

A COMPARATIVE ASSESSMENT OF AZINPHOSMETHYL BIOACCUMULATION AND TOXICITY IN TWO ESTUARINE MEIOBENTHIC HARPACTICOID COPEPODS

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Abstract—Aqueous, pore-water, and whole-sediment bioassays were conducted with meiobenthic copepods with different infaunal lifestyles to assess the acute and chronic toxicity of the organophosphorous pesticide azinphosmethyl (APM) and its bioaccumulation potential in sediments. Biota sediment accumulation factors were an order of magnitude higher for the deeper burrowing *Amphiascus tenuiremis* (26.6) than the epibenthic *Microarthridion littorale* (2.2). The female *A. tenuiremis* APM median lethal concentration (LC50; 3.6 µg/L) was twice the male LC50 (1.8 µg/L), in straight seawater exposures, and nearly 20% higher than males in whole-sediment exposures (540 vs 456 ng/g dry weight). *Amphiascus tenuiremis* were 17 times more sensitive to sediment-associated APM than *M. littorale*. In pore-water-only exposures, the adult mixed-sex *A. tenuiremis* LC50 (5.0 µg/L) was nearly twice the seawater mixed-sex LC50 (2.7 µg/L). Dissolved organic carbon in pore water was five times higher (20 mg/L) than in seawater-only exposures (4 mg/L). Differences in acute toxicity within exposure media were driven by species- and sex-specific differences in lipid content. *Amphiascus tenuiremis* likely experienced greater exposure to sediment-associated toxicants via longer periods of direct contact with pore water than *M. littorale* and, therefore, exhibited correspondingly higher bioaccumulation and acute toxicity. Copepod reproduction was significantly reduced (>60%) in 14-d sediment culture exposures at sublethal APM levels, suggesting that chronic field exposure to sediment-associated APM would result in sharp declines in copepod population growth.

Keywords—Azinphosmethyl Meiobenthos Sediments Copepod Bioaccumulation

INTRODUCTION

Through 1999, the acetylcholinesterase-inhibiting pesticide azinphosmethyl (APM; 0,0-dimethyl-*s*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] phosphorodithioate; trade names Guthion® and Gusathion® [Chemagro, New York, NY, USA]) was believed to have been responsible for more aquatic incidents (i.e., fish kills) than any other pesticide in the U.S. Environmental Protection Agency's (U.S. EPA) ecological incident database, representing approximately 21% of all reported aquatic incidents [1]. During this time, more than 140 incidents were reported in the United States that were probable or highly probable to be associated with the routine agricultural use of APM [1]. Aerial spraydrift and surface runoff during rain events can transport APM to aquatic environments adjacent to agricultural fields where APM is applied [2]. The majority of reported APM incidents were associated with cotton and sugarcane farming, including some incidents associated with sugarcane farming that affected areas spanning 2 miles or more; in 10 incidents, more than 10,000 fish were killed [1]. In 1999, the U.S. EPA prohibited the use of APM on sugarcane, and its use on cotton was restricted and reduced [1]. In 2000, no major aquatic incidents were reported to the U.S. EPA for which APM was associated [1]. Although the use of APM has been dramatically reduced in the United States, the use of APM continues in other countries for lowland rice production (<http://ace.orst.edu/info/extoxnet/pips/azinpho.htm>) and on fruit crops [3]. Specific information regarding the

intensity of international use is largely unreported in the public domain.

Azinphosmethyl toxicity results from the metabolic conversion of the parent compound to its oxygen analog, which can subsequently inhibit cholinesterase enzymes [4]. Azinphosmethyl toxicity assessments have primarily focused on fish and water column invertebrates inhabiting both freshwater and marine environments. The lethal concentration for 50% of the population (LC50) for fish exposed to aqueous APM in laboratory toxicity tests are widely variable (2–1,900 µg/L) [5]. In exposures to APM in estuarine mesocosms, Atlantic silverside minnows (*Menidia menidia*) were even more sensitive, with a 96-h LC50 of 1.2 µg/L [6]. Studies have indicated that APM is generally more toxic to crustaceans than fish. The adult grass shrimp, *Palaemonetes pugio* exhibited a 96-h LC50 of 1.05 µg/L [7], whereas the LC50 for the mysid shrimp, *Mysidopsis bahia* (now *Americamysis bahia*), was as low as 0.29 µg/L [8]. Newly hatched and 18-day-old larval *P. pugio* were much more sensitive to the effects of APM, with LC50s of 0.52 and 0.38 µg/L, respectively [9]. In 24-h bioassays, LC50 values ranged from 1.4 µg/L for the freshwater amphipod *Paramelita nigroculus* [10] and 4.4 to 16.8 µg/L for the freshwater grass shrimp *Palaemonetes kadiakensis* [11]. In studies investigating the sublethal effects of APM, acetylcholinesterase (AChE) inhibition occurred in the mummichog *Fundulus heteroclitus* and juvenile red drum *Sciaenops ocellatus* at concentrations as low as 0.81 and 5.2 µg/L, respectively [5,12]. Measurements from field studies of APM in surface waters adjacent to agricultural fields ranged from 0.06 to 420 µg/L [2,7,10,13–15,16 and references therein], suggesting that

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significant mortality, inhibition, or both from AChE could occur in many fish and crustacean populations following heavy rainfall in these study systems. Azinphosmethyl surface water concentrations of more than 0.57 $\mu\text{g/L}$ were associated with significant brain AChE inhibition in mummichogs during in situ bioassays [15], and concentrations of more than 17 $\mu\text{g/L}$ were measured during several fish kills in South Carolina (USA) estuaries [2].

