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Acetylcholinesterase Inhibition and Behavioral Changes of Crucian carp (*Carassius auratus*) Exposed to the Waterborne Parathion

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Parathion에 노출된 붕어(*Carassius auratus*)의 Acetylcholinesterase 억제와 행동변화

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Abstract
The investigation of the Acetylcholinesterase (AChE) activity in tissues (brain, eye, muscle and serum) of crucian carp (*Carassius auratus*) exposed to the waterborne parathion was carried out for application as biomarker of organophosphate pesticides. The AChE activities were significantly inhibited in the experimental organs of *C. auratus* treated $\geq$ 63 μg/L of concentrations of parathion. The AChE activity of *C. auratus* was significantly reduced in response to brain (79.1～92.4%), eye (76.0～91.5%), muscle (89.7～97.6%) and serum (68.9～78.0%) after 30 days exposure. No significant mortality occurred during the experiment duration but behavioral changes occurred in the carp after exposure to the parathion were erratic swimming and convulsions. The anomaly in the carp exposed to parathion were observed in the form of scoliosis. The use of AChE activity and other adverse responses of the carp might be use as a reliable monitoring tool to detect parathion in aquatic ecosystem which might produce significant population changes.

keywords : Acetylcholinesterase, *Carassius auratus*, Parathion, Scoliosis, Sublethal concentration

1. 서 론

Organophosphate insecticides (OPs) are a group of wide spectrum pesticides which is entering into the environment in great quantity (Coppage et al., 1974), especially afterward the banning of many ubiquitous chlorinated insecticides. Because of their toxic effects on organisms, most pesticides may produce serious detrimental effects on aquatic ecosystems seriously. Levels of pesticides measured in surface waters generally are very low from the dose of lethal concentrations for aquatic organisms, but sublethal adverse effects may result from chronic exposure of pesticides at environmentally relevant concentrations (Lampert et al., 1989). Parathion has a broad spectrum as OP used for controlling many insects and mites. Because of its highly toxic characteristics, parathion is classified as a Restricted Use Pesticide (RUP) by USEPA (Extonet, 1993).

OPs are anticholinergic agents that bind to the esteratic site of the enzyme acetylcholinesterase (AChE) (Matsumura, 1985). Due to inhibition of AChE, the neurotransmitter acetylcholine (ACh) is not hydrolysed in nerve synapses and in neuromuscular junctions, causing an abnormal amount of ACh at these sites, which leads to overactivation of tissue. Thus, measurements of AChE activity is routinely used as a biomarker of exposure to group of OPs (Ferrari et al., 2002). Although AChE inhibition has widely monitored in terrestrial aquatic environment as a biomarker of toxicants, the effect of parathion on AChE has not yet been clear.

Inhibition of this enzymatic activity could disrupt the integrated ecosystems at several levels of complexity, influencing individual behavior, and consequently fish populations could decrease (Heath, 1995).

Numerous causes of anomaly in fishes have been identi-

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fied, including genetics, pollutants, water quality, nutritional deficiencies, infectious agents, and physical and electrical shocks (Hickey, 1972; Couch et al., 1979). Experimental field studies of USEPA (1999) documented the malformation of the skull and degeneration of fin in inhabiting fish and other aquatic organism of upper Klamath Lake caused by the pentachlorophenol (PCP) discharged as the main constituent of wood treatment plant's effluent, situated nearby the lake area.

The objectives of present study were to determine the toxic responses in terms of AChE inhibition, the rate of behavior changes and certain other physical disorder anomaly of crucian carp (*Carassius auratus*) exposed to sublethal concentrations of parathion.

2. Material and Methods

2.1. Experimental animals

C. auratus were obtained from the Chungcheongbuk-do Inland Fisheries Research Institute, South Korea. Healthy *C. auratus* (body weight, 27.04±5.58 g; body length, 9.72±1.87 cm) were acclimated to the laboratory condition. Temperature was kept at 23±1°C. Fish were fed twice a day with commercial fish feed containing 45% protein, 7.0% lipid, and 20% carbohydrate (Purina Ltd., Korea). Glass chamber (300 L capacity) were filled with 200 L of freshwater.

