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Acute Toxicity of PCB Congeners to *Daphnia magna* and *Pimephales promelas*

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Commercial mixtures of polychlorinated biphenyls (PCBs) are composed of individual molecules called congeners. They differ only in the number and position of chlorine atoms on the biphenyl molecule. McFarland and Clarke (1989) identified 36 individual PCB congeners as toxicologically important. Their prioritization was based on environmental occurrence as well as the ability of the congener to induce mixed-function oxidase (MFO) enzymes. The MFO system is involved in a variety of metabolic processes including the synthesis of gonadotropic hormones. Induction of the MFO system by chlorinated contaminants is associated with pathological conditions in mammals such as body weight loss, thymic atrophy, enlarged "fatty" liver, cellular hyperplasia and immunosuppression (Parkinson and Safe 1981).

The acute toxicity (EC50/LC50) of commercial PCB mixtures has been reported to range from 2.0 to 283 ug/L (Mayer et al. 1977). Because PCBs are very hydrophobic most biological studies have utilized a carrier solvent to facilitate introduction of PCBs into aqueous solution. As a result, biological effects are often reported at exposure concentrations exceeding water solubility (USEPA 1980). The purpose of this work was to evaluate the comparative toxicity of selected PCB congeners without carrier solvents. These tests were conducted on early life stages of two sensitive freshwater organisms, *Daphnia magna* and *Pimephales promelas*.

MATERIALS AND METHODS

A variety of PCB congeners were evaluated in these experiments (Table 1). There was at least one PCB from each isomer group from trichlorobiphenyls through octachlorobiphenyls. The three major MFO induction types are represented; phenobarbital (PB), 3-methylcholanthrene (3-MC) and mixed (Chambers and Yarbrough 1976). PCBs considered to have no MFO inducing capabilities were also evaluated. Finally, toxicologically important congeners from the first three priority groupings identified by McFarland and

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Table 1. PCB congeners selected for evaluating acute toxicity.

IUPAC Number	Structure	Environmental Occurrence[a]	MFO Activity[ab]	Toxicological Priority[a]
18	2,2',5	Frequent	IA	3rd
28	2,4,4'	Infrequent	IA	3rd
52	2,2',5,5'	Frequent	weak PB	3rd
77	3,3',4,4'	Frequent	3-MC	1st
101	2,2',4,5,5'	Frequent	PB	2nd
116	2,3,4,5,6	Infrequent	IA	NP
128	2,2',3,3',4,4'	Frequent	Mixed	1st
153	2,2',4,4',5,5'	Frequent	PB	2nd
171	2,2',3,3',4,4',5	Infrequent	PB*	NP
194	2,2',3,3',4,4',5,5'	Frequent	PB	2nd

[a]From McFarland and Clarke 1989
[b]MFO = Mixed-Function Oxidase
IA = Inactive
3-MC = 3-methylcholanthrene-type
PB = phenobarbital-type
PB* = phenobarbital-type theoretical
Mixed = characteristics of both PB-type and 3-MC-type induction
NP = not prioritized

Clarke (1989) were tested. PCB nomenclature follows that proposed by Ballschmiter and Zell (1980) and later adopted by the International Union of Pure and Applied Chemists (IUPAC).PCB congener standards (99% purity) were purchased from Ultra Scientific Laboratories (Hope, Rhode Island). Aqueous solutions of individual PCBs were prepared using a modification of the generator column procedure described by Veith and Comstock (1975). This approach has been used by others in preparing dosing stocks of hydrophobic compounds for biological tests (Shiu et al. 1988; Black and McCarthy 1988). Three millimeter glass beads were coated via roto-evaporation with an amount excess to that required to achieve nominal water solubility in the total volume of test solution needed for each congener. Dechlorinated tap water was then recirculated for 96 hr through a 20-cm stainless steel column filled with 11.5 g of PCB-coated beads. The resultant solution is operationally defined as 100% saturated.

Toxicity tests for *Daphnia magna* neonates and *Pimephales promelas* larvae were performed according to the U.S. Environmental Protection Agency (USEPA) methods for determining the acute toxicity of effluents to freshwater organisms (Peltier and Weber 1985). Briefly, 10 *Daphnia* neonates (<24 hr old) were placed in 100-mL beakers containing 80 mL of solution. For *Pimephales*, 10 larvae (<24 h old) were placed in 1000-mL beakers containing 500 mL of solution. There were 3 replicate beakers per treatment for both species. All beakers were thoroughly rinsed with the appropriate concentration before a test was begun to minimize loss of PCBs to the glass surface. Tests were static with no renewal as per Peltier and Weber (1985). Temperature was maintained at 21°C and 25°C for *Daphnia* and *Pimephales*, respectively. Food was withheld during all exposures. Dead organisms were removed every 24 hr. *Daphnia* were exposed for a total of 48 hr and fish for 96 hr. Water quality characteristics were determined at the beginning of each test and ranged from 7.6-8.3 for pH, 92-108 for alkalinity, 118-158 mg/L as $CaCO_3$ for hardness and 7.6-8.6 mg/L for dissolved oxygen.

