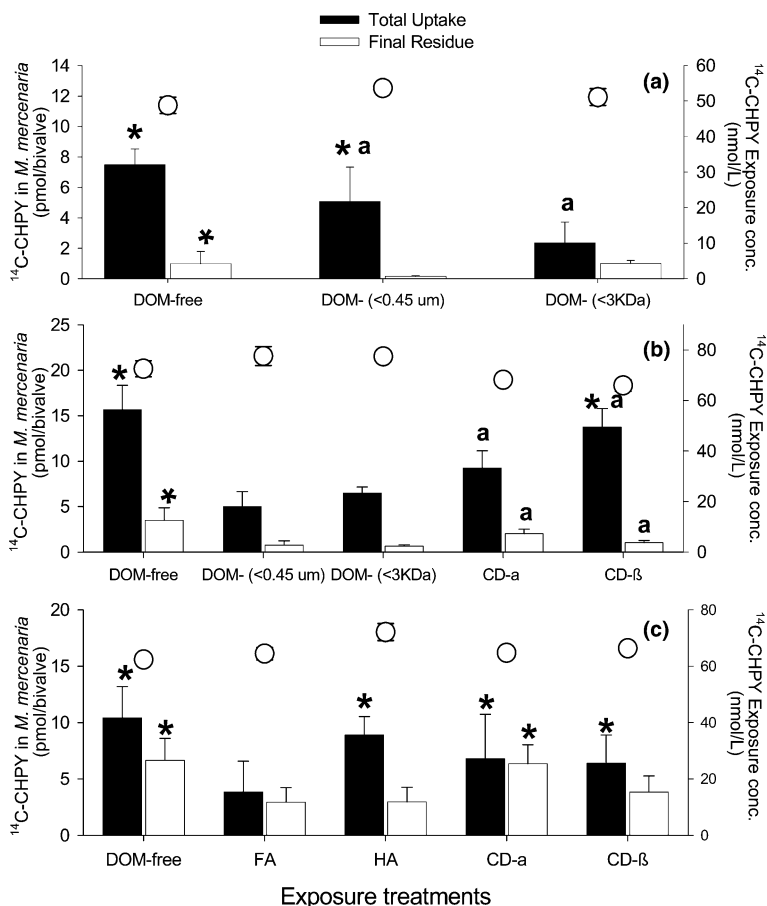


Fig. 1. Total chlorpyrifos (^{14}C -CHPY) uptake and final residues (mean \pm standard deviation) in three different experiments (a) to (c) exposing juvenile *Mercenaria mercenaria* (left axis; bars) to constant ^{14}C -CHPY concentrations (right axis; symbols). Symbols represent significant difference ($\alpha = 0.05$) within bars sharing colors. Treatments correspond to filtered seawater (DOM-free), two salt-marsh sediment DOM filtrate fractions: DOM-(<0.45 μm) and DOM-(<3 kDa), fulvic and humic acids (FA and HA); and cyclodextrins (CD- α and CD- β).

ves exposed to DOM-free seawater was nearly 60% faster than bivalves exposed to DOM-(<0.45 μm) and DOM-(<3 kDa), resulting on a much higher total pesticide uptake. In contrast, elimination rates (K_e) were similar across treatments, and predicted body residues similar to measured ^{14}C -CHPY final residue concentrations.

A second experiment with juvenile bivalves included DOM-free seawater as well as the DOM treatments containing DOM-(<3 kDa) and DOM-(<0.45 μm), and cyclodextrins (CDs) α and β . FTIR analysis (Fig. 3) shows the presence of the host (CDs) and guest (CHPY) molecules alone and in an inclusion complex. The broad hydroxyl band (O-H stretch) of CD alone at ca. 3343 cm^{-1} is shifted to a lower frequency at ca. 3315 cm^{-1} in the inclusion of CHPY. Also, changes in the peak at ca.

