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A RAPID ASSESSMENT OF THE TOXICITY OF THREE CHLORINATED CYCLODIENE INSECTICIDE INTERMEDIATES TO FATHEAD MINNOWS

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A RAPID ASSESSMENT OF THE TOXICITY OF THREE CHLORINATED CYCLODIENE INSECTICIDE INTERMEDIATES TO FATHEAD MINNOWS

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FOREWORD

This report describes the toxicity of three organochlorine compounds used as intermediates in the manufacture of pesticides. The U.S. Food and Drug Administration noted residues in fish samples collected below a pesticide manufacturing plant and alerted the Environmental Protection Agency for necessary action. This research demonstrates the application of current state of the art methods to measure chronic toxicity and residue forming potential in a 30-day test.

A broad base of data accumulated over the past 10 years suggests that the results of embryo-larval and early juvenile tests with associated residue measurements will provide results within a factor of two of the values that would be obtained in full life-cycle chronic tests.

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ABSTRACT

A rapid assessment study to determine the toxicity and bioaccumulation of three chlorinated cyclodiene insecticide intermediates; hexachlorocyclopentadiene, hexachloronorbornadiene, and heptachloronorbornene to fathead minnow larvae and early juveniles was conducted for 30 days under flow-through conditions.

A concentration of 7.3 µg/liter of hexachlorocyclopentadiene caused significant decreases in survival after 4 days. Growth of fish exposed for 30 days was not significantly decreased at any of the concentrations tested. The highest concentration of hexachlorocyclopentadiene having no adverse effect was 3.7 µg/liter.

Concentrations of 122 and 226 µg/liter of hexachloronorbornadiene caused significant decreases in survival after 4 days. Growth of 30 day-old larvae was significantly decreased at 38.4 µg/liter and was the most sensitive indicator of toxicity. The highest concentration having no adverse effect was 20.0 µg/liter. The average bioconcentration factor for fish exposed to less than 38.4 µg/liter of this compound was 6400.

Survival of fathead minnows exposed to heptachloronorbornene was significantly decreased at 83.5 µg/liter after 4 days. Growth was significantly reduced at 40 µg/liter after 30 days and was the most sensitive indicator of toxicity. The highest concentration having no adverse effect was 25.9 µg/liter. The average bioconcentration factor for fish exposed to less than 40 µg/liter of this compound in water was 11,200.

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ACKNOWLEDGMENTS

The authors wish to thank Mr. D. Tanner for making routine water analysis, constructing test equipment, and for daily assistance; and the Environmental Research Laboratory-Duluth's staff for technical assistance, advice and manuscript review.

SECTION 1

INTRODUCTION

The purpose of this study was to make a rapid assessment of the toxicity and bioaccumulation of three chlorinated cyclodiene insecticide intermediates; hexachlorocyclopentadiene ("hex"), hexachloronorbornadiene ("X"), and heptachloronorbornene ("Y") using larval and early juvenile stages of the fathead minnow. The latter two compounds have been referred to as "X" and "Y", respectively, in the literature (Barthel et al., 1969).

The study was initiated upon request for assistance from the Regional Administrator, Region IV, to aid the U.S. Environmental Protection Agency (EPA) in carrying out its responsibilities under the Federal Water Pollution Control Act of 1972 concerning the discharge of these chemicals into the Mississippi River near Memphis, Tennessee.

Results of a study by the U.S. Food and Drug Administration (1976) in 1972-1974 indicate that significant amounts of "X" and "Y" along with an epoxy derivative (a metabolite of "X") were found in edible fish from the Mississippi River near Memphis. The data showed that the most frequent occurrence and highest residues of these chemicals were found in fish from the Memphis area near a primary manufacturer of endrin and heptachlor, in contrast to low amounts found in fish from other parts of the United States. This agrees with work by Barthel et al. (1966, 1969) who found that pesticide manufacturing operations near Memphis was a source of significant pesticide contamination in the sediments and water during studies conducted in 1964, 1966, and 1967. These results showed that high concentrations (parts per thousand) of several insecticide residues and residues of "X" and "Y" occurred in the bottom sediments, spoils, and flood plain deposits.