2.2. Experimental conditions

Fishes were acclimatized to laboratory conditions for 20 days in 300 L glass tanks before they were used for the experiments. They were supplied with continuous aeration under natural photoperiod (about light 12 hr : dark 12 hr). They transferred to 15 test aquaria (30 L volume with 25 L of water, each 27 fish). Fishes were exposed in a flow- through system, which was situated in a climate chamber. Physico-chemical characteristics of the experimental water measured by the method described in APHA et al. (1998) (Table 1).

Table 1. Experimental conditions of the water used in the test

Parameter	Value
Temperature (°C)	22.4±1.3
pH	7.0±0.7
Dissolved oxygen (mg/L)	7.53±0.54
Ammonia-nitrogen (mg/L)	0.31±0.14
Nitrite-nitrogen (mg/L)	0.011±0.008
Nitrate-nitrogen (mg/L)	2.10±1.05
Chemical oxygen demand (mg/L)	2.04±0.33
Total hardness as $CaCO_3$ (mg/L)	1.88±0.09
Hg	N.D.
Cu	N.D.
Pb	N.D.
Parathion	N.D.

Value indicate mean±SD; N.D.: not detected.

Parathion-ethyl, 99.9% pure was purchased from Supelco (USA). This stock solution was appropriately diluted with the test water to achieve the desired concentrations of parathion. The nominal concentrations of parathion were 0, 63, 95, 190 and 380 μg/L. The experiment was designed to expose the *C. auratus* to the parathion for 30 days.

2.3. AChE analysis

AChE activity was determined in (1:50) brain, eye, muscle and serum homogenate in 0.1 M phosphate buffer containing 0.1% Triton ×100 (Sigma), pH 8. Muscle (1:10) and whole eye homogenates (1:100) were also prepared in the same buffer. The crude homogenates were then decanted into eppendorf tubes and centrifuged 10,000 g for 20 min at 4°C. The supernatants were removed and used to test AChE activity. AChE activity was measured by an automated adaptation of the Ellman assay (1961) using a spectrophotometer (HACH 4000, USA). AChE activity was normalized to protein content and expressed as μmoles min-1 mg protein-1. Protein concentration was determined using Bradford's method (1976), with a bovine serum albumin (Sigma, USA) as standard.

2.4. Statistical analysis

Statistical analysis was performed using SPSS/PC statistical package. Significant differences between groups were determined using one-way ANOVAs and the Student's t-test for two groups. Significance level was established at $P<0.05$.

3. Results

3.1. AChE activity

Effects of parathion on the inhibition of AChE activity in the brain *C. auratus* are presented in Fig. 1. Exposed fishes exhibited a significant reduced brain AChE activity as value of ≥63 μg/L concentrations of parathion ($P<0.05$). Parathion concentrations higher than 63 μg/L caused much more inhibition in the enzyme. Mean inhibition rate of AChE according to concentration of parathion in *C. auratus* was recorded 79.1% (63 μg/L); 84.9% (95 μg/L); 84.0% (190 μg/L) and 92.4% (380 μg/L).

The results of AChE activity measured in the eye of *C. auratus* are presented in Fig. 2(a) significant inhibition was found in the eye in response to ≥63 μg/L concentrations ($P<0.05$). Mean inhibition rate of eye AChE of *C. auratus* for parathion was 76.0% (63 μg/L), 78.2% (95 μg/L), 89.9% (190 μg/L), 91.5% (380 μg/L).

