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Use of Chinese Bleak, *Aphyocypris chinensis*, in Embryo and Sac-Fry Stages Toxicity Test

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왜몰개 (*Aphyocypris chinensis*)를 이용한 Embyo, Sac-fry stages Toxicity Test

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요 약

ESS (Embryo and sac-fry stage) 독성시험에서 시험어종으로서의 국내토착종인 왜몰개 (*Aphyocypris chinensis*)의 적용성을 평가하기 위하여 아연(Zn)을 사용하여 국제적인 추천시험 어종인 송사리 (*Oryzias latipes*)와 감수성을 비교하였다. 시험기간은 대조군에서 아사가 관찰되는 시기 즉, 왜몰개는 수정 후 8일, 송사리는 수정 후 16일로 하였으며, 시험기간 동안 수정란의 부화율, 수정란 및 난황단계의 치어(sac-fry)의 사망률, 형태적인 발달, 치어의 성장 등을 관찰 및 측정하였다. 두 종 모두 수정란의 생존율에 아연의 영향을 받았으며, LOEC는 모두 14.5 mg/L 이었다. 난황단계 치어의 사망률을 관찰한 결과, 왜몰개는 1.4 mg/L 부터 급격히 증가된 반면에 송사리는 14.5 mg/L에서 100% 사망률이 관찰되었다. 시험물질에 노출된 왜몰개와 송사리 모두 척추변형이 관찰되었으며, 체장을 측정한 결과는 왜몰개가 송사리에 비해 민감하게 반응하는 것으로 나타났다. 위와 같은 결과들은 종합해 볼 때, ESS 독성시험에서 왜몰개가 대체 시험어종으로서 사용될 수 있는 가능성을 보여주었다.

Key words : Chinese bleak, medaka, embryo and sac-fry toxicity test, sensitivity

INTRODUCTION

Attempts to develop rapid tests that be used as a screening test for either a full early life stage toxicity test or chronic toxicity of chemicals to fish have resulted in the development of the embryo and sac-fry stages (ESS) toxicity tests. In this test, the fish are exposed to pollutants from the newly fertilized egg to the end of the sac-fry stage. Various fish species have been recommended for ESS toxicity testing, e.g., zebrafish (*Brachydanio rerio*), rainbow trout (*Oncorhynchus mykiss*), carp (*Cyprinus carpio*), medaka (*Oryzias latipes*), and fathead minnow

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(*Pimephales promelas*) (OECD, 1998). Although the time and effort required to conduct ESS toxicity tests with these species are much less than those of full early life stage toxicity tests, the duration of tests, however, still ranges from weeks to months.

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 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. The purpose of this study was to investigate its applicability as a toxicity indicator in the short-term ESS toxicity test. For that purpose, zinc ($ZnSO_4 \cdot 7H_2O$) was used as test substance on *A. chinensis* because zinc was recommended as a suitable reference toxicant (Environment Canada, 1990). The results of this ESS toxicity test were compared with those obtained from the toxicity test with medaka, *Oryzias latipes*, fish species recommended for short-term embryo and sac-fry toxicity testing (OECD, 1998).

MATERIALS AND METHODS

The experimental testing procedure used in this study is based on the principles outlined in the standard protocols for conducting ESS toxicity test with fish (OECD, 1998). *A. chinensis* and *O. latipes* in the simulated condition can produce fertilized eggs daily once provided with adequate nutrients in the laboratory. Prior to test initiation, the embryos were inspected using a stereomicroscope and those that appeared abnormal or fungus-infected were discarded. Normal embryos were used to set the test.

A flow-through system designed to provide up to five test concentrations and a dilution water control was used during the test. Each glass test aquarium measured approximately 20 × 10 × 13H cm, had a 40 mesh Nybolt (Switzerland) screen-covered drain guard, and held a test volume of approximately 2 L. Embryos were incubated in chambers suspended in the treatment and control aquaria. These egg incubation chambers were constructed from approximately 7-cm diameter glass jars with Nybolt screen glued with silicon to the bottom. To ensure exchange of water, the egg cups were oscillated in the test solution by means of a rocker arm apparatus driven by a low rpm electric motor. During the test, the flow-through system provided at least ten volume turnovers in each test vessel during a 24-h period.

The test solution of zinc was prepared from reagent grade $ZnSO_4 \cdot 7H_2O$ (Junsei Chemical Co. Ltd.). The dilution water in all tests was activated carbon filtered tap water with hardness: 50 mg/L of as $CaCO_3$. This water has been used successfully in the in-house fish culture system. Nominal exposure concentrations were set at 0.1, 0.4, 1.4, 4.5, and 14.5 mg/L, but chemical analysis of the test solutions was not conducted. The range of the chemical concentrations for the tests with *A. chinensis* and *O. latipes* was selected from the preliminary tests. For both species, groups of thirty-six embryos were exposed to each treatment level and control (12 embryos/replicate, 3 replicates per concentration). Tests were terminated when the mortality of larvae by starvation started in controls, i.e., 8 and 16 days after fertilization for *A. chinensis* and *O. latipes*, respectively. Main test conditions were: temperature 23.7±0.5°C, dissolved oxygen (DO) 7.8±0.2, pH 6.6±0.1 and photoperiod 16 h light : 8 h dark. No food was provided during the test period.

