

LABORATORY ANALYSES OF THE POTENTIAL TOXICITY OF
SEDIMENT-ASSOCIATED POLYDIMETHYLSILOXANE TO
BENTHIC MACROINVERTEBRATES

KEVIN S. HENRY,*† WILLEMEN H. WIELAND,‡ DAVID E. POWELL,§ and JOHN P. GIESY†

†Department of Zoology, National Food Safety and Toxicology Center, Institute for Environmental Toxicology, Michigan State University,
East Lansing, Michigan 48824, USA

‡Wageningen University, Toxicology Section, 6700 EA Wageningen, The Netherlands

§Environmental Sciences (C03101), Dow Corning Corporation, Midland, Michigan 48686, USA

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Abstract—Polydimethylsiloxane (PDMS) is widely used in a number of industrial processes and consumer products that result in down-the-drain disposal. The log *p* value for the PDMS used in the present study was 10, and the vapor pressure and water solubility values were below detection limits. These physicochemical characteristics and a measured degradation rate of 3% after six months in moist soils suggest that PDMS may accumulate in aquatic sediments. Sediment toxicity tests with the amphipod *Hyalalella azteca* and with larvae of the midge *Chironomus tentans* were used to assess the potential for toxicity of PDMS-amended sediments to benthic invertebrates in short-term (10-d) and whole-life-cycle (28 d for *H. azteca*, 50–65 d for *C. tentans*) exposures. Endpoints for short-term tests included survival and growth, while life-cycle assays considered survival, growth, reproduction, and, for *C. tentans* only, emergence. Short-term and life-cycle exposures to concentrations of $\geq 1,000$ mg PDMS/kg sediment (dry wt) indicated that PDMS will not reduce survival, growth, or reproduction in *H. azteca* or *C. tentans*.

Keywords—*Chironomus tentans* *Hyalalella azteca* Chronic toxicity testing Polydimethylsiloxane

INTRODUCTION

Polydimethylsiloxane (PDMS) fluids are synthetic polymers consisting of an alternating silicon–oxygen backbone with methyl side groups (Fig. 1). Polydimethylsiloxane is used in a variety of industrial and consumer applications, including heat transfer fluids, antifoams, cosmetics, waxes, and polishes [1]. Many of these uses result in down-the-drain disposal. The most common linear siloxanes are insoluble in water and extremely hydrophobic; therefore, siloxanes become associated with sludge solids on reaching sewage conduits and wastewater treatment plants [2,3].

Approximately 3% of the PDMS disposed to wastewater treatment plants in the United States is released to surface waters adsorbed to sludge particles in the treated effluent, representing a total annual loading of approximately 294,000 kg [4]. On entering surface waters, the majority of the suspended sludge and associated PDMS will eventually deposit into bottom sediments. The small fraction of PDMS not sorbed to suspended particles in the effluent would partition to suspended solids in the river or lake water and also eventually become deposited into the bottom sediments [4].

Degradation of PDMS has been demonstrated in soils and is believed to occur by hydrolysis to yield monomeric dimethylsilanediol [2], though the rate of this reaction is inversely related to soil moisture content [5]. The potential for PDMS degradation in freshwater sediments was demonstrated in the laboratory [6]. After a year of incubation in aerated sediments, 5 to 10% of the PDMS present was hydrolyzed to dimethylsilanediol (DMSD)—results similar to those for deg-

radation studies in moist soils, where 3% of the PDMS was hydrolyzed to DMSD in six months [5]. The rate of PDMS degradation was less in sterile sediments, suggesting that this process may be related to sediment microflora [6]. The mobility of PDMS in aquatic systems will be limited primarily to resuspension and deposition of sediment particles with which it is associated. Concentrations of PDMS decrease with depth in sediments and are not detectable at depths that correspond to periods before the production and use of PDMS fluids [7,8]. While PDMS tends to accumulate in sediments, concentrations can decrease in response to mass dilution and degradation [6] or if inputs are reduced or discontinued, for example, by enhanced wastewater treatment technology [9]. Mean measured PDMS concentrations in sediments from depositional areas downstream from wastewater treatment plant (WWTPs) range from less than the detection limit to 78 mg/kg (dry wt) at sites receiving point source inputs [9]. However, 90% of the sediments analyzed contained less than 26 mg PDMS/kg (dry wt) [9]. The biota exposed to PDMS in these areas would primarily be benthic invertebrates intimately associated with these sediments.

