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Differences in Sensitivity to Developmental Toxicants as Seen in *Xenopus* and *Pimephales* Embryos

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Amphibian and fish tests are useful for evaluating the developmental toxicity of chemicals and environmental mixtures (Birge et al. 1985; Dawson et al. 1988). A developmental toxicant should have similar effects on amphibians and fish if the organisms are exposed during the time homologous vertebrate structures are formed (Cameron et al. 1985).

The Frog Embryo Teratogenesis Assay--*Xenopus* (FETAX) was developed by Dumont et al. (1983) to provide a standard screening test for developmental toxicants. FETAX has been used to evaluate surface waters, sediment extracts, groundwater, pure chemicals, solvents, and compounds requiring metabolic activation (Dawson et al. 1985; Dawson et al. 1988; Fort and Bantle 1990).

The fathead minnow, *Pimephales promelas*, is a member of Cyprinidae--the largest family of freshwater fish in North America. Fathead minnows are used as standard bioassay fish to assess the toxicity of complex environmental mixtures and pure compounds (Devlin et al. 1985; Holcombe et al. 1982). Fathead minnows have been tested for survival, growth, and developmental responses (Birge et al. 1985).

Birge et al. (1983) (time to hatching plus 4 d) as well as Dawson et al. (1988) used differing exposure periods to compare sensitivities between different species of frogs and fish. Dawson et al. (1988) exposed *Xenopus laevis* (4 d) and *Pimephales promelas* (6 d) to sediment extracts from Tar Creek and the Neosho River, Oklahoma. While the test samples containing heavy metals including zinc were teratogenic to both species, fish were found to be more sensitive than frogs. This may have been due to a longer exposure time for fish. Several studies have attempted to explain toxicological differences between aquatic organisms using rate of uptake, bioconcentration, and toxicant distribution and elimination as the basis for comparison (Muir et al. 1985; Ingebrigsten 1988).

In this study an attempt was made to compare species sensitivity using the same environmental conditions. *Xenopus laevis* (frog) and *Pimephales promelas* (fish)

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embryos were exposed to sodium acetate, caffeine, and 5-fluorouracil. A modification of standard FETAX protocol (ASTM 1991) was followed in that FETAX exposure was extended from 96 to 120 hr. The frog and fish embryos underwent organogenesis, hatched and became free-swimming during the 120 hr exposure period. The developmental stages for both species are similar through this period of time.

MATERIALS AND METHODS

Xenopus culture, breeding procedures, and egg sorting were as described previously (ASTM 1991). *Pimephales* embryos were obtained from the Water Quality Research Laboratory, Oklahoma State University, Stillwater, Oklahoma. Frog embryos at small-cell blastula stage and fish embryos at high blastula stage (Devlin 1982) were chosen for testing. Fish embryos were separated according to Gast (1973).

Sodium acetate CAS# 127-09-3 (SA), caffeine CAS# 58-08-2 (CAF), and 5-fluorouracil CAS# 51-21-8 (5-FU) were chosen as test compounds based on their range of teratogenicity, cost, and available mammalian data. Test compounds, all 99% pure, were obtained for initial testing from Sigma (St. Louis, Missouri).

Dilutions of test materials were made with modified FETAX solution (MFS) which allowed normal development of both frog and fish embryos (Dawson et al. 1988). This reconstituted water medium contained 400 mg NaCl, 96 mg $NaHCO_3$, 30 mg KCl, 14 mg $CaC1_2$, 60 mg $CaSO_4 2H_20$, and 75 mg $MgSO_4$ per L of deionized distilled water. A range test and at least two definitive tests were conducted for each compound. Forty frog embryos and 30 - 40 fish embryos were exposed per dilution in separate dishes. Tests consisted of four control dishes and two dishes per dilution (8 mL total solution in each dish). Static-renewal tests were conducted for 120 hr at 24± 2 °C for both species. Test material was replaced every 24 hr during the test, Test organisms were incubated with a photoperiod (16 hr light, 8 hr darkness) to allow for maximum fish hatching. During the tests, pH was measured daily and dead embryos were counted and removed. At 120 hr, surviving embryos were examined for malformations using a dissecting microscope. Head-to-tail lengths were measured using an IBM-compatible computer equipped with Sigma Scan digitizing software (Jandel Scientific, Corte Madera, California).

The EC50 (median concentration inducing malformation in 50% of surviving embryos) and the LC50 at 120 hr were determined using Litchfield-Wilcoxon probit analysis. The Minimum Concentration to Inhibit Growth (MCIG) was the LOEC as determined by the t-test for grouped observations ($p = 0.05$). Frog and fish responses (i.e. how much greater one responded over the other) could then be compared.

RESULTS AND DISCUSSION

FETAX results are used to determine the teratogenic hazard of test materials based on embryo growth, Teratogenic Index values [TI=LC_{50}/EC_{50} (malformation)], and the type and severity of induced malformations. In general, TI values < 1.5 indicate low teratogenic hazard. At higher TI values, the mortality and malformation concentration-response curves are separated and the potential for the survival of deformed embryos increases (Dawson and Bantle 1987). Mortality and malformation curves for most test compounds are parallel allowing the TI to be used. This study tested one nonteratogen (SA), one moderate teratogen (CAF) and one strong teratogen (5-FU). Representative concentration-response curves for SA, CAF, and 5-FU are presented in Figure 1A. Results for the concentration-response studies are found in Table 1.

