Relative Toxicity of PCB Congeners to Sea Urchin Embryos

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ABSTRACT

The toxicity of three PCB congeners having different structural characteristics was studied using water exposures and the embryos of two sea urchin species, *Strongylocentrotus purpuratus* (purple sea urchin) and *Lytechinus pictus* (white sea urchin). Biological effects were assessed using two endpoints: percentage of normal embryo development and mitotic rate. Differential toxicity of the congeners was observed, with Congener 47 producing much stronger effects than Congeners 77 and 153 at comparable body burdens. The effects of PCBs on embryo development were similar in both species investigated. It was found that mitotic rate measurement was a more sensitive indicator of effects than was embryo development. The results of this study illustrate that different PCB congener structure activity relationships exist for various animal groups and/or endpoints. Widely used estimates of congener relative toxicity derived from mammalian research are not accurate for estimating effects on marine organisms due to the physiological differences of these two groups.

INTRODUCTION

Polychlorinated biphenyls (PCBs) persist in the environment and continue to cause concern, even though their use has been discontinued since 1977 in the United States (Smith and Johnston 1992). In the Southern California Bight, detectable levels of PCBs are present in sediments, with concentrations ranging from a few parts per billion (µg/kg) in reference areas (Thompson *et al.* 1987) to several parts per million (mg/kg) in highly contaminated locations (SCCWRP 1995). Sediment PCBs are bioavailable, as demonstrated by previous research that measured PCB bioaccumulation in local fish and invertebrates (SCCWRP 1994). Contaminated field sediments containing PCBs and other chemicals have been shown to be toxic in laboratory studies using sea urchins (SCCWRP 1995 and Thompson *et al.* 1989). Determining the relative influence of PCBs on sediment toxicity is hindered by several factors, including a poor understanding of the relative toxicity of individual PCB congeners.

Much of our understanding of the biological effects of these congeners is based on mammalian research. These studies show that the potency of different congeners can vary by several orders of magnitude (Safe 1990). Differences in congener potency also exist among animal groups, as shown by comparisons between fish and mammals (Walker and Peterson 1991).

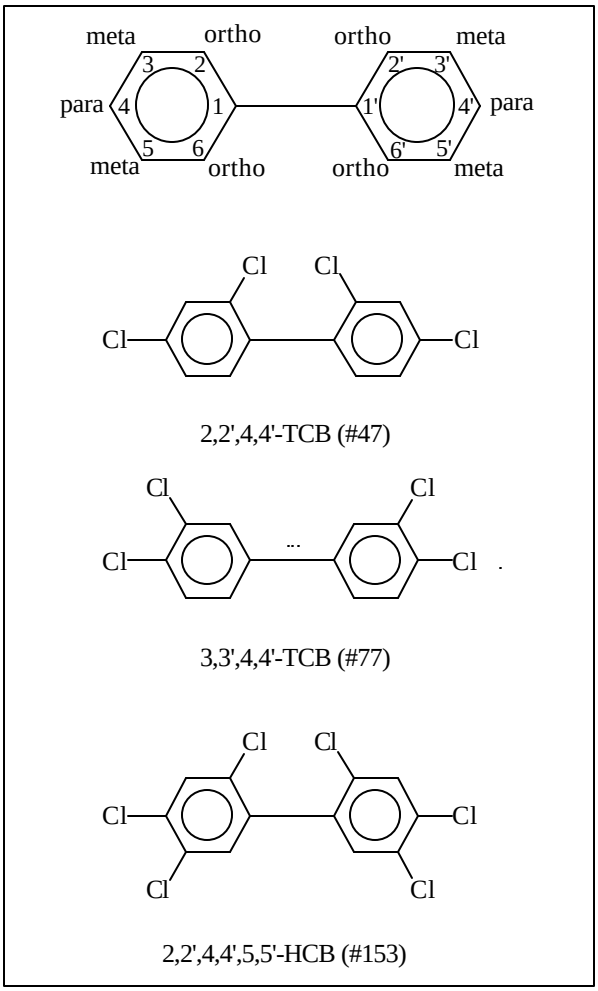
Mammals and marine invertebrates are substantially different in physiological composition, making the application of mammalian toxicity data to invertebrates inappropriate. A few studies have documented differential congener toxicity to invertebrates (Dillon and Burton 1991, Smith and Johnston 1992), but the lack of corresponding tissue chemistry measurements limits the usefulness of these data. Consequently, scientists trying to evaluate the risk of PCBs to marine life are often limited to expressing concentration data as total PCB or Aroclor equivalent concentrations (e.g., Long *et al.* 1995 and SCCWRP 1995). These methods, however, ignore potentially important variations in congener composition and toxicity. Improved data on the bioaccumulation patterns and toxicity of PCB congeners in invertebrates are needed to improve the ability to assess ecological risk.