Because minimal acute toxicity was observed under static conditions, additional tests were carried out using static-renewal procedures. Test species were transferred to new containers containing PCB solutions at the same time each day. In addition, all beakers were covered with aluminum foil to minimize volatilization. Otherwise static-renewal procedures were identical to those used in the static toxicity tests.

Aliquots of the initial exposure media from the static tests were analyzed for PCBs. Water samples were extracted twice with hexane, combined, dried over Na_2SO_4 and evaporated to about 0.5 mL. Volume was brought back up to 2 mL with hexane. Extracts were analyzed on a Hewlett-Packard GC equipped with a 30 m DB-5 fused capillary column and EC detector. Samples were not replicated. Percent recovery ranged from 94% to 120% for all congeners. Detection limit was 0.05 ug/L for all congeners except numbers 77 (0.07 ug/L) and 128 (0.01 ug/L).

Effects on percent survival was evaluated using one-way analysis of variance (SAS 1985). Mean separation was achieved by the Waller-Duncan k-ratio t test. All differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Mean percent survival of *Daphnia magna* exposed for 48 h under static conditions to 9 PCB congeners (28, 52, 77, 101, 116, 128, 153, 171 and 194) was high and ranged from 97% to 100% (Fig 1). However, *Daphnia* survival was significantly lower (53%) in the 100% saturated solution of the lowest chlorinated PCB tested; congener 18. A similar pattern was observed in static tests with *Pimephales promelas* fry (Fig 1). Fish survival was high (93%-100%) in all PCB treatments except congener 18 where only 17% of the animals were alive after 96h in the 100% saturated solution. Results of the static-renewal tests were not appreciably different from the static tests (Fig 1). Survival was very high for both *Daphnia* and *Pimephales* exposed to all PCBs except congener 18. Fathead minnow fry appear to be slightly more sensitive to congener 18 than *Daphnia* neonates. Control survival was high (97%-100%) for both species in all tests.

Except for congener 194, detectable amounts of all PCBs were found in the exposure water from the static tests (Table 2). Concentra-

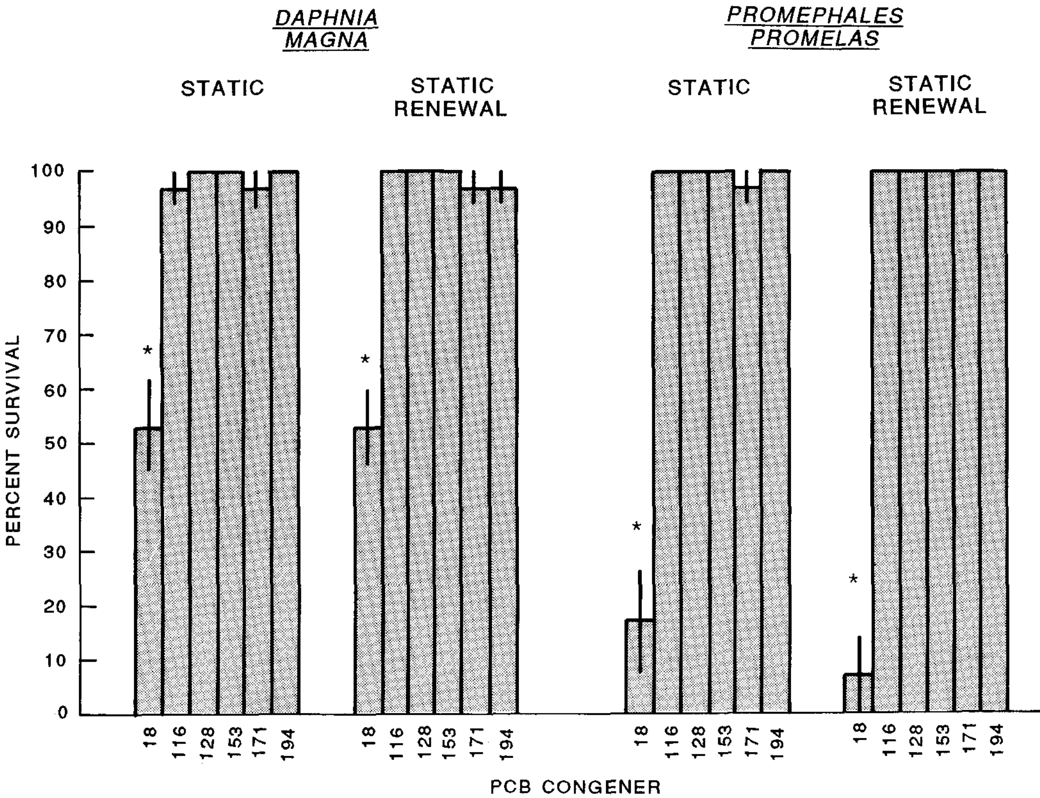

Figure 1. Mean percent survival of *Daphnia magna* neonates and *Pimephales promelas* fry exposed to 100% saturated aqueous solutions of individual PCB congeners under static and static-renewal conditions. vertical bars = SE, n = 3, star (*) = significantly different from controls. Control survival was 97% to 100%.

tion of congener 18, the only PCB causing acute toxicity, was an order of magnitude greater than that observed in other treatments.

