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Bioaccumulation, Maternal Transfer, and Sublethal Effects of a PCB in the Sea Urchin, *Lytechinus pictus*

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ABSTRACT

The effects of maternal transfer of polychlorinated biphenyl (PCB) Congener 47 on sea urchin offspring were investigated and compared to the toxicity produced by exposure of sea urchin embryos to this PCB dissolved in seawater. Adult sea urchins were exposed to sediments spiked with the PCB Congener 47 (2,2',4,4'-tetrachlorobiphenyl) at concentrations of 0, 0.5, 5, and 20 mg/kg dry wt. The sea urchins were spawned after a 35-d exposure to the PCB, and the embryos were measured for bioaccumulation and toxic effects. Toxicity was also evaluated in adult sea urchins by measuring growth rate and gonadal somatic index (GSI, percent of weight which is gonad) at the different doses. Maternal transfer of Congener 47 was substantial, approximately 30% of the mass of PCB in adults was contained within eggs. No toxic effects were detected in the PCB-exposed embryos or adults. The results of this experiment indicate that sea urchin embryos exposed to PCB from maternal transfer are no more sensitive to Congener 47 than embryos exposed from the water.

INTRODUCTION

The PCB Congener 47 (2,2',4,4'-tetrachlorobiphenyl) has been shown to cause toxicity in developing sea urchin embryos when exposed directly through the water phase (Schweitzer *et al.* 1997). However, aqueous exposure is unlikely to be an important exposure mechanism because seawater concentrations of PCBs in the environment are typically lower than the levels that have produced effects in the laboratory. While embryo development for *L. pictus* is affected at approximately 23 µg/L PCB Congener 47 (Schweitzer and Bay 1997), maximum concentrations of total PCBs in the water column of the Southern California Bight are less than 0.01 µg/L (Tran and Zeng 1997).

A more likely exposure route for *L. pictus* embryos is through the transfer of contaminants from the mother during egg maturation, since adult sea urchins ingest contaminated sediments and can accumulate high concentrations of PCBs in gonad tissue. Hydrophobic contaminants such as PCBs and PAHs have been shown to cause early life stage mortality in fish from exposure by maternal transfer (Walker *et al.* 1994, Black *et al.* 1988, vonWesternhagen *et al.* 1981). Similarly, exposure of male fish to polycyclic aromatic hydrocarbon- (PAH-) and PCB-contaminated sediments has been shown to result in reduced numbers of hatched eggs, even when females are raised on clean sediments (Nagler and Cyr 1997). Effects of PCBs on sea urchins by maternal transfer have not been reported.

The main objective of this study was to determine whether the maternal transfer of PCB Congener 47 would result in toxicity in developing sea urchin embryos and if

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these embryos would display the same types of effects produced in embryos exposed directly through water at comparable body burdens. The effects of PCB exposure from maternal transfer may differ from those produced by water exposure because of differences in exposure timing. The PCB concentrations in embryos exposed by maternal transfer are highest at the onset of development, while embryos exposed from the water begin development with no contaminant load and accumulate higher levels with time. If the early stages of sea urchin embryo development are more sensitive to PCBs, then the maternal transfer of PCBs may produce toxicity at a lower body burden than that predicted from water exposure data. Congener 47 was chosen for this project because it was the most toxic of three congeners to *L. pictus* in previous water exposure tests.

A secondary objective of this study was to investigate the effects of Congener 47 on the adult sea urchins used in the maternal transfer experiment. In a previous study (Zeng *et al*. 1997), sea urchin growth was not affected following exposure to sediments spiked with a mixture of congeners. Since the present study used a congener shown to be toxic to sea urchins, we had the opportunity to improve our understanding of the relationship between PCB bioaccumulation and toxicity in adults.

METHODS

Sediment Handling and Exposure

Sediment and sea urchins were collected near Dana Point, California. Sediment was collected with a Van Veen grab sampler and sea urchins were collected with an otter trawl. Initial (background) concentrations of PCBs in the sediment or sea urchins were not measured, but prior research (Zeng *et al*. 1997) has demonstrated that animals from this site contain low levels of contaminants (<10 ng/g total PCB).

