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Effects of Cartap on the Early-Life Stages of Medaka (*Oryzias latipes*)

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Tests with the early-life stages of fish are intended to define the lethal and sub-lethal effects of chemicals on the stages and species tested (Gary and Metcalfe 1999). The first aquatic vertebrate life cycle toxicity study was performed with the fathead minnow (*Pimephales promelas*), and now it is considered to be the ultimate test for the establishment of long-term safe environmental concentrations of toxic chemicals. Medaka has been widely used in aquatic toxicity tests including the early life stage (Tadocoro and Maeda 1984), because this species is easy to breed and medaka developmental stages are easy to observe due to the transparent chorion.

Cartap (*S,S'*-[2-(dimethylamino)-1,3-propanediyl] dicarbamothioate), a synthetic analog of nereistoxin (4-*N,N*-dimethylamino-1,2-dithiolane), is an insecticide reported in 1967. Later, hydrochloride was introduced to increase the efficacy of this chemical. Currently, cartap is used for the control of chewing and sucking insects, at almost all stages of development, on many crops, including rice, potato, cabbage, and other vegetables; also on soya beans, peanuts, sunflowers, maize, sugar beet, wheat, pearl barley, pome fruit, stone fruit, citrus fruit, vines, chestnuts, ginger, tea, cotton, and sugar cane, *etc*. Cartap can cause the paralysis of muscle via ganglionic blockage on the central nervous system. Insects stop feeding upon contact, and die of starvation (Tomlin 1994). Several organophosphorus and carbamate insecticides and nereistoxin bind to the nicotinic acetylcholine (ACh) receptor of the electric organ of Torpedo with high affinity. Insect brain contains more nicotinic than muscarinic ACh receptors, while the reverse is found in mammalian brain (Eldefrawi 1983). The LC_{50} of cartap for carp is 1.6 mg l^{-1} in 24 hours and 1.3 mg l^{-1} in 48 hours. The toxicity class of cartap is WHO II and EPA II, and DT_{50} in soil is 3 days (Tomlyn 1994), which means cartap is neither highly toxic, nor highly persistent in the environment. However, thorough evaluation of lethal and sublethal toxicity of cartap should be required since cartap is one of the most widely used insecticides and present in the environment with other similar insecticides. In this study, toxic effects of cartap were determined using the medaka early life stage test in order to re-evaluate potential lethal and sublethal toxicity, then, to determine the lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC).

MATERIALS AND METHODS

Cartap hydrochloride of greater than 98% purity was obtained from Norvatis Agro Korea, Seoul, Korea. MS222 was purchased from Sigma (USA). Adult medaka were maintained at a 3:2 female/male ratio with a density of about five fish per liter in UV disinfected, dechlorinated tap water. Brood tanks were held at 25 ℃ and provided a daily regimen of

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RESULTS AND DISCUSSION

Cartap caused significant teratogenic effects on the early life stages of medaka. The occurrence rates of the terata at each concentration group are summarized in Table 1.

Table 1. The occurrence rates of abnormal larvae by the cartap treatment. Medaka embryos and larvae at the early stages were exposed to various concentrations of cartap and daily observations were made by examining each embryo. See the methods for the detailed instructions.

Cartap (ppb)	No. of hatched	No. of abnormal larvae	Rate of Abnormality (%)
250	51	51	100
100	53	38	71.7
40	56	3	5.4
14	55	0	0
6	54	0	0
0	58	0	0