Effects of parathion on AChE activity in the muscle of *C. auratus* are presented in Fig. 3. Recorded mean inhibi-

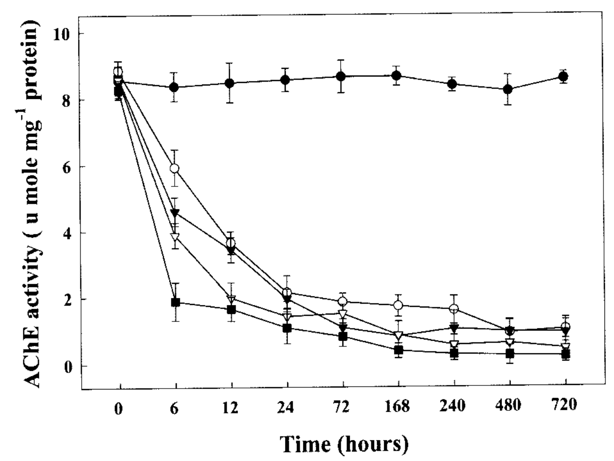

Fig. 1. Changes of acetylcholinesterase (AChE) activity in brain of *Carassius auratus* exposed to parathion for 30 days. Values are mean±S.E. (n=7). (● Control; ○ 63µg/L; ▼ 95µg/L; ▽ 190µg/L; ■ 380µg/L).

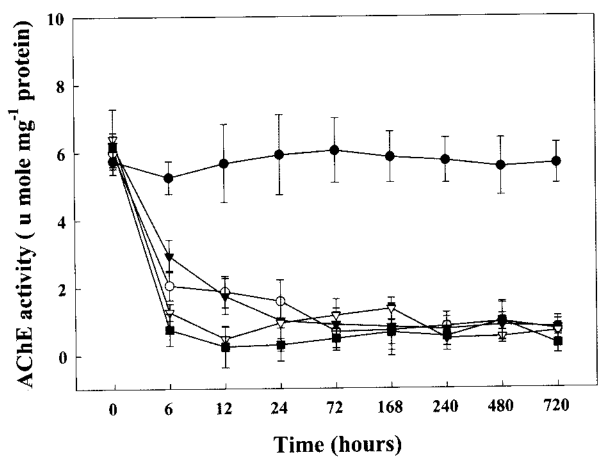

Fig. 2. Changes of acetylcholinesterase (AChE) activity in eye of *Carassius auratus* exposed to parathion for 30 days. Values are mean±S.E. (n=7). (● Control; ○ 63µg/L; ▼ 95µg/L; ▽ 190µg/L; ■ 380µg/L).

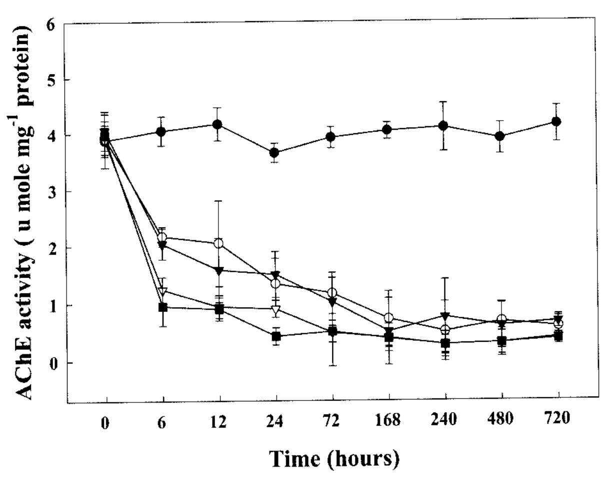

Fig. 3. Changes of acetylcholinesterase (AChE) activity in muscle of *Carassius auratus* exposed to parathion for 30 days. Values are mean±S.E. (n=7). (● Control; ○ 63µg/L; ▼ 95µg/L; ▽ 190µg/L; ■ 380µg/L).