Although APM is transported to aquatic environments primarily in the dissolved phase [1], it adsorbs to suspended organic material upon entering the water column because of its affinity for sediment carbon (log octanol water partitioning coefficient, $K_{ow} = 2.7$) [17]. Once adsorbed to particulate matter, APM suspended sediment concentrations can well exceed those in the water column immediately after runoff [10]. Azinphosmethyl degrades rapidly in water (half-life = 77 h) [1], is moderately persistent in soils (half-life = 180 d) [1], and can persist for over three months in suspended sediments [10]. Despite the well-characterized acute toxicity of APM and the likelihood for chronic APM exposure of fauna in sediments and soils, no published data exist for the toxicity of sediment-bound or pore-water APM, and only one study exists on the bioaccumulation of APM by a nontarget organism [18]. Persistence in soils and sediments for extended periods makes APM a potential source of chronic toxicity to nontarget benthic communities, especially to those benthic fauna with life cycles approximating the persistence period of APM.

Most temperate to tropical benthic harpacticoid copepods have life cycles of three to six weeks [19]. As such, they are at risk to APM and other moderately persistent hydrophobic contaminants because of their intimate association with sediments during their entire life cycle. Harpacticoid copepods have a cosmopolitan distribution in both freshwater and marine systems and are typically the second most abundant metazoan in marine sediments, often occurring at densities of 10 to 50 individuals/cm² [20]. Benthic copepods are an important food source for a variety of estuarine species and regularly dominate the guts of soft-bottom benthic-feeding predators, particularly juvenile fishes [21]. *Amphiascus tenuiremis* (Mielke) is an ampho-Atlantic diosaccid harpacticoid found in the United States in muds near oyster reefs of salt marsh estuaries. It is sensitive to low levels of toxicants [e.g., 22] and has consequently been used in multiple toxicity assessments of contaminated sediments [e.g., 7,23]. *Microarthridion littorale* (Pope) is ubiquitous in U.S. Atlantic and Gulf coast estuaries and occurs frequently as the predominant copepod species in both relatively pristine [24] and contaminated salt marsh sediments [25]. In contaminated estuaries, harpacticoid copepods are exposed to toxicants to varying degrees depending on their sediment burrowing lifestyles, feeding behaviors, and swimming abilities. In this study, the copepods *A. tenuiremis* and *M. littorale* were subjected to acute and chronic exposures of APM in an effort to examine the acute and chronic toxicity and lipid-normalized bioaccumulation of APM and to investigate the relative importance among aqueous, sediment, and pore-water APM exposure routes to these copepod species.

MATERIALS AND METHODS

The artificial seawater used in all experiments was prepared using 18 mega-ohm deionized, carbon-filtered water and Instant Ocean[®] seasalts (Aquatium Systems, Mentor, OH, USA). Seawater was prepared to a salinity of 30 ppt for all experiments with *Amphiascus tenuiremis*. For the more euryhaline

Microarthridion littorale, a salinity of 15 ppt was used to coincide with field salinities at the time of copepod collection. Seawater was aerated and filtered (0.45 μm) prior to use. Water quality measurements (pH, dissolved oxygen, temperature, and salinity) were performed at test initiation and termination in 96-h bioassays and every 3 d in the 14-d APM chronic toxicity experiments. All measured water quality parameters were within American Society for Testing and Materials recommended guidelines [26]. Technical-grade APM obtained from Mobay Chemical (Kansas City, MO, USA) was used in all experiments. The ¹⁴C-APM had a specific activity of 140 mCi/mmol. All solvents were pesticide-grade or better.

Copepod collection

Amphiascus tenuiremis were collected 24 h prior to experiments from stock sediment cultures in a laboratory flow-through seawater system [27]. Thousands of *M. littorale* were collected and sieved from the top centimeter of intertidal sediments during low tide at North Inlet (Georgetown, SC, USA; 33°19.0'N, 79°11.6'W). North Inlet is a relatively pristine estuarine site with low ng/g total polycyclic aromatic hydrocarbon and nondetectable levels of polychlorinated biphenyls (PCBs) and pesticides. *Microarthridion littorale* were collected by retaining the 125 to 500 μm sieved fraction in natural seawater for immediate transport to the laboratory and then allowed to acclimate under aeration for 24 to 36 h prior to experimentation. Experiments were performed during the winter and spring months to coincide with high *M. littorale* field densities.

Experimental sediments

Relatively pristine sediments for APM spiking were collected from an intertidal mud flat (Bread and Butter Creek) during low tide at North Inlet. Sediments were sorted (<63 μm) and washed as described in Chandler and Green [27]. Total organic carbon of prepared sediments was 3.85%. Unmanipulated field sediments were analyzed for the presence of APM and found to be below detection limits (5 ng/g).