The sediment was stored in the refrigerator at 4°C. Approximately 16 L of sediment was sieved with a 1 mm mesh sieve to remove macrofauna. One week later, seawater (approximately 60% by volume) was added to facilitate stirring. The sediment mixture was gently stirred with an overhead stirrer and divided into four parts. Three of these parts were spiked with a mixture of unlabeled and radiolabeled (^{14}C) PCB Congener 47 (2,2',4,4'-tetrachlorobiphenyl) in 4.5 mL acetone to nominal PCB concentrations of 1, 10, and 50 µg PCB/g dry sediment. Each sediment had approximately the same amount of radioactivity (14 µCi). A control batch of sediment was spiked with an equivalent amount of acetone. The spike solution was slowly added to a continuously stirred batch of sediment over a period of 2 h, and then stirred an additional 2 h (for a total of 4 h of mixing).

The sediment was allowed to equilibrate for 10 d, at which time the excess overlying water was removed, samples were taken for PCB measurement, and the sediment was distributed into triplicate plastic exposure chambers (12" x 14" x 6" depth). Each chamber contained a 2 cm layer (approximately 1.5 L) of sediment covered by 5 cm of overlying water. Each chamber received filtered (0.45 µm pore size) natural seawater at a rate of 10 mL/min (equivalent to the addition of three times the overlying water volume per day). Measured concentrations of Congener 47 for the three treatments were 0.6, 7.4, and 22 mg/kg.

Sea urchins (*L. pictus*) were measured for wet weight and diameter and then added in groups of 10 to each exposure chamber. Diameter was determined using a computer image analysis system. The sea urchins were fed once every other day with a suspension of hatchfry encapsulon (Argent Chemical Laboratories, Redmond, WA) in seawater at a concentration of 0.018 g/mL, equivalent to 0.014 g per sea urchin. The exposure period was 35 d.

Bioaccumulation and Maternal Transfer of PCB

At the end of 35 d, all sea urchins were spawned using an injection of potassium chloride. Samples of egg and sperm were immediately processed for assessing effects on offspring, and adults were processed for assessing growth endpoints (see following sections). Next, the adult sea urchins were dissected and samples of gut and gonad were removed for PCB measurement.

The PCB concentrations were calculated from measurements of ^{14}C PCB using liquid scintillation counting (LSC). Tissue samples (gonad, embryos, or eggs) were prepared for LSC measurement by digesting the tissue with Scintigest tissue solubilizer (Fisher Scientific, Pittsburgh, PA), adjusting the pH with acetic acid, and adding Ecolite scintillation cocktail (ICN Biomedicals, Aurora, OH). Radioactivity was measured using a Wallac LKB 1214 Rackbeta scintillation counter equipped with quench correction. Calculations of the mass of Congener 47 in each sample were based upon the relationship between radioactivity and PCB mass in the stock solution used to spike the sediments.

The PCB concentrations were reported on a molar basis (mmol/kg wet weight), which was calculated by dividing the mass per unit weight (mg/kg) by the formula weight for Congener 47 (292). The total mass of PCB in each tissue type was calculated from the measured

concentration and total tissue weight in individual sea urchins. Biota sediment accumulation factors (BSAFs) were determined as the concentration of Congener 47 in the tissues normalized to lipid content divided by the concentration of Congener 47 in the sediment normalized to the organic carbon content (Boese *et al.* 1995). Lipids were extracted with 1:1 chloroform:methanol and were measured gravimetrically (Herbes and Allen 1983).

Effects on Offspring

The sperm from all males within an exposure chamber were pooled and used for fertilizing the eggs of individual females from that replicate, except for the control group in which all eggs from a replicate were pooled. Embryos from each female were reared separately in replicate glass vials (250 embryos per vial) containing 10 mL filtered seawater. Three methods were used to assess toxic effects in the embryos.

The first assessment method consisted of an examination for cytogenetic and cytologic effects using a method previously described by Hose (1985). This analysis was conducted on three replicate vials of embryos, which were preserved with buffered formalin at 48 h of development. At least 10 embryos per replicate vial were removed, squashed into a monolayer, stained, and examined with a light microscope (1,000x magnification) for cytologic abnormalities (degeneration of cell nucleus) and cytogenetic aberrations (micronucleus formation, anaphase aberrations). The number of cells per embryo undergoing mitosis (division) was also measured. The frequency of occurrence of cytologic and cytogenetic abnormalities was compared to samples of control embryos (unspiked sediment exposure) in order to determine if a PCB treatment effect was present.

