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Toxicity of PCBs (Aroclor-1221, 1254) to Embryos and Larvae of *Xenopus laevis*

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Polychlorinated biphenyls (PCBs), as persistent organic pollutants (POPs), are global environmental contaminants. Although their manufacture was banned about 30 years ago in most countries, PCBs are routinely detectable pollutants in air, water, sediments, fish, wildlife, and human tissues. The toxic and biochemical effects of various commercial PCB mixtures have been extensively investigated in various laboratory animals, fish, and wildlife species (Safe 1994). It was reported that they can alter endocrine, immune, and nervous system functions and cause adverse effects on reproduction and development of animals including humans. The lethal toxicity of PCBs varies with the PCB formulation, the organism species and stage of development, and the test conditions employed. Toxic effects on embryos and larvae of fish exposed to PCBs are equivocal. PCBs and other chemical pollutants are also suspected to be one of the causes for the increasing malformation and population decline of amphibians worldwide in the last 20 years (Ouellet et al. 1997). However, only limited data are available concerning the effects of PCBs on amphibians (Gutleb et al. 1999; Rosenshield et al. 1999; Jelaso et al. 2002; Fisher et al. 2003) and the knowledge of effects of PCBs on the embryos and larvae of amphibians is still scarce.

The main objective of the present study was to investigate the developmental toxicity of two commercial PCB mixtures-Aroclor 1221 (A-1221) and Aroclor 1254 (A-1254) using the Frog Embryo Teratogenesis Assay —*Xenopus* (FETAX) (ASTM 1998), a standardized and well-validated developmental toxicity model. In addition, the acute toxicity of these two commercial PCB mixtures on *Xenopus laevis* tadpoles was evaluated.

MATERIALS AND METHODS

Adult *X. laevis*, obtained from the Institute of Developmental Biology of the Chinese Academy Sciences, were maintained separately in dechlorinated tap water at 22±2°C with a 12 hr light: 12 hr dark cycle, and were fed on ground swine liver twice a week. Breeding was induced by injection of human chorionic gonadotropin subcutaneously.

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FETAX testing was conducted according to ASTM E1439-98 (ASTM 1998). Briefly, 25 normally cleaving embryos (stage 8~11 of Nieuwkoop and Faber, 1956) were placed in 60-mm glass dishes with 10 mL FETAX medium, which is composed of 625 mg NaCl, 96 mg $NaHCO_3$, 30 mg KCl, 15 mg $CaCl_2$, 60 mg $CaSO_4 \cdot 2H_2O$, and 75 mg $MgSO_4$ per liter of distilled water (Dawson and Bantle 1987). Each experiment included four dishes for the control group and two for each test group. The two commercial PCB mixtures, A-1221 and A-1254, were purchased from the Supelco Company U.S.A. They were added to FETAX medium using DMSO as a solvent, resulting in nominal concentrations from 10 μg/L to 10 mg/L. The final concentration of DMSO in FETAX medium was 1/10,000 (v/v). During the FETAX assay, the temperature was maintained at 23±1°C, the solutions were changed every 24 hr and dead embryos were removed and counted simultaneously. The number of hatched embryos was recorded at 48 hr postfertilization. At 96 hr, surviving tadpoles were counted, fixed in 3% formalin (v/v), and scored for developmental malformations and head-to-tail lengths using a dissecting microscope. Each experiment was repeated three times with tadpoles obtained from different spawnings.

For the acute larval toxicity test, five-day postfertilization tadpoles (stage 46~47) were exposed to either 1.0, 1.5, 2.0, 2.5, or 3.0 mg/L of A-1221 and to either 0.10, 0.25, 1.0, 5.0, or 10.0 mg/L of A-1254 in 2-L glass beakers containing 1-L FETAX medium for 96 hr. A static renewal system was used and the medium was renewed every 24 hr. Each group contained 20 tadpoles. The final concentration of carrier solvent, ethanol, was less than 1/10,000 (v/v). During the time of exposure, tadpoles were not fed. Tadpoles were examined for death and malformation at 24, 48, 72 and 96 hr, and the dead tadpoles were removed simultaneously. Each exposure was repeated three times with tadpoles obtained from different spawnings. The actual exposure concentrations and tissue sample residues in the FETAX assay and acute larval toxicity test were not measured.