Whole-sediment acute toxicity tests

Amphiascus tenuiremis adult females, *A. tenuiremis* adult males, and a random mixture of *M. littorale* adult males and females were exposed separately in whole-sediment assays. Preliminary rangefinder experiments revealed strong differences in APM toxicity between the species; thus, *A. tenuiremis* and *M. littorale* were exposed to different ranges of sediment-associated APM in definitive APM toxicity tests. All *A. tenuiremis* were exposed in triplicate (50 copepods per replicate) to nominal sediment concentrations of 10, 20, 40, 60, 80, 120, 160, 240, and 480 ng APM/g sediment wet weight. *Microarthridion littorale* were exposed in triplicate (50 copepods per replicate) to nominal sediment concentrations of 100, 500, 1,000, 1,250, 2,000, 2,500, 4,000, 5,000, and 10,000 ng APM/g sediment wet weight. Azinphosmethyl analyses were not performed on sediments from the *M. littorale* assay; therefore, nominal concentrations were used in LC50 calculations for that species only. Sediment treatments were prepared by spiking appropriate volumes of APM in acetone into 125-ml glass beakers containing 50 ml of sediment slurry under vortex on a magnetic stir plate. Each sediment treatment was stirred for 24 h prior to loading into toxicity test chambers to ensure homogeneity. Toxicity test chambers were prepared by adding 10-ml aliquots of APM-contaminated sediment slurry to the

bottom of 50-ml glass crystallizing dishes containing 35 ml of artificial seawater. Uncontaminated sediment control and solvent-sediment control chambers were similarly prepared. Copepod-free chambers containing only contaminated sediments and artificial seawater were also prepared for chemical analysis to investigate organism effects on APM partitioning and degradation. Chambers were loosely covered and placed in an environmental chamber (20°C, 12:12-h light:dark cycle). *Amphiascus tenuiremis* assays were conducted static for 96 h without feeding; however, *A. tenuiremis* stock cultures were heavily fed 24 h prior to test initiation. Unlike *A. tenuiremis*, *M. littorale* preferentially feed on microphytobenthos suspended in the water column and on the sediment surface [28]; therefore, they were fed a concentrated algal mixture of 1 ml per replicate (*Dunaliella tertiolecta*, *Isochrysis galbana*, *Phaeodactylum tricornutum*; 1:1:1; $\sim 10^7$ cells) at $t = 0$ h to avoid starvation mortality observed in rangefinder controls. After 96 h, the contents of each chamber were poured over a 63- μm stainless steel sieve placed on top of a glass jar. The sieve was gently tapped on the jar to allow the sediments to pass through. This procedure ensured that all copepods, including naupliar stages, were retained on the sieve while sediments were collected in the jar below for chemical analyses. The contents of the sieve were checked for dead individuals and then preserved in 5% buffered formalin with Rose Bengal (Sigma Aldrich, Saint Quentin, Fallavier, France) stain. Copepods were sorted and enumerated using a stereo dissection microscope.

Aqueous acute toxicity tests

On the basis of results from preliminary rangefinder experiments, adult male and female *A. tenuiremis* were exposed in separate experiments to measured water concentrations of 1.8, 3.6, 6.1, 10.9, and 85.2 μg APM/L in the absence of sediments. Unlike most other infaunal copepods, this species will survive well in the absence of a sediment substrate and has proven ideal for phase comparisons of different routes of toxicant exposure and uptake [22]. Appropriate volumes of APM in acetone were added to clean glass beakers containing artificial seawater while the water was vortexing on a magnetic stir plate. Azinphosmethyl-water mixtures were allowed to mix for an additional 15 min before use. Fifty adult copepods (25 females and 25 males; three replicates per treatment) were added to 50-ml glass crystallizing dishes containing 35-ml of APM-spiked seawater. Uncontaminated control dishes and acetone carrier control dishes were similarly prepared. Chambers were loosely covered and placed in an environmental chamber (20°C, 12:12-h light:dark cycle). The tests were conducted static for 96 h without feeding. Copepod prefeeding, collection, and enumeration methods were the same as those used for the sediment assay.

Pore water acute toxicity test

Amphiascus tenuiremis were exposed in triplicate to pore water extracted from APM-contaminated whole sediments via centrifugation (16,000 g for 10 min). Fifty randomly selected adult copepods were added to 10-ml glass crystallizing dishes containing 4 ml of pore water. Measured pore-water treatment concentrations at $t = 0$ h were 0, 3.4, 11.0, 18.2, and 41.2 μg APM/L and corresponded to measured sediment concentrations of 0, 435, 864, 1,728, and 3,460 ng/g dry weight, respectively. Chambers were loosely covered and placed in an environmental chamber (20°C, 12:12-h light:dark cycle). The

test was conducted static for 96 h without feeding. Copepod prefeeding, collection, and enumeration methods were the same as those used for the sediment assay.

Whole-sediment chronic toxicity test

Because *A. tenuiremis* mature from egg to adult in 19 to 20 d at 21°C [27], a 14-d exposure to ^{14}C -APM-spiked sediments was sufficient to study reproductive effects in this species. In addition to an uncontaminated control treatment, *A. tenuiremis* were exposed to spiked sediments containing measured concentrations of 125 and 183 ng APM/g sediment dry weight, the approximate LC10 and LC15 from the acute tests, respectively. Sediment APM spikes were prepared as described previously for the acute sediment assay. Adult *A. tenuiremis* (50 nongravid females and 30 males per replicate; four replicates per treatment) were added to 150-ml glass test chambers containing artificial seawater and 10 ml spiked sediments. Test chambers consisted of a 150-ml glass Erlenmeyer flask drilled with two opposing 63 μm Nitex® mesh-covered holes (1 cm diameter; Aquatic Eco-Systems, Apopka, FL, USA) to allow water to flow out yet retain the copepods. The experiment was conducted using a single-pass microflow-through seawater culturing system built within an environmental chamber (20°C, 12:12-h light:dark cycle, ~ 4 water changes/d). Copepods were fed 2-ml of an algal mixture ($\sim 10^7$ cells/ml) every three days. Copepod and sediment collection and copepod enumeration methods were the same as those used for the acute sediment assay.

