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Effects of Pentachlorophenol on *Galba pervia*, *Tubifex sinicus* and *Chironomus plumousus* Larvae

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Abstract The 24-h median lethal concentrations of pentachlorophenol to *Chironomus plumousus*, *Tubifex sinicus* and *Galba pervia* were 0.302, 0.977 and 0.293 mg/L, respectively. Bioconcentration factors of *C. plumousus*, *T. sinicus* and *G. pervia* to pentachlorophenol were 108, 367 and 85 at 0.02 mg/L pentachlorophenol, respectively. As pentachlorophenol concentration increased, the *G. pervia* egg hatching rates became lower, and the total hatched time became longer. Pentachlorophenol teratogenesis was demonstrated by observing the deformation of *C. plumousus* larvae mentum.

Keywords Pentachlorophenol · *Galba pervia* · *Tubifex sinicus* · *Chironomus plumousus* larvae

Pentachlorophenol (PCP) is a phenol substituted pesticide with insecticide, fungicide and defoliant activity (Abuknesha and Griffith 2004). PCP is often found in contaminated aquatic environments, and represents one broad class of aquatic contaminants (water-soluble organic compounds) (Besser et al. 2005). PCP is highly toxic to humans, and causes injury to major organs including the lung, liver, kidneys, heart, and brain (Dorsey et al. 2004). PCP not only directly impairs human body, but also shows potential damaging effects on genetics (Yang et al. 2005). At low level of exposure, PCP is found to be mitogenic, showing a strong dose- and time-dependent response with regard to cell proliferation (Dorsey and Tchounwou 2004). Bluegill (*Lepomis macrochirus*) cough rate and movement are responded to PCP at the 96-h LC_{50} within an hour or less, (Van der Schalie et al. 2004). Changes in phototoxic behavior of *Daphnia magna* clone C1 242 can be observed at 0.80 mg/L of PCP, much lower than LC_{50} (48 h) (Yuan et al. 2003).

The purpose of this paper is to study the toxic effects of PCP on flash water snail *Galba pervia*, *Chironomus plumousus* larvae and oligochaetes *Tubifex sinicus*. First, BCFs of these animals to PCP at low concentrations were determined. Second, the effects of PCP on snail egg hatching were investigated. At last, deformation of *C. plumousus* larvae mentum was observed, which showed PCP teratogenesis.

Material and Methods

Chironomus plumousus larvae and *T. sinicus* were purchased from Nanshan flower-bird-fish market in Qingdao city, China. A total of 500 mL distill water were put into 30 cm × 20 cm × 3 cm vitreous enamel plate as culture medium.

Snails (*G. pervia*) were collected from Zhongshan park in Qingdao city, China. Snails were cultured in distill water with cooked cabbage. Healthy snails (average weight and length were 153.63 mg and 1.16 cm, respectively) were selected for test.

The 24-h LC_{50} test was conducted in 200 mL test solution in a 250 mL beaker. The concentrations of PCP (Sigma-Aldrich company, 98% purity) used in tests were shown in Table 1.

Ten *C. plumousus* larvae, ten *G. pervia*, or ten *T. sinicus* were put into a beaker, respectively. The beaker was lighted at 2,500 Lx for 12 h, then was kept in the dark for 12 h each day during the experiment. Each test was

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repeated three times. For bioconcentration test of *C. plumousus* larvae or *T. sinicus*, 500 *C. plumousus* larvae or 500 *T. sinicus* were put into 500 mL test solution with PCP concentration at 0.02 mg/L in a 30 cm × 20 cm × 3 cm vitreous enamel plate. For biocencentration test of *G. pervia*, 20 *G. pervia* were put into 2 L test solution at 0.02 mg/L PCP in an aquarium (diameter 20 cm and height 30 cm). Each test lasted for 7 days, and replicated for three times. At the end of test, 400 *C. plumousus* larvae, all *T. sinicus*, or 10 *G. pervia* were homogenized with 50 mL *n*-hexane to extract PCP. The extraction solution was centrifuged at 3,000*g* for 30 min. The clear solution was dried with N_2, and ethanol was added to 5 mL.

