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Effects of Kepone Tolerance on Liver Alkaline Phosphomonoesterase and Glutamic-oxaloacetic Amino Transferase from *Fundulus heteroclitus*

Charles D. Rice[1] and Richard R. Mills[2]

[1]Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, Virginia 23062 and [2]Virginia Commonwealth University, Department of Biology, 918 Park Avenue, Richmond, Virginia 23284

Hepatotoxicity and hepatopathological damage are the most common responses to various xenobiotic agents in fish (Couch 1975). Methods to evaluate toxicity, function, and metabolic activities of fish livers have followed those employed clinically in assessing mammalian systems. These include monitoring the rate at which the liver detoxifies foreign agents such as drugs, pesticides, and other xenobiotics (Lech and Bend 1980; Nebert 1979; Burns 1975), and those which monitor the physiochemistry associated with hepatic function (Bell 1968; Lane and Scura 1970).

Levels of both aspartate aminotransaminase (GOT; E.C.2.6.1.1.) and alkaline monoester phosphatase (AP; E.C.3.1.3.1.), enzymes utilized during increased metabolic activity and tissue synthesis, increase in response to hepatotoxicity following xenobiotic contamination. They are found in both mammalian and fish systems (Jackim et al., 1970; Cvancara and Huang, 1978), and are located easily in the cytoplasmic fraction of hepatic homogenates.

Kepone was manufactured by Allied Chemical Corporation's Hopewell, Virginia facility from 1966 until 1974 and by Life Science Inc. in Hopewell, Virginia from 1974 until 1976. Kepone was subsequently discharged into the air, soil, and water environments of the James River estuary system.

This research investigated possible hepatotoxic effects of kepone, an organochlorine insecticide, and the possible resistance to liver damage in an endemic population of the minnow, Fundulus heteroclitus. Fish were taken from the exposed area of Bailey Creek, Hopewell, Virginia, located in the James River estuary, and from an unexposed area in The Great Wicomico River, Northumberland County, Virginia.

Send reprint requests to RR Mills at the above address

MATERIALS AND METHODS

Minnows, Fundulus heteroclitus, were collected from two streams differing in sediment kepone concentrations. Control fish were collected in Northumberland County, Virginia, at the kepone-free headwaters of the Great Wicomico River which empties into the Cheasepeake Bay at Reedville, Virginia. Experimental fish were collected from the overflow pools of Bailey Creek, near Hopewell, Virginia, where sediments contain high levels of kepone (VWCB, 1982). This creek feeds into the James River at Jordans Point 1.0 km south of Allied Chemical Corporations Hopewell plant.

At both sites, fish were trapped using standard minnow traps baited with cracked blue crab, Callinectes sapidus, removed, and placed in an ice cooler for transportation to the laboratory. Fish were acclimated for 10 days prior to experimentation at ambient photoperiod and temperature, with 0.0 salinity. Fish were maintained on a tropical fish food diet (Tetramed), and water changed weekly using dechlorinated and aged tapwater. Water quality at the beginning of experiments included pH of 7.8, 160 mg/L Calcium Carbonate, and DO above 80 %.

Relative kepone tolerance was determined by conducting 96 h LC-50 static toxicity bioassays following procedures described by Stephan et al. (1975). Data were subjected to a computerized program of PROBIT (Finney 1971).

Enzyme activity was measured in naturally occuring fish from both sites exposed to in situ kepone concentrations, and in fish from both populations exposed to 0.0, 50.0. and 100.0 ug/L kepone in aquaria. Each aquaria contained ten adult fish in 60 L of water with a 12:12 photoperiod and a temperature of 25 C. Kepone solutions were changed at 2 day intervals for a total of 10 days. Fish were sacrificed in a methanol/dry-ice bath and stored at -80 C (less than 2 weeks), followed by hepatic homogenation in a 2.50 mL Thompson animal tissue grinder with 1.0 mL 1.15 % KCl solution. Previous experimentation using subsamples of hepatic homogenates yielded no significant effect on enzyme activity due to sacrifice method or storage temperature. Homogenates were centrifuged at 10,000 g for 10 min, and supernatants were stored at 4.0 C in an ice bath.