Table 2. Initial concentration (ug/L) of PCB congeners in exposure media from static toxicity tests. sl = sample lost.

	PCB Congeners									
Treatment	18	28	52	77	101	116	128	153	171	194
Exposed	86	1.5	sl	0.3	1.2	2.8	0.4	1.3	1.7	<dl
Controls	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl

While these water concentrations appear low, they are much higher than found in the highly contaminated waterways. For example, concentrations of congener 18, the only acutely toxic PCB, range from 70 pg/L (Oliver and Niimi 1988) to 2-14 ng/L (Bush et al. 1985). Because environmental concentrations are several orders of magnitude lower than those observed in these laboratory tests the potential toxicological risk to water column organisms in the field is relatively low.

The concentration of PCBs produced by the column generator method were at or below the range of published solubilities for individual PCB congeners (Table 3). These concentrations are lower presumably due to manipulation of test solutions while setting up the bioassays. Similar results have been reported by other investigators using the same generator column technology (May et al. 1978; Billington et al. 1988; Black and McCarthy 1988). Direct evaluation of the efficiency of the column generator methods is hampered by the variability in published solubility values which may differ by up to an order of magnitude (Table 3). This variability is due to wide methodological differences in techniques used to generate solubility data. For example, some investigators shake aqueous mixtures of hydrophobic compounds while others stir vigorously. Equilibration times may range from a few hours to many weeks. Unfiltered particles or colloidal dispersions may exist in solution. And analytical chemistry methods differ among laboratories.

Table 3. Aqueous solubilities of PCB congeners reported in summaries by Shiu and Mackay (1986) and Opperhuizen et al. (1988).

Solubility (ug/L)									
18	28	52	77	101	116	128	153	171	194
61	67	6	0.6	4.2	5.5	0.1	0.9	2.2	0.1
135	85	15	0.8	4.2	6.5	0.3	1.0	6.2	0.3
248	148	18	1.8	10	6.8	0.4	1.1		0.7
407	260	22	3.0	10	6.8	0.4	1.2		1.5
640	266	26	11	12	9.0	1.0	1.3		2.7
		27	17	13	14.0	9.9	2.8		3.0
		36	175	15	21.0		5.0		
		41		16			8.8		
		46		19			10.0		
		74		31					

Of the ten PCB congeners evaluated in these experiments, only one (number 18) was acutely toxic to *Daphnia magna* neonates and *Pimephales promelas* fry. Comparable data are only available for studies which have utilized carrier solvents. Mayer et al. (1977) determined LC50s for five PCBs (8, 15, 22, 155, 101) using the amphipod *Gammarus pseudolimnaeus*. The LC50s ranged from 70 ug/L to

210 ug/L. Except for the lowest LC50, all concentrations exceed the aqueous solubility for individual congeners. Dill et al. (1982) exposed *Daphnia magna* to congeners 1, 2, 3 and 47 and reported 24-h LC50s of 710 ug/L , 430 ug/L, 420 ug/L and 30 ug/L, respectively. While these concentrations are all less than water saturation the comparative value of the toxicity data is limited since survival in the control treatment was not reported.

Other than congener 18, survival was high in both static or static-renewal exposures to all PCB congeners. There are several possible reasons why these PCBs were not acutely toxic. The first possibility, insensitive test species, can be discounted. *Daphnia* neonates and fathead minnow fry were selected for USEPA's effluent testing program precisely because they are sensitive test species (Peltier and Weber 1985).

A second possibility is that the most toxic congeners were not evaluated in this study. Although that remains a possibility, we selected congeners to represent a variety of PCBs including those considered toxicologically important (Table 1). For example, congener 77 was tested specifically because it is one of only three PCB congeners (77, 126, 169) which are pure 3-MC-type inducers. McFarland and Clarke (1989) considered these congeners to be the most toxicologically important PCBs. Based on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents, these highly coplanar 3-MC-type inducers are believed to be the most toxic constituents in commercial PCB mixtures; even more so than polychlorinated dibenzofurans (Safe 1987; Kannan et al. 1988). Therefore, the lack of any toxicity from congener 77 on early life stages of two sensitive freshwater species is particularly puzzling.

The only congener producing significant mortalities (number 18) was one of the three "inactive" congeners tested (Table 1). Abernethy et al. (1986) have shown that acute toxicity of a wide variety of aromatic and chlorinated hydrocarbons to *Daphnia magna* is a simple function of aqueous solubility. As the solubility increased so does the acute toxicity. Other factors such as molecular weight and the degree or position of chlorination had no demonstrable effect. Results presented here tend to support the findings of Abernethy et al. (1986). Additional toxicity tests conducted without carrier solvents are needed to determine whether this is a general phenomenon for low molecular weight PCBs or is something unique to congener 18.

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