Student's *t*-test was used to compare the significant ($p<0.05$) difference between treated and control groups.

RESULTS AND DISCUSSION

No significant differences ($p>0.05$) were found in hatching success, mortality, malformation and body size in all groups in the FETAX assay except that the survival of tadpoles exposed to 10 mg/L A-1221 was only 4.7% and the tadpoles at 10 mg/L A-1254 showed depigmentation. The average percentages of hatchability, survival and malformation are shown in Table 1. Because the concentration of 10 mg/L is much higher than their water solubility, we did not perform further FETAX testing. This result was similar to that of Gutleb et al. (1999), who reported A-1254 and PCB 126 have no significant effects on the mortality, malformation, and development of *X. laevis* in FETAX assay. It was reported that PCBs might reduce egg hatchability in lake trout and the double-crested cormorant in field studies (Mac and Swartz 1992; Tillitt et al. 1992). However, opposite results were also reported. Rosenshield et al (1999)

Table 1. Average percentages of hatching, survival and malformation (mean±SEM) of *X. laevis* embryos exposed to A-1221 and A-1254 at 96 hr in FETAX assay.

Groups	Hatchability (%)	Survival (%)	Malformation (%)
Control	94.7±2.7	94.0±2.7	1.7±2.7
A-1221			
10 ng/L	94.0±3.3	93.3±2.1	0.7±1.7
100 ng/L	95.3±3.9	94.0±2.2	1.3±3.3
1 μg/L	94.0±2.2	93.3±2.1	0.7±1.6
10 μg/L	94.0±2.7	93.3±2.1	2.0±3.3
100 μg/L	94.7±3.3	92.7±1.6	2.7±2.1
1 mg/L	96.0±3.6	95.3±3.9	2.0±2.2
10 mg/L	94.7±3.3	4.7±3.0*	0.7±1.6
A-1254			
10 ng/L	94.7±2.1	93.3±2.1	0.7±1.6
100 ng/L	96.7±2.1	95.3±3.9	1.3±2.1
1 μg/L	94.7±4.1	93.3±2.1	0.7±1.6
10 μg/L	94.0±2.2	94.0±2.2	1.3±2.1
100 μg/L	95.3±3.0	95.0±3.5	1.3±2.1
1 mg/L	96.7±3.0	94.7±2.1	2.7±2.1
10 mg/L	94.7±3.3	93.3±2.1	4.0±3.6

* $p<0.05$

reported that there was no significant effect on hatchability of green frogs and leopard frogs when exposed to 50 μg/L PCB 126. Matta et al (1997) also reported that the hatchability of trout was not affected by 2',4',6'-trichloro-4-biphenylol, a polychlorinated biphenyl metabolite, even at concentrations up to 95 mg/L. It is likely that other environmental factors such as temperature, water quality or PCBs acting synergistically with other pollutants cause variation in hatching success (Rosenshild et al. 1999). Taken together, these data suggest that PCBs alone have no observable adverse effects on the hatchability in laboratory-based experiments.

The results of acute toxicity to the tadpoles are shown in Figure 1 and Figure 2. During the first 24 hr, the survivorship of A-1221 groups was shown to be dose-dependent, but there were no significant differences of survival rate in all A-1254 groups, even at concentrations up to 10 mg/L. With the time of exposure prolonged and the concentration increased, the mortality increased. At 96 hr, tadpoles all died at concentrations above 1 mg/L A-1254, tadpoles exposed to 0.1 mg/L A-1254 also exhibited lethal toxic effects. The survivorship of tadpoles exposed to A-1254 in our experiment is lower than that reported previously (Jelaso et al. 2002; Fisher et al. 2003). They used a static exposure system, however, we used a renewal-static exposure system. The high adsorption on the