Bioaccumulation experiments

Adult *A. tenuiremis* and *M. littorale* were exposed to sublethal levels of sediment-associated ^{14}C -APM in separate 96-h bioaccumulation experiments. Because *M. littorale* exhibited a twofold, significantly higher tolerance to APM than *A. tenuiremis* in the whole-sediment acute assays, different exposure concentrations were targeted for each species. Each species was exposed to their projected median LC5 (*A. tenuiremis*) or LC10 (*M. littorale*), with slight differences in the design of each experiment. Two experiments were conducted in which *A. tenuiremis* were exposed to a nominal concentration of 83 ng APM/g sediment dry weight and a carrier control treatment. Sediment treatments were prepared as described previously for the chronic sediment assay. Ten-milliliter aliquots of the APM-contaminated sediments were dispensed into 50- by 35-mm glass crystallizing dishes containing 35 ml of artificial seawater. Two hundred randomly selected adult *A. tenuiremis* were added to each dish (three replicates for APM exposure and one control replicate) without disturbing the bottom sediment layer. Dishes were incubated for 96 h in an environmental chamber at 20°C under a 12:12-h light:dark cycle. The results of a trophic transfer feeding experiment [18], in which *M. littorale* were similarly exposed to a nominal sediment concentration of 750 ng APM/g dry weight for 96 h and then fed to a fish predator, were used for comparison with *A. tenuiremis* APM bioaccumulation in this study.

Chemical analyses for acute toxicity tests

Sediments. Azinphosmethyl concentrations were determined from two replicate sediment samples per treatment at $t = 0$ and 96 h. Five grams of test sediments were ground with sodium sulfate, and the mixture was Soxhlet-extracted with 250 ml dichloromethane for 12 h. Dichloromethane was

Table 1. *Amphiascus tenuiremis* and *Microarthridion littorale* dry weight and percent (%) total lipid on a dry weight basis (± 1 SD)^{ab}

	<i>A. tenuiremis</i> dry wt (μ g)	<i>A. tenuiremis</i> % total lipid	<i>M. littorale</i> dry wt (μ g)	<i>M. littorale</i> % total lipid
Gravid female	1.4 (± 0.10)	2.9 (± 0.2)	1.6 (± 0.05)	7.9 (± 0.4)
Nongravid female	1.1 (± 0.04)	2.0 (± 0.2)	1.1 (± 0.06)	5.8 (± 0.3)
Grand mean female ^c	1.2	2.4	1.4	6.8
Male	0.6 (± 0.02)	1.6 (± 0.3)	1.2 (± 0.04)	3.3 (± 0.2)
Grand mean ^d	1.0	2.2	1.3	5.6

^a *M. littorale* dry weight and lipid values previously published by Klosterhaus et al. [29].

^b Standard deviations represent the variability among pools of individuals (50 individuals for dry wt; 50 gravid females, 200 nongravid females, or 300 males for lipids; $n = 4$ for each sex and reproductive stage).

^c The grand mean female was calculated from the individual measurements (pools of 50–300 individuals) for each reproductive stage ($n = 8$).

^d The grand mean was calculated using individual measurements (pools of 50–300 individuals) for each sex and reproductive stage ($n = 12$).

evaporated from the extracts under nitrogen and exchanged into iso-octane to a final volume of 1 ml.

Waters. Overlying water and pore water from the whole-sediment tests and pore water and seawater from their respective acute exposures were collected from each exposure treatment at test initiation and termination for APM quantification (two replicates per treatment). Pore water from the sediment tests was separated from whole sediments by centrifugation (16,000 g for 10-min). Azinphosmethyl was extracted from waters using C_{18} extraction columns. Extracts were eluted from columns using ethyl acetate/hexane (15:85); otherwise, APM was extracted from water samples using previously published methods [5].

APM quantification. Sediment, pore water, and overlying water samples were analyzed by gas chromatography with electron capture detection using a Hewlett-Packard model 5890 gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a 5% phenyl methyl-silicone capillary column (25 m). Sample injection was in the splitless mode, and helium was the carrier gas with a flow rate of 1 ml/min. The injector temperature was 200°C and the detector temperature was 300°C for the analysis of APM. The temperature program consisted of a 10°C/min ramp from 80 to 185°C, a 5°C/min ramp from 185 to 270°C, with a 15 min hold at 270°C, and a 20°C/min ramp from 270 to 300°C, with a 10 min hold. Dissolved organic carbon (DOC) in overlying water and pore water was measured (two replicates per treatment) using a Rosemount Analytical Dohrman DC-190 total organic carbon analyzer (Mason, OH, USA).

Chemical analyses for chronic toxicity test and bioaccumulation experiments

Copepods from the bioaccumulation experiments were sieved (63 μ m) from sediments, washed vigorously three times with clean seawater, and placed in depuration chambers [29] for 4 h to allow for complete gut clearance. Batches of copepods (150 *A. tenuiremis* per replicate, 200 *M. littorale* per replicate) from the bioaccumulation experiments and test sediments (triplicate 100- μ l samples for each treatment at test initiation and termination) from both the bioaccumulation and chronic tests were analyzed for APM using a liquid scintillation counter method [18].

