Comparative Sensitivity of Embryo–Larval Toxicity Assays With African Catfish (*Clarias gariepinus*) and Zebra Fish (*Danio rerio*)

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ABSTRACT: Embryo–larval toxicity tests with the African catfish (*Clarias gariepinus*) were conducted with five chemicals (Cr, Cd, Zn, NaPCP and malathion) and three environmental samples. The sensitivity of the 5-day assay was compared to that of the 12-day embryo–larval toxicity tests with the zebra fish (*Danio rerio*). The ratios of the *C. gariepinus* and *D. rerio* LC_{50} values ranged from 0.4 for Cr to 8.9 for Zn. The ratios of subchronic values ranged from 0.25 for NaPCP to 3.1 for Cd indicating a more comparable sensitivity of the two species. For the three sediment pore waters, the ratios were 0.6, 1.1, and 2.4 and the subchronic values were identical for the two species. The results suggest that, considering the short-test duration and its sensitivity, the 5-day embryo–larval tests with *C. gariepinus* may be a potential alternative for short-term embryo–larval toxicity testing with fish. Environ Toxicol 16: 566–571, 2001

Keywords: *Clarias gariepinus*; *Danio rerio*; embryo–larval toxicity tests; sensitivity

INTRODUCTION

Early life stage (ELS) toxicity tests with fish are increasingly being used for the hazard assessment of chemicals and aquatic wastes. In this test, a continuous toxicant exposure from the embryonic stage until the exogenous feeding stage of the test organism is examined. Various fish species have been recommended for ELS toxicity testing, e.g., fathead minnow (*Pimephales promelas*), rainbow trout (*Oncorhynchus mykiss*), medaka (*Oryzias lapites*), and zebra fish (*Danio rerio*) (OECD, 1992). Although the time and effort required to perform ELS toxicity tests with these species are much less than that of full life cycle tests, the duration of the tests still ranges from weeks to months. This limits their application, especially for toxicity monitoring of environmental samples. In response to the need of more rapid assays for the routine toxicity evaluation, short-term ELS tests with some selected fish species have been developed, which are currently used in various regulatory frameworks (US EPA, 1985; Environment Canada, 1992; OECD, 1998). The reduction of the exposure period to only one or two (i.e., embryo and/or larva) developmental stages of the fish renders short-term ELS toxicity testing both practical and economic (Van Leeuwen et al., 1985; Norberg-King, 1989; Pickering and Lazorchak, 1995; Cooney, 1995).

Among catfish of the genus *Clarias, C. gariepinus* is recognized as one of the most promising aquaculture

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species (Richter et al., 1995). Besides being an excellent candidate for aquaculture, this species has also been used in fundamental research, e.g., as a model for the regulation of gene expression and endocrinology. The important characteristics of *C. gariepinus* that make it such a suitable model includes: a well-documented general biology; short developmental time (embryo–larval period lasting 4–5 days after fertilization); transparent eggs; easy culturing; and year round reproduction (Volckaert et al., 1994a). These characteristics also qualify *C. gariepinus* to be considered as a suitable test organism for ELS toxicity testing. The aim of this study was to develop a short-term embryo–larval toxicity test with *C. gariepinus*. For that purpose, toxic effects of various chemicals on *C. gariepinus* exposed for five days, i.e. from the embryonic stage until the end of the yolk sac stage, were examined. The results of this embryo–larval toxicity assay were compared with those obtained from the 12-day embryo–larval toxicity tests with *D. rerio*, a fish species recommended for short-term embryo–larval toxicity testing (OECD, 1998). Next to the tests with the five chemicals, side-by-side experiments with the two species exposed to three environmental samples were also performed.

MATERIALS AND METHODS

Test Organisms

Fertilized eggs of *C. gariepinus* were obtained by artificially fertilizing hand-stripped eggs with 1:10 diluted sperm taken from the adults, which were bred under the standard laboratory conditions (Volckaert et al., 1994b). Immediately after fertilization, the eggs (2- to 4-cell stage) were exposed to test chemicals. For the assays with *D. rerio*, fertilized eggs were also obtained from fish cultured in laboratory. The procedure proposed by Kristensen et al. (1988) was followed to obtain fertilized zebra fish eggs. Eggs in the blastula stage (Hisaoka and Battle, 1958) were used to initiate the experiments.