Polydimethylsiloxane appears to be relatively nontoxic to benthic invertebrates (Table 1). In part, this nominal level of toxicity is due to the fact that little bioaccumulation occurs (Table 1). This low potential to bioaccumulate is due to the high molecular weight of PDMS molecules, which are too large to cross biological membranes [10,11]. While PDMS has been found to be nontoxic in short-term studies, little information was available on the chronic toxicity of PDMS to sediment-dwelling benthic invertebrates. Since PDMS is surface active, it was postulated that PDMS in sediments could change the physical characteristics of the matrix. For instance, it has been reported that PDMS can reduce the bioavailability of

* To whom correspondence may be addressed (kshenry@dow.com). The current address of K.S. Henry is Dow Chemical Company, 1803 Building, Midland, MI 48674, USA.

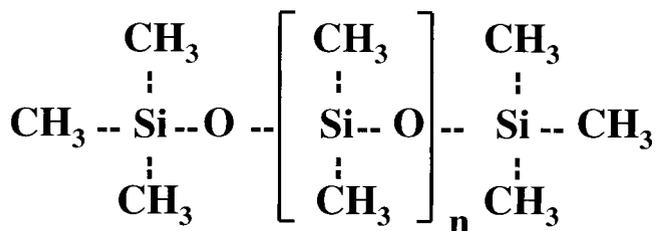


Fig. 1. Generic structure of polydimethylsiloxane (PDMS). The value of n can range from 10 to >10,000 [35].

organic contaminants such as polycyclic aromatic hydrocarbons (PAHs) from sediments [12]. Because a number of benthic invertebrates extract organic compounds from sediments, either as an energy source or in the form of fat-soluble vitamins, it was postulated that PDMS might affect the availability of the organic compounds by acting as a cosolvent. Finally, because PDMS may affect the texture of sediments with respect to their viscosity or degree of consolidation, concern arose that PDMS contamination could change the suitability of the sediment matrix for benthic invertebrates. If the degree of consolidation of the sediment is altered by PDMS, bioturbation and the production of cases or burrows may be adversely impacted.

The objectives of this study were to determine if sediment-bound PDMS would affect the survival, growth, or reproduction of benthic invertebrates. This was investigated by the use of controlled laboratory toxicity tests with freshwater macroinvertebrates. Test methodologies used included standardized short-term tests as well as multiple life-stage protocols recently developed by the U.S. Environmental Protection Agency (U.S. EPA) [13].

MATERIALS AND METHODS

Test organisms and sediment

Two species of freshwater invertebrates were used in laboratory exposures: an amphipod (*Hyalella azteca*, Crustacea) and a midge (*Chironomus tentans*, Insecta). Both of these species

demonstrate characteristics of ideal test species [14]. Standard test methods using these organisms exist, and survival and growth are the primary endpoints [13,15,16]. Therefore, these organisms were used as surrogates for insects and crustaceans in natural communities, as the two organisms selected represent a range of possible tolerances and life histories.

Both species were reared and maintained according to methods adapted from several sources [13,15,17–19]. Briefly, *H. azteca* were cultured at $23 \pm 1^\circ\text{C}$ in 1-L plastic breeding chambers in an incubator. Nylon bolting cloth was used as a substratum. Amphipods were fed daily a suspension of yeast, cereal leaves, and trout chow (yeast-cerophyl-trout-chow [YTC]) as well as the green alga *Selanastrum capricornutum*. Partial water replacements were performed two times per week. *Chironomus tentans* were cultured in 19-L aerated glass aquaria to which shredded and presoaked unbleached paper towels were added as a substratum. *Chironomus tentans* were fed daily with a suspension of TetraMin blended fish food flakes (Tetra Sales, Blacksburg, VA, USA). Water for both cultures consisted of a mixture containing 30% well water and 70% Barnstead E-Pure (Dubuque, IA, USA), resulting in a final hardness of approximately 90 mg CaCO_3/L and a final alkalinity of approximately 100 mg CaCO_3/L .

Test sediment for all studies except those conducted with low organic carbon sediment was collected from a small pond in Williamston, Michigan, USA, which receives no regular surface water inputs. Low organic carbon sediments from Lake Michigan were obtained from Peter Landrum at the National Oceanic and Atmospheric Administration (NOAA)/Great Lakes Environmental Research Laboratory (Ann Arbor, MI, USA). Sediment from Williamston contained 4% organic carbon (OC), while that from Lake Michigan contained 0.5% OC. Sediments were stored in high-density polyethylene buckets in the dark at 4°C until use.