Lipid analyses and copepod dry weights

Total percent lipid was determined several years after APM experiments were conducted with a microtechnique for tissue samples in the low microgram range [29]. This technique was not developed until 1999 and thus was unavailable for use at the time of the APM experiments. For *A. tenuiremis*, lipid and APM experiments were performed with copepods from the same laboratory-reared stock populations. For *M. littorale*, lipids were measured from a population located in similar habitat but approximately 35 km away from the population used in the current investigation. Both *M. littorale* populations were located in pristine intertidal *Spartina alterniflora* salt marshes and thus were likely exposed to food supplies of similar quality and quantity. Lipid measurements were performed in the winter months for both species; thus, within- and between-species differences in lipid content as a result of seasonal interactions were avoided. The methods for determining individual copepod dry weights and the *M. littorale* dry weight and lipid values used for lipid normalization in this study have been previously published [29].

Statistical analyses

For all statistical tests, an a priori α level of 0.05 was used. Differences in mean copepod mortality both within (e.g., sex) and between species in all experiments were analyzed by analysis of variance (ANOVA) and Proc GLM [30]. Whenever the overall ANOVA general linear model was significant, Tukey's studentized range test was used to test for significant differences between controls and treatments. Two-way ANOVA was used to test for significant differences in sediment recovery efficiencies between experiments and among treatments within experiments. Dunnett's one-tailed t test was used to judge when controls and carrier solvent controls were not significantly different, so that corresponding mortalities could be pooled. Relative APM median lethal concentrations were estimated using trimmed Spearman-Kärber [31] in sediment tests and Probit analysis [30] in aqueous and pore-water tests.

RESULTS

Total lipid and dry weight

Mean female *A. tenuiremis* total lipid was 1.5 times higher than male *A. tenuiremis* total lipid (Table 1). Mean female *M.*

Table 2. Azinphosmethyl 96-h sediment median lethal concentrations (LC50s) and 95% confidence intervals for the infaunal copepods *Amphiascus tenuiremis* and *Microarthridion littorale*

	Nominal sediment LC50 (ng/g sediment)	Measured sediment LC50 (ng/g sediment)
<i>A. tenuiremis</i> females	946 (862–1,038)	540 (499–584)
<i>A. tenuiremis</i> males	735 (625–865)	456 (430–497)
<i>A. tenuiremis</i> mixed adults ^a	841	498
<i>M. littorale</i> mixed adults	14,250 (9,758–20,808)	—

^a Estimated from the mean of *A. tenuiremis* female and male 96-h experiments.

littorale total lipid was twice the male *M. littorale* total lipid. The mean *M. littorale* total lipid was approximately 2.5 times higher than the mean *A. tenuiremis* total lipid.

Whole-sediment acute toxicity tests

Carrier control and control data were pooled after Dunnett's one-tailed *t*-test revealed no significant differences in copepod mortality between these APM-free sediment manipulations. *Amphiascus tenuiremis* exhibited a 17-fold, significantly higher overall sensitivity to sediment-associated APM than *M. littorale* ($p < 0.01$), when compared on a nominal concentration basis, and significantly different between-sex sensitivities ($p < 0.01$; Table 2). Male *A. tenuiremis* were significantly more sensitive than female *A. tenuiremis*, with an LC50 value 84 ng/g lower than females (Table 2).

Comparisons of measured APM concentrations in sediments containing *A. tenuiremis* at $t = 0$ and 96 h of exposure show losses ranging from 33 to 59.6%, averaging $46.4 \pm 5\%$. Comparisons of measured APM concentrations in copepod-free sediments at $t = 0$ and 96 h of exposure indicated losses averaging only 19%, a 27% lower reduction than sediments containing copepods. Empirically, $1.0 \pm 0.21\%$ and $0.5 \pm 0.15\%$ of initial APM measured dry sediment concentrations partitioned into pore water and overlying seawater, respectively, at $t = 96$ h in the whole-sediment bioassays. The bulk of the APM remained associated with sediment particles (>98% on a dry weight basis). Recovery efficiencies in the whole-sediment *A. tenuiremis* exposures ranged from 75 to 104% of nominal APM concentrations, averaging $89.8 \pm 13.2\%$ for all sediments at $t = 0$ h, and were not significantly different between experiments using two-way ANOVA ($p = 0.91$).

Aqueous and pore-water acute toxicity tests

Between-sex differences in *A. tenuiremis* APM sensitivity were most dramatic in the aqueous exposures (Fig. 1). In contrast to whole-sediment exposures (Table 2), aqueous concentrations of 3.62 and 1.83 $\mu\text{g/L}$ caused nearly 50% mortality in *A. tenuiremis* females and males, respectively. Only 10.9 $\mu\text{g APM/L}$ caused 84% mortality of females and 95% mortality of males. The aqueous LC50 for combined male and female *A. tenuiremis* was 1.99 $\mu\text{g APM/L}$ (Fig. 2). Mean DOC concentration in the aqueous exposures was 4 ± 0.5 mg/L. Recovery efficiencies in the aqueous exposures ranged from 88 to 99%, averaging $91 \pm 7\%$. Final APM concentrations in the aqueous tests ranged from 63 to 81% of initial exposure concentrations, with a mean of 71.8%.

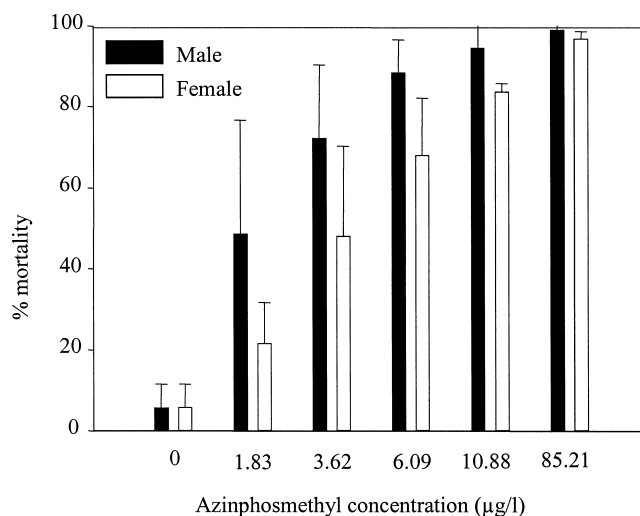


Fig. 1. The 96-h effects of seawater-solubilized azinphosmethyl on *Amphiascus tenuiremis* survival by sex. Bars represent percent mortality (± 1 SD). All concentrations are measured concentrations at time 0 h.