Table 1
Estimated chlorpyrifos (^{14}C -CHPY) rates and predicted concentration in whole juvenile *Mercenaria mercenaria*

^{14}C -CHPY	DOM-free	DOM-($<0.45 \mu\text{m}$)	DOM-($<3 \text{ kDa}$)	CD- α	CD- β	FA	HA
Runs test (p -val)							
EXP1	0.042	0.050	0.875	NA	NA	NA	NA
EXP2	0.542	0.050	0.577	0.014	0.011	NA	NA
EXP3	0.051	NA	NA	0.177	0.460	0.934	0.065
Uptake rate estimate (K_u) (/h)							
EXP1	0.36 ± 0.04^a	0.15 ± 0.04^b	0.13 ± 0.02^b	NA	NA	NA	NA
EXP2	1.27 ± 0.18^a	0.36 ± 0.06^b	0.47 ± 0.06^b	0.66 ± 0.08^c	0.76 ± 0.14^c	NA	NA
EXP3	0.92 ± 0.08^a	NA	NA	0.40 ± 0.06^b	0.45 ± 0.06^b	0.20 ± 0.03^c	0.45 ± 0.05^d
Elimination rate estimate (K_e)(/h)							
EXP1	0.021 ± 0.001^a	0.017 ± 0.003^a	0.022 ± 0.003^a	NA	NA	NA	NA
EXP2	0.036 ± 0.003^a	0.036 ± 0.003^a	0.040 ± 0.004^a	0.033 ± 0.002^a	0.035 ± 0.004^a	NA	NA
EXP3	0.030 ± 0.002^a	NA	NA	0.020 ± 0.002^b	$0.028 \pm 0.003^{a,b}$	0.020 ± 0.002^c	$0.028 \pm 0.002^{a,c}$
Maximum uptake (pmol/bivalve)							
EXP1	6.42 ± 0.76^a	3.33 ± 1.01^b	2.12 ± 0.46^b	NA	NA	NA	NA
EXP2	13.06 ± 1.36^a	3.69 ± 1.38^b	4.26 ± 1.37^b	7.15 ± 1.61^c	7.84 ± 1.38^c	NA	NA
EXP3	11.47 ± 0.97^a	NA	NA	7.09 ± 1.03^b	6.07 ± 0.96^b	3.62 ± 0.54^c	6.04 ± 0.83^d
Whole clam-body residue (pmol/bivalve)							
EXP1	1.33 ± 0.48^a	1.13 ± 0.84^a	0.33 ± 0.24^a	NA	NA	NA	NA
EXP2	3.95 ± 1.01^a	1.08 ± 0.97^a	0.95 ± 0.96^a	2.45 ± 1.02^a	2.43 ± 0.98^a	NA	NA
EXP3	3.89 ± 0.93^a	NA	NA	5.33 ± 1.24^a	3.11 ± 0.98^a	3.1 ± 0.80^a	2.74 ± 0.65^a

Values represent mean estimates (\pm standard deviation). Within experiments, treatments sharing same letter are not statistically significant from each other ($\alpha = 0.05$). The Runs test for model fitness was performed at $\alpha = 0.01$. Treatments correspond to filtered seawater (DOM-free), two salt-marsh sediment DOM filtrate fractions: DOM-($<0.45 \mu\text{m}$) and DOM-($<3 \text{ kDa}$), fulvic and humic acids (FA and HA); and cyclodextrins (CD- α and CD- β).