PDMS fluid

Polydimethylsiloxane fluid with a viscosity of 350 cs was obtained from Dow Corning Corporation (Midland, MI, USA). The average molecular weight of this fluid was 17,970, and

Table 1. Effects of polydimethylsiloxane (PDMS) on benthic invertebrates

Test organism	Maximum concentration tested	Endpoint and result	Reference
Polychaete, <i>Nereis diversicolor</i>	10,000 mg/kg in sediment	No reduction in growth or survival to 28 d, though burrowing activity was affected, perhaps because of pretreatment of sediments rather than to PDMS	[29]
Polychaete, <i>Nereis diversicolor</i> ; Mussels, <i>Mytilus edulis</i> and <i>Mytilus galloprovincialis</i> ; Crab (Crustacean), <i>Carcinus maenas</i>	10,000 mg/L in water	Low toxicity and bioaccumulation/biomagnification potential in marine trophic chains	[30,31]
Oligochaete, <i>Lumbriculus variegatus</i>	150 mg/kg in sediment	No toxicity or bioaccumulation; rapid depuration of PDMS. PDMS in sediments reduced the bioaccumulation of benzo[a]pyrene	[12]
<i>Chironomus tentans</i>	560 mg/kg (organic content $\geq 4\%$) 450 mg/kg (4% > organic content $\geq 2\%$) 350 mg/kg (organic content < 1%)	No bioaccumulation or adverse effects (survival or growth) observed during 14-d exposure.	[32]
<i>Ampelisca abdita</i>	2,300 mg/kg (4% > organic content $\geq 2\%$)	No mortality observed during 10-d exposure.	[33]
<i>Hyalella azteca</i>	2,200 mg/kg (2% > organic content $\geq 1\%$)	No adverse effects on survival or growth observed during 28-d exposure.	[34]

the log *p* was approximately 10 [3]. Vapor pressure and water solubility are not measurable for PDMS of this viscosity [3].

Sediment dosing and analyses

Application of PDMS to sediments was intended to be as realistic as possible, with target concentrations selected to range from those typically measured at wastewater treatment plant outfalls (about 26 mg/kg dry wt) to concentrations an order of magnitude greater than the greatest measured concentration (about 300 mg/kg dry wt) reported in the literature [9]. The PDMS-amended treatments were prepared by spinning PDMS-free sludge, 350-cs PDMS fluid, and water in a round-bottomed flask on a rotary evaporator in order to mix these components. This spiked sludge was then stirred into homogenized sediments using a drill-mounted stainless-steel propeller. The sediment/sludge mixture was shaken for 2 h on a platform shaker, then permitted to equilibrate undisturbed for 7 to 40 d at 4°C before it was homogenized again for use in toxicity tests.

Concentrations of PDMS in sediments were determined on duplicate or triplicate subsamples collected for analysis. Wet sediment samples were extracted three times with tetrahydrofuran. The resulting extracts from each sample were pooled, evaporated to 1 ml, and analyzed by inductively coupled plasma atomic emission using the method described in [4]. Variability in PDMS concentration among replicates was typically ≤10%.

Short-term laboratory exposures

Short-term sediment toxicity tests were conducted using procedures adapted from those developed by the U.S. EPA [13,15] and Environment Canada [17,18]. Tests with *C. tentans* were conducted on cohorts reared from eggs oviposited on the same date. Larvae of uniform age (13 d postoviposition) and approximately uniform size were selected for use in testing. Assays with *H. azteca* were performed with 7- to 8-d-old organisms. Ten-day toxicity tests were conducted using a Benoit exposure manifold [20] in which each 300-ml test chamber contained 10 test organisms and received two volume replacements per day. Test organisms were fed daily with a suspension of Tetramin (*C. tentans*) or YCT (*H. azteca*).

At the end of a test, the contents of each test vessel were sieved through a 425- or 710-μm sieve and rinsed with culture water to separate *H. azteca* and *C. tentans*, respectively. The number of survivors in each chamber was recorded and the weight of surviving *C. tentans* determined to calculate growth. *Chironomus tentans* individuals were pooled by replicate in pre-ashed aluminum weigh boats and dried for 24 h at 60°C to determine dry weight. Ash-free dry weight (AFDW) was also measured to reduce the bias introduced by gut contents [21]. To measure AFDW, the weigh pans containing the dry larvae were ashed at 550°C for 2 h. The pans with the ashed larvae were then reweighed, and the tissue mass of the larvae was determined as the difference between the weight of the dried larvae plus pan and the weight of the ashed larvae plus pan.

