Effect of Phthalate Esters on Shrimp

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Environmental pollution by phthalate esters has become intensified with the increasing production of plastics. Phthalate esters have been detected in not only water, soil, air and foods, but also in animal organs. It has been reported(1) that phthalate esters have been detected in jelly-fish in the deep sea.

To estimate the toxicity of phthalate esters in fresh-water invertebrates, the effect of phthalate esters on the hatching of shrimp eggs and the mortality of the nauplius or larvae stage was tested. The toxic order of three phthalate esters was DBP$\gg$ DEP $>$DMP.

MATERIALS AND METHOD

Brine Shrimp(*Artemia Salina*)eggs and chemicals were obtained commercially. Water which was distilled from deionized water was used in the experiment. For the hatching test, these eggs were weighed on a direct reading balance and from 1 to 2 mg were placed into petri dishes(9×2cm). The control dishes were filled with 2 % NaCl solution adjusted to pH 8.3 with $NaHCO_3$. In the experimental dishes, a suitable amount of phthalate ester was added to 2 % NaCl solution. These dishes were placed in an incubator at 26 °C. After 24 hours the number of nauplius was counted.

To observe the effect of phthalate esters on the larvae stage, eggs were hatched as described above, and 10 larvae per dish were examined. The control larvae were placed into 2 % NaCl solution and the experimental larvae were placed in 2 % NaCl solution containing a phthalate ester. After 24 hours the number of dead larvae, those for which no movement was detected under the microscopic field, was counted.

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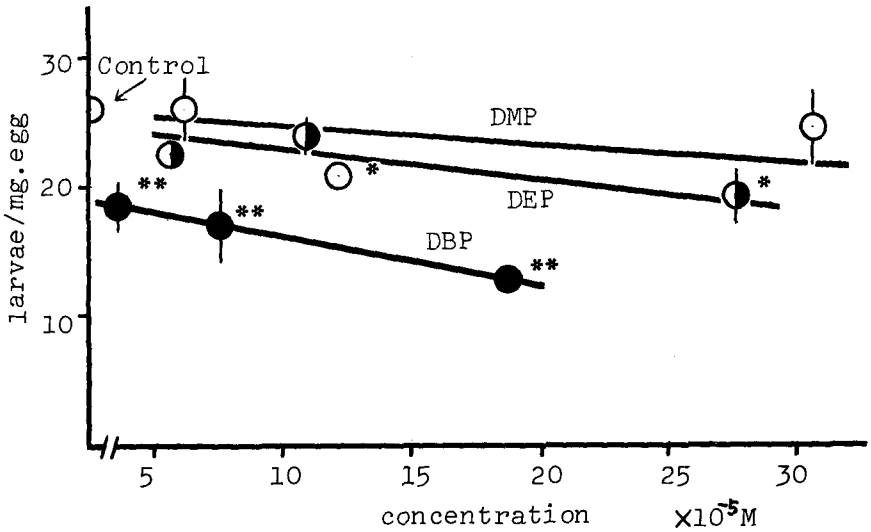
RESULTS

Effect of Phthalate Esters on Hatching. Fig. 1 shows the number hatched at the various concentrations of DMP (Dimethyl phthalate), DEP(Diethyl phthalate) or DBP(Di-n-butyl phthalate). The effect of DMP(6.6×10^{-5}M) or DEP (5.5×10^{-5}M) did not vary from that of the control, but at 27.7×10^{-5}M of DEP, the number hatched was significantly lower ($p<0.05$) than that of the control. On the other hand, DBP had more effect on hatching than DMP or DEP. At 3.7×10^{-5} M the number hatched was significantly less($p<0.01$) than that of the control.

Fig. 1 Toxic Effect of Phthalate Esters on Hatching



All data is represented by M±SD and analyzed according to the t-test (*$p<0.05$, **$p<0.01$).

Effect of Phthalate Esters on Nauplius. Table 1 shows the effect of DMP, DEP or DBP on the mortality of nauplius. DMP and DEP did not effect the death of larvae at the order of 10^{-4}M, but DBP had an effect at 3.7×10^{-5}M.

Table 1 Toxic Effect of Phthalate Esters on Nauplius

group	concentration $X10^{-5}$ M	mortality number
Control		1.3±0.7
DMP	30.7	1.3±0.4
	61.5	2.6±0.9
DEP	27.7	2.0±0.8
	55.5	2.3±0.4
	3.7	6.6±0.9
DBP	7.5	6.6±1.2
	18.8	8.0±0.8

Each dish contained 10 larvae and was maintained at 26 °C for 24 hours. All data are given as M±SD and represent 3-6 experiments.

DISCUSSION

Nematollahi et al(2)investigated the toxicity of phthalate esters with alkyl groups C_1 to C_{12} and reported that phthalate esters with alkyl groups C_1 to C_5 showed a toxic effect on chick embryos or L-cells, but the toxic order from C_1 to C_5 was not clear. Calley et al(3) reported that the LD_{50}s of DMP, DEP and DBP were 1.58 g/kg, 2.83 g/kg and 4.00 g/kg, respectively, using mice injected intraperitoneally. Singh et al(4) also reported that the LD_{50}s of DMP, DEP and DBP were 3.37 ml/kg, 5.05 ml/kg and 3.04 ml/kg in rats. From the results of these reports, differences of toxicity may be due to the solubility of the chemicals in water.

In this experiment the toxic order of phthalate esters on the hatching of eggs and nauplius was DBP$\gg$ DEP > DMP. The toxic order was inversely proportional to their solubility in water. The discrepancy between this experimet and others is not yet clear, but solubility may be unimportant to toxicity.

Kasuya(5) reported that the toxic order of phthalate esters(DMP, DEP and DBP) was DBP>DEP>DMP using fibroblast cultures from newborn rat cerebellar tissue; ester interaction with the membrane may play an important role in toxicity.

At the concentration which has been detected in the environment(6), the hatching of eggs and the mortality of nauplius were affected. Aquatic invertebrates are the main food source of many fishes and wildlife, and thus growth and reproduction of these predatory vertebrates could be adversely affected in an indirect manner whether or not phthalate esters directly affected fish and wildlife.

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