In this study, the relative toxicity of individual PCB congeners to sea urchin embryos was determined. The results were compared with those predicted from mammalian studies. The long-term goal of this research effort is to understand the relative contribution of PCB toxicity to marine organisms so that we will improve our ability to assess risk to these organisms from environmental exposure to contaminants.

MATERIALS AND METHODS

The uptake, accumulation, and toxicity of three PCB congeners were examined using embryos of two local sea urchin species, *S. purpuratus* and *L. pictus*. Toxicity was measured by examining the embryos for abnormal development and the presence of cytogenetic alterations. The three congeners chosen for this study were 2,2',4,4',5,5'-hexa-chlorobiphenyl (IUPAC #153), 3,3',4,4'-tetrachlorobiphenyl (#77), and 2,2',4,4'-tetrachlorobiphenyl (#47).

FIGURE 1. Chemical structures of the polychlorinated biphenyls used for toxicity tests. Different PCB congeners arise from varying levels of chlorine substitution at the ortho, meta, and para positions.



These congeners were chosen for their different structural characteristics, environmental significance, and availability as radioisotopes. Congeners 77 and 47 each contain four chlorines but have different substitution patterns (Figure 1). Congeners 153 and 47 have different levels of chlorination but a similar substitution pattern, and are predicted to have less biological activity than the coplanar configuration of Congener 77.

Exposure

Developing sea urchin embryos were exposed to each of the three PCB congeners over a 72-h period at 15 °C (Chapman *et al*. 1995). Sea urchin embryos were obtained by spawning field-collected individuals that had been held in the laboratory for less than one yr. Experiments were conducted with ^{14}C radiolabeled congeners. Each PCB was first dissolved into filtered seawater (using acetone as a carrier) at several different concentrations and placed in exposure vials. All glassware was pretreated overnight with seawater spiked with the target concentration of PCB in order to mitigate PCB losses from adsorption onto glass. At least five replicate exposure vials were prepared for each test concentration. Each day, about 50% of the test solution was replaced. A glass syringe with a 20 µm Nitex screen affixed to its tip (to exclude embryos) was used to remove water from the vials. At the end of exposure, sea urchin embryos were either removed from some replicates for bioaccumulation measurement or preserved for evaluation of toxic effects.

Bioaccumulation

Embryo PCB concentrations were measured in two or three replicates at each exposure concentration. Embryos were immobilized with 0.1 mM chromium chloride, then removed from the bottom of the container by pipette with minimal water and placed in a clean vial. Residual water was removed with the glass syringe/Nitex screen apparatus. Embryos were washed with seawater until PCBs were undetectable in the wash. Embryos were counted under a microscope and transferred to a plastic or glass scintillation vial. Ecolite® scintillation fluid (ICN Biomedicals, Aurora, Ohio) was added to embryo samples, water aliquots, and empty exposure containers. Radioactivity was measured with a Wallac LKB 1214 Rackbeta scintillation counter (Wallac-EG&G Co., Gaithersburg, Maryland) equipped with automatic quench-correction.

Tissue concentrations were based on egg wet weight, which was determined gravimetrically on several representative samples. Bioconcentration factors (BCFs) for the three PCB congeners in sea urchin tissues were calculated as the concentration (µg/kg) of PCB in the tissues divided by the concentration in the water (µg/L). Water concentrations were also measured by liquid scintillation counting. A distinction was not made between total water concentration and the concentration of the truly dissolved phase. Therefore, water concentrations sometimes exceeded published solubility limits.

Developmental Effects

Toxicity tests included a seawater control and a carrier control (equivalent concentration of acetone in clean seawater). A reference toxicant test with copper chloride was also conducted simultaneously to monitor changes in test performance among experiments.

Sea urchin embryos (about 250 per vial) from three or four replicates were preserved with buffered formalin after 72 h and evaluated microscopically to determine developmental effects. Five types of embryo abnormalities were recorded: pre-hatch malformations, post-hatch malformations, skeletal abnormalities, gut abnormalities, and developmental retardation.