Whole-life-cycle assays

Whole-life-cycle assays with *C. tentans* and *H. azteca* were conducted on the basis of draft protocols under development by the U.S. EPA and U.S. Fish and Wildlife Service that have since been issued [13]. Minimum average data quality objectives for survival, growth, and reproduction have been suggested [13] and are provided here when available.

Table 2. Mean ± standard error (SE) of *Chironomus tentans* and *Hyalella azteca* responses in short-term (10-d) exposures with polydimethylsiloxane (PDMS)-spiked sediments. No significant differences in response were identified between PDMS-treated and control sediments

PDMS concn. (mg/kg; measured)	Survival (% ± SE)	Dry weight (mg/larva)	Ash-free dry weight (mg/larva)
<i>Chironomus tentans</i> (n = 16)			
0	95 (2.7)	1.45 (0.152)	0.769 (0.138)
26	99 (3.1)	1.36 (0.036)	1.24 (0.212)
250	94 (2.2)	1.44 (0.045)	1.03 (0.210)
2,590	95 (2.7)	1.37 (0.040)	1.05 (0.179)
<i>Hyalella azteca</i> (n = 12)			
0	83 (4.3)		
21	87 (4.0)		
195	94 (1.9)		
1,900	88 (3.2)		

C. tentans whole-life-cycle assay

The life-cycle test for *C. tentans* was modified from Benoit et al. [22] and a U.S. EPA guidance manual [13]. Tests were initiated with 12 newly hatched larvae per exposure vessel. A total of 12 beakers per treatment were used in these tests, and an additional four beakers were prepared and newly hatched midge larvae introduced on day 10 for production of males to be used in reproduction. These beakers were necessary because peak female emergence can occur up to one week later than that of male *C. tentans* [22,23]. On day 20 of the test, the sediment from four randomly selected beakers per treatment was sieved to recover larvae for determination of growth, as ash-free dry weight, and survival. An average data quality objective of 0.48 mg/control individual has been suggested for the growth endpoint [13]. After 20 d, screened lids were placed over the remaining eight reproductive replicates for collection of emerging adults.

Adults were collected one to three times a day from each test chamber and transferred to a corresponding reproduction chamber for breeding. Each reproduction unit was checked daily for dead adults and for egg cases, and dead adults were removed. An average data quality objective for percentage control emergence of 50% has been suggested [13]. Egg masses were collected from the reproduction chambers, and the number of eggs present and hatching success were determined as described in [13]. Average quality assurance objectives for control treatments of ≥800 eggs per female and 80% hatch have been suggested by the U.S. EPA [13].

In nontoxic sediments, the test typically requires 50 to 65 d for completion [13,22]. However, test duration increases in the presence of stressors, which act to reduce growth and delay emergence. Therefore, the test was terminated when no emergence occurred from the control treatments for 7 d. At test termination, all beakers were sieved to recover remaining larvae, pupae, or pupal cases. The endpoints for the test were thus 20-d growth and survival, emergence, reproduction (as the number of eggs per female), and percentage hatch.

H. azteca whole-life-cycle assay

Whole-life-cycle testing with *H. azteca* was initiated with amphipods that were 7 to 8 d old. A total of 12 test beakers were set up for each treatment, and test organisms were pipetted directly into the overlying water of the exposure bea-

Table 3. Mean \pm standard error (SE) of *Chironomus tentans* and *Hyalella azteca* responses in short-term (10-d) exposures with polydimethylsiloxane (PDMS)-spiked sediments of low organic carbon content (0.5% OC). No significant differences in response were identified between PDMS treated and control sediments

PDMS concn. (mg/kg; measured)	Survival (% \pm SE)	Dry weight
<i>Chironomus tentans</i> (n = 16)		
0	95 (2.9)	2.47 (0.089) ^a
1,246	90 (3.8)	2.69 (0.174) ^a
<i>Hyalella azteca</i> (n = 12)		
0	87 (3.1)	0.180 (0.011) ^b
1,156	89 (5.1)	0.180 (0.007) ^b

^a Expressed as milligrams per larva.

^b Expressed as milligrams per individual.

kers. Details on the testing methodology are provided elsewhere [13].

Endpoints monitored included 28-d survival and growth of *H. azteca* and 35- and 42-d survival, growth, and reproduction (number of young/female). On day 28 of the test, the contents of four of the replicate beakers per treatment were separated with a stainless-steel sieve to collect surviving *H. azteca*, and dry weights for these amphipods were measured. Control survival and dry-weight average data quality objectives of 80% and 0.15 mg/individual have been suggested [13].