Growth was the most sensitive indicator of stress in a study of the effects of phenolics on the early life stages of the fathead minnow (Holcombe et al. 1982). Growth was also more sensitive than survival in early life stage tests by Norberg and Mount (1985) with fathead minnows. Representative growth curves for SA, CAF, and 5-FU are presented in Figure 1B. The teratogenicity of SA, CAF, and 5-FU was assessed by considering the Minimum Concentration to Inhibit Growth (MCIG; also expressed as % compound LC50). Rates of growth inhibition (i.e., slope) and overall reduction in embryo growth vary with the severity of the teratogen. Dawson et al. (1989) suggest that compounds with significant teratogenic potential generally inhibit growth at concentrations < 30% of the respective LC50 values. Growth data were used to make comparisons between species responses and were important indicators of effects that occurred at low levels of exposure.

Frog control mortality and malformation rates were 50 out of 560 (8.9%) and 16 out of 510 survivors (3.1%), respectively. Fish control mortality and malformation rates were 18 out of 460 (3.9%) and 14 out of 442 survivors (3.2%) respectively. Acceptable rates of control mortality and malformation in FETAX were less than 10% each (ASTM, 1991).

Frogs were more sensitive to SA than fish at all endpoints tested (growth, EC50, and LC50) possibly due to an increased uptake. The most common malformations induced by SA in frogs were failure of the gut to coil, optic and facial malformations, and edema at concentrations > 2.0 mg/mL. At concentrations > 3.5 mg/mL spinal kinking and stunting was common. In these experiments, any type of spinal curvature is referred to as spinal kinking whether it be scoliosis or lordosis. At concentrations > 4.5 mg/mL severe kinking, optic and facial malformations, and edema occurred. The most common malformations induced by SA in fish were spinal kinking and stunting at concentrations > 7.0 mg/mL. Heart edema and facial malformations were also common at these concentrations. At

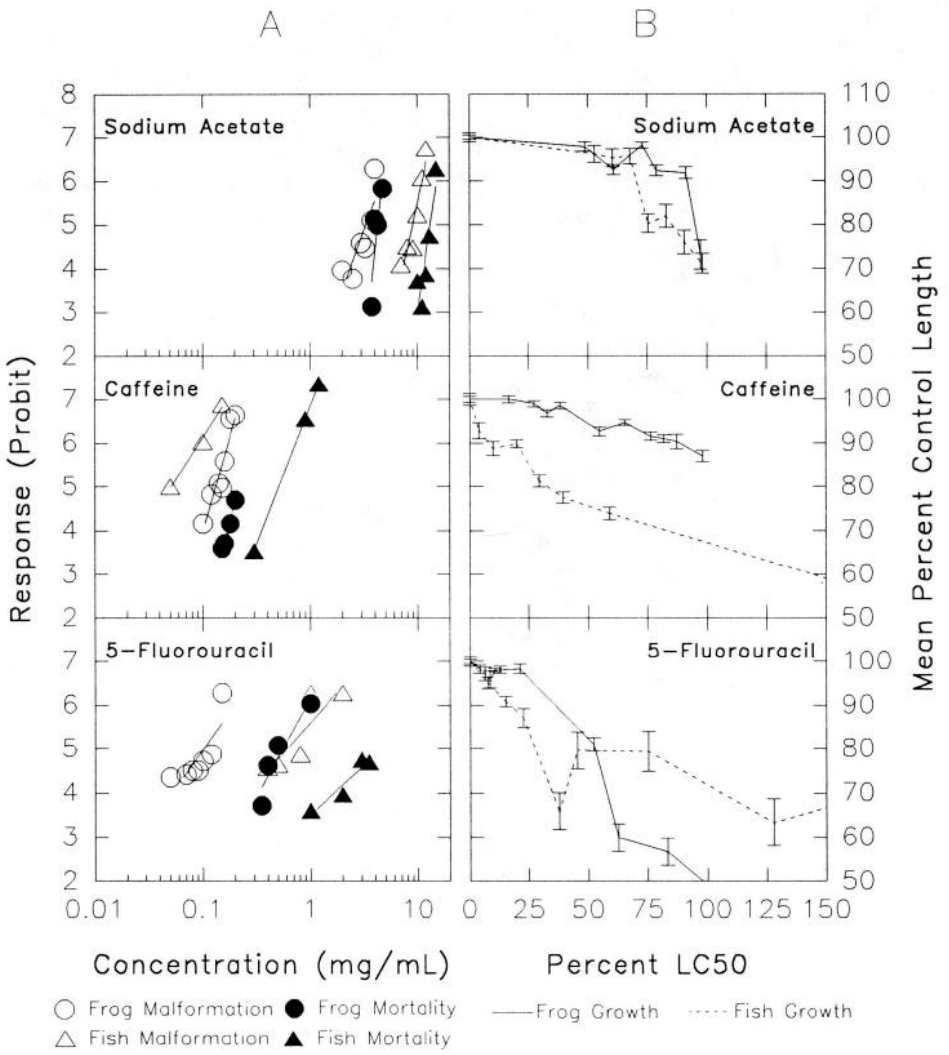

Figure 1. Representative concentration-response curves(A) and growth curves(B) for three compounds tested with Xenopus and Pimephales embryos.

