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Toxicity and Bioaccumulation of Hexachlorocyclopentadiene, Hexachloronorbornadiene and Heptachloronorbornene in Larval and Early Juvenile Fathead Minnows, *Pimephales promelas*

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Hexachlorocyclopentadiene is the key intermediate in the synthesis of stable chlorinated cyclodiene insecticides including aldrin, dieldrin, endrin, endosulfan, heptachlor, chlordane, isodrin and mirex (BROOKS 1974). Some other products derived from hexachlorocyclopentadiene are nonflammable resins, fungicides, heat resistant and shock proof plastics, acids, esters, ketones and fluorocarbons. Using the Diels-Alder reaction, hexachlorocyclopentadiene can be transformed by the addition of vinyl chloride to heptachloronorbornene and further converted by dehydrochlorination to hexachloronorbornadiene, a key intermediate in the synthesis of isodrin and endrin (BROOKS 1974).

Hexachlorocyclopentadiene was qualitatively identified as a contaminant in the discharge of pesticide production plants in Memphis, Tennessee (December, 1975) and Michigan (May, 1977) (U.S. EPA 1977). Results of a study by the U.S. FOOD AND DRUG ADMINISTRATION (1976) in 1972-1974 indicate that significant amounts of hexachloronorbornadiene and heptachloronorbornene along with an epoxy derivative were found in edible fish from the Mississippi River near Memphis. BARTHEL et al.(1966, 1969) similarily found that pesticide manufacturing operations near Memphis was a source of significant pesticide contamination in the sediments and water as determined by studies conducted in 1964, 1966, and 1967. Only limited information is available in the literature concerning the toxicity of these three chemical intermediates to aquatic life (COLE 1954; DAVIS and HARDCASTLE 1959; U.S. DEPARTMENT OF HEALTH EDUCATION AND WELFARE 1956; and MOUNT AND PUTNICKI 1966).

The purpose of this study was to determine the toxicity and bioaccumulation of these compounds using 30 day flow through tests with larval and early juvenile stages of the fathead minnow. MCKIM (1977) conducted an extensive review of the literature on life-cycle toxicity tests with fish and showed that embryo-larval and early-juvenile stages were the most or among the most sensitive to chemical pollutants. It was concluded that tests utilizing these stages can be used to estimate the maximum acceptable toxicant concentration (MATC) within a factor of two and should be useful in screening large numbers of chemicals. HANSEN et al. (1971) and BLAU et al. (1975) discussed that the bioconcentration potential and steady state concentrations of chemicals can be predicted from relatively short exposure periods.

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MATERIALS AND METHODS

Unfiltered Lake Superior water was heated and used in all tests at 25 ± 2 C. Chemical characteristics of the test water were determined weekly according to methods described by the AMERICAN PUBLIC HEALTH ASSOCIATION et al. (1975). Ranges for these measurements were (mg/L): dissolved oxygen, 7.2-8.6; hardness, 45-47 as $CaCO_3$; alkalinity, 42-43 as $CaCO_3$; and acidity, 1.5-2.5 as $CaCO_3$. The pH ranged from 7.2 to 7.7.

The 3 tests were conducted with intermittent-flow exposure systems consisting of a multi-toxicant injection system (DEFOE 1975) which delivered 5 toxicant concentrations with equal amounts of acetone (4 mg/L) and a control to duplicate exposure chambers. The test chambers were glass aquaria, 45x16x18 cm, with a water volume of 8.9 L. Water depth was 13.5 cm. Flow rate to each chamber was 500 mL every 3 min providing a 95% replacement of the test water every 2.7-h (SPRAGUE 1969). Flourescent bulbs provided a light intensity of 18-28 lumens at the water surface. An automatically controlled 16-h photoperiod was used.

Chemicals used in this study were supplied by Velsicol Chemical Corporation, Memphis, Tennessee. Water samples were collected daily from the test chambers and analyzed on a Hewlett Packard 5730 H gas chromatograph equipped with an auto sampler and Ni-63 electron capture cell. The column was 1.8 m x 2 mm (ID) glass coil filled with 4% SE-30 and 6% OV-210 on 80/100 mesh Gas Chrom Q. The carrier gas was argon containing 5% methane and all chromatograms were produced at a column temperature of 150 C.