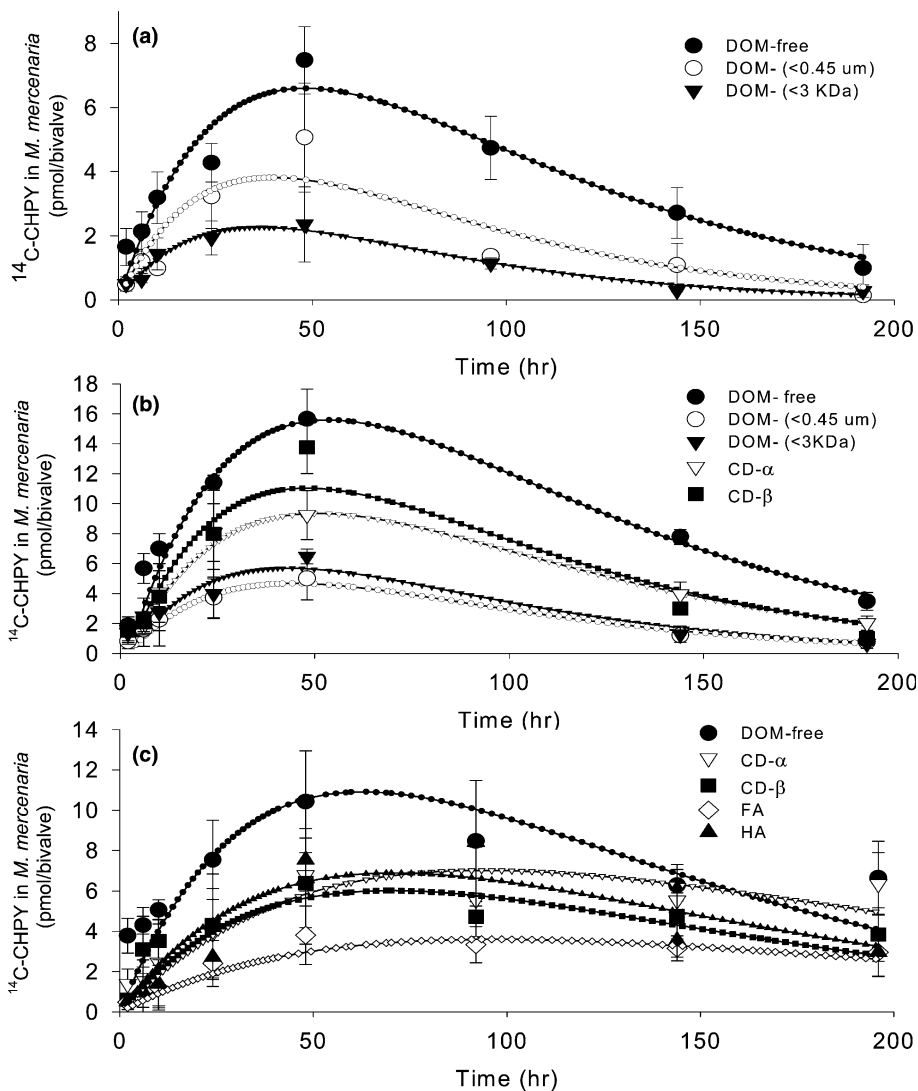


Fig. 2. Measured (mean \pm standard deviation; symbols) and estimated (small symbols and lines) total chlorpyrifos (^{14}C -CHPY) concentration in three different experiments (a) to (c) assessing uptake and elimination with juvenile *Mercenaria mercenaria*. Treatments correspond to filtered seawater (DOM-free), two salt-marsh sediment DOM filtrate fractions: DOM-(<0.45 μm) and DOM-(<3 kDa), fulvic and humic acids (FA and HA); and cyclodextrins (CD- α and CD- β).

1645 cm^{-1} in the CD spectra shifted to ca. 1624 cm^{-1} in the inclusion complex likely due to vibration modification in the carbonyl groups. In this experiment the mean initial ^{14}C -CHPY exposure concentration across treatments was on average $74.3 \pm 7\text{ nmol/L}$. Due to differences in total organic carbon content between salt-marsh sediment derived DOM (DOM-(<0.45 μm) and DOM-(<3 kDa)) and

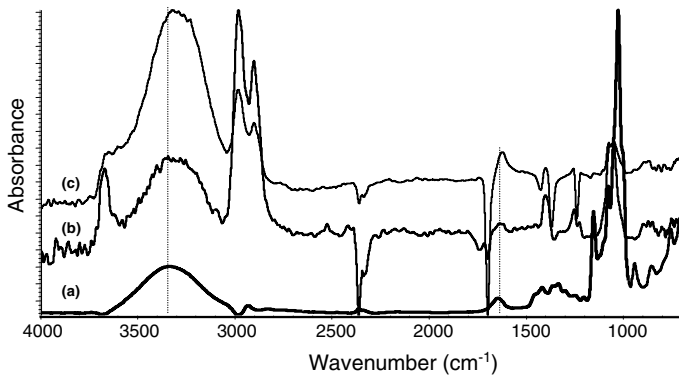


Fig. 3. ATR-FTIR absorbance spectra of β -CD alone (a), CHPY alone (b), and β -CD/CHPY inclusion complex (c) dispersed in a KGe crystal.

cyclodextrins (CD- α and CD- β), these two groups were compared only against DOM-free seawater. Total ^{14}C -CHPY bivalve uptake over the 48 h of exposure was reduced by 68% (5 ± 1.65 pmol/bivalve) and 59% (6.48 ± 0.69 pmol/bivalve) in the DOM-(<0.45 μm) and DOM-(<3 kDa) treatments, respectively, compared to DOM-free seawater bivalves (15.66 ± 2.69 pmol/bivalve; $p < 0.05$; Fig. 1(b)). Similarly, ^{14}C -CHPY bivalve uptake was reduced by 41% (9.25 ± 1.89 pmol/bivalve) in the CD- α treatment relative to DOM-free bivalves ($p = 0.023$). ^{14}C -CHPY uptake in the CD- β was not statistically different (13.76 ± 2.02 pmol/bivalve; $p > 0.05$) from DOM-free bivalves. However, total ^{14}C -CHPY uptake was not statistically different between DOM-(<0.45 μm) and DOM-(<3 kDa), nor between CD- α and CD- β ($p > 0.05$). Final residues after the 144 h elimination period followed similar patterns as total uptake. Final residues were reduced by 78% (0.77 ± 0.47), 81% (0.65 ± 0.13), 25% (2.02 ± 0.5) and 70% (1.04 ± 0.23) in the DOM-(<0.45 μm), DOM-(<3 kDa), CD- α and CD- β respectively, compared to DOM-free seawater bivalves (3.47 ± 1.41 pmol/bivalve; $p < 0.05$).