There were no abnormal larvae in the control and 6 or 14 ppb of cartap-treated test groups. However, larvae exposed to 40, 100, 250 ppb of cartap showed distinct abnormal external appearance of 5.4, 71.7 and 100 % occurrence rates, respectively (Table 1). Affected fish showed an inflexible, extremely wavy structure of the vertebral column and a lack of movements in a concentration-dependent manner. The representative figures of abnormal larvae in each group are showed in Fig. 1 (A), (B), and (C) (40, 100 and 250 ppb, respectively). Most of the affected fish in the treated group didn't show movement, while normal larva actively swam around immediately after hatch. The occurrence of the abnormal larvae from the cartap-treated group before they hatched was expected since the abnormal embryos were found under the dissecting microscopic examination. Embryos showed significant external abnormality in a concentration-dependent manner. Compared with the negative control (Fig. 2 (A)), the embryos from 100 (Fig. 2 (B)) and 250 ppb (Fig. 2 (C)) cartap-treated groups showed significant malformations such as loss of the roundness of egg shells and severely bent vertebral columns. Based on the results, LOEC and NOEC for teratogenic effects of cartap were 40 and 16 ppb, respectively.

Observations on hatching and survival were made once a day and numbers recorded. Most embryos hatched between 9 and 12 days post-fertilization in all groups. Cartap did not cause a significant influence on the duration to hatch. There were no significant differences in time to hatch among any treatments ($P>0.05$,data not shown). Cartap didn't seem to have a serious influence on hatching; hatching rates in all groups, even the highest concentration (250 ppb) showed more than 85 % of hatching rate. However, when compared with control, the groups exposed to 100 and 250 ppb cartap showed significant differences of hatching rates ($P<0.05$) (Fig. 3). Post-hatch success was also significantly reduced by cartap treatment in all treated groups ($P<0.05$) (Fig. 3).

Post-hatch success rates of control, 6, 16, 40, 100, and 250 ppb groups are 91.7 ± 2.9, 85, 83.3 ± 7.6, 78.3 ± 2.9, 75 ± 5, and 40 ± 5 %, respectively. Based on the result, LOEC for hatching and survival of cartap was 6 ppb. At the end of the test, all surviving fish were killed by overdose of MS222. Total lengths and dried weights were measured to detect the potential influence of cartap on the growth of fish. Results showed that cartap reduced

growth of surviving fish significantly. Body lengths and weights are summarized in Table 2 and 3, respectively. Based on the result, LOEC for growth of cartap was 14 ppb and NOEC was 6 ppb.

In the past decade, the agricultural use of insecticides in combination, such as organophosphates and carbamate including cartap, has become increasingly popular. Exposure to cholinesterase-inhibiting agents has been considered a major health problem for farmworkers in the USA (Queiroz MLS et al. 1999) and other countries. Extremely diverse clinical symptoms from such exposure has been reported, including several types of cancer, genotoxic effects, teratogenic effects, immunosuppression, sterility, and spontaneous abortion, primarily resulting from the inhibition of cholinesterase (ChE) activity (Kahn 1976; Midtling et al. 1985; Sullivan 1989; Ciesielski et al. 1994; Lander and Ronne 1995). Nereistoxin and cartap have been known to block the neuromuscular transmission (Deguchi et al. 1971) and inhibit the nicotinic acetylcholine receptor channel (AchR) system (Sherby et al. 1986). Nereistoxin was reported to cause membrane depolarization in rat diaphragm muscle, and cockroach postsynaptic membrane (Eldefrawi et al. 1980; Satelle et al. 1985). And Nakata et al. (1997) reported that cartap acted as an open channel blocker at the nicotinic acetylcholine receptor channel. In human, cartap causes dizziness, headache, and memory loss as CNS effects; visual disturbance, increased sweating, and abdominal pain as muscarinic effects; and muscular weakness and myalgias as nicotinic effects (Queiroz MLS et al. 1999). In the present study, fish in cartap-exposed groups showed a lack of movement, weak heartbeat, and slow bloodstream and the fish at 100 and 250 ppb didn't show eating action at the observation. Cartap increased mortality of medaka lavae and decreased the growth rate. In addition, cartap induced a significant teratogenic effect. It appears to be an osteolathyric effect. Ghate HV and Mulherkar L (1980) showed that osteolathyrism (failure of normal collagen and elastin cross- linking) was caused by diethyldithiocarbamate on developing embryos of the frog (*Microhyla ornate*).