Table 1. Concentration Response Results for *Xenopus* and *Pimephales* Embryos Exposed to Three Compounds

Compound		EC50[a]	LC50[b]	MCIG[c]	TI[d]
Sodium Acetate [*127-09-3*]					
	Frog	3.29 (3.10-3.49)	4.24 (4.02-4.46)	2.5	1.29
	Fish	9.13 (8.56-9.73)	13.33 (12.43-14.31)	7.5	1.46
Caffeine [*58-08-2*]					
	Frog	0.13 (0.12-0.13)	0.19 (0.18-0.21)	0.08	1.46
	Fish	0.07 (0.04-0.11)	0.72 (0.50-0.11)	0.02	10.29
5-Fluorouracil [*51-21-8*]					
	Frog	0.08 (0.06-0.10)	0.53 (0.42-0.62)	0.2	6.63
	Fish	0.40 (0.17-0.91)	2.42 (1.28-4.56)	0.2	6.05

[a] Mean 120-hr EC50 (malformation) with (95% confidence interval), mg/mL.
[b] Mean 120-hr LC50 with (95% confidence interval), mg/mL.
[c] Mean Minimum Concentration to Inhibit Growth (LOEC), mg/mL.
[d] Mean Teratogenic Index (Mean LC50/ Mean EC^{50}(malformation)).

concentrations > 13.0 mg/mL, severe spinal kinking, optic and facial malformations and edema occurred.

CAF was moderately teratogenic to frogs but was strongly teratogenic to fish. Both organisms demonstrated similar types of malformations, but fish malformations were more severe. Fish were more sensitive at growth and malformation endpoints than frogs possibly due to a difference in uptake. The most common malformations induced by CAF in frog embryos was spinal kinking at concentrations > 0.03 mg/mL. At concentrations > 0.14 mg/mL spinal kinking, facial malformations, and improper gut coiling were common. At concentrations > 0.16 mg/mL moderately severe spinal kinking, stunting, and edema were observed. The most common malformations induced by CAF in fish were spinal kinking and stunting at concentrations > 0.02 mg/mL along with occasional facial and eye malformations. At concentrations > 0.1 mg/mL moderate spinal kinking, heart defects, and edema were noted. At concentrations > 0.2 mg/mL severe curling of the tail and growth stunting occurred.

Smith et al. (1983) reported 5-FU as a strong teratogen in humans, mice, rats, and in chicks. In previous 4-d tests with FETAX, 5-FU exhibited strong teratogenic potential in *Xenopus* with a TI value of 11.8 (Dawson and Bantle 1987). Teratogenic data was not found for *Pimephales*. 5-FU was strongly teratogenic to both frogs and fish, and severely malformed and stunted embryos were observed with both. Fish were more sensitive to 5-FU than frogs at growth endpoints and had more severe malformations at low concentrations, possibly due to an increased uptake. Malformations in frogs exposed to 5-FU consisted of slight to moderate abnormalities of the gut, face, eye, brain, heart and spine at 0.01 mg/mL. At concentrations > 0.15 mg/mL similar malformations occurred with greater severity in addition to stunting, edema and blistering. At concentrations > 0.5 mg/mL embryos were so severely stunted that vitality was difficult to determine. Malformations in fish exposed to 5-FU consisted of moderate to severe spinal kinking, optic, and facial abnormalities at 0.05 mg/mL. At concentrations > 0.1 mg/mL the severity of brain, optic, spinal kinking, heart defects, and edema increased. At concentrations > 0.5 mg/mL vitality was difficult to determine and embryos had large yolk sacs, severe multiple malformations and stunted growth.

In order to conduct a species comparison assay and to determine sensitivity differences, as many test factors as possible must be kept constant. Sensitivity to a toxicant, and thus the test results, can be affected by species, strain, previous exposure, age, size, health, and animal handling procedures (Adelman and Smith 1976). In addition, exposure time variations can influence bioassay results (McCarty 1986). A testing protocol that allows for similar developmental stages to be exposed over the same time period is beneficial for understanding species differences. However, as organogenesis rates and order of development differs among organ systems and species, differences in development are still a factor in species comparison tests.

The results of this study showed that *Xenopus* embryos were more sensitive than *Pimephales* for two of the three compounds tested in this study when environmental and exposure conditions were held constant. The FETAX malformation endpoint was more sensitive in two of three cases while the FETAX mortality endpoint was always more sensitive. The FETAX MCIG was more sensitive in one case, the same sensitivity in the second case, and less sensitive in the third case. Because organisms respond differently to different compounds, it is not possible to conclude that *Xenopus* embryos are generally more sensitive than *Pimephales.* More compounds must be tested to establish this relationship.

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