The concentration of PCP was determined with a Waters Associates high-pressure liquid chromatograph system (HPLC Model 244), a Model 481 variable-wavelength UV detector (wavelength set at 280 nm), a Model 680 system flow controller, and a Model 730 data station. Separation was performed by isocratic elution with a binary mixture containing 85% methanol and 15% acetic acid buffer (15 g acetate sodium/100 mL, pH value at 4.0 adjusted by glacial acetic acid) at a flow rate of 1.0 mL/min (Chi et al. 1999). The analytical column was a 4.6 mm × 250 mm, 10 μm Irregular-H C18 column and was maintained at R.T. Five standard PCP solutions (concentrations were 0.1, 0.2, 0.4, 0.6, 0.8 mg/L) and control were analyzed to make a standard curve. PCP concentrations were regressed with area integral of peaks so as to calculate the standard curve function.

The function was calculated as follows:

$$Y = 530114\ X + 7659.8$$

where Y was area integral of peak, X was PCP concentration (mg/L). The correlative coefficient was 0.9995, and the limit of detection was 0.04mg/L.

At the same time, recovery test was studied and replicated three times. The mean recoveries of samples of water and animal were 96% and 87%, respectively.

T-test statistical method was used to study the data of PCP treatment.

Table 1 Concentrations of PCP in tests (mg/L)

	PCP concentration (mg/L)					
Galba pervia	0	0.5	1.0	2.0	3.0	4.0
Tubifex sinicus	0	1.0	2.0	4.0	6.0	8.0
Chironomus plumousus larvae	0	0.05	0.10	0.15	0.20	0.25

The mentum and mandibles deformity observation of *C. plumousus* larvae

At the end of test, 100 *C. plumousus* larvae in each test were killed in boiling water. The larvae was treated with increased concentration (%) of ethanol for dehydration. The incubation time with each ethanol concentration was 30 min. Finally, the larvae was treated with 100% ethanol twice. Before separating mentum and mandible from larvae, larvae were soaked in 25 mL xylene for 5 min. Hundred mentums and mandibles were separated from larvae under anatomization microscope.

G. pervia Snail Egg Hatching Rate Test

Galba pervia snail egg hatching rate test was conducted in 200 mL test solution in 250 mL beaker. In each test, 12 eggbags were treated with test solution without PCP (control) or solution with different concentrations of PCP (0.1, 0.2, 0.3, 0.4 mg/L). Each test was replicated three times. The test solution was replaced every day. The test stopped until no egg hatched. The time of the first snail hatched was recorded, and the total number of *G. pervia* snail hatched was counted.

Results and Discussion

The LC_{50}s of PCP on *G. pervia*, *T. sinicus* and *C. plumousus* larvae were shown in Table 2, and BCFs of *G. pervia*, *T. sinicus* and *C. plumousus* larvae to PCP were shown in Table 4.

From Table 2, it showed that the sensitivity of *C. plumousus* larvae to PCP was similar to that of *G. pervia* , and *T. sinicus* was least sensitive among three species. LC_{50} of PCP to *D. magna* was 0.68 mg/L (Milam et al. 2005), which suggested that sensitivity of *C. plumousus* larvae or *G. pervia* to PCP was higher than that of *D. magna*, and *T. sinicus* was less sensitive than *D. magna*. Mosquito larvae was recommended as a good bioindicator for the risk assessment of aquatic environment (Farah et al. 2004), but mosquito larvae appeared more resistant to PCP than fish did. The concentration of PCP inhibiting reproduction and causing almost 100% adult mortality in *D. magna* only reduced the reproduction of *Chironomus prasinus* slightly (Sanchez et al. 2005).

Table 2 The 24-h LC_{50}s of PCP on *Chironomus plumousus*, *Tubifex sinicus* and *Galba pervia*

	Chironomus plumousus larvae	*Tubifex sinicus*	*Galba pervia*
PCP mg/L	0.302 (0.201–0.389)	0.977 (0.801–1.192)	0.293 (0.266–0.322)

Table 3 Acute toxicity of pentachlorophenol to six fish species and one amphibian species including 96-h LC_{50}s (Dwyer et al. 2005)

Species	Fathead minnow	Sheepshead minnow	Rainbow trout	Shortnose sturgeon	Boreal toad	Fountain darter	Gila topminnow
LC_{50} (mg/L)	0.25	0.05	0.16	0.07	0.37	0.11	0.34

Table 4 BCFs of *Chironomus plumousus*, *Tubifex sinicus* and *Galba pervia* to PCP

	Chironomus plumousus larvae	*Tubifex sinicus*	*Galba pervia*
BCF	108	367	85

Table 3 shows the acute toxicity of PCP to six fish species and one amphibian species. The results of Table 3 indicated that *T. sinicus* was less sensitive to PCP compared with these organisms. The LC_{50}s of PCP to *C. plumousus* larvae or *G. pervia* were lower than that of Gila topminnow or Boreal toad, but were higher than those of the other species shown in the Table 3.