After 28 d, the remaining eight of the original 12 beakers per sediment were sieved and the surviving *H. azteca* in each beaker placed into water-only beakers. Reproduction of amphipods was measured after 35 and 42 d in the water-only beakers by removing and counting the adults and young in each beaker. Adult amphipods surviving at 42 d were preserved, and the number of adult males and females in each beaker was determined. The number of surviving females was used to determine the number of young per female per beaker produced between 28 and 42 d. An average data quality objective for control vessels of two young per female has been suggested [13]. Dry weight for the surviving *H. azteca* was also measured.

Statistics

Mean treatment effects of PDMS were examined statistically by a combination of parametric and nonparametric methods. Percentage data were arcsine square root transformed before testing. All data were assessed for homogeneity of variance using Bartlett's test and for normality by Shapiro-Wilk's test. If the data met these assumptions, parametric statistics were used. If one or more assumptions were not met, non-

parametric statistics were used. Data were evaluated for significance by analysis of variance, followed by Williams test for pairwise comparison. When necessary, nonparametric analyses were performed using the same tests on rank-transformed data. Type I (α) error for all tests was set at 0.05.

RESULTS AND DISCUSSION

Short-term laboratory exposures

Survival of *C. tentans* or *H. azteca* was not affected during 10-d exposures to PDMS-spiked sediments. Survival of *C. tentans* larvae exposed to sediment concentrations of up to 2,590 mg PDMS/kg (dry wt) was not different from that of individuals in control sediments (Table 2). Similarly, survival of *H. azteca* was not significantly less in sediments containing up to 1,900 mg PDMS/kg (dry wt) than that of controls (Table 2). Each of these values represents the greatest concentration tested. No significant differences in *C. tentans* growth were identified between any of the PDMS-spiked sediments and control sediment with respect to both dry weight and ash-free dry weight (Table 2).

No differences in growth or survival of *C. tentans* or *H. azteca* were observed in 10-d exposures to Lake Michigan sediments of low OC content that were amended to approximately 1,200 mg PDMS/kg (dry wt) in a one-treatment exposure (Table 3). Virtually all particles in aquatic systems carry an organic coating that can potentially sorb chemical contaminants [24], and the partitioning of hydrophobic compounds to this coating is often great [25,26]. Sediment OC content has been reported to influence the bioavailability and toxicity of some compounds [27]. Therefore, the potential exists that organic carbon content of sediments may affect the degree of sorption and perhaps the toxicity of PDMS. Kukkonen and Landrum [12] found no reduction in growth or survival of *L. variegatus* in Lake Michigan sediments of 0.5% OC content treated with 150 mg PDMS/kg (dry wt).

Whole-life-cycle assays

While short-term tests can be used to study the potential for acute toxicity, they cannot be used to predict subtle effects from long-term exposures at the community level [22,28]. In order to better evaluate the certainty that PDMS does not cause adverse effects in aquatic environments, assessments of the potential for chronic, sublethal impacts rather than acute toxicity were made by use of whole-life-cycle studies with *C. tentans* and *H. azteca*.

Survival, growth, emergence, and reproduction of *C. tentans* in PDMS-treated sediments at concentrations up to 2,600 mg PDMS/kg (dry wt) were not significantly different than from that of *C. tentans* on untreated sediment (Table 4). Suggested day 20 average data quality objectives of 70% survival

Table 4. Mean \pm standard error of *Chironomus tentans* responses in whole-life-cycle toxicity tests with polydimethylsiloxane (PDMS)-spiked sediments. No significant differences in response were identified between PDMS-treated and control sediments

PDMS concn. (mg/kg; measured)	Survival at 20 d (%; n = 4)	Ash-free dry weight at 20 d (mg/larva; n = 4)	% Emergence (n = 8)	Number of eggs/female	% Hatch
0	75 (5.9)	1.00 (0.039)	45 (4.1)	1,040 (91)	93 (3.0)
25	81 (5.2)	0.933 (0.074)	55 (3.1)	1,071 (49)	91 (2.4)
260	73 (9.2)	0.947 (0.144)	53 (4.1)	1,096 (89)	93 (4.1)
2,600	77 (4.0)	0.821 (0.030)	55 (5.6)	1,125 (58)	90 (4.5)