Cytogenetic Effects

Cytogenetic evaluation of the embryos (Hose 1985) was used to better define mechanisms of PCB toxicity and to provide a more sensitive endpoint of toxicity. Following examination of the preserved embryos for developmental effects, a subsample of embryos (about 30) was removed, squashed into a monolayer, and stained with an aceto-orcein to make the chromosomes visible. The preparations were examined with a light microscope (1,000x magnification) to determine the number of anaphase aberrations and mitotic activity. Embryos were examined in three or four replicates from each exposure level.

Anaphase aberrations are abnormalities that occur in chromosome configurations when the chromosomes divide and migrate to opposite poles within the cell during mitosis. For example, chromosomes may divide unequally, or fragments of chromatin may become detached from the rest of the chromosomes. Micronuclei formation can occur when detached fragments form a miniature (secondary) nucleus. All cells were examined for each sea urchin embryo analyzed, and the total number of anaphase aberrations found per embryo was determined. Mitotic activity was calculated as the average number of mitotic figures (prophase through early telophase stages) per embryo.

Congener toxicity was determined by calculating EC50s for both developmental and cytogenetic endpoints. The EC50s were calculated by either probit analysis or nonlinear interpolation.

RESULTS

Bioaccumulation

Extensive uptake of PCBs into the developing sea urchin embryos occurred during the 72-h exposures. Exposure of *S. purpuratus* embryos to Congener 77 produced tissue concentrations of 7.0 to 72 mmol/kg (equivalent to 2,044 to 21,024 mg/kg). Similar tissue concentrations were produced in embryos exposed to each congener, although different exposure (water) concentrations were used.

Embryo tissues contained PCB concentrations that were 4 to 5 orders of magnitude greater than water levels, producing BCFs ranging from 51,000 to 186,000 (Table 1). In both sea urchin species, Congener 77 had a lower bioconcentration factor than Congeners 47 and 153. BCF estimates were variable among test concentrations, resulting in coefficients of variation that ranged from 21 to 65%. The variability did not follow a consistent pattern with exposure concentration.

TABLE 1. Bioconcentration factors determined from sea urchin embryo toxicity tests of PCB congeners.

	Average BCF		Octanol-Water Partition Coefficient
Congener	*S. purpuratus* (%CV)	*L. pictus* (%CV)	Log K_{ow}
47	186,000 (29%)	81,000 (39%)	5.3[a]
77	51,000 (29%)	42,000 (65%)	6.1[b]
153	87,000 (21%)	137,000 (41%)	6.4[a]

[a]Girvin *et al.* 1990.
[b]Clark *et al.* 1990.

Developmental Effects

Exposure to Congener 47 produced toxic effects on embryo development in both sea urchin species (Figures 2 and 3). Nearly all affected embryos in the Congener 47 test group hatched, but did not undergo gastrulation. Most of the affected embryos consisted of an irregular mass of lysed or undifferentiated cells. Other types of malformations (e.g., pre-hatch malformations, skeletal abnormalities, gut abnormalities, and developmental retardation) were infrequently observed.

Embryo development was not significantly affected by exposure to Congeners 77 and 153. This pattern was observed even at tissue burdens 2 to 4 times greater than concentration of Congener 47, which caused nearly 100 % abnormal development. The percentage of normal embryo development in *L. pictus* embryos exposed to Congener 153 was 20 to 30% lower (not statistically significant) than the control (Figure 3). This response was not dose-dependent and appeared to be the result of difficulties encountered during water changes. The screen covering the syringe used for water became clogged during the experiment, requiring the use of greater suction to remove the water. This situation may have caused physical damage to the PCB-exposed embryos. A separate syringe apparatus, which did not become clogged, was used for the control group.

Cytogenetic Effects

For both *S. purpuratus* and *L. pictus*, cytogenetic aberrations were found in less than 10 percent of the embryos at the highest dose examined. Micronucleus formation was the most commonly encountered aberra-

tion, with the exposed and control samples reporting the same rate of occurrence.

Reduced mitotic activity was present in *S. purpuratus* embryos exposed to Congeners 77 and 153 (Figure 4). Control embryos contained on average about 7.2 mitoses per embryo while mitotic activity in the highest exposure groups declined to 0.8 (Congener 77) or 2.2 (Congener 153). Mitotic activity rates were highly variable within some groups, but all exposure groups were significantly different from the control groups.