In December, 1975, "hex" was qualitatively identified as a contaminant in the discharge of a pesticide production plant in Memphis (U.S. EPA, 1977). In May, 1977, "hex" was also qualitatively identified at a pesticide production plant in Michigan. Concentrations of "hex" were identified in the air, in the plant's aqueous discharge and in fish tissue in the receiving stream.

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). The accumulation of residues of these insecticides in higher trophic levels has been demonstrated by several authors and is presently being used as a guideline for water quality criteria (National Academy of Sciences and National Academy of Engineering, 1973). Some other products derived from "hex" are nonflammable resins, fungicides, heat resistant and shock proof plastics, acids, esters,

ketones and fluorocarbons. It is a clear, yellow, slightly soluble liquid that is produced by two companies in the United States; Hooker Chemical Corporation (at Montague, Michigan and Niagara Falls, New York) and Velsicol Chemical Corporation (at Memphis, Tennessee). Using the Diels-Alder reaction, hexachlorocyclopentadiene can be transformed by the addition of vinyl chloride to heptachloronorbornene and further converted by dehydro-chlorination to hexachloronorbornadiene, a key intermediate in the synthesis of isodrin and endrin (Brooks, 1974) (Figure 1). The estimated production of hexachlorocyclopentadiene in the U.S. is approximately 7 to 15 million pounds per year (U.S. EPA, 1977).

The toxicity of chlorinated cyclodiene insecticides to aquatic life has been studied by several authors: Henderson et al. (1959), Mount (1962), Mount et al. (1966), Mount and Putnicki (1966), Johnson (1967), Reinert (1967), Brungs and Mount (1967), Macek et al. (1969), Grant (1976), Cardwell (1977) and numerous others. These compounds and their metabolites have been shown to accumulate in aquatic systems and are directly toxic to various aquatic organisms at water concentrations of less than 1 µg/liter. However, only limited information is available in the literature concerning the toxicity of these three chemical intermediates. Cole (1954) investigated the germicidal effects of a commercial "hex" preparation called P-162 in sewage effluents and found that this chemical was more toxic to bacteria, coliform and Salmonella typhasa than was chlorine. Results showed that 10 mg/liter of "hex" reduced bacteria counts by at least 90% in 2-hr while chlorine over the same time period and concentration reduced the total count by about 45%. Similar results were observed with coliform and Salmonella at 5 and 10 mg/liter. Davis and Hardcastle (1959) determined the 24, 48, and 96-hr median tolerance limit (TL_m) of "hex" for bluegills (Lepomis macrochirus) and largemouth bass (Micropterus salmoides). The results of static bioassays for these time periods were respectively, for bluegills, <500, 30, and 25 mg/liter and for bass <500, 35, and 20 mg/liter (average water hardness, 77 mg/liter). The toxicity of "hex" to fathead minnows was tested by the U.S. Department of Health, Education and Welfare (1956) using two dilution waters and two formulations for preparing test concentrations. The results demonstrated that "hex" was slightly less toxic in an acetone solution than in a water emulsion and more toxic in hard water. Recorded 24, 48, and 96-hr TL_m values in hard water (emulsion) were 0.075, 0.059 and 0.059 mg/liter, respectively. A model ecosystem study by Lu et al. (1975) showed that "hex" has considerable ecological stability and moderate biomagnification potential in algae (Oedogonium cardiacum), snails (Physa sp.), mosquito larvae (Culex pipiens quinquefasciatus) and fish (Gambusia affinis). Studies reported by Mount and Putnicki (1966) showed that the chemical intermediates "X" and "Y" and endrin accumulated in all samples of fish dying in the Mississippi River in 1963. Laboratory studies by these authors indicated that these two intermediates were approximately 1,000 to 10,000 times less toxic than endrin to guppies (Poecilia reticulata).

Studies by Ingle (1953), Treon et al. (1955) and Naishstein and Lisovskaya (1965) have shown that hexachlorocyclopentadiene can produce toxicity in mammals via ingestion, inhalation, or dermal exposures. Degenerative changes in the brain, heart, adrenals, liver, kidney and lungs were observed in severly poisoned animals by all routes of administration.