and 0.48 mg/kg AFDW [13] were achieved or exceeded in the controls and all PDMS treatments (Table 4). The PDMS-treated sediments did not significantly affect percentage emergence of adult *C. tentans*. Control emergence was 45%, while the suggested average data quality objective was 50% [13]. Midge emergence from all three of the PDMS-treated sediments exceeded 50% (Table 4). During recent definitive round-robin testing with a control sediment from West Bearskin Lake (MN, USA), a total of 50% of the participating laboratories met acceptability criteria for both survival and emergence [13]. *Chironomus tentans* in PDMS-spiked sediment exhibited no significant differences in the number of eggs produced per female, and mean production for all treatments was greater than the suggested egg production acceptability criterion of 800 eggs per control egg case (Table 4). Percentage hatch of the eggs was not significantly different in any of the spiked sediments from the control, and all treatments exceeded the 80% suggested average data quality level (Table 4). Polydimethylsiloxane in sediments did not influence either egg production or percentage hatch, which suggests that reproduction of *C. tentans* will not be adversely impacted by sediment concentrations of up to 2,600 mg PDMS/kg (dry wt).

The PDMS-spiked sediments with concentrations as great as 994 mg PDMS/kg (dry wt) did not significantly reduce survival, growth, or reproduction of *H. azteca* during the whole-life-cycle assays (Table 5). Survival of amphipods at 28, 35, and 42 d in PDMS-treated sediments was not significantly different from that in control sediments, although the percentage survival in the control treatment decreased from 87% after 28 d to 77% and 63% after 35 and 42 d, respectively (Table 5). While the average data quality objective of 80% survival after 28 d was achieved, survival values were somewhat less than interlaboratory means of 94% (28 d), 92% (35 d), and 92% (42 d) in the control sediment from West Bearskin Lake during the round-robin assay [13]. These interlaboratory values represent survival data from laboratories that met the control survival test acceptability at 28 d of 80% survival. Differences between round-robin results and those observed in the present study may be due to differences in physicochemical parameters of the sediments, such as grain size, which make the Williamston Pond sediment less ideal for amphipod colonization. Growth of *H. azteca* was not significantly different between PDMS-spiked and control sediments after 28 or 42 d (Table 5), although variability was high in the 9-mg/kg (dry wt) PDMS treatment after 28 d (coefficient of variation [CV] = 43%). This is likely due to the few replicates for this endpoint ($N = 4$) and could be improved with increased replication. The control average data quality objective of 0.15 mg/individual after 28 d was achieved in all treatments. Length of survivors was not measured in this study.

Amphipod reproduction in all treatments exceeded the suggested control average data quality value of two young per female, and no difference was observed between any individual PDMS treatment and the control (Table 5). The suggested acceptability criterion for mean control reproduction of two young per female was exceeded in all treatments. However, the reproduction endpoint was more variable than either growth or survival, with CVs of 64, 90, 71, and 64% in the control, 9-, 104-, and 994-mg-PDMS/kg (dry wt) treatments, respectively. This great variability suggests that reproduction may not be as useful a measure of contaminant effect as survival or growth, or increased replication may be needed to improve statistical resolution of this endpoint.

Table 5. Mean \pm standard error of *Hyalella azteca* responses in whole-life-cycle toxicity tests with polydimethylsiloxane (PDMS)-spiked sediments. No significant differences in response were identified between PDMS-treated and control sediments

PDMS concn. (mg/kg; measured)	Survival at 28 d (%; $n = 4$)	Survival at 35 d (%; $n = 8$)	Survival at 42 d (%; $n = 8$)	Dry weight at 28 d (mg/individual; $n = 4$)	Dry weight at 42 d (mg/individual; $n = 8$)	Young per female
0	88 (2.5)	78 (3.7)	63 (6.2)	0.253 (0.033)	0.536 (0.031)	2.4 (0.53)
9	83 (3.5)	69 (4.8)	60 (3.8)	0.307 (0.066)	0.569 (0.042)	2.5 (0.80)
104	82 (3.7)	73 (4.5)	63 (4.9)	0.254 (0.024)	0.621 (0.064)	2.8 (0.70)
994	82 (3.9)	71 (4.4)	56 (4.2)	0.349 (0.011)	0.585 (0.057)	2.1 (0.48)

In summary, the present study corroborates and expands on previous research (Table 1) that indicates minimal toxicity of PDMS to sediment-dwelling organisms. The PDMS in sediments did not affect growth, survival, or reproduction of *C. tentans* or *H. azteca* during laboratory exposure to concentrations that greatly exceed those observed in the environment. Results of this study indicate that PDMS in the aquatic environment will not induce significant, adverse effects on *H. azteca* or *C. tentans* populations or, presumably, other benthic invertebrates.

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