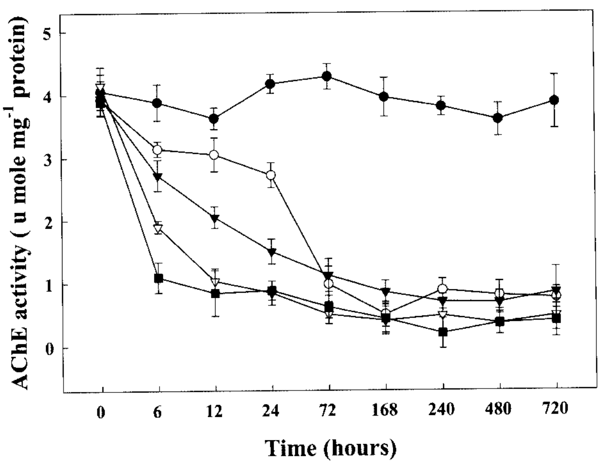

Fig. 4. Changes of acetylcholinesterase (AChE) activity in serum of *Carassius auratus* exposed to parathion for 30 days. Values are mean±S.E. (n=7). (● Control; ○ 63µg/L; ▼ 95µg/L; ▽ 190µg/L; ■ 380µg/L)

tion rate of muscle AChE in *C. auratus* (Fig. 5.3. B) was 89.7% (63 µg/L), 93.9% (95 µg/L), 97.6% (190 µg/L), and 96.8% (380 µg/L).

Impact on various concentrations of parathion on the inhibition of AChE activity in the serum of *C. auratus* are elaborated in Fig. 4. Significant inhibition was found in the serum AChE of experimental species in response to ≥ 63 µg/L concentrations of parathion. Mean inhibition rate of serum AChE of *C. auratus* in parathion 63 µg/L was 68.9%; 95 µg/L (70.8%); 190 µg/L (78.0%) and at 380 µg /L inhibition rate was 76.5%.

3.2. Behaviors

Table 2 shows the survival rate of experimental organisms for the different treatment groups of parathion. No significant mortality occurred during the experiment duration. But after addition of exposure the fishes are erratic swimming and convulsions.

Table 2. Percentage survival and scoliosis rates in *Carassius auratus* exposed to different levels of parathion

Measured parameters	Parathion concentration (µg/L)	Day 1～10	Day 11～20	Day 21～30
Survival rate	0	100	100	100
	63	100	100	95
	95	100	100	100
	190	100	95	95
	380	100	100	95
Scoliosis rate	0	0	0	0
	63	0	0	0
	95	0	0	0
	190	0	0	15
	380	0	40	75

3.3. Malformation

Anomalies like scoliosis, abnormalities in vertebrate were found in *C. auratus* exposed to parathion (Fig. 5). The estimated percentage of exposed-animals having scoliosis were 15% at 190 μg/L and 75% at 380 μg/L, till the end of experiment. The ratio of parathion-enhanced abnormality increased according to increase concentration and duration of exposure (Table 2).

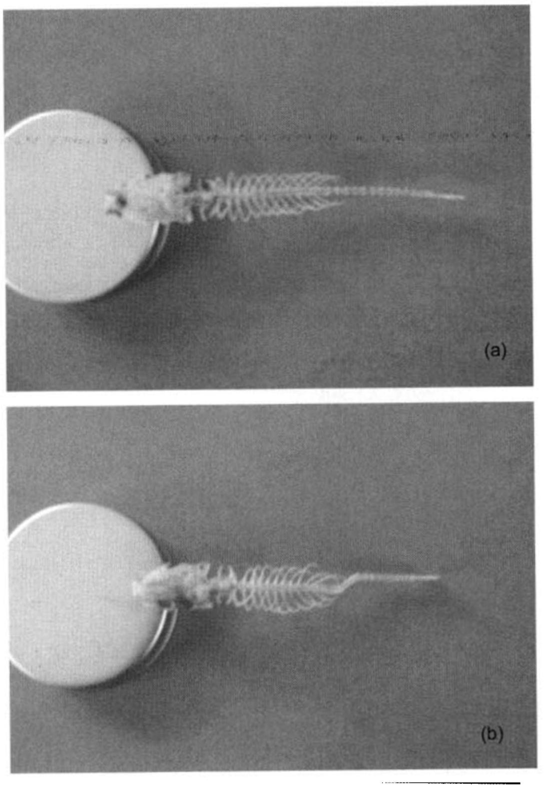

Fig. 5. Bone structures of the normal tail in parathion-unexposed male *Carassius auratus* (a) and the scoliosis tail in parathion-exposed male *C. auratus* (b).