The endpoint of mitotic activity could not be evaluated for *S. purpuratus* embryos exposed to Congener 47. Satisfactory cell preparations were obtained from only the two lowest Congener 47 exposure groups. These low-dosed embyros had mitotic rates similar to the control groups. The cells in the embryos of the higher exposure groups were either too damaged to evaluate, or they disintegrated during slide preparation.

Evaluation of mitotic activity changes was also not successful in experiments with *L. pictus*. Control embryos contained an abnormally low number of mitoses (3 to 4 per embryo), greatly reducing the ability to detect effects resulting from PCB exposure.

Relative Toxicity of PCB Congeners

Comparisons of relative toxicity are complicated by the lack of precise EC50s for some of the congener-species combinations. The most complete data are available for embryo development (Table 2). Embryo development EC50s for Congeners 77 and 153 can only be reported as a lower limit because no dose response was obtained at the highest exposures. No estimate of the mitotic activity EC50 is provided for *S. purpuratus* exposed to Congener 47 because we were unable to evaluate any of the exposed embryos for this endpoint.

FIGURE 2. Dose-response relationship for *S. purpuratus* where response is measured as percent normal embryo development. Data are normalized to the control group. All test groups which were statistically different (ANOVA, Dunnett's Test, p<0.05) from the controls are noted with an asterisk. Error bars are standard error of the mean.

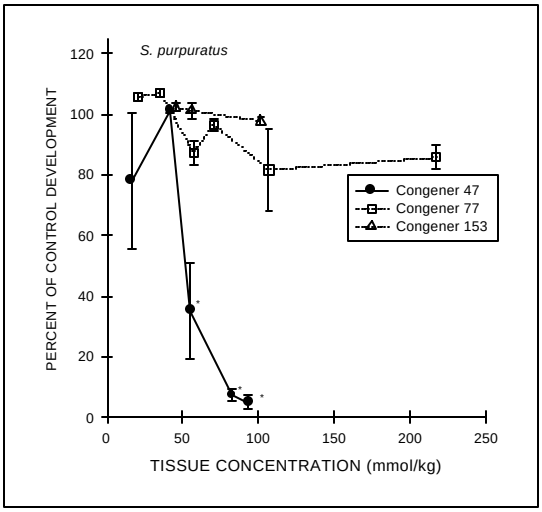
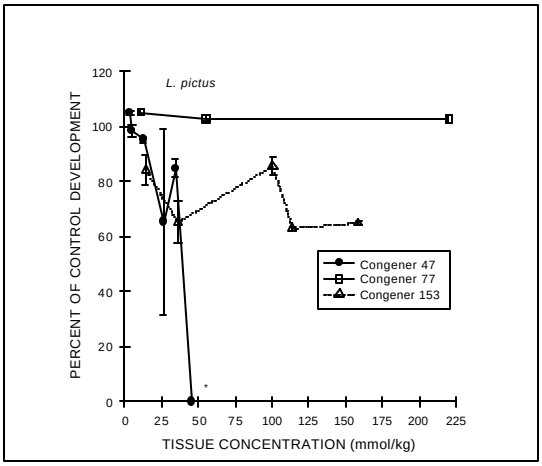


FIGURE 3. Dose-response relationship for *L. pictus* where response is measured as percent normal embryo development. Data are normalized to the control group. The test group which was statistically different (ANOVA, Dunnett's Test, p<0.05) from the controls is noted with an asterisk. Error bars are standard error of the mean.



The EC50s for Congener 47 effects on embryo development were slightly different between species, ranging from 47 mmol/kg (*S. purpuratus*) to 25 mmol/kg (*L. pictus*). Embryos from both sea urchin species were similar in being much less sensitive to Congeners 77 and 153.

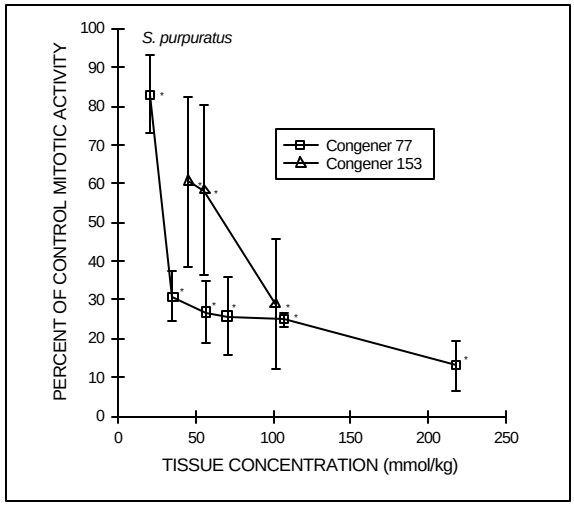
The mitotic activity endpoint did provide discrimination between Congeners 77 and 153, however (Table 2). This analysis indicated Congener 77 to be significantly more toxic to *S. purpuratus* embryos than Congener 153.