The wide use of cartap and disposal in water and soil may have a significant impact on the environment. The noteworthy problems are that cartap is discharged directly to rivers. Even though cartap is moderately toxic in other toxicity tests, and is not persistent in the environment, the results in this study showed that the relatively low concentration of cartap could, in a short period of time, cause significant effects in medaka. When considering that cartap is one of the most widely used insecticides and present in the environment with other similar insecticides, further studies should be carried out to investigate 1) whether and/or to what extent cartap occurs, 2) with what other chemicals it is present in the aquatic environment, and 3) on how much potency cartap and its mixture with other chemicals have.

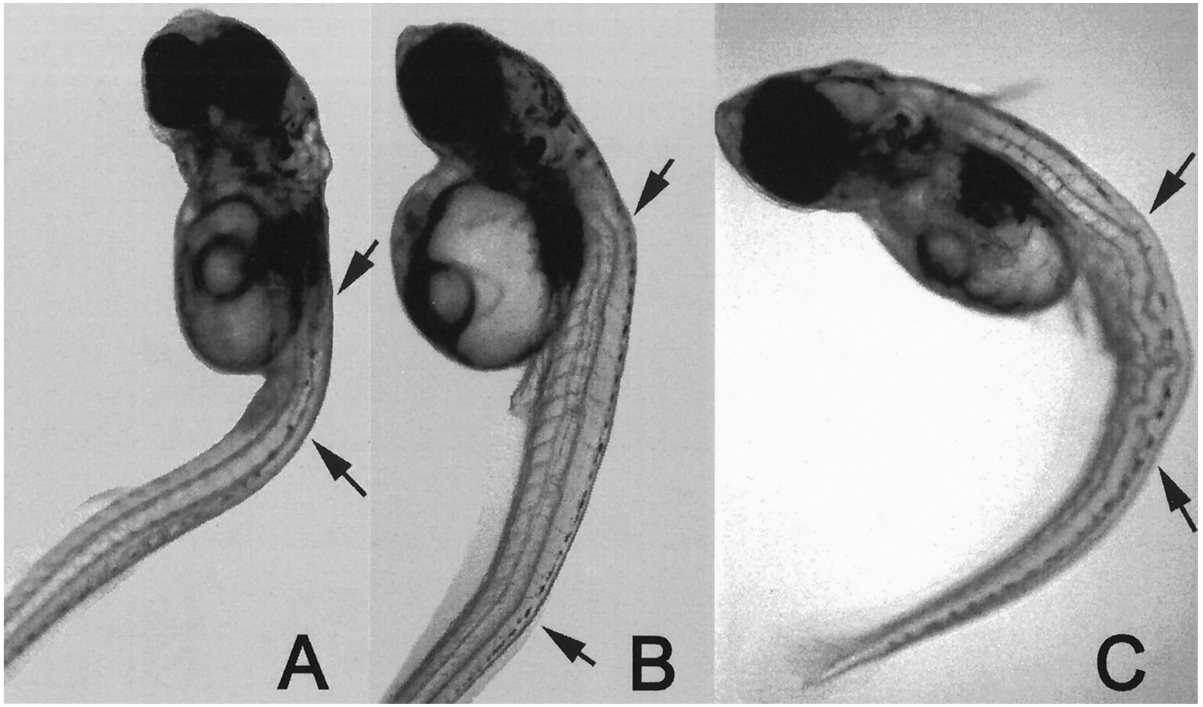

Figure. 1 Abnormal medaka larvae hatched from eggs treated with 40 (A), 100 (B), and 250 ppb (C) cartap, respectively. Each abnormal Larva showed an inflexible, extremely wavy structure of the vertebral column (arrows) (×40).