The second assessment involved the microscopic examination of embryos preserved after 72 h for developmental abnormalities. The percent of embryos showing evidence of abnormal development (e.g. pre-hatch malformations, post-hatch malformations, skeletal abnormalities, gut abnormalities, and developmental retardation) was calculated. At least 100 embryos in 4 replicates of each exposure group were examined and compared to control samples.

Finally, the response of embryos to a reference toxicant was examined in order to evaluate the embryos' ability to tolerate additional contaminant stress. Control and PCB-exposed embryos were added to triplicate vials of seawater which had been spiked with copper to concentrations of 5.6, 18, 32, and 64 µg/L. The occurrence of developmental abnormalities after 72 h was measured. Changes in embryo sensitivity to copper were gauged by determining the median response concentration (EC50) for each PCB exposure group.

Leftover embryos were reared in 1 L beakers of seawater and evaluated for depuration of PCB over time. For this, aliquots of embryos were removed, placed into clean containers of seawater, counted under a microscope, and then measured for PCB content by LSC.

Adult Endpoints

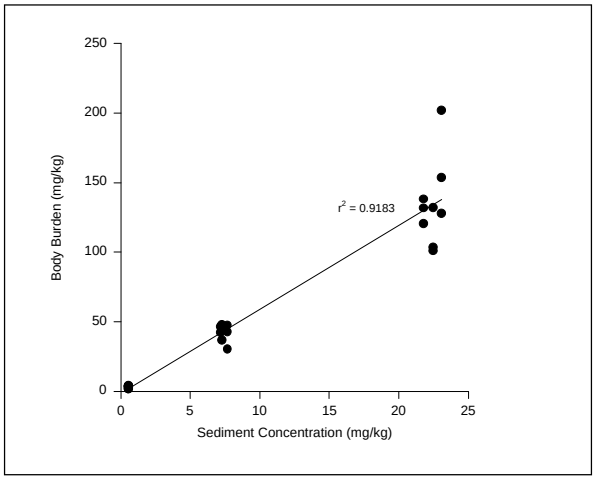
After spawning and before dissecting, all adult sea urchins were weighed and imaged as described previously for determining diameter. Upon dissection, each individual gonad was weighed. Data for individuals within a replicate were averaged. Adult endpoints assessed were growth (change in total weight and diameter) and GSI, calculated as gonad weight/whole organism weight times 100.

RESULTS

Bioaccumulation and Maternal Transfer of PCB

After 35 d of exposure, adult sea urchins accumulated PCB Congener 47 from the spiked sediments in proportion to the exposure concentration (Figure 1). Whole body (gonad + eggs + gut) concentrations ranged from 0.005 to 0.69 mmol/kg (1.5 to 202 mg/kg). The lipid and organic carbon-normalized BSAF for different tissue types varied significantly, with eggs showing the highest relative accumulation (Table 1). The eggs had a lipid content of 3% (solvent extractable), whereas gonad and gut tissue contained 4 and 6% lipid, respectively.

FIGURE 1. Accumulation of PCB Congener 47 in gonad of adult *Lytechinus pictus* from sediment.



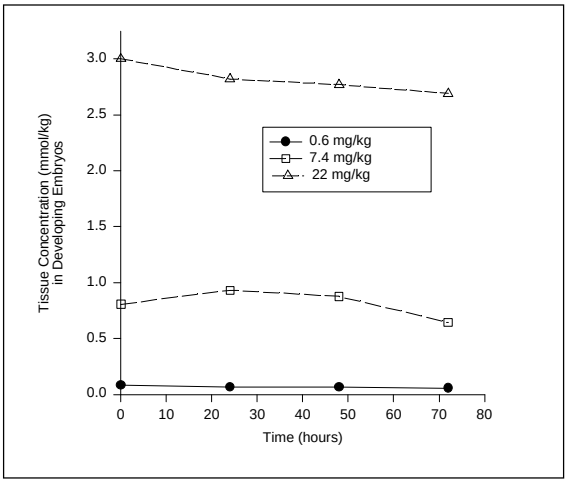
Gonad contained the largest mass of Congener 47, although the eggs had the highest concentrations (Table 1). An average of 29% of the total PCB contaminant load in the sea urchins was contained in the eggs and thus was lost during spawning. This loss

TABLE 1. Distribution of PCB Congener 47 by environmental compartment. The biota sediment accumulation factor (BSAF) is the lipid and TOC normalized accumulation factor and mass is the total amount of congener present in the tissue per individual *L. pictus*. Values for eggs and gonad are means of multiple individuals per replicate. Single composite samples of gut tissue were analyzed for each exposure level.