The simple two-parameter model showed an overall good fit between predicted and observed data except for the treatment containing CDs (Table 1; Fig. 2(b)). Mean predicted total ^{14}C -CHPY uptake after the 48 h exposure period was under-predicted for all treatments relative to measured concentrations. Based on our model, estimated uptake rates (K_{in}) in bivalves exposed to DOM-free seawater were between 60% and 70% faster than those of bivalves exposed to DOM-(<0.45 μm) and DOM-(<3 kDa), and between 40% and 50% faster than those of bivalves exposed to CD- α and CD- β . However, elimination rates (K_{e}) were similar across treatments. Predicted body residues were slightly higher but similar to measured ^{14}C -CHPY concentrations and not statistically different across treatments. Nevertheless, bivalves exposed to the DOM-(<0.45 μm) and DOM-(<3 kDa) treatments were predicted to accumulate less ^{14}C -CHPY than DOM-free seawater bivalves.

In the last experiment with juvenile bivalves, individuals were exposed to DOM-free seawater and to solutions containing CD- α , CD- β , and natural fulvic (FA) and

humic acids (HA). In this experiment the mean ^{14}C -CHPY exposure concentration across treatments was on average 65.63 ± 3.24 nmol/L. Due to differences in total organic carbon content between cyclodextrins (CD- α and CD- β) and humic substances (FA and HA), these two groups were compared only against DOM-free seawater. Total ^{14}C -CHPY uptake was reduced by 63% in bivalves exposed to FA (3.86 ± 2.73 pmol/bivalve) relative to DOM-free seawater bivalves (10.43 ± 2.76 pmol/bivalve, Fig. 1(c)). In contrast with the previous experiment, bivalves in DOM-free seawater accumulated a similar amount of pesticide (10.43 ± 2.76 pmol/bivalve) as bivalves in the CD- α treatment (6.79 ± 3.95 pmol). Also, final residues were reduced by 56% (2.95 ± 1.28), 55% (2.96 ± 1.29) and 42% (3.84 ± 1.42) in the FA, HA and CD- β relative to the DOM-free seawater treatment (6.65 ± 1.94 pmol/bivalve; $p < 0.05$). Even though bivalves in the DOM-free, CD- α and CD- β treatments were exposed to similar ^{14}C -CHPY concentrations as bivalves from the previous experiment, bivalves from this experiment eliminated less pesticide over the same amount of time.

The simple two-parameter model showed an overall good fit between predicted and observed data (Table 1; Fig. 2(c)). Mean predicted total ^{14}C -CHPY uptake after the 48 h uptake period was similar to measured concentrations. Based on our model, estimated ^{14}C -CHPY uptake rates (K_u) in bivalves exposed to DOM-free were between 50% and 80% faster than those of bivalves exposed to FA and HA, and 50–60% faster than bivalves exposed to CD- α and CD- β . Elimination rates (K_e), on the other hand, were slightly faster in bivalves exposed to DOM-free compared to bivalves in the CD- α and FA treatments. Although predicted body residues in DOM-free seawater bivalves were much lower than measured concentrations, the model indicates a similar ^{14}C -CHPY residue concentration across treatments.

3.1.2. Pulse-chase exposures

The ^{14}C -CHPY exposure concentration in the experiment with larger bivalves was slightly higher in the DOM-(<3 kDa) (93.36 ± 5.56 nmol/L) than in DOM-free seawater (82.25 ± 2.08 nmol/L). ^{14}C -CHPY accumulation in bivalve soft tissues exposed to DOM-(<3 kDa) (44.82 ± 7.01 pmol/bivalve) was 28% that of bivalves exposed to DOM-free seawater (158.31 ± 45.11 pmol/bivalve; Fig. 4). These results are consistent with a much faster ^{14}C -CHPY uptake rate (0.154/h vs. 0.124/h) of ^{14}C -CHPY in bivalves exposed to DOM-free seawater. Similarly, after the elimination period final ^{14}C -CHPY soft tissue residue in bivalves exposed to DOM-(<3 kDa) was only 14% (10.12 ± 9.26 pmol/bivalve) of the residue in DOM-free bivalves (70.83 ± 10.61 pmol/bivalve). These patterns were consistent with those observed in juvenile bivalves suggesting that the mechanisms of pesticide uptake and elimination are independent of bivalve size.