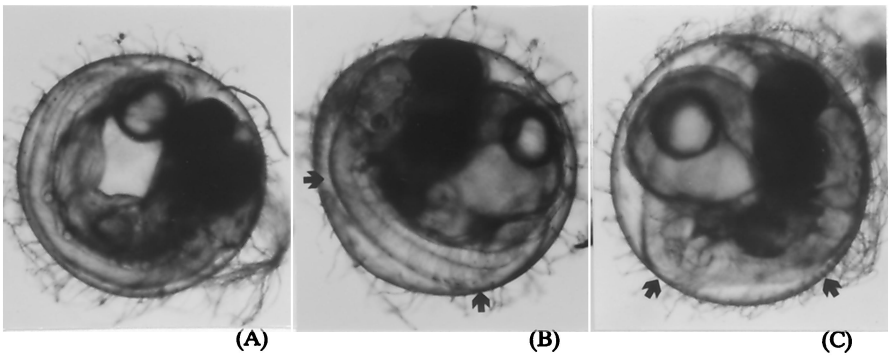

Figure. 2 (A) Normal medaka embryo. Eggshell is round and Embryo is not bent. (B) and (C) Abnormal medaka embryos treated with 100 and 250 ppb, respectively. Notice that egg shell lost its roundness and embryo is bent (arrows) (×40).

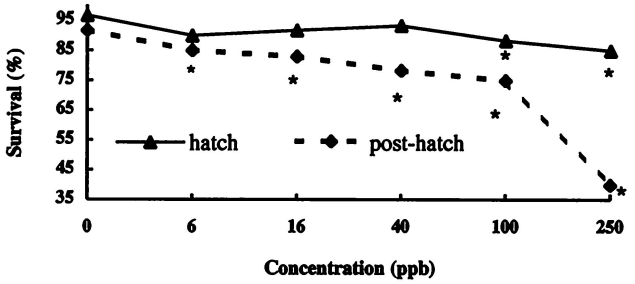

Figure. 3. Percentage of medaka larva which successfully hatched and survived through the test period. * Significantly different from control ($P<0.05$, χ^2 analysis).

Table 2. Changes of body length in medaka exposed to cartap. Medaka eggs and larvae at the early stages were exposed to various concentrations of cartap and total lengths of individual fish were measured and recorded. See the methods for the detailed instructions.

Cartap (ppb)	Replication**			Average ± S.D. (cm)
	1	2	3	
250 *	1.09 ± 0.17	1.14 ± 0.10	1.15 ± 0.15	1.13 ± 0.14
100	1.32 ± 0.13	1.29 ± 0.12	1.30 ± 0.14	1.30 ± 0.13
40	1.31 ± 0.15	1.35 ± 0.14	1.36 ± 0.14	1.34 ± 0.14
14	1.36 ± 0.20	1.35 ± 0.13	1.36 ± 0.13	1.36 ± 0.16
6	1.36 ± 0.13	1.36 ± 0.12	1.36 ± 0.15	1.36 ± 0.13
0	1.36 ± 0.14	1.38 ± 0.13	1.36 ± 0.12	1.36 ± 0.13

* Significantly different from control (P<0.05, Dunnett's test)
**Each concentration of cartap was tested in triplicate and all the tests were repeated three times (S.D. means standard deviation).

Table 3. Changes of body weight in medaka exposed to cartap. Medaka eggs and larvae at the early stages were exposed to various concentrations of cartap. At the end of test, fish were dried and weighed. See the methods for the detailed instructions.

Cartap (ppb)	Replication**			Average ± S.D. (mg)
	1	2	3	
250 *	9.7	9.9	11.2	10.3 ± 0.81
100 *	11.4	9.5	11	10.6 ± 0.58
40 *	10.7	11.1	9.8	10.5 ± 0.38
14 *	10	13.8	11.7	11.8 ± 1.09
6	19.1	18.9	19.5	19.2 ± 0.18
0	20	20	18	19.3 ± 0.67

*Significantly different from control ($P<0.05$, Dunnett's test)
**Each concentration of cartap was tested in triplicate and all the tests were repeated three times (S.D. means standard deviation).

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