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Acute Toxicity of Four Pesticides on the Chinese Bleak (*Aphyocypris chisnensis*) Indigenous to Korea

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In this study, acute toxicity of four pesticides (diazinon, iprodione, fenproparthrin, and myclobutanil) to Chinese bleak, *Aphyocypris chinensis*, and to study their adverse effects were conducted in static systems. The 96-hr LC_{50} values were determined, as well as 95% confidence limits. The 96-hr LC_{50} values of these chemicals derived for Chinese bleak were rank ordered from the most toxic to the least toxic technicals as follows: Fenproparthrin (0.003 mg L^{-1}) > Myclobutanil (9.1 mg L^{-1}) > Diazinon (14.1 mg L^{-1}) > Iprodione (31.8 mg L^{-1}). The data presented in this study indicate the acute toxicity tests carried out on only one fish species may lead to erroneous determination of water quality criteria and classification of test chemicals for environmental management and regulatory purpose. Therefore, more studies comparing the susceptibility of a variety of fish species to various toxicants are needed.

Key words : Chinese bleak, fish acute toxicity, pesticide, LC_{50}

INTRODUCTION

Standard ecotoxicological study based on the official test guidelines has been one of the primary approaches in order to assess the effects of toxic chemicals on aquatic organisms (fish, crustacean, and algae) and to classify these chemicals with respect to their potential hazard to the environment. According to the guidelines developed by the Organization for Economic Cooperation and Development (OECD), for example, acute toxicity test on fish using juvenile or adult stage is to be carried out on only one of the eight recommended species. This would imply an equivalent susceptibility of the difference species of fish to the chemicals, under the conditions described in the official guidelines (Vittozzi and De Angelis, 1991). However, Gallo *et al*. (1995) indicated that toxicity testing with only one fish species may be inadequate to classify chemicals belonging to these classes for their environmental impact. In addition, toxicity of chemicals to aquatic organisms has shown to be effected by age, size and health of the species, species, and the duration of exposure (Farah *et al*., 2004; Köprücü *et al*., 2006). Therefore, the knowledge on the difference in susceptibility to various fish species may help to improve the determination of water quality criteria and the classification of chemicals, according to the regulations devoted to environmental protection.

The Chinese bleak, *Aphyocypris chinensis*, is a small cyprinid widely distributed in the small streams and ponds of Korea, and is also distributed in other East Asian countries such as Taiwan, China, and Japan (Kim, 1997). The important characteristics of *A. chinensis* are short developmental time, transparent eggs, ease of culturing, and year-round reproduction (Park *et*

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al., 1998; Yeom *et al*., 2001). With these attractive features, *A. chinensis* can also be considered as a suitable test organism for ecotoxicological purpose, particularly for embryo-larval toxicity testing (Yeom *et al*., 2005). These days, however, the numbers of Chinese bleak are decreasing due to environmental changes such as destroying of natural waterways and ponds as oriental weatherfish's habitat, and increased use of agricultural chemicals in paddy fields. Despite this, there is little information on the effects of short-term exposure to agricultural pesticides.

The present study was performed to determine acute toxicity of four pesticides currently in use, such as diazinon, iprodione, fenpropathrin, and myclobutanil, to Chinese bleak, *Aphyocypris chinensis*. This species was selected for bioassays because it can easily be raised under laboratory conditions. It fulfills most of the requirements of a model species and is available throughout the year.

Table 1. Specification of the test chemicals.

Chemicals	CAS No.	Purity (%)	Supplier
Diazinon	333-41-5	93.9	Syngenta Korea (Seoul, Korea)
Iprodione	36734-19-7	95.5	Bayer CropScience Korea (Seoul, Korea)
Fenpropathrin	64257-84-7	91.3	Kyung Nong Corporation (Seoul, Korea)
Myclobutanil	88671-89-0	93.6	Kyung Nong Corporation (Seoul, Korea)

Table 2. Physicochemical properties of the test water.

Parameter	Value
Temperature (°C)	23.7±0.8
pH	6.48-8.02
Dissolved oxygen (mg L^{-1})	5.8-8.3
Total hardness (as $CaCO_3$, mg L^{-1})	36-50

MATERIALS AND METHODS

Fishes used in this study were reared in this laboratory from eggs. After hatching, the larvae were fed daily with Baby Fish Food (Tetra, Germany) until reaching juvenile stage within 2 months. Juvenile fishes were fed daily with live brine shrimp until they were used in toxicity testing. The adult fishes, approximately four month old, were used for the toxicity test. Fishes were cultured with tap water dechlorinated through membrane and charcoal (residual chlorine, <0.01 mg L^{-1}; hardness, 36-50 mg L^{-1} as $CaCO_3$; alkalinity, 19-39 mg L^{-1} as $CaCO_3$; pH 7.1-7.9). Fish were kept at kept at 24-26°C with a 16 : 8 hour light : dark photoperiod.