The embryos were observed and counted daily throughout the exposure period. For both species, hatching was defined as the rupture of the egg membrane. Partially and completely hatched larvae were counted as hatched. Survival and malformation of larvae were observed and recorded everyday. Embryos and larvae were considered dead when no heartbeat was observed. The growth of the fish was

estimated at the end of tests by measuring the body length and weight of surviving larvae. The body length of the fish was taken as standard length (from the top of premaxilla to the center of the caudal fin) of each individual. After measuring the length, the body wet weight was determined.

Data on survival and growth of surviving fish were subjected to one-way analysis of variance (ANOVA), after being tested for the normality distribution using the Kormogolov-Smirnov test and for homogeneity of variance using the Barlett test (US EPA, 1994). Comparisons between the controls and the treatments were made at $p \leq 0.05$. The LC50 values (larval survival) were calculated using the Trimmed Spearmen-Karber method (US EPA, 1994).

RESULTS AND DISCUSSION

Zn affected embryo survival of the two species at the same concentration. The LOEC value for embryo survival obtained for *A. chinensis* and *O. latipes* was 14.5 mg/L (Fig. 1). The similarity in embryo survival of the two species may be linked to the developmental stage of the embryo at exposure. As *A. chinensis* and *O. latipes* were exposed after the chorionic hardening, the permeability of the chorion may be one of the reasons for the similar sensitivity to Zn of the two species (Dave *et al.*, 1987). Nguyen and Janssen (2001) also reported that the embryos of *Clarias gariepinus* and *Danio rerio* exposed to Zn showed similar LOEC values.

Reduced survival of *A. chinensis* sac-fry was observed at 1.4 mg/L. For *O. latipes* sac-fry the LOEC value of 14.5 mg/L was noted (Fig. 1). Conversely, sac-fry survival of *O. latipes* was less sensitive than that of *A. chinensis*. The vertebral deformation was observed in *A. chinensis* and *O. latipes* exposed to Zn. The proportion of this abnormality was 2.8% in *A. chinensis*, while abnormality of *O. latipes* sac-fry ranged from 2.9% to 6.0%. However, no clear concentration-response relationship between the percentage of sac-fry with malformation and Zn concentrations was observed.

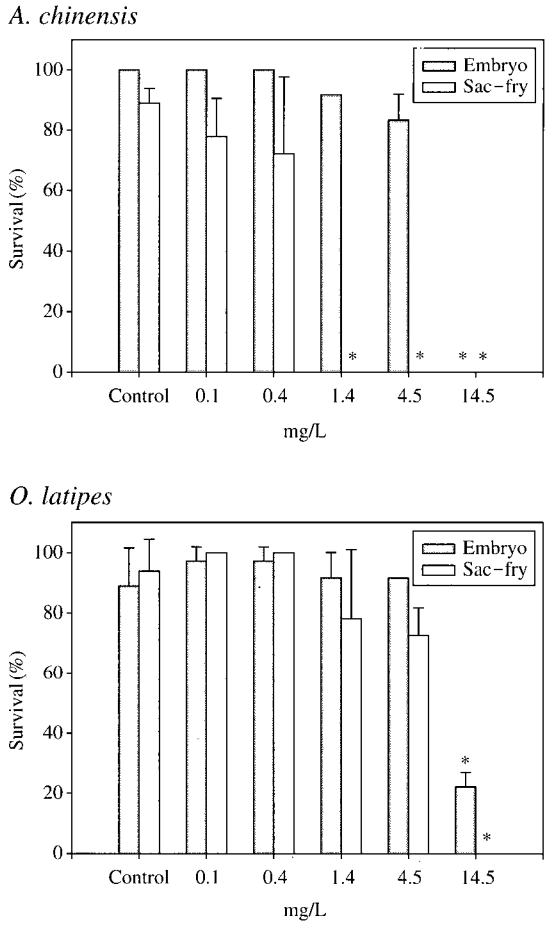

Fig. 1. Embryo and sac-fry survival of *A. chinensis* and *O. latipes* exposed to zinc. Data are given as mean± SD; n = 3. *Significantly different from the control ($p \leq 0.05$).

The body length of the *A. chinensis* sac-fry exposed to Zn was significantly reduced at ≥ 0.1 mg/L, whereas the weight was not affected (Table 1). In contrast, growth of survivors of *O. latipes* was not influenced by Zn (Table 2). The results of the growth measurements showed that *A. chinensis* was more sensitive to Zn stress in comparison with *O. latipes*.