Higher concentrations of APM in pore water were required to produce the same mortalities observed in the aqueous-only exposures (Fig. 2). The pore-water LC50 for combined adult male and female *A. tenuiremis* was more than double the combined adult LC50 for aqueous exposures. Additionally, the pore-water LC75 was more than three times the aqueous LC75. Mean DOC concentrations in each pore-water treatment ranged from 19 to 22 mg/L, approximately five times the DOC concentration in the aqueous exposures.

Bioaccumulation experiments

There were no significant differences in initial sediment, final sediment, or copepod APM concentrations between the two *A. tenuiremis* bioaccumulation experiments ($p > 0.05$); therefore, mean values were used. On a dry weight basis, initial and final sediment concentrations averaged 116 ± 22 and 50 ± 9 ng/g, respectively in the *A. tenuiremis* experiments and

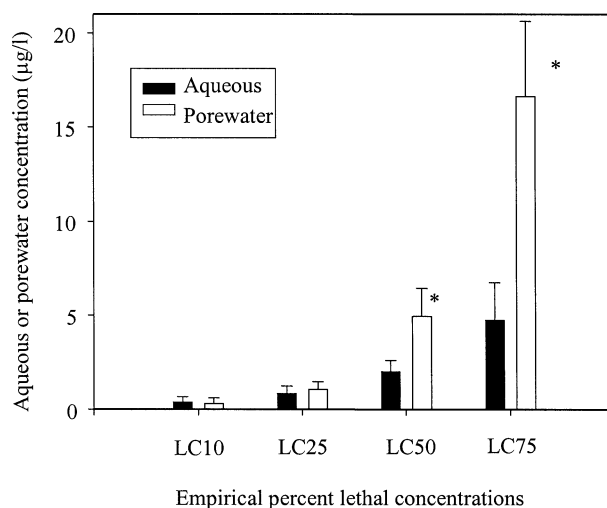


Fig. 2. Comparative 96-h toxicity of aqueous versus pore water-associated azinphosmethyl to *Amphiascus tenuiremis* based on log₁₀ Probit analysis. *Lethal concentration (LC)50 and LC75 estimates significantly different between aqueous and pore-water exposures ($p < 0.05$).

1,421 ± 122 and 1,130 ± 22 ng/g, respectively in the *M. littorale* experiment. Mean 96-h sediment exposure concentrations, tissue concentrations after 96-h exposure, and biota sediment accumulation factors ([BSAFs]; lipid-normalized tissue concentration/carbon-normalized sediment concentration) for both copepod species are given in Table 3. *Microarthridion littorale* and *A. tenuiremis* lipid-normalized tissue concentrations were similar after 96 h despite an order of magnitude difference in APM sediment exposure concentration. Consequently, the BSAF for *A. tenuiremis* was more than an order of magnitude higher (26.8) than the BSAF for *M. littorale* (2.2). The mean APM sediment concentration over 96 h was used in BSAF calculations.

Whole-sediment chronic toxicity test

Based on day 0 and 14 measurements, the mean sediment APM concentrations were 85 ng/g dry weight for the 125 ng/g treatment and 129 ng/g dry weight for the 183 ng/g treatment. The degradation/loss of APM over the 14-d period ranged from 59.5 to 63.3% of initial concentrations.

Mean clutch sizes produced in the 0, 125, and 183 ng/g treatments were not significantly different (5.48, 5.93, and 6.06, respectively; $p > 0.05$). Potential offspring (total number of eggs, nauplii, and copepodites per surviving adult female copepod on day 14) and realized offspring (total number of nauplii and copepodites per surviving adult female copepod on day 14) were significantly depressed in the 183 ng/g treatment relative to controls ($p < 0.05$; Fig. 3). Realized offspring in the 183 ng/g treatment was significantly more depressed than in the 125 ng/g treatment ($p < 0.05$). In the 125 ng/g treatment, both potential and realized offspring were depressed, but not significantly, relative to controls. Normalization of data to the number of surviving adult females eliminates bias from replicates where differential female survival occurs and gives a more conservative comparison of effects than raw total production values.

DISCUSSION

Acute toxicity

Decreased sensitivity was associated with increased total body lipid in the acute experiments in this study, suggesting that differences in APM tolerance between copepod species and sexes were primarily driven by copepod lipid content. Female *A. tenuiremis*, which have twice the amount of lipid as male *A. tenuiremis* (Table 1), tolerated twice the concentration of APM as the males in aqueous exposures (Fig. 1) and nearly 20% higher concentrations in whole-sediment exposures. *Microarthridion littorale*, which have over 2.5 times as much lipid as *A. tenuiremis* (Table 1), tolerated concentrations of sediment-associated APM nearly twice as high as *A.*