In pulse chase exposures with large *M. mercenaria* exposure ^{14}C -CHPY concentration was on average 140.96 ± 4.33 nmol/L in the first experiment (i.e., DOM-free, CD- α and CD- β treatments) and 65.66 ± 3.24 nmol/L in the second experiment (i.e., DOM-free, DOM-(<0.45 μm), DOM-(<3 kDa), CD- α , CD- β , FA and HA treatments). In these two pulse chase exposures total ^{14}C -CHPY

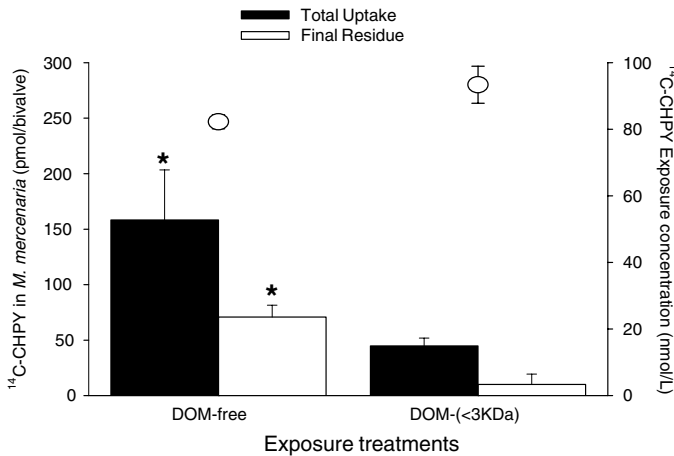


Fig. 4. Total chlorpyrifos (^{14}C -CHPY) uptake and final residues (mean \pm standard deviation) in exposures with large *Mercenaria mercenaria* (left axis; bars) to constant ^{14}C -CHPY concentrations (right axis; symbols). Symbols (*) represent significant difference ($\alpha = 0.05$) within bars sharing colors. Treatments correspond to filtered seawater (DOM-free) and a salt-marsh sediment DOM filtrate (DOM-(<3 kDa)).

uptake was on average 21.47 ± 3.48 pmol/bivalve across all treatments in the first experiment and 13.28 ± 2.94 pmol/bivalve across all treatments in the second experiment. The majority of total ^{14}C -CHPY uptake (>80%) was lost over the 24 h elimination period as shown by low ^{14}C -CHPY amounts in bivalve tissues (Table 2). In neither of these experiments, was ^{14}C -CHPY tissue assimilation efficiency of bivalves exposed to DOM sources significantly different from that of bivalves exposed to DOM-free seawater ($p > 0.05$). Tissue assimilation (%) ranged from 1% to 7% in the first pulse-chase exposure and from 7% to 20% in the second pulse-chase exposure. Likewise estimated elimination ^{14}C -CHPY rates were not statistically different across treatments ($p > 0.05$). Overall elimination rates for experiments 1 and 2 with large bivalves ($L = 7.44 \pm 0.48$ mm, $W = 108.3 \pm 23.32$ mg/whole bivalve) were $0.701 \pm 0.01/\text{h}$ and $0.35 \pm 0.03/\text{h}$, respectively. Taking into account the amount of ^{14}C -CHPY in the exposure media, all large bivalves across treatments eliminated ^{14}C -CHPY at a very similar rate.

Based on the results from the 48-h constant exposure, bivalves exposed to ^{14}C -CHPY in DOM-(<3 kDa) accumulated over 50% less ^{14}C -CHPY than bivalves exposed to DOM-free. In the pulse-chase exposure, however, ^{14}C -CHPY tissue accumulation in large bivalves was similar across treatments. These findings indicate that the short length of exposure (1.5 h as opposed to 24 h) was not long enough to allow for the previously observed ^{14}C -CHPY uptake differences across treatments. Hence, pulse-chase exposures using DOM may underestimate uptake because pesticide uptake via filtration across gills might be a slow process compared to digestive processes. Consequently, we recommend longer exposures (>1.5 h) in bioconcentration studies dealing with DOM.