Exposure System

The experimental testing procedure used in this study is based on the principles outlined in the standard protocols for conducting ELS toxicity tests with fish (ASTM, 1990; OECD, 1998). For both species, tests were terminated when the larvae started exogenous feeding period, i.e., 5 and 12 days after fertilization for *C. gariepinus* and for *D. rerio*, respectively. No food was provided during the test period. The 24-well polystyrene plates (Nunc, Wiesbaden, Germany) were used as exposure vessels, with one organism per well containing 2 mL of the test solution. To avoid evaporation during incubation, the plates were covered with a Parafilm® strip. For each test concentration, four replicate plates were used. *C. gariepinus* was incubated at 27 ± 1°C with a light–dark cycle of 0–24 h. For *D. rerio*, a temperature of 25 ± 1°C and a light–dark cycle of 16–8 h were employed.

Test Chemicals and Samples

All the test solutions were prepared with aerated tap water with hardness of 200 mg/L as $CaCO_3$; dissolved oxygen (DO) 8.3 ± 0.3 mg/L, and pH 7.2 ± 0.14 (mean $\pm$ s.d.). Test solutions were renewed every 24 h; DO, pH and temperature were measured daily. Five test substances: Cr (99% $K_2Cr_2O_7$; Fluka, Switzerland), Cd ($CdCl_2 \cdot 2.5\,H_2O$; UCB, Belgium), Zn (99.5% $ZnSO_4 \cdot 7H_2O$; Ferak, Germany), pentachlorophenol (98% NaPCP; Merck, Germany), malathion (98 analytical standard; Riedel-de Haën, Germany) and three sediment pore water samples were evaluated. The chemical concentrations for the tests with *C. gariepinus* were selected on a logarithmic basis with a 0.3 or 0.5 interval. The range of the chemical concentrations for the tests with *D. rerio* was selected based on data obtained from literature. Pore waters from three contaminated sediments (coded as SPW1, SPW2 and SPW3) were extracted using a pressurized squeeze-extraction device (Carr et al., 1989). Pore waters were subsequently filtered (0.45-μm polyethylene filter) and stored in glass bottles at 4°C prior to testing.

Test Criteria

The effects of toxicants on *C. gariepinus* and *D. rerio* were observed daily throughout the exposure period. For *C. gariepinus*, the number of dead eggs/embryos was recorded 24 h after fertilization. It should be noted that this figure included the unfertilized eggs, which cannot be distinguished from those dead through toxicant exposure. The total hatching percentage was calculated based on the number of surviving embryos. In the *D. rerio* assay, dead eggs resulting from nonfertilization (initial mortality) were removed from the exposure wells on day 1 and were eliminated in the further analysis of the test data (Dave et al., 1987; Görge and Nagel, 1990; OECD, 1998). For both species, hatching was defined as the rupture of the egg membrane. Partially and completely hatched larvae were counted as hatched. Embryos and larvae were considered dead when no heartbeat was observed. At the end of the assay, the growth of larvae was evaluated by measuring the standard body length.

Statistical Analysis

Data on hatching, survival, abnormalities and body length of surviving fish were subjected to one-way analysis of variance, after being tested for the normality of

distribution using the Kormogolov–Smirnov test and for homogeneity of variance using the Barlett test (US EPA, 1985). Duncan's multiple comparison test was used to compare concentration means to control means. Statistical significance was set at $p \leq 0.05$. The geometric mean of the NOEC and the LOEC for the most sensitive endpoint, was calculated to obtain subchronic values (SCV) (Pickering, 1988). The LC_{50} values (larval survival) were calculated using the Trimmed Spearmen–Karber method (Hamilton et al., 1977).

RESULTS

A summary of the embryo–larval toxicity tests with both species is given in Table I. Cr affected survival of *C. gariepinus* embryos at concentrations ≥36 mg/L, while no mortality of *D. rerio* embryos was observed up to the highest concentration tested (86 mg/L). No effect of Cd on embryo survival of the two species was observed. The LOEC values for embryo survival obtained for *C. gariepinus* and *D. rerio* embryos were 2.3 and 1.4 mg/L Zn, respectively. In the assays with NaPCP, the toxicant affected *C. gariepinus* embryos at a concentration 10 times higher than that producing an adverse effect on *D. rerio.* For malathion, no mortality of *C. gariepinus* embryos was observed at 5 mg/L, while the LOEC value of 10 mg/L was noted for *D. rerio*.