In this study, static acute toxicity tests were conducted according to standard methods (OECD, 1993). The concentration range of the agrochemicals was determined with a series of range finding tests. Thereafter, definitive acute toxicity tests were conducted by exposing fish to different concentrations of test chemicals. Each test consisted of exposing groups of 7 fish to a series of six or seven toxicant concentrations, one dilution water control, and one solvent control exposure. The four agrochemicals used in the present study had the specification listed in Table 1. Since all test substances have low solubility, a stock solution of the test chemicals was prepared by dissolving desired amount in dimethylsulphoxide (DMSO, Merck, Germany) and then added to the same water as that used for fish culture to give the desired exposure concentrations. The concentration of DMSO was less than 100 mg L^{-1}. Tests were conducted in 5 L glass test vessels containing 4 L of test solution.

Mortality and abnormal behavior were monitored and recorded at 24-hour intervals during the tests, and dead fish were removed at those intervals. Cessation of opercular movement was the criteria for mortality. At the termination of each test, all control fish were weighed and total length was measured. The average wet weight and the average total length (±SD) of fish used in experiment were 0.28 (±0.09) g and 3.1 (±0.4) cm, respectively. Dissolved oxygen and pH were measured and recorded in the control, solvent control, low, medium, and high concentrations of each test with live fish present at 0, 48, and 96 hr of exposure. Dissolved oxygen was measured with a YSI Model 57 dissolved oxygen meter and probe with a standard membrane. pH was measured with an Orion 720A pH meter with a Ross pH electrode and automatic temperature compensation electrode. A 16 : 8 hour light : dark photoperiod was provided throughout the test. The physiochemical characteristics of test water are listed in Table 2.

The median lethal concentrations (LC_{50} values) with 95% confidence limits were calculated using either the Probit or Moving average angle methods as described by Stephan (1977). All LC_{50} values are expressed as nominal concentration of each test chemical.

RESULTS AND DISCUSSION

Temperature ranged from 22.0 to 24.8°C during the exposure period. The concentrations of dissolved oxygen in all tests were maintained at grater than 60% saturation, as recommended by OECD test guidelines (1992). There were no mortalities and adverse effects observed in any of the fish exposed to control and solvent control during the experiments. The 96-hr LC_{50} values with 95% confidence limits determined with the four agrochemicals are presented in Table 3.

In the present study, the 96-hr LC_{50} value of diazinon, an organophosphate insecticide, to Chinese bleak was 14.1 mg L^{-1} (Table 3). The 96-hr LC_{50} values of diazinon were reported as 1.5 mg L^{-1} for common carp (*Cyprinus carpio*) larvae (Aydin and Köprücü, 2005), 3.9 mg L^{-1} for Japanese medaka (*Oryzias latipes*) (Oh *et al.*, 1991), 0.1-0.5 mg L^{-1} for fry bluegill sunfish (*Lepomis macrochirus*), 1.5 mg L^{-1} for sheepshead minnow (*Cyprinodon variegates*), 1.7 mg L^{-1} for fry rainbow trout (*Oncorhynchus mykiss*), and 7.8 mg L^{-1} for fathead minnow (*Pimephales promelas*) (Office of Pesticide Programs, 2000). These results showed that Chinese bleak was approximately 2-141 times more tolerant than any other fish species reported above. These results suggest that diazinon has greater margin of safety to Chinese bleak in comparison to other fish. The symptoms of intoxication observed at all concentrations higher than 4.0 mg L^{-1} of diazinon were loss of equilibrium, swimming at the water surface, and dark discoloration. In addition to these, Chinese bleak exposed to 8.0 mg L^{-1} of diazinon showed vertebral deformity. McCann and Jasper (1972) observed haemorrhaging and fractures of the caudal vertebrae of bluegill fingerings exposed to organophosphorus and triazine formulations and they reported that most of this injury was due to a complex fracture of the caudal centrum and the haemal arch of a number of the vertebrae. Kanazaw (1975) also found that up to 40% of an experimental population of motsugo (*pseudorasbora pava*) suffered vertebral damage after exposure to diazinon.

Table 3. Calculated LC_{50} values (mg L^{-1}) and 95% confidence intervals for diazinon, iprodione, fenpropathrin, and myclobutanil in Chinese bleak.