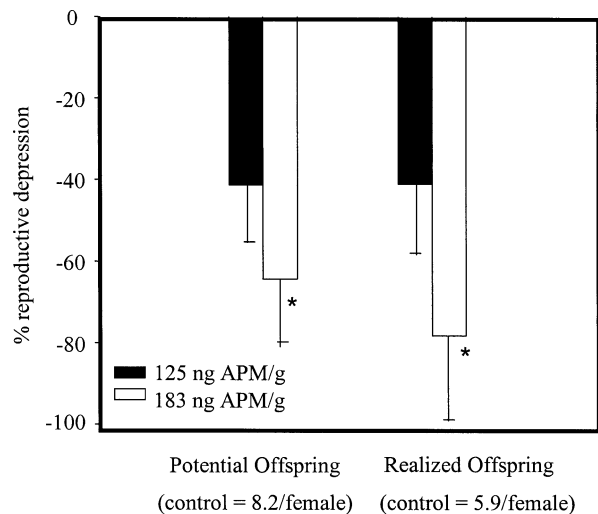


Fig. 3. Azinphosmethyl (APM) effects on reproduction of *Amphiascus tenuiremis* after 14 d in contaminated sediments. Bars represent mean percent depression (± 1 SD). * Percent depression significantly different from controls (potential offspring) or controls and 125 nL/L treatment (realized offspring; $p < 0.05$).

tenuiremis (Table 2). Sequestering hydrophobic organic chemicals in body lipid has been suggested as a primary mechanism for enhanced tolerance to contaminants in acute exposures [32] and is supported by the results in this study. Similar results were observed from an exposure to the sediment-associated PCB mixture Aroclor® 1254 (Monsanto, St. Louis, MO, USA), in which the female *M. littorale* LC50 concentration was more than double the LC50 for the male [33]. Similarly, Strawbridge et al. [34] found that adult female *A. tenuiremis* survival was significantly higher than adult male survival when exposed to the sediment-associated fenvalerate, a lipophilic pyrethroid insecticide. The addition of algal food in the *M. littorale* whole-sediment acute exposures, but not in the *A. tenuiremis* acute exposures, might have contributed to the reduced sensitivity to sediment-associated APM observed for *M. littorale*. The presence of an uncontaminated food source in the water column and sediment surface could have resulted in a lower APM exposure to *M. littorale* compared to *A. tenuiremis* because of the ingestion of relatively uncontaminated particulate matter rather than sediments; however, this alternative food source likely does not account for the 17-fold difference in tolerance to APM-contaminated sediments. In pore-water-only APM exposures in this study, DOC likely complexed with otherwise bioavailable APM, reducing uptake and subsequent toxicity in *A. tenuiremis* in comparison to aqueous exposures at five times lower DOC (Fig. 2).

Different salinities used in the *A. tenuiremis* (30 ppt) and

Table 3. Mean accumulation of sediment-associated azinphosmethyl (APM) by *Amphiascus tenuiremis* and *Microarthridion littorale* after a 96-h exposure. All concentrations are on a dry weight basis

	Mean 96-h sediment exposure ^a (ng/g)	Carbon-normalized sediment exposure (ng/g carbon)	APM accumulated (pg/copepod)	APM accumulated (ng/g copepod)	Lipid-normalized APM accumulation (ng/g lipid)	BSAF ^b (g carbon/g lipid)
<i>A. tenuiremis</i>	83	2,160	1.3 ± 0.3	1,270	57,700	26.8
<i>M. littorale</i>	1,276	33,140	5.3 ± 2.1	4,080	72,800	2.2

^a Determined from the mean measured sediment APM concentrations at $t = 0$ and 96 h.

^b Biota sediment accumulation factors.

M. littorale (15 ppt) whole-sediment exposures cannot be discounted as playing a role in the large discrepancy in APM sensitivity exhibited between species. Although not demonstrated for APM specifically, in general, the sorption of hydrophobic organic chemicals to sediment phases has been shown to increase with salinity [e.g., 35]. If salinity-driven sorption was indeed controlling exposure to APM in this study, the copepods in the higher salinity exposure (*A. tenuiremis*) would thus be expected to experience a lower exposure to APM in the pore water than copepods in the lower salinity exposure (*M. littorale*). As a result, copepods in the higher salinity exposure would be expected to exhibit a higher sediment LC50 than those in the lower salinity exposure. The opposite, however, was observed in this study. Copepods in the high-salinity exposure (*A. tenuiremis*), which were exposed to sediment APM concentrations an order of magnitude lower, had an LC50 an order of magnitude lower than the copepods in the low-salinity exposure (*M. littorale*). If the salinity difference was a factor controlling APM toxicity to these species, a much higher sediment LC50 for *A. tenuiremis* would be expected.