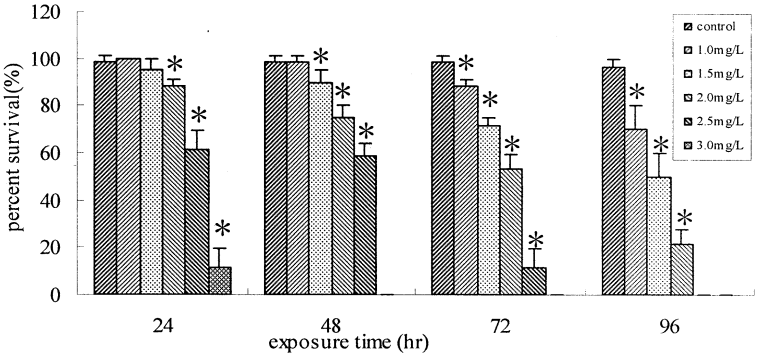

Figure 1. Survivorship (mean±SD) of *X. laevis* tadpoles (stage 46~47) exposed to A-1221 for 24, 48, 72, and 96 hr * $p<0.05$

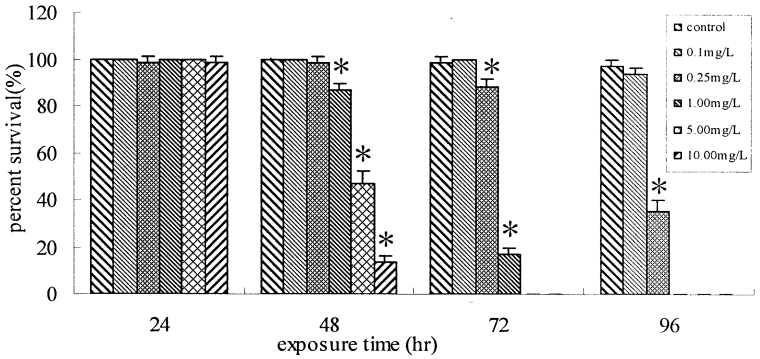

Figure 2. Survivorship (mean±SD) of *X. laevis* tadpoles (stage 46~47) exposed to A-1254 for 24, 48, 72, and 96 hr * $p<0.05$

surface of glass vessel may cause a decline of actual concentration of PCBs, and therefore underestimate the toxicity of PCBs in the static system.

The lethal concentration of A-1254 decreased more than 100 times from 24 hr to 96 hr, however, it decreased slowly for A-1221. This may be explained by their different characteristics of bioaccumulation and metabolism. The rate and extent of PCB biotransformation are dependent upon the animal species, and the number and position of chlorines on the biphenyl molecule. Higher chlorinated PCB tends to be bioaccumulated, while lower chlorinated PCB are more easily metabolized. A-1221 is mainly composed of 1~2 chlorine substituted congeners, however, the main congeners of A-1254 are 5~6 chlorine substitutions. A-1254 is more likely to be bioaccumulated than A-1221 by these tadpoles, but we did not carry out tissue sample analysis. The difference in acute toxicity of A-1254 and A-1221 also can be explained by the difference in their solubility. 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 increases so does the acute toxicity. Dillon and Burton (1991) also found that the toxicity of PCB 18 is the highest among 10 different tested PCB congeners. Because the water solubility of A-1221, 0.59 mg/L, is higher than that of A-1254, 0.021 mg/L, the acute toxicity of A-1221 is great than that of A-1254 at 24 hr exposure. With increased time of exposure, the acute toxicity of A-1254 increased sharply because the cumulative toxicity of A-1254 was manifested.

Tadpoles exposed to A-1254 developed dose-dependent depigmentation at concentrations above 1 mg/L. However, this phenomenon is not manifested in tadpoles exposed to A-1221. The size of the pigment patches is under nervous system control. Agents that affect these nerves cause smaller pigment patches and the overall color of the larvae will be pale (ASTM 1998). This may reflect the different neurotoxicity between the two Aroclors: A-1254 has higher neurotoxicity than A-1221.

The results of the above study indicate that PCBs have minimal effects on embryo development and are not acutely toxic to embryo and larvae of *X. laevis*. However, the toxicity of PCBs is often manifested in upper trophic level consumers after bioaccumulation from chronic exposure (Fontenot et al. 2000). More investigations should be conducted to detect the effects of chronic and environmentally relevant concentrations on amphibians.

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