For both species, hatching was not influenced by the highest Cr and Cd concentrations that were tested. In the Zn assays, hatching success of *C. gariepinus* was reduced at 13 mg/L, with *D. rerio* this occurred already at 2.8 mg/L. As mentioned, hatching results presented include complete and partial hatching, the latter occurring when the embryos could not completely free themselves from the enveloping chorion (Sharp, 1991). Of the 28% hatching of *C. gariepinus* at 13 mg/L Zn, only 20% embryos had hatched completely. Similar to embryo survival, NaPCP effect on *C. gariepinus* hatching was at a much higher concentration than in the *D. rerio* assays. No effect on hatching of *C. gariepinus* was observed up to the highest concentration of malathion tested, while hatching of *D. rerio* was inhibited at 3.0 mg/L.

Reduced survival of *C. gariepinus* larvae was observed at 20 mg/L Cr and for *D. rerio* larvae the LOEC value of 43 mg/L was noted. Conversely, larval survival of *C. gariepinus* was less sensitive to Cd and NaPCP compared to that of *D. rerio*. For malathion, survival of *C. gariepinus* and *D. rerio* larvae exhibited a similar sensitivity. Cr, Cd and NaPCP induced abnormalities in *C. gariepinus* larvae at ≥36, 0.5, and 0.3 mg/L, respectively. No deformations were observed in *D. rerio* exposed to these toxicants. The highest Zn concentration did not induce adverse effects on the morphological development in both species. Abnormal body axis development was observed in *C. gariepinus*, and pericardial edema was noted in *D. rerio* exposed to 1.25 mg/L and 3.0 mg/L malathion, respectively. Adverse effects on the growth of *C. gariepinus* were detected at 11 mg/L Cr, while this metal did not affect *D. rerio* growth up to 43 mg/L. Growth of survivors of the two species was not influenced by Cd. For Zn, the LOEC value for *D. rerio* growth was 2.8 mg/L and no effect on *C. gariepinus* growth was found up to 13 mg/L. In contrast, the effect of NaPCP on *C. gariepinus* growth was found to be more pronounced than that on the growth of *D. rerio.*

Table II summarizes the results of *C. gariepinus* and *D. rerio* embryo–larval tests with the three sediment pore water samples. Embryo survival of the two species was not affected by any of the samples up to the highest concentration tested. Hatching of the two species was not influenced by samples SPW1 and SPW3. In the test with SPW2, hatching of *C. gariepinus* was significantly inhibited at 50%, while no effect on hatching of *D. rerio* was observed. Larval survival of the two species was significantly affected at equivalent concentrations of SPW1 (LOEC= 50%) and SPW3 (LOEC= 12.5%). For SPW2, survival of *D. rerio* larvae was more affected (LOEC= 6.25%) than was survival of *C. gariepinus* larvae (LOEC= 12.5%). No effect of the samples on morphological development of the two species was observed. Samples SPW1 and SPW2 inhibited growth of the two species at similar concentrations: 50% and 6.25%, respectively. No effect of SPW3 on growth of *C. gariepinus* and *D. rerio* larvae was noted.

TABLE I. Summary of the results of *C. gariepinus* and *D. rerio* embryo–larval toxicity tests with various chemicals

	LOEC (mg/L)				
	Cr	Cd	Zn	NaPCP	Malathion
Embryo survival					
C. gariepinus	36	>5[a]	2.3	1.0	>5[a]
D. rerio	>86[a]	>1.5[a]	1.4	0.1	10
Hatching					
C. gariepinus	>114[a]	>5[a]	13	1.0	>5[a]
D. rerio	>86[a]	>1.5[a]	2.8[a]	0.1	3
Larval survival					
C. gariepinus	20	0.5	>13	1.0	2.5
D. rerio	43	0.15	>2.8[a]	0.1	3
Abnormality					
C. gariepinus	36	0.5	>13	0.3	1.25
D. rerio	>86[a]	>1.5[a]	>2.8[a]	>0.1	3
Growth					
C. gariepinus	11	>1.5[a]	>13	0.01	1.25
D. rerio	>43	>0.05	2.8[a]	0.1	>1

[a]Highest concentration tested.