		Sea Urchin Tissue								
Sediment	Pore Water	Eggs			Gonad			Gut		
		Conc.	BSAF	Mass	Conc.	BSAF	Mass	Conc.	BSAF	Mass
mg/kg	mg/L	mg/kg		µg	mg/kg		µg	mg/kg		µg
0.6	0.01	16	10	0.16	4	1.6	1	0.0003	0.0001	0.002
7.4	0.03	215	13	2.60	61	2.8	9	0.003	0.0001	0.04
22	0.10	704	11	9.75	153	1.8	20	0.023	0.0002	0.40

equates to a transfer of contaminant to offspring. Depuration of Congener 47 occurred slowly during embryo development (Figure 2). Concentrations declined by 10 to 30% during the first 72 h of development.

FIGURE 2. Depuration of PCB Congener 47 from *Lytechinus pictus* embryos over time.



Effects on Offspring

Like gonad, Congener 47 body burdens in embryos increased in proportion to sediment concentration (Figure 3). A maximum mean embryo concentration of 3.4 mmol/kg was obtained from maternal transfer, which was lower than the concentration associated with toxicity (EC50) in prior water exposure experiments (Figure 3).

The embryos that had acquired their contaminant loads through maternal transfer did not have significantly higher percentages of developmental abnormalities than did control embryos reared in seawater. All groups had >90% normally developed embryos after 72 h of development.

Embryos from each PCB exposure group showed a similar response to the copper reference toxicant (Table 2). Although a trend was observed towards higher sensitivity to copper (i.e., lower EC50) with increased PCB exposure, no significant differences were found between groups (ANOVA, p=0.637).

The cytogenetic/cytologic analysis of the embryos exposed to the PCB via maternal transfer and then reared in clean seawater did not reveal any significant differences between exposure groups. The most common cytologic abnormalities in all *L. pictus* groups were dissolution and/or fragmentation of the cell nucleus (karyolysis and/or karyorrhexis, respectively). The control group had an average of 0.42 cytologic abnormalities per embryo (Table 2), which was similar to the overall mean of 0.33 cytologic abnormalities per embryo obtained when the data for all treatments (471 embryos) were combined. A low frequency of cytogenetic aberrations (micronucleus formation or abnormal chromosome

FIGURE 3. Accumulation of PCB Congener 47 in *Lytechinus pictus* eggs from maternal transfer. Also shown is the EC50 for developmental effects in water-borne exposures.

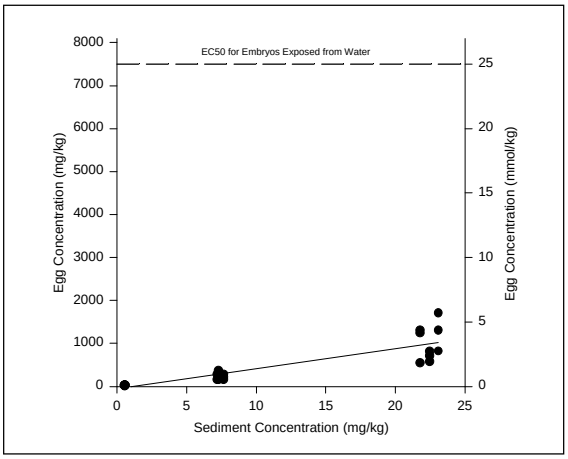


TABLE 2. Comparison of *L. pictus* embryo cytologic/mitotic abnormalities and sensitivity to copper between PCB exposure groups. Data are mean and standard deviation for three replicates, except for control cytologic and mitotic abnormalities (single composite analyzed).