Procedures for protein determinations followed those described by Lowry et al. (1951). Procedures for GOT assays followed those described by Mills and Cochran (1963). Activity of GOT was measured as micromoles of

oxaloacetate produced per mg protein per min. The reaction mixture contained 0.2 mL 0.05 M aspartate, 0.2 mL 0.1 M tris-acetate buffer pH 7.6, 0.1 mL 0.005 M alpha ketoglutarate, 0.1 mL of 0.0001 M pyridoxal phosphate, and 0.2 mL of liver homogenate. The pH optimum was 7.6 and optimum temperature was determined to be 35-40 C. Zero order reactions occurred for 40 min and the reaction was stopped by the addition of 0.4 mL of 3,4 dinitrophenylhydrazine. After 20 min, 6 mL of 0.4 N NaOH were added, and the absorption was determined at 505 nm, 10 min later. Enzyme-no substrate and substrate-no enzyme controls were used for each assay. An oxaloacetate standard curve provided a conversion to umoles.

Procedures for AP assays followed those described by Mills et al.(1966). Activity of AP was measured as micromoles of inorganic phosphorus produced from beta-glycerolphosphate per mg protein per min. The reaction mixture contained 0.2 mL of 0.05 M beta-glycerophosphate, 0.2 mL of 0.10 M tris-acetate buffer pH 9.6, 0.1 mL of 0.001 M MgCl, and 0.2 mL liver homogenate. The pH optimum was 9.6 and the optimum temperature was determined to be 35-40 C. After a reaction time of 40 min, 0.4 mL of 10 N sulfuric acid, 0.8 mL of ammonium molybdate, and 6.0 mL distilled water were added. The resulting mixture was centrifuged at 600 g for 10 min to remove precipitated protein. After the addition of 0.4 mL Fisk-SubbaRow reducing agent, absorption was determined at 660 nm. Enzyme-no substrate and substrate-no enzyme controls were used for each assay. Known concentrations of inorganic phosphorus were used as standards.

A Student's t-test was used to compare the means of basal enzyme activity in fish from both rivers, and an ANOVA was used to compare enzyme activities between the two treatment groups and three concentrations of kepone for the aquaria exposure experiments (Steele and Torrie, 1980). Any significant differences due to the effects of kepone, as determined by ANOVA, were subjected to Tukey's multiple contrast test (Steele and Torrie, 1980).

RESULTS AND DISCUSSION

Ninety six-h LC-50 bioassays showed a higher tolerance for kepone in Fundulus obtained from previously exposed waters than for Fundulus obtained from unexposed waters. Using PROBIT analysis, the LC-50 value for Bailey Creek fish was 233 ug/l, and the value for Wicomico fish was 138 ug/l. The 95 % fiducial limits did not overlap.

The effects of in situ kepone levels on liver GOT and AP activity are described as follows. Statistical differences were not apparent ($p>.05$) for either GOT or AP between populations. Specific GOT activity expressed as uM oxaloacetic acid produced / mg protein / min, was 0.012 (S.E.=0.001, n=21) and 0.016 (S.E.=0.002, n=21) for Wicomico fish and Bailey Creek fish, respectively. Specific AP activity expressed as uM inorganic phosphate produced / mg protein / min, was 0.096 (S.E.=0.012, n=21) and 0.119 (S.E.=0.013, n=21) for Wicomico fish and Bailey Creek fish respectively.

Increasing kepone concentrations increased enzyme activity in both Wicomico and Bailey Creek minnows. Aminotransaminase levels were significantly higher ($p<.05$) at 50.0 ug/L and 100.0 ug/L when compared to 0.0 ug/L treated Wicomico fish (Table 1). However, increases in GOT activity approached significance ($p<.055$) only at 100.0 ug/L for exposed fish.

Table 1. Levels of GOT expressed as specific activity (uM oxaloacetic acid / mg / min) measured at treatments of 0.0, 50.0, and 100.0 ug/L kepone in an unexposed population (Wicomico) and an exposed population (Bailey Creek) of Fundulus heteroclitus.

	Treatment		
	0.0 ug/L	50.0 ug/L	100.0 ug/L
Wicomico	0.016 (SE=0.001)	0.024 * (SE=0.002)	0.031 * (SE=0.003)
Bailey Creek	0.018 (SE=0.001)	0.023 (SE=0.005)	0.026 (SE=0.004)

All sample sizes are n=18. SE = standard error
* denotes significant difference ($p<.05$) when compared with controls using Tukey's multiple contrast.