TABLE II. Summary of the results of *C. gariepinus* and *D. rerio* embryo–larval toxicity tests with three samples of sediment pore water

	LOEC (%)		
	SPW1	SPW2	SPW3
Embryo survival			
C. gariepinus	N.E[a]	>50[b]	>50[b]
D. rerio	N.E	>50[b]	>50[b]
Hatching			
C. gariepinus	N.E	50	>50[b]
D. rerio	N.E	>50[b]	>50[b]
Larval survival			
C. gariepinus	50	12.5	12.5
D. rerio	50	6.25	12.5
Abnormality			
C. gariepinus	N.E	>50[b]	>50[b]
D. rerio	N.E	>50[b]	>50[b]
Growth			
C. gariepinus	50	6.25	>12.5
D. rerio	50	6.25	>12.5

[a]N.E: no effects observed.
[b]Highest concentration tested.

DISCUSSION

Only few differences in terms of sensitivity between *C. gariepinus* and *D. rerio* were observed in this study. The dissimilarity in embryo survival of the two species may be linked to the developmental stage of the embryo at exposure. As *C. gariepinus* was exposed prior to the hardening of the chorion, and *D. rerio* was exposed after the hardening, the permeability of the chorion may be one of the reasons for higher sensitivity to Cr of the former species. Stevens and Chapman (1984) reported that *O. mykiss* when exposed to Cr during a later embryonic stage, hatching and survival of fish were reduced at a higher concentration, showing a considerable reduction in toxicity. Moreover, embryo mortality of *C. gariepinus* exposed to potassium dichromate before water hardening has been attributed not only to Cr toxicity but also to the presence of K^+ in the ambient environment (Nguyen et al., 1999). However, despite the different developmental stages at exposure, Zn affected embryo survival of the two species at similar concentrations. Toxicity of Zn on fish embryo has been demonstrated in previous studies. Somasundaram et al. (1984) found that about 70% of accumulated Zn entered *Clupea harengus* eggs exposed before chorionic hardening. Nevertheless, Rombough (1985) reported that after hardening the chorion prevents metals with high-binding affinities (e.g., Hg, Cu, Ag) from entering the egg, whereas metals with low-binding affinities such as Zn and Pb can pass through. Developmental time of the embryo is also known as a factor influencing its susceptibility to toxicants. Based on his review of ELS toxicity tests with different fish species, Kristensen (1990) noted that increased sensitivity to toxicants such as pentachlorophenol (PCP) and disulfiram, was observed in species with a relatively long embryonic developmental time. The lower sensitivity to NaPCP of *C. gariepinus* embryo (survival) compared to *D. rerio* seems to support this observation. Indeed, the former species has a shorter embryonic period (1 day) than has *D. rerio* (4 days).

In general, morphological development of *C. gariepinus* appeared to be more susceptible to the chemicals tested than that of *D. rerio*. Several types of *C. gariepinus* malformations were observed in the Cr, Cd, NaPCP and malathion exposure. With *D. rerio*, only malathion seems to induce deformation at sublethal concentrations. Skeletal deformities are one the most frequent effects that have been reported for fish ELS exposed to metals (Rombough and Garside, 1982; Hiraoka and Okuda, 1983; Weis and Weis, 1991) and to malathion (Weis and Weis, 1989; Srivastava and Srivastava, 1990; Alam and Maughan, 1992). Indeed, the most pronounced effect of Cr and malathion on *C. gariepinus* was abnormal body axis. However, in Cd-exposed *C. gariepinus*, no skeletal deformation was observed and a common type of malformation noted was a reduction of pigmentation. As Cd-induced vertebral deformities have been attributed to decreased calcium and phosphorus in the bones (Muramoto, 1981), the absence of vertebral deformities in Cd exposed *C. gariepinus* may probably due to the absence of bony elements in the 5-day old larvae. PCP-induced pericardial edema, which has been observed in other fish species (Holcombe et al., 1982; Spehar et al., 1985), was also found in *C. gariepinus*. It has also been reported that PCP caused a significant reduction in hatching success and influenced growth of fish larvae and juveniles (Holcombe et al., 1982; Dominguez and Chapman, 1984). In this study, the effects on hatching of *C. gariepinus* were observed at a concentration of PCP, which delayed the embryonic development and/or inhibited hatching completely. The results obtained also show that growth of *C. gariepinus* larvae was reduced at concentration 100 times lower than which affected survival.