4. Discussion

The primary mechanism of action of OPs involves the inhibition of AChE by oxygenated metabolites (-oxon). Therefore, possible hazardous effect of AChE-inhibiting pesticides in the aquatic environment should not be ignored, since these pesticides act as a nerve poison for the respiration centers of the brain and neuromuscular junctions of the respiratory apparatus (Coppage et al., 1976). In our study, AChE activity was significantly inhibited in the experimental organs treated with higher concentration more than 63 μg/L of parathion.

Straus et al. (1995) showed the higher AChE activity than other organs in brain of *Ictalurus punctatus*. Similarly, we observed brain exhibited highest AChE inhibition. Therefore, brain AChE inhibition of fish by OPs has been proposed as the main determinant of the toxicity *in vivo* (Coppague et al., 1974). Brain AChE activity in *C. auratus* was respectively reduced 79.1~92.4% at sublethal concentrations of parathion than the control group. A significant depression of brain AChE activity was observed from 6 hour of exposure at ≥63 μg/L concentrations of parathion. This early inhibition has also been reported by various investigators (Cerón et al., 1996; Sancho et al., 1998). Several studies have shown that AChE inhibition caused by OPs increases with increasing exposure concentration and exposure time (Legierse et al., 1999), but this was not the case in our experiment with, probably caused by the higher exposure concentration of parathion than the previous studies.

The high values for AChE activity in whole eye in comparison with to other tissues might be influenced by its high level in the retina as reported in most animal species (Harlin, 1991). Therefore, it seems that the retina is a good tissue for AChE determination in fish because of its high nerve activity. Sclera, cornea, chorid, iris, ciliary body, and optic nerve also contain acetylcholine (Harlin, 1991). The AChE activities in the eye derived from the present study are also very sensitive. In this study, eye AChE activity of *C. auratus* was deeply reduced 76.0~91.5% at sublethal concentrations of parathion than the control group. Present results are similar to the result of AChE activity with eye of *A. anguilla* (Cerón et al., 1996). Sancho et al. (2000) found AChE activity in sensory organs because eye was reduced up to 70% after 96hr LC_{50} value of carbamate thiobencarb.

Parathion concentrations higher than 63 μg/L, caused a nearly complete inhibition of muscle AChE activity. Muscle offers some advantages over brain tissue, including ease of collection and the availability of a large quantity of material, and different perspective on neurotoxicity test (Kirby et al., 2000).

Generally, serum AChE is highly sensitive to inhibition reported in many of the laboratory studies (Cerón et al., 1996). The serum AChE activity of *C. auratus* in this work was reduced by 68.9~78.0% in parathion-treated groups than the control. According to Fernández-Vega et al. (1999), the AChE activity decreased 50% from the first hours of contact with the toxicant and maintained this depletion for the entire exposure time of 0.22 mg/L of thiobencarb measured in the serum of eel. But in our experimental condition, relatively the exposure of high concentration for longer period did not show any differences in the AChE inhibition among the treated groups of *C. auratus*.

The successive physiological disturbances induced by pesticides including hyperactivity, loss of equilibrium, tremors and convulsions are avoidance mechanism, causing population movements. This mechanism in fish has been reported frequently by some researchers (Alabaster et al., 1980; Baier et al., 1985). For instance, *Salmo gairdneri* tend to remain motionless for several minutes and *Coregonus clupeaformis* show consistent swimming patterns (Murty, 1986). AChE inhibition attribute alterations in nerve impulse frequency following exposure to test chemicals. Consequently, vital behavioral responses affected by such alterations. Since it involved visual function, integration of information by the central nervous system, coordinated moter activity, and swimming capacity, all of which might be sensitive to toxicants in the environment (Heath, 1995).