Table 2
Chlorpyrifos (^{14}C -CHPY) concentration in *Mercenaria mercenaria* following exposure to dissolved/colloidal sources

^{14}C -CHPY	DOM-free	DOM-($<0.45\ \mu\text{m}$)	DOM-($<3\ \text{kDa}$)	CD- α	CD- β	FA	HA
Exposure media (nmol/L)							
EXP1	147.07 \pm 0.63	NA	NA	137.37 \pm 0.43	138.47 \pm 0.00	NA	NA
EXP2	62.31 \pm 0.68	65.78 \pm 0.07	63.95 \pm 0.52	64.72 \pm 0.43	66.29 \pm 0.74	64.46 \pm 2.22	72.08 \pm 3.00
Total uptake (pmol/bivalve) ^a							
EXP1	23.79 \pm 2.19	NA	NA	20.22 \pm 4.34	19.99 \pm 1.0	NA	NA
EXP2	14.03 \pm 1.01	14.26 \pm 2.27	12.95 \pm 1.79	12.21 \pm 1.69	13.54 \pm 3.09	14.47 \pm 5.25	10.80 \pm 1.59
Tissue (pmol/bivalve)							
EXP1	1.07 \pm 0.79 (4.26 \pm 2.86)	NA	NA	0.2 \pm 0.19 (0.95 \pm 0.79)	1.26 \pm 0.93 (7.24 \pm 4.19)	NA	NA
EXP2	1.53 \pm 0.78 (10.65 \pm 5.08)	0.94 \pm 0.68 (6.83 \pm 4.98)	1.72 \pm 0.94 (13.40 \pm 7.17)	0.90 \pm 0.51 (7.45 \pm 4.42)	2.55 \pm 1.26 (19.02 \pm 8.68)	2.62 \pm 2.41 (15.09 \pm 9.29)	1.46 \pm 0.90 (12.82 \pm 5.81)
Shell (pmol/bivalve)							
EXP1	0.61 \pm 0.25	NA	NA	0.4 \pm 0.17	0.76 \pm 0.29	NA	NA
EXP2	1.047 \pm 0.17	0.843 \pm 0.23	1.022 \pm 0.27	0.81 \pm 0.27	1.06 \pm 0.57	1.25 \pm 0.6	0.63 \pm 0.29
Elimination (pmol/bivalve)							
EXP1	22.11 \pm 1.38	NA	NA	19.62 \pm 4.17	18.38 \pm 1.51	NA	NA
EXP2	11.46 \pm 0.60	12.48 \pm 2.54	10.21 \pm 2.09	10.5 \pm 1.70	9.93 \pm 2.53	10.61 \pm 2.77	8.71 \pm 0.86
Estimated elimination rate (/h)							
EXP1	0.714 \pm 0.068	NA	NA	0.6958 \pm 0.140	0.694 \pm 0.08	NA	NA
EXP2	0.335 \pm 0.106	0.328 \pm 0.112	0.371 \pm 0.118	0.349 \pm 0.132	0.370 \pm 0.312	0.338 \pm 0.429	0.406 \pm 0.187

Values represent mean ^{14}C -CHPY concentration (\pm standard deviation). Parenthesis represents the mean ^{14}C -CHPY tissue assimilation (AE%) ($n = 4-7$). Treatments correspond to filtered seawater (DOM-free), two salt-marsh sediment DOM filtrate fractions: DOM-($<0.45\ \mu\text{m}$) and DOM-($<3\ \text{kDa}$), fulvic and humic acids (FA and HA); and cyclodextrins (CD- α and CD- β).

^a Total uptake was corrected for lost by respiration, and passive binding of ^{14}C -CHPY to bivalve surfaces.

4. Discussion

Dissolved organic matter (DOM) contains a variety of ligands (Stangroom et al., 2000) that facilitate binding of organic contaminants, thus potentially reducing their bioavailability to exposed organisms. Since contaminant absorption across body surfaces is one of the major routes of contaminant uptake in bivalves, it is reasonable to assume that the presence of various DOM forms with varying chemical structures, compositions, and even steric conformations may influence contaminant uptake. In fact, studies have shown that the origin, quantity, quality and composition of DOM influence the uptake and bioconcentration of hydrophobic contaminants (Gourley, Tusseau-Vuillemin, Garric, & Mouchel, 2003; Haitzer et al., 1999).