	Mean (Standard Deviation)			
PCB Exposure Group (mg/kg)	Cytologic Abnormalities[a] #/Embryo	Anaphase Aberrations[b] % of Mitoses	Micronuclei % of Mitoses	Copper EC50 µg/L
Control	0.42	0.31	1.23	31.3 (7.1)
0.6	0.30 (0.20)	0.2 (0.29)	0	29.0 (4.5)
7.4	0.39 (0.15)	0.59 (0.82)	0.50 (0.62)	27.1 (7.2)
22	0.40 (0.07)	0.91 (0.68)	0.44 (0.52)	24.4 (3.3)

[a]Pycnosis, karyolysis, or karyorrhexis.
[b]Lagging/stray chromosome, acentric fragment, translocation/side-arm bridge, or unequal chromosome distribution.

arrangements during anaphase) was observed among the treatment groups (Table 2). A small (2-3x) increase in anaphase aberrations was observed in embryos exposed to the higher PCB concentrations, but the mean values were not significantly different between groups. No differences in mitotic rate were present between PCB exposure groups.

Effects on Adults

Sea urchins in all exposure groups increased in weight and diameter during the 35-d experiment (Table 3). Differences in growth between treatments were small and not related to dose level. No significant differences in growth were found between treatments.

Differences in GSI, a measure of reproductive state, were also small between groups. A trend was observed in the GSI data, with smaller relative gonad sizes being found in the highest exposure group, but no significant differences were noted relative to the control groups.

DISCUSSION

The results of the experiment show that maternal transfer has a significant effect on bioaccumulation of PCBs in sea urchins. Approximately 30% of the body burden of Congener 47 was transferred to offspring in a single spawning event.

The absence of developmental or cytologic/cytogenetic effects in this study is consistent with the results of prior studies with water-exposed embryos (Schweitzer and Bay 1997), which detected effects only when tissue concentrations higher than 13 mmol/kg were present. These results indicate that the higher exposure to PCBs in the first 24 h of embryo development did not produce a marked increase in toxic effects. Estimates of tissue PCB concentrations associated with toxicity that were obtained using water exposures appear to be appropriate for other exposure routes.

The lack of effects on sea urchin embryos from exposure to Congener 47 is contrary to predictions based upon the critical body residue model of narcosis (McCarty and Mackay 1993; Van Wezel and Opperhuizen 1995). Narcosis, a nonspecific reversible disturbance of the functioning of the membrane, is considered to represent a minimum level of toxicity exerted by any chemical. The critical body residue theory predicts that the narcotic effects of hydrophobic organic chemicals (including PCBs) should be essentially constant among aquatic organisms, producing death at tissue residues of 2-8 mmol/kg (40-160 mmol/kg lipid).

TABLE 3. Growth and gonadal somatic index (GSI) of *Lytechinus pictus* following 35 day exposure to PCB contaminated sediments.

	Mean (Standard Deviation)		
PCB Exposure Group (mg/kg)	Change in Weight (g)	Change in Diameter (mm)	GSI %
Control	0.15 (0.05)	0.65 (0.13)	3.56 (0.81)
0.6	0.20 (0.03)	0.36 (0.29)	3.59 (0.25)
7.4	0.25 (0.07)	0.46 (0.10)	3.47 (0.68)
22	0.17 (0.10)	0.57 (0.16)	3.11 (0.23)
ANOVA p value	0.38	0.26	0.78

Embryo PCB body burdens averaged 1.57 mmol/kg (52 mmol/kg lipid) for the highest exposure group in the present study, yet no toxicity was detected. A similar lack of toxicity was observed in previous experiments, where embryo development was unaffected at tissue concentrations of 100-200 mmol/kg of Congeners 77 and 153 (Schweitzer and Bay 1997). Certainly, if not lethal, these PCB residues should have manifested toxic effects such as inhibited development based upon the critical body residue theory.

Differences in the body burden causing toxicity among chemicals or species can be caused by many factors, such as differences in the mode of toxicity or the internal distribution of the toxicant. The PCBs cause toxicity through several mechanisms, producing differences in toxic potency of up to four orders of magnitude between congeners (Safe 1990). Differences in mode of toxicity cannot account for the disparity between the narcosis model predictions and the lack of effects on embryos, however, since the presence of a different mode of action would reduce the critical body burden needed to produce toxicity. It is more likely that the lack of agreement with the narcosis model is related to differences in test species. Most data used to refine the narcosis model were derived from studies of fish or adult invertebrates (Van Wezel and Opperhuizen 1995). Sea urchin embryos have a higher surface area:volume ratio and probably have a different lipid composition compared to fish. Variations in these characteristics may influence the distribution of PCBs within the cell, leading to a different relationship between the measured body burden and the concentration at the site of toxic action (membrane lipids).

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