DISCUSSION

The relative toxicity of the three congeners was different between sea urchin embryos and mammals. Relative toxicity data for mammals (expressed as toxic equivalency factors: (TEF)) were compared to similarly scaled embryo toxicity data (inverse of the EC50) as shown in Table 2. The TEF data from the literature identified Congener 77 as being three orders of magnitude more potent than Congeners 47 and 153. The sea urchin results indicate that Congener 47 is more potent by at least a factor of five. The rank order of toxicity in this study therefore contradicts the predicted structure-activity relationships (SARs) based on mammalian data.

The TEFs for mammals are calculated as a ratio of the potency of the individual congener to the potency of a reference chemical, 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD), which has a similar structure to PCBs. In vertebrates, the toxicity of TCDD and PCBs is based on a common mechanism. These chemicals bind to the aryl hydrocarbon (Ah) receptor, inducing several P450 enzymes that elicit a

FIGURE 4. Dose-response relationship for *S. purpuratus* where response is measured as mitotic activity. Data are normalized to the control group. All test groups which were statistically different from the controls (ANOVA, Dunnett's or Dunn's Test, p<0.05) are noted with an asterisk. Error bars are standard error of the mean.



wide array of effects (Davis and Safe 1990, Safe 1991, Ganey *et al.* 1993, Sericano *et al.* 1994). Congener structure affects the binding affinity for this receptor and thus the ability to induce P450 enzymes and cause toxic effects. Congeners lacking chlorine substitution in the ortho position (termed coplanar), but with substitution in meta/para positions, are predicted to be the most toxic.

While evidence suggests that marine invertebrates have the Ah receptor (Porte and Albaiges 1993, Porte *et al.* 1991, Livingstone 1988), it is not clear whether the expression of toxic effects in invertebrates bears the same relationships with PCB structure that have been developed for mammals. Furthermore, alternative mechanisms of PCB toxicity may exist that are unrelated to the binding of this receptor (Smith and Johnston 1992). For these reasons, it was not surprising that the rank order of toxicity of the PCB congeners to developing sea urchin embryos in this study were different than what was predicted from the mammalian data.

The BCFs determined in this study did not fit predicted SAR models where PCB congeners with the highest degree of chlorination or highest octanol-water partition coefficient are predicted to have the highest bioconcentration factors. This pattern was only found for Congener 153 in *L. pictus*. However, physical damage (Congener 153, *L. pictus*) or toxicity (Congener 47) to embryos can alter uptake and bioaccumulation. In addition, inaccuracies in the BCFs may have been caused by uncertainty in the water concentration measurements. In this study, the total water concentration was measured, but presumably only the freely dissolved fraction is bioavailable.

Estimates of relative toxicity (i.e., TEF) and bioaccumulation are important components of risk assessment calculations. In human health risk assessments, the TEF for an individual congener is multiplied by the tissue concentration of the congener to give a toxic equivalent quantity (TEQ) (Safe 1990). The TEQs for all the individual PCB congeners are then summed up and the total represents the measure of risk to the consumer. The research described in this report indicates that existing TEF values are not sufficiently accurate to enable use of the TEQ approach to evaluate PCB risk to marine organisms.

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TABLE 2. Summary of toxicity of PCB Congeners 47,77, and 153 to sea urchin embryos. Relative toxicity for sea urchins was calculated as the inverse of the EC50.

	EC50 (mmolkg)		Relative Toxicity		
Congener	Embryo Development (95% CI)	Mitotic Activity (95% CI)	Embryo Development	Mitotic Activity	Mammals[a]
47					
S. purpuratus	47 (42-55)	-	0.021	0	0.00002
L. pictus	25 (9-31)	-	0.040	-	
77					
S. purpuratus	>218	30 (21-35)	<0.005	0.034	0.01
L. pictus	>147	-	<0.007	-	
153					
S. purpuratus	>102	67 (58-79)	<0.010	0.015	0.00002
L. pictus	>106	-	<0.009	-	

[a]TEF values from Safe 1990.

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