In estuarine waters DOM comprises a significant organic carbon pool with concentrations ranging from 1.2 to 2.6 mg organic carbon/L (Dafner & Wangersky, 2002; Guo & Santschi, 1997). Also, pore water DOM concentrations in marine and estuarine sediments are one order of magnitude greater than those from the overlying water (Dafner & Wangersky, 2002). In this study, the presence of different forms of natural (salt-marsh sediment derived, HA and FA; CD- α and CD- β) DOM, at concentrations ranging from a low 1.4 ± 0.1 mg organic carbon/L in DOM-like forms (cyclodextrins α) to a high of 11.57 ± 0.18 mg organic carbon/L in estuarine-pore water (DOM-($<0.45 \mu\text{m}$)), in most cases reduced the bioconcentration of ^{14}C -CHPY to the juvenile bivalve *M. mercenaria*, relative to DOM-free seawater. Generally, bivalves in DOM-free seawater accumulated up to 81% more pesticide (i.e. final pesticide residue) than bivalves in the presence of different DOM types. These higher ^{14}C -CHPY in tissues may have resulted from much faster (at least 40%) pesticide uptake rates in DOM-free seawater bivalves.

Studies have shown the importance of natural DOM in reducing organic contaminant bioconcentration to aquatic organisms (Akkanen et al., 2001; Haitzer et al., 1999). For example, nematodes (*Caenorhabditis elegans*) exposed to B[a]P in the presence of marsh and swamp-DOM showed reduced bioconcentration compared to nematodes exposed to B[a]P in the presence of pond-DOM (Haitzer et al., 1999). Despite similar nominal dissolved organic carbon (DOC) concentrations across DOM sources, reduced B[a]P bioconcentration in the presence of marsh and swamp-DOM may be attributed to greater B[a]P accessibility to DOM-hydrophobic domains (Haitzer et al., 1999). In the present study, most experiments conducted with estuarine salt-marsh sediment DOM (DOM-($<0.45 \mu\text{m}$) and DOM-(<3 kDa) fractions) at environmentally realistic organic carbon concentrations (DOM-($<0.45 \mu\text{m}$) = 11.57 ± 0.18 mg organic carbon/L and DOM-(<3 kDa) = 9.08 ± 0.09 mg organic carbon/L), resulted in lower ^{14}C -CHPY uptake and bioconcentration by bivalves than those exposed to DOM-free seawater. The similar effect of both of these DOM filtrates on pesticide uptake and bioconcentration in *M. mercenaria* suggests that the most sorptive moieties on this DOM fraction are likely contained in the truly dissolved DOM and lower molecular weight (<3 kDa) colloids, rather than larger molecular weight colloids (>3 kDa). Consequently, a future

characterization of the truly dissolved salt-marsh sediment DOM (i.e., elemental composition, polarity and hydrophobicity of constituents), will elucidate the myriad of binding macromolecules and other chemical properties in natural DOM responsible for reducing pesticide bioavailability.

Humic substances represent a significant portion of the DOM pool accounting for 50–80% and 5–15% of the dissolved organic carbon in riverine and marine waters, respectively (Malcolm, 1985; Mayer, 1985). Natural humic substances comprise a variety of heterogeneous and polymorphic organic acids responsible for increasing organic contaminant solubility (Chiou et al., 1987), and potentially decreasing the bioavailability and toxicity of pollutants. In the present study, the presence of FA at a concentration of 7.83 ± 0.02 mg organic carbon/L reduced both uptake and bioconcentration of ^{14}C -CHPY in *M. mercenaria* by 63% and 55%, respectively, relative to bivalve bioconcentration in DOM-free seawater. In contrast, HA had only significant effects on ^{14}C -CHPY bioconcentration relative to in bivalves exposed to DOM-free seawater. Although HA used in this study had a much larger molecular weight and aromaticity (i.e., hydrophobicity) than FA (Averett et al., 1989), bivalves exposed to ^{14}C -CHPY in the presence of HA showed a much higher pesticide uptake than FA. These results are comparable with a previous study with *M. mercenaria* (Bejarano et al., 2003), where bivalves exposed to humic acid-coated silica showed a much higher ^{14}C -CHPY uptake than other dissolved/colloidal sources. Overall, lower pesticide uptake in the presence of Suwannee River FA might be due to higher pesticide complexation, thus less free-pesticide in solution and/or lower affinity of these particular FA with gill membranes resulting in reduced pesticide uptake. Regardless of differences between HA and FA on pesticide uptake, final ^{14}C -CHPY residues were lower in these two treatments than in DOM-free seawater, indicating a reduced pesticide bioconcentration in the presence of humic substances. Other studies also have recognized the importance of humic substances in the binding of highly hydrophobic compounds such as PAHs (McCarthy & Jimenez, 1985), PCBs (Chiou et al., 1987), and pesticides (i.e., DDT; Carter & Suffet, 1982). De Paolis & Kukkonen (1997) found that humic acids (HA) isolated from river and marine water and sediments showed a greater affinity for binding hydrophobic compounds (e.g., higher K_{oc} values) such as benzo[*a*]pyrene (B[*a*]P) than fulvic acids (FA) isolated from the same sources. Likewise, a study with HA and FA extracted from terrestrial and aquatic environments showed higher partitioning coefficients benzo[*a*]pyrene (Ba P) for HA ($\text{Log } K_{ow} = 4.12\text{--}4.29$) than for FA ($\text{Log } K_{ow} = 3.48\text{--}3.86$) (Alberts, Filip, & Leversee, 1989). However, it should be noted that comparisons with the present study are not practical due to inherent differences in experimental designs and humic substances sources.