An overview of main results concerning the comparative sensitivity of the two fish species is given in Table III. The ratios of LC_{50} values obtained with the chemicals ranged from 0.4 (Cr) to 8.9 (Zn). The subchronic values indicate more comparable sensitivity of the two species. For the chemicals tested, the ratios of the *C. gariepinus* subchronic values to the *D. rerio* subchronic values range from 0.25 (NaPCP) to 3.1 (Cd). The LC_{50} ratios of the pore water samples

TABLE III. Comparison of the sensitivity to various chemicals (in mg/L) and environmental samples (in %) of *C. gariepinus* 5-day embryo–larval tests with that of *D. rerio* 12-day embryo–larval tests

Chemical Sample	LC_{50} (95% Confidence Limits)	Effect Concentration			*C. gariepinus*/*D. rerio*	
		NOEC	LOEC	SCV[c]	LC_{50}	SCV[c]
Cr						
C. gariepinus	20.1 (35.2–62.5)	11	20.0	14.8		
					0.4	0.5
D. rerio	47.7 (41.9–54.3)	21	42	29.7		
Cd						
C. gariepinus	0.67 (0.5–0.82)	0.15	0.5	0.236		
					6.7	3.1
D. rerio	0.1 (0.07–0.15)	0.05	0.15	0.086		
Zn						
C. gariepinus	18.7 (16.4–21.3)	<2.3[a]	2.3	<2.3		
					8.9	2.3
D. rerio	2.1 (1.80–2.40)	0.7	1.42	1.0		
NaPCP						
C. gariepinus	0.55 (0.47–0.55)	0.003	0.01	0.005		
					5.0	0.25
D. rerio	0.11 (0.10–0.13)	0.056	0.01	0.02		
Malathion						
C. gariepinus	3.42 (2.91–4.01)	0.63	1.25	0.9		
					1.9	0.5
D. rerio	1.80 (1.50–2.08)	1.0	3.0	1.7		
SPW1						
C. gariepinus	7.7 (6.1–9.8)	25.0	50.0	34.4		
					0.6	1.0
D. rerio	13.6 (11.5–16.0)	25.0	50.0	34.4		
SPW2						
C. gariepinus	8.8[b]	3.1	6.25	4.4		
					2.4	1.0
D. rerio	3.6 (1.5–9.0)	3.1	6.25	4.4		
SPW3						
C. gariepinus	39.9 (35.8–44.5)	6.25	12.5	8.8		
					1.1	1.0
D. rerio	35.2 (27.7–44.8)	6.25	12.5	8.8		

[a]Lowest concentration tested.
[b]95% confidence limits are not detectable.
[c]Subchronic value.

were 0.6 (SPW1), 2.4 (SPW2) and 1.1 (SPW3). The subchronic ratios obtained were 1.0 for all samples tested.

The toxicity of the tested chemicals to ELS of various fish species has been reported. Dave et al. (1987) conducted 16-day embryo–larval assay with *D. rerio* and recorded an LOEC value of 21.2 mg/L Cr, which is similar with the LOEC value for *C. gariepinus* (20 mg/L). The toxicity of Cd and PCP has been evaluated using 8-day *P. promelas* embryo–larval tests (Birge et al., 1985; Pickering, 1988). The LOEC values (1.0 mg/L Cd and 0.512 mg/L PCP) reported by the authors are comparable with that obtained in the present study (0.5 mg/L Cd and 0.01 mg/L PCP). Somasundaram et al. (1984) investigated the influence of Zn on embryo and larva of *Clupea harengus.* The authors noted that embryo development was affected at 2.0 mg/L Zn, which is also similar to the LOEC for embryo survival of *C. gariepinus* (2.3 mg/L Zn). For malathion, an LOEC value of 1.0 mg/L was obtained in the 32-day ELS with *D. rerio* (Kumar and Ansari, 1984). Crawford and Guarino (1985) when exposed *Fundulus heteroclitus* to malathion for 55 days found that a concentration of 10 mg/L caused abnormal axis formation in the embryos. The sensitivity of *C. gariepinus* to malathion (LOEC = 1.25 mg/L) thus appears to fall within the range of other fish species exhibited in the tests with a longer exposure period.

In conclusion, the 5-day embryo–larval toxicity test with *C. gariepinus* provided similar subchronic values for the chemicals and environmental samples tested to those obtained from the 12-day standard embryo–larval toxicity tests with *D. rerio*. The sensitivity of the *C. gariepinus* embryo and larva was also found to be comparable with that of other fish species early life stages reported in the literature. Taking into consideration the short duration of the *C. gariepinus* embryo–larval test and its sensitivity, it can be concluded that the African catfish is a good test organism to evaluate sublethal toxicity of chemicals and environmental samples.

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