Behavioral effects of OPs have been determined in several studies, even at concentrations that are much lower than those which cause other overt effects. Decreased activity and food consumption upon exposure to parathion are common effect, both in fish (Rand, 1977) as well as in invertebrates (Detra et al., 1991).

These studies showed also a positive correlation between AChE inhibtion and the obseved behavioral responses. However, other studies have shown an increased activity upon exposure to sublethal concentrations of OPs (Kumar et al., 1998). According to Henry et al. (1984), the hyperactivity in bluegill is associated with relatively low concentrations of OPs.

In our study, malformation were observed in the form of scoliosis in *C. auratus* exposed to parathion. Scoliosis in fish is caused by several diverse agents that possibly act on the central nervous system, neuromuscular junctions, or ionic metabolism (Couch et al., 1977). In general, the rate of deformities in fish populations in a good water quality is expected to be quite low. For example, Hughes et al. (1987) reported that anomaly rates of fish, including the large scale sucker and the mountain sucker, averaged less than 1% in the upper Willamette River of good water quality but approximately 6.5% in the lower river where contaminants and poorer water quality were identified. Couch et al. (1979) found the herbicide trifluralin caused severe cases of vertebral dysplasia in young *Cyprinodon variegatus*. Similarly, the herbicide 2,4-D is also known to reduce vertebral collagen concentrations in *Pimephales promelas and Ictalurus punctatus*, potentially resulting in broken or deformed backbones (Mayer et al., 1986). This observation was also recorded by Kennedy et al. (2001), who studied the effects of methyl parathion on *Rana tigrina*. The macroscopic results of Wofatox 50 EC (50% methylparathion) on pheasant embryos showed a dose-dependent maldevelopment (generally cervical lordosis and scoliosis, cyllosis and sporadic thoraco-gastroschisis) (Deli et al., 1985). The anomalies described by them is impaired swimming, adverse effect on feeding rates and avoidance of predators.

Therefore, the use of AChE activity, behavior and scoliosis of *C. auratus* might be used to as some detecter for the sublethal levels of parathion.

5. CONCLUSIONS

The use of AChE activity, behavior and scoliosis of *C. auratus* might be used to as some detecter for the sublethal levels of parathion.

1) The AChE activities were significantly inhibited in the experimental organs of *C. auratus* treated ≥63 μg/L of concentrations of parathion.
2) No significant mortality occurred during the experiment duration but behavioral changes occurred in the carp after exposure to the parathion were erratic swimming and convulsions.
3) The anomaly in the carp exposed to parathion were observed in the form of scoliosis.

국문요약

파라치온에 노출된 붕어의 뇌, 안구, 근육 및 혈청의 AChE 활성 변화를 조사한 결과 파라치온 63 μg/L 농도 이상에서 유의하게 감소하는 경향을 보였다. 실험종료 시 AChE 활성은 뇌에서 79.1~92.4%, 안구에서 76.0~91.5%, 근육에서 89.7~97.6% 및 혈청에서 68.9~78.0% 감소하였다. 실험기간동안 유의한 생존율 변화는 관찰되지 않았으나, 유영이상 및 경련을 보였다. 또한 파라치온에 노출된 붕어의 형태변화는 척추측만(scoliosis)이 관찰되었는데 이러한 변화는 파라치온 190 μg/L 농도는 15%, 380 μg/L에서는 75%로 관찰되었다. 따라서 붕어의 AChE 활성과 그 밖의 행동 및 형태적 특성은 자연수계에서 파라치온 오염을 추정할 수 있는 생물학적 모니터링 도구로 활용할 수 있다.

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