Bioaccumulation

After a 96-h whole-sediment exposure, adult *A. tenuiremis* and *M. littorale* accumulated similar concentrations of APM on a gram lipid basis as would be predicted by the equilibrium partitioning bioaccumulation model (Table 3) [36]. However, *M. littorale* was exposed to sediment concentrations an order of magnitude higher than *A. tenuiremis*, which resulted in a BSAF for *A. tenuiremis* that was an order of magnitude higher than for *M. littorale*. The BSAF for *M. littorale* (2.2) was similar to BSAFs observed for other pesticides with similar $\log K_{ow}$ [37], however the relatively low K_{ow} for APM would not have predicted such high bioaccumulation in *A. tenuiremis*. Large differences in measured bioaccumulation factors suggest that *A. tenuiremis* experienced a higher exposure to sediment-associated APM, exhibited higher rates of active APM uptake than *M. littorale*, or both. Because size differences can be discounted as a source of exposure variation (Table 1), species-specific differences in behavior in sediments might cause large differences in contaminant exposure. *Microarthridion littorale* is an epibenthic species that lives predominantly in the top 1 to 2 mm of the sediment surface layer and makes frequent excursions into the water column to pursue phytoplankton food [38]. *Amphiascus tenuiremis*, however, is a deeper burrowing species that only occasionally leaves its infaunal habitat (G.T. Chandler, personal observation); thus, it might experience a greater exposure to sediment-associated toxicants than *M. littorale* because of longer periods of direct contact with sediment particles and pore water. Results from this study revealed that $1.0 \pm 0.2\%$ of sediment-associated APM partitioned into pore water after a 96-h equilibration period, with only half as much ($0.5 \pm 0.2\%$) partitioning into overlying water. The equilibrium partitioning model would have predicted approximately 7% in pore water on the basis of the organic carbon content of our test sediments and the $\log K_{ow}$ for APM (e.g., [39]). Interestingly, 1% of the whole-sediment LC50 for *A. tenuiremis* (5.0 ng/g) is extremely close to the pore-water-only LC50 of 4.95 ng/g, strongly suggesting that pore water is the primary route of APM exposure and uptake for *A. tenuiremis*. If sediment ingestion was an important route for this compound, then the sediment LC50 would likely be lower because of additive uptake from the ingestion route. *Microar-*

thridion littorale might have been more resistant to sediment-sorbed APM as a result of its closer association to less APM-concentrated overlying water than *A. tenuiremis*. Another study using these same copepod species found that *A. tenuiremis* accumulated almost twice as much PCB (Aroclor 1254) as the more lipid-rich *M. littorale* when exposed to identical sediment concentrations [40]. They speculated this was similarly a result of enhanced exposure via pore water in *A. tenuiremis*. The enhanced uptake by *A. tenuiremis* in their PCB study likely was not a result of elevated lipid content because it is now known that *A. tenuiremis* have less lipid than *M. littorale*, and metabolism was not a likely source of interspecies variation in PCB body burdens because of a general lack of evidence for significant PCB elimination pathways in crustaceans. Pore water has been considered to be a significant route of exposure in other studies investigating the effects of other sediment-associated contaminants on infaunal organisms [41,42].

In addition to an increase in APM exposure because of a more interstitial lifestyle in sediments, enhanced tissue concentrations in *A. tenuiremis* relative to sediments and greater sensitivity in *A. tenuiremis* in APM-contaminated sediments compared with *M. littorale* could be the result of preferential ingestion of smaller sediment particles, which contain more bacteria and microphytoplankton and higher APM concentrations than larger particles with smaller surface to volume ratios. Additionally, selective feeding on particles with higher organic carbon concentrations could lead to a higher ingested dose than predicted from the whole-sediment concentration [43]. Unfortunately, preferential ingestion of sediment particles by benthic copepods on the basis of size and quality has not been well studied.

Reductions in APM concentration in sediments without copepods suggest that normal degradation processes (i.e., microbial, photolysis, etc.) and partitioning into the overlying waters and pore waters might account for a 19% loss in azinphosmethyl sediment concentration within the first 96 h of exposure. The twofold increase in this APM degradation rate in the presence of copepods suggests an important role for benthic copepods and meiobenthic-level bioturbation in the degradation of pesticides such as APM. Green and Chandler [44] reported enhanced mobilization of cadmium from sediments to pore waters because of meiofaunal bioturbation; perhaps similar enhancements occur with APM.

Chronic toxicity

Significant reductions in potential and realized offspring indices (Fig. 3) without reductions in clutch size strongly suggest that the reproductive effects observed during the chronic exposure to sediment-associated APM were most likely due to naupliar, copepodite (juvenile), or combined mortality. Significant reductions in offspring production were also observed in contaminated sediment bioassays with meiobenthic copepods exposed to the organophosphorous pesticide chlorpyrifos [23,45] and the PCB Aroclor 1254 [33]. Conversely, exposure to sublethal levels of the pyrethroid insecticide fenvalerate in sediments depressed both egg production and mean clutch size in *M. littorale* without observed effects on survival of any copepod life stages [46]. In a more detailed investigation of the effect of sublethal levels of organophosphate pesticides on *A. tenuiremis* populations, chronic chlorpyrifos exposure resulted in significant reductions in fecundity and, consequently, population growth (as indicated by reductions in population

growth rate, r , and net reproductive rate, R_0) using a life table approach [47]. Results from these previous investigations of population-level effects of chronic pesticide exposure and declines in fecundity observed in this study for *A. tenuiremis* suggest a potential for considerable reductions in meiobenthic copepod populations in estuaries receiving azinphosmethyl runoff or spraydrift. Furthermore, because of their small body masses, meiobenthic copepods are easily transported, essentially as passive particles, over centimeter to meter spatial scales in estuaries via tidal currents [48,49]. Thus, active avoidance of contaminated sediments is difficult for these organisms because many species are continuously resuspended and dispersed passively to new locations with every tide.

Most APM studies to date have focused on fate and effects in the water column and have neglected the potential for transport and toxicity to benthic organisms. Yet, the presence of relatively high levels of APM on suspended particles in field observations (216–1,247 ng/g dry weight) [10], its persistence months after a single runoff event [10], and the strong affinity for sediments observed in this study demonstrate that APM's adsorption behavior is characteristic of a more hydrophobic, high- K_{ow} compound. These observations suggest that even a single, relatively high-volume runoff event could result in exposures sufficient to affect multiple generations of the short-lived harpacticoid copepods and potentially other similarly sensitive meiobenthic organisms.

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