Alkaline phosphatase activity approached significance ($p<.057$) only at 100.0 ug/L when compared with 0.0 ug/L treated Wicomico fish (Table 2). There were no significant differences ($p>.05$) in AP activity at 50.0 ug/L or 100.0 ug/L when compared to 0.0 ug/L treated Bailey Creek fish.

Table 2. Levels of AP expressed as specific activity (uM inorganic phosphorus / mg / min) measured at treatments of 0.0, 50.0, and 100.0 ug/L kepone in an unexposed population (Wicomico) and an exposed population (Bailey Creek) of Fundulus heteroclitus.

	Treatment		
	0.00 ug/L	50.0 ug/L	100.0 ug/L
Wicomico	0.106 (SE=0.018)	0.127 (SE=0.014)	0.176 (SE=0.031)
Bailey Creek	0.119 (SE=0.013)	0.172 (SE=0.003)	0.177 (SE=0.026)

All sample sizes are n=18
SE = standard error

Comparing enzyme levels in the two fish populations, using ANOVA, at three levels of kepone treatment demonstrated no significant difference in GOT acitivity ($P>.05$) between the two populations of Fundulus. The slope of GOT acitivity increase was determined to be greater than zero in both populations; however, when compared between populations, there was no significant difference ($p>.05$). ANOVA demonstrated no significant difference ($p>.05$) in AP activity between populations at the three levels of kepone treatment. The slope of activity increase for each population was determined to be zero; however, when compared between populations, there was no significant difference ($p>.05$).

Although kepone toxicity has been investigated in representative esturaine phytoplankton, zooplankton, benthic, and fish species, there is little published data on kepone toxicity to the minnow, Fundulus heteroclitus. These fish are responsible for a large percent of high marsh secondary production (Meredith and Lotrich 1979), and knowledge of their tolerance to xenobiotics is important in estimating the impact of pollutants on the estuarine ecosystems. Ninety-six hr static toxicity bioassays resulted in values which are much higher than those reported for spot, Leiostomus xanthurus (6.6 ug/L), and the sheepshead minnow, Cyprinodon variegatus (70 ug/L), (Schimmel and Wilson, 1977). This further demonstrates the resistance of Fundulus to environmental stress.

Kepone concentrations range between non-detectable and 0.80 ug/L in the water column and between 1.0 and 9.99 mg/L in sediments at Bailey Creek sampling stations (VWCB, 1982). Low water-column concentrations of kepone are misleading; high tidal flux and other flooding conditions re-suspend kepone ladened organics in the water column, allowing continuous exposure to the organisms. Phytoplankton can accumulate as much as 6,000 X the ambient concentration of kepone (Bahnner et al. 1977). Therefore, because of food chain bioconcentration, Fundulus may be exposed to high levels of the contaminant. As a result, finfish body burdens in Bailey Creek have reached levels of 300 ug/L or greater in migratory species and as high as 570 ug/L in resident species (VWCB 1982).

The clear separation of kepone tolerance between exposed (Bailey Creek) and unexposed (Wicomico River) minnows and the correlation between xenobiotic contamination and hepatotoxicity (Couch, 1975), would suggest that liver enzyme activity may correlate with the organismic kepone tolerance. Hepatic GOT and AP activity, indicative of hepatotoxicity at elevated levels, measured in fish in situ indicated no significant difference ($p>.05$) between populations of Fundulus.

The total GOT activity increased in response to increasing kepone concentrations. Significant differecnces ($p<.05$) between treatment groups were observed only in previously unexposed Fundulus. Activities of hepatic enzymes increase in response to other xenobiotics in fish (Bell, 1968; Jackim et al.,1970; Lane and Scura, 1970; Racicot et al., 1975; Kendall, 1977), but this is the first known study reporting elevated hepatic enzymes in response to Kepone toxicity in Fundulus. Higher kepone tolerance and lower levels of hepatic GOT may indicate physiological adaptation to this xenobiotic in previously exposed populations of Fundulus. Elevated tissue levels of hepatic enzymes are indicative of increased metabolism and tissue repair following hepatotoxicity (Bell 1968).

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