According to Table 4, BCF of *T. sinicus* was nearly three times higher than that of *C. plumousus* larva. The BCF of *G. pervia* to PCP was the lowest among three tested organisms. There were few data on BCF of PCP in aquatic organisms. The BCF of PCP in duckweed *Lemna minor* was 274.2 at 0.2 mg/L PCP (Song and Huang 2005). These data illustrated that PCP would follow from plant and animal food web to higher nutrition levels, and exhibited the characteristic of biomagnification.

The shapes of mentum and mandible of *C. plumousus* larvae were normal in control after 7 days, which were shown in Figs. 1 and 2. Mentum of larvae showed regular arc, and the teeth grew symmetrically, and were thick. The six teeth in mandible were sharp. In contrast, after 7 days' exposure of PCP solution, 30% of mentum and mandible of the *C. plumousus* larvae exhibited abnormal morphology, which were shown in Figs. 3 and 4. The teeth in the mandible became smoother, as shown in Fig. 3. The mentum became unsymmetrical, and teeth almost disappeared, as shown in Fig. 4.

The characteristics of chironomid deformities, which could be associated with indirect or direct effects caused by cadmium, were split medial mentum teeth (more frequent in 9 and 27 μg Cd/L) and premandible deformities (especially in 3 μg Cd/L). Previous study demonstrated a concentration–response relationship between deformities and sub lethal levels of cadmium (de Bishoven et al. 2001). Deformities of the mentum and the mandibles were also recorded in second, third, and fourth instars exposed to copper and lead test solution (de Bisthoven et al. 1998). Recent study demonstrated that larvae with mentum deformities had significantly higher active nucleoli in their polytenic chromosomes than normal larvae (Meregalli et al. 2002). Biomonitoring sediment stress studies based on deformity analysis could be carried out in a greater range of cases (Servia and Gonźalez 1998).

The effects of PCP on snail *G. pervia* egg hatching were shown in Table 5.

Fig. 1 The normal mentum of *Chironomus plumosus* larva grow (400×)

Fig. 2 The normal mandible of *Chironomus plumosus* larva grow (400×)

Fig. 3 The mandible of *Chironomus plumosus* larva exposed to PCP of 0.02 mg/L for 7 days (400×)

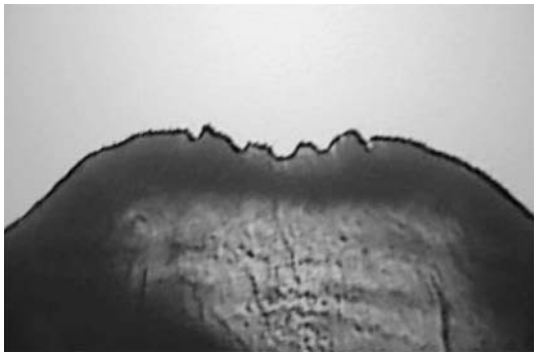

Fig. 4 The mentum of *Chironomus plumosus* larva exposed to PCP of 0.02 mg/L for 7 days (400×)

Table 5 Effects of PCP on *Galba pervia* snail egg hatching

PCP concentration (mg/L)	0	0.1	0.2	0.3	0.4
Hatching rate (%)	92.05	93.28	88.91	79.85	74.45
Total hatched time (Day)	7	7	8	9.33	11.33

According to Table 5, as PCP concentration increased, the hatching rates became lower, and at the same time, the total hatched time became longer. At 0.4 mg/L of PCP, the hatching rate was 74.45%, which was about 20% lower than the hatching rate of control, and total hatched time was 11.33 days, which was 1.6 times longer relative to control. The data showed that snail *G. pervia* eggs' hatch was delayed in the presence of low concentration of PCP. It has been reported that PCP could remarkably inhibit the development of *Brachydanio rerio* embryo, and cause its malformation and even death (Zheng and Zhu 2005). Pentachlorophenol in the range of 37.5–75 mM contributed to oxidative DNA damage, fluidity changes and peroxidation activity in the plasma membrane of *Unio tumidus* (Milowska et al. 2003). Lysosome destabilization was an early cytotoxic response that preceded the mitochondrial dysfunction when mammalian cells were exposed to moderate doses of pentachlorophenol (Fernandez et al. 2005). Combined with previous research, the current studied demonstrated that PCP could induce various harmful effects on aquatic organisms.

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