In this study we attempted to use other forms of DOM (cyclodextrins, CD- α and CD- β) to determine patterns of pesticide uptake, bioconcentration and elimination in *M. mercenaria*. We found that despite the higher molar concentration of CD- α and CD- β relative to chlorpyrifos, CDs did not effectively reduce pesticide uptake or bioavailability. This may have occurred partially due to low organic carbon concentrations in cyclodextrin treatments (CD- $\alpha = 1.4 \pm 0.01$ mg organic

carbon/L and $CD-\beta = 2.28 \pm 0.01$ mg organic carbon/L). Although chlorpyrifos complexation to CDs was confirmed with FTIR analysis, the strength and stability of pesticide-CDs complexes needs to be further studied. Despite our findings, CDs are known to bind organic compounds such as pesticides, thus potentially reducing their toxicity (Oláh, Cserhádi, & Szejtli, 1988; Szejtli, 1998; Wen-Lu, Qing-Guo, & Lian-Sheng, 1999). For example, CD- β reduced the acute toxicity of low-polarity benzene compounds and pesticide intermediates on the luminescent bacteria *Photobacterium phosphoreum* (Wen-Lu et al., 1999). Regardless of our findings, these macromolecules could be potentially used in studies assessing pesticide transport across surfaces, as well as in bioavailability studies with a variety of contaminants and target organisms. Furthermore, studies using CDs coupled with FTIR analysis will provide valuable information regarding specific bond linkages between host and guest molecules.

It has been suggested that binding of hydrophobic contaminants to DOM results in the formation of complexes that are minimally transported or diffused across biological membranes such as gill cells (Black & McCarthy, 1988), resulting in reduced contaminant uptake. Consequently, DOM might not only reduce pesticide transport through animal surfaces but also facilitates contaminant desorption from membranes. This hypothesis is supported by data showing that final ^{14}C -CHPY body residues were much lower in bivalves exposed to ^{14}C -CHPY in the presence of any natural DOM treatments compared to DOM-free seawater. However, the mechanisms of pesticide desorption and elimination at the membrane level are difficult to determine empirically.

Finally, the current study used a simple two parameter model to estimate simultaneous uptake and elimination rates of ^{14}C -CHPY in *M. mercenaria*. Although the model, in most cases, under-predicted total ^{14}C -CHPY uptake, it provided a precise quantitative measures of pesticide uptake rates, where high rates resulted in elevated total ^{14}C -CHPY concentration. Despite our use of large sample sizes and selection of similar bivalve class sizes for experiments, we conclude that the high variability in measured ^{14}C -CHPY bioconcentration in bivalves through time hindered a suitable model fit for each of the DOM treatments. Regardless, modeling the uptake and elimination processes of ^{14}C -CHPY has provided useful information concerning the roles of various DOM forms on pesticide bioavailability.

5. Conclusions

The bioavailability of chlorpyrifos to *M. mercenaria* was influenced by various forms of DOM relative to the DOM-free seawater. Natural estuarine salt-marsh sediment DOM and the soluble fulvic acids reduced pesticide uptake and bioconcentration, while cyclodextrins, at the organic carbon concentration tested, did not efficiently reduce pesticide bioconcentration. Future studies should address the characterization of natural DOM and the role of chemical composition and functional groups on hydrophobic contaminant uptake, elimination and bioconcentration.

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