Kristensen (1991) reported that length and weight measurements were equally influenced. However, body length was more sensitive to zinc stress in comparison with body weight in *A. chinensis*. This result

Table 1. Body length and weight of *A. chinensis* exposed to zinc

Concentration (mg/L)	Sac-fry survival (%)	Length (mm/ individual)	Weight (mg/ individual)
Control	88.9±4.9	4.46±0.20	0.15±0.05
0.1	77.8±12.7	4.33±0.17*	0.18±0.06
0.4	72.2±25.5	4.26±0.18*	0.19±0.06
1.4	0*	-	-
4.5	0*	-	-
14.5	0*	-	-

Data are expressed as mean±SD; n≥3
*Significantly different from the control ($p \leq 0.05$)

Table 2. Body length and weight of *O. latipes* exposed to zinc

Concentration (mg/L)	Sac-fry survival (%)	Length (mm/ individual)	Weight (mg/ individual)
Control	93.9±10.5	4.54±0.23	0.30±0.07
0.1	100±0	4.62±0.17	0.29±0.11
0.4	100±0	4.57±0.22	0.29±0.09
1.4	78.2±22.8	4.57±0.22	0.26±0.09
4.5	72.7±9.1	4.56±0.21	0.25±0.08
14.5	0*	-	-

Data are expressed as mean±SD; n≥3
*Significantly different from the control ($p \leq 0.05$)

may be linked to the difficulties in measuring the weight due to the small size of the 8-day-old *A. chinensis* sac-fry and the variation of the weight caused by differential yolk sac resorption (Kristensen 1991). It may be concluded that preference should be given to body length measurements as a growth endpoint.

The LC_{50} values of Zn on *A. chinensis* and *O latipes* sac-fry survival were 0.7 and 4.8 mg/L, respectively. The maximum-acceptable toxicant concentration (MATC) using the most sensitive endponts was < 0.1 mg/L for *A. chinensis* and 8.1 mg/L for *O. latipes* (Table 3).

The LOEC values for the most sensitive endpoint obtained using 5-day *C. gariepinus* and 12-day *D. rerio* embryo-larval assays were 2.3 and 1.42 mg/L Zn, respectively (200 mg/L as $CaCO_3$, pH 7.2±0.14) (Nguyen and Janssen, 2001). Despite the limits in reference data, these data showed the range of embryo-larval toxicity of zinc to different species of fish was large and the most sensitive fish was *A. chinensis*. These differences in the sensitivity of fish species may be due to the binding affinity and permeability of metal to the chorion, water quality, especially water hardness, embryonic developmental period and hatching time, (Dave *et al.*, 1987; Nguyen and Janssen, 2001). Taking into consideration of the short duration of *A. chinensis* embryo and sac-fry assay and its sensitivity, this species may be a potential alternative for short term embryo-larval toxicity testing with fish.

Table 3. Comparison of the sensitivity to zinc (mg/L) in embryo-sac fry toxicity tests of *A. chinensis* and *O. latipes*

Species	LC_{50} (95% confidence limits)	Effect concentration		MATC
		NOEC	LOEC	
A. chinensis	0.7 (0.5~0.8)	<0.1	0.1	<0.1
O. latipes	4.8 (3.8~6.1)	4.5	14.5	8.1

REFERENCES

Dave G, Damgaard B, Grande M, Martelin JE, Rosander B and Viktor T. Ring test of an embryo-larval toxicity test with zrbrafish (*Brachydanio rerio*) using chromium and zinc as toxicants, Environ Toxicol Chem 1987; 6: 61-71.

Environmental Canada. Environmental Protection Series: guidance document on control of toxicity test precision using reference toxicants, Canada. 1990.

Kim IS. Encyclopedia of fauna and flora of Korea, Vol. 37. Freshwater fish. Ministry of Education, Seoul. 1997.

Kristensen P. Evaluation of the sensitivity of short-term fish early life stage tests in relation to other fish early life stage test method, Final report, Water Quality Institute, Denmark. 1991.

Nguyen THL and Janssen CR. Comparative sensitivity of embryo-larval toxicity assays with African catfish (*Clarias gariepinus*) and zebra fish (*Danio rerio*),

Environ Toxicol 2001; 16: 566-571.

OECD. Guideline for testing of chemicals: fish, short-term toxicity test on embryo and sac-fry stages. OECD 212, Paris. 1998.

Park DS, Yeom DH, Lee SK and Choi SS. Development of eggs and larvae *Aphyocypris chinensis* GUNTHER (Cyprinidae: Leuciscinae) reared in the laboratory, Koran Journal of Environmental Biology 1998; 16(3): 245-251.

US EPA. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 3d ed. EPA/600/4-91/002, Cincinnati, OH. 1994.

Yeom DH, Lee SK and Choi SS. Influence of water temperature on spawning of Chinese bleak, *Aphyocypris chinensis*, Korean J Limnol 2001; 34: 337-341.