Measured concentrations of each chemical are included in Tables 1, 2 and 3. The recovery of hexachlorocyclopentadiene from 12 spiked samples was 93.7 ± 6.6%, of hexachloronorbornadiene from 11 spiked samples was 102 ± 4%, and of heptachloronorbornene from 11 spiked samples was 101 ± 3%.

All three 30-day tests were conducted simultaneously beginning in April and ending in May, 1977. To begin each test, 25 one-day-old fathead minnow larvae were randomly selected and distributed to each duplicate exposure chamber. All fish were fed brine shrimp nauplii 3 to 4 times a day. Mortalities were recorded after the fourth day and then once a week for the remainder of the test. Death was defined as complete immobilization and failure of the animals to respond to probing.

After the 30-day period whole fish were analyzed on a wet weight basis using the methods described by VEITH and LEE (1971) for residue analysis. The recoveries of hexachloronorbornadiene and heptachloronorbornene were 98.0 ± 2.3% and 93.0 ± 2.9%, respectively. Hexachlorocyclopentadiene extracts were concentrated using a Kuderna-Danish vaporator which resulted in a 89.0 ± 4.0% recovery. Bioconcentration factors (BCF) for each compound were determined by dividing the residue tissue concentration by the corresponding exposure concentration.

TABLE 1

Survival and growth of fathead minnows exposed to various concentrations of hexachlorocyclopentadiene. Asterisk (*) denotes values significantly less than controls (analysis of variance, Dunnett's test, P=0.05).

Item	Measured concentration (µg/L)											
	9.1[a] ± 1.8		7.3 ± 4.7		3.7 ± 1.2		1.7 ± 0.78		0.78 ± 0.31		<0.04	(control)
	A	B[b]	A	B	A	B	A	B	A	B	A	B
						4-day						
Survival (%)	4	* 0	64	* 76	100	88	100	96	96	96	100	96
						30-day						
Survival (%)	0	* 0	60	* 72	96	84	100	92	92	96	92	96
Length (mm)		----	25.6 ± 2.9		24.6 ± 2.9		24.7 ± 2.3		25.1 ± 2.3		24.8 ± 2.5	
Weight (g)		----	0.13 ± 0.04		0.11 ± 0.04		0.11 ± 0.03		0.12 ± 0.03		0.12 ± 0.04	

[a] Mean ± S.D. of duplicate chambers.

[b] Duplicate chamber.

TABLE 2

Survival and growth of fathead minnows exposed to various concentrations of hexachloronorbornadiene. Asterisk (*) denotes values significantly less than controls (analysis of variance, Dunnett's test, P=0.05).

Item	Measured concentration (µg/L)											
	226[a] ± 26.3		122 ± 8.8		56.9 ± 10.2		38.4 ± 3.1		20.0 ± 3.9		<0.04 (control)	
	A	B[b]	A	B	A	B	A	B	A	B	A	B
						4-day						
Survival (%)	28	* 48	84	* 72	96	96	96	96	100	100	100	100
						30-day						
Survival (%)	0	* 0	72	* 60	96	96	92	92	100	100	96	92
Length (mm)	---		19.0 ± 2.3*		24.5 ± 1.6*		24.6 ± 2.3*		25.0 ± 2.5		25.9 ± 1.7	
Weight (g)	---		0.06 ± 0.02*		0.11 ± 0.03*		0.11 ± 0.03*		0.12 ± 0.03		0.13 ± 0.03	

[a] Mean ± S.D. of duplicate chambers.

[b] Duplicate chamber.

TABLE 3

Survival and growth of fathead minnows exposed to various concentrations of heptachloronorbornene. Asterisk (*) denotes values significantly less than controls (analysis of variance, Dunnett's *test*, P=0.05).

	Measured concentration (µg/L)											
	180.3[a] ± 14.8		164.9 ± 36.6		83.5 ± 7.1		40.0 ± 10.8		25.9 ± 3.4		<0.04 (control)	
Item	A	B[b]	A	B	A	B	A	B	A	B	A	B
					4-day							
Survival (%)	0	0	0	0	44	76	100	76	84	96	100	100
					30-day							
Survival (%)	0	0	0	0	8	36	92	76	84	96	96	96
Length (mm)	---		---		19.2 ± 3.3*		24.2 ± 2.2*		24.7 ± 2.8		25.4 ± 1.9	
Weight (g)	---		---		0.07 ± 0.03*		0.11 ± 0.03		0.12 ± 0.03		0.12 ± 0.04	

[a] Mean ± S.D. of duplicate chambers.