Pesticide	Duration (hr)	LC_{50} (95% confidence limits)
Diazinon	96	14.1 (10.9-17.4)
Iprodione	96	31.8 (27.2-38.8)
Fenpropathrin	96	0.003 (0.002-0.004)
Myclobutanil	96	9.1 (7.4-11.7)

US EPA (1998) reported the 96-hr LC_{50} values of iprodione, a dicarboximide fungicide, on various fish species as follows: 3.7-7.8 mg L^{-1} for bluegill sunfish (*L. macrochirus*), 4.1-4.2 mg L^{-1} for rainbow trout (*O. mykiss*), 3.1 mg L^{-1} for channel catfish (*Ictalurus punctatus*). In terms of relative sensitivity, Chinese bleak appeared generally resistant and their 96-hr LC_{50} values were higher than those in bluegill sunfish, rainbow trout, and channel catfish. For example, the corresponding 96-hr LC_{50} value for the present study was 31.8 mg L^{-1} (Table 3).

The fenpropathrin, a pyrethroid insecticide, appeared to be the most toxic to Chinese bleak with 96-hr LC_{50} value was 0.003 mg L^{-1} among the agrochemicals tested (Table 3). The results of the present study were similar to other report for fish exposed to fenpropathrin. For example, the 96-hr LC_{50} value was 0.002 mg L^{-1} for bluegill sunfish (*L. macrochirus*), 0.002 mg L^{-1} for rainbow trout (*O. mykiss*), and 0.003 mg L^{-1} for sheepshead minnow (*C. variegates*) (http://www.epa.gov/tri/chemical/hazard_enviro95.pdf). Pyrethroids have low toxicity in mammals, but they can be highly toxic to other animals, especially fish (Glickman and Lech, 1982). Yilmaz *et al.* (2004) reported the hypersensitivity of fish to pyrethroid intoxication is partly due to species' specific difference in pyrethroid metabolism, but principally to the increased sensitivity of the piscine nervous systems to these pesticides. Although under field conditions synthetic pyrethroids are considered to pose less risk due to its high adsorption to soil (Köprücü *et al.*, 2006), these results indicate that fenpropathrin contamination is very dangerous to fish. Therefore, this fact should be taken into consideration when pyrethroid insecticides including fenpropathrin are used in agriculture.

The British Crop Protection Council (2003) reported the acute toxicity of myclobutanil, a triazole fungicide, on bluegill sunfish and rainbow trout as 2.4 mg L^{-1} and 2.0 mg L^{-1}, respectively. The 96-hr LC_{50} value (9.1 mg L^{-1}) reported in the present study for myclobutanil, however, were higher than those of British Crop Protection Council (Table 3). Since myclobutanil is rapidly excreted by animals, myclobutanil is classified as a moderately toxic compound (Athanasopoulos *et al*., 2003). The toxicity difference of myclobutanil among fish species may be related to host metabolism. Therefore, additional study is needed to confirm the contribution of metabolism, such as absorption, detoxification rate, and excretion, to toxicity difference in the Chinese bleak.

Acute toxicity studies are the very first step in determining the water quality requirements of fish. These studies obviously reveal the toxicant concentrations (LC_{50}) that cause fish mortality even at short exposure. The data presented in this study suggest that there are differences in the acute toxicity of pesticides for various fish species. The acute toxicity tests carried out on only one fish species may lead to erroneous determination of water quality criteria and classification of test chemicals for environmental management and regulatory purpose. Therefore, more studies comparing the susceptibility of a variety of fish species to various toxicants are needed in order to predict the potential risk of contaminants.

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< **국문적요** >

국내 토착종인 왜몰개(*Aphyocypris chinensis*)에 대한 4개 농약의 급성독성 생물검정

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본 연구에서는 국내 토착종인 왜몰개(*Aphyocypris chinensis*)을 사용하여 4가지 농약(diazinon, iprodione, fenpropathrin, myclobutanil)의 급성독성을 평가하기 위하여 지수식으로 독성실험을 실시하여 96-hr LC_{50} 값과 95% 신뢰한계를 구하였다. 각 실험농약의 96-hr LC_{50} 값을 비교하여 왜몰개에 대한 급성독성이 높은 순서를 정리한 결과는 Fenproparthrin (0.003 mg L^{-1}) > Myclobutanil (9.1 mg L^{-1}) > Diazinon (14.1 mg L^{-1}) > Iprodione (31.8 mg L^{-1}) 순으로 나타났다. 본 연구에서 사용된 4가지 농약에 대한 왜몰개의 급성독성 값은 현재까지 보고된 다른 어종의 급성독성 값과 차이가 있었다. 그러므로 환경관리 및 규제목적으로 어류에 대한 급성독성실험 결과를 수질기준 또는 물질분류에 이용하기 위해서는 다양한 어류에 대한 급성독성 평가를 통하여 종합적 판단이 필요하다고 사료된다.