[b] Duplicate chamber.

Median lethal concentrations (LC50) and 95% confidence limits were estimated by a computerized procedure utilizing the trimmed Spearman-Karber method (HAMILTON et al. 1977). In addition, survival and bioaccumulation data were subjected to one-way analysis of variance (P=0.05) and Dunnett's one-sided comparison of treatment means to control means (P=0.05) (STEEL and TORRIE 1960).

RESULTS

Thirty-day exposures with larval and early juvenile fathead minnows showed that concentrations of 7.3, 38.4, and 40.0 µg/L and above of hexachlorocyclopentadiene, hexachloronorbornadiene, and heptachloronorbornene, respectively, would be deleterious to this species (Tables 1, 2, and 3). The 96-h LC50 and 30-day LC50 values for these compounds, respectively, were: 7.0 and 6.7 µg/L, 188 and 123 µg/L, and 85.6 and 60.1 µg/L.

Residues of hexachlorocyclopentadiene in 30 day-old fathead minnows were less than 0.1 µg/L in all tanks resulting in a BCF of less than 11. The concentration of hexachloronorbornadiene accumulated by fathead minnows was directly proportional to the mean exposure concentration up to a concentration of 38.4 µg/L which decreased the growth of the fish. Residues were highest in fish exposed to concentrations of 38.4 µg/L and below and resulted in a BCF of approximately 6400. The concentration heptachloronorbornene accumulated by fathead minnows was directly proportional to the mean exposure concentration up to the "effect" concentration of 40 µg/L. Residues were highest in fish exposed to concentrations of 40 µg/L and below and resulted in a BCF of approximately 11,200.

DISCUSSION

Comparison of the results of this test to earlier studies show that all 3 chemical intermediates were more toxic to fathead minnows in this study than to this same species and others exposed to these compounds for similar time periods. The 96-h LC50 value of hexachlorocyclopentadiene reported for fathead minnows in this test was 8 times lower than the value reported for this species by HENDERSON (1956). The 96-h LC50 values of hexachlorocyclopentadiene in static bioassays reported for bluegills and bass (DAVIS and HARDCASTLE 1959) were approximately 2800 to 3600 times higher than 96-h values reported in this test. Results of the present test with hexachloronorbornadiene and heptachloronorbornene indicate that these compounds are at least 4 times more toxic to fathead minnows than to guppies as reported by MOUNT and PUTNICKI (1966). The lower values obtained in this test for all three compounds are probably due to the utilization of intermittent-flow exposure systems and/or the use of the most sensitive life stages of development for testing (MOUNT 1962; MCKIM 1977).

Toxicity results for hexachlorocyclopentadiene showed that a median lethal threshold (the concentration at which acute toxicity of 50% of the test animal ceases) was attained within 4 days. The presence of a threshold level for this exposure time would indicate that this compound was noncumulative. This is substantiated by the fact that hexachlorocyclopentadiene did not accumulate to a high degree (<0.1 μg/g) in fathead minnows after 30 days of exposure. LU et al. (1975) similarily found that fish accumulated low amounts of this compound (0.11 μg/g) in a model ecosystem study after a 33 day exposure period. The authors showed that hexachlorocyclopentadiene reached a maximum level in the water phase after 14 days and decreased rapidly thereafter. The substantial volatility of the compound was indicated as the probable cause for the relatively low amounts in fish and other organisms including algae, snails, and mosquito larvae.

Toxicity data for hexachloronorbornadiene and heptachloronorbornene showed that these compounds may have had a cumulative action. This is suggested by the fact that adverse effects on growth were observed at concentrations much lower than those which decreased survival (Table 2 and 3) and by their high bioaccumulation in whole body tissue. The ability of these compounds to accumulate in fish is similar to that of the persistent organochlorine insecticides including endrin, one of the most toxic of all economic poisons to fish (GRANT 1976). Their accumulation in fathead minnows indicate both a real and potential hazard to higher food chain organisms. Both hexachloronorbornadiene and heptachloronorbornene have been found in edible fish such as catfish, carp, and others (U.S. FOOD AND DRUG ADMINISTRATION 1976).

This research demonstrates the application of current state of the art methods to measure toxicity and residue forming potential utilizing sensitive life stages of fish in 30-day tests and provides a close approximation of the values that would be obtained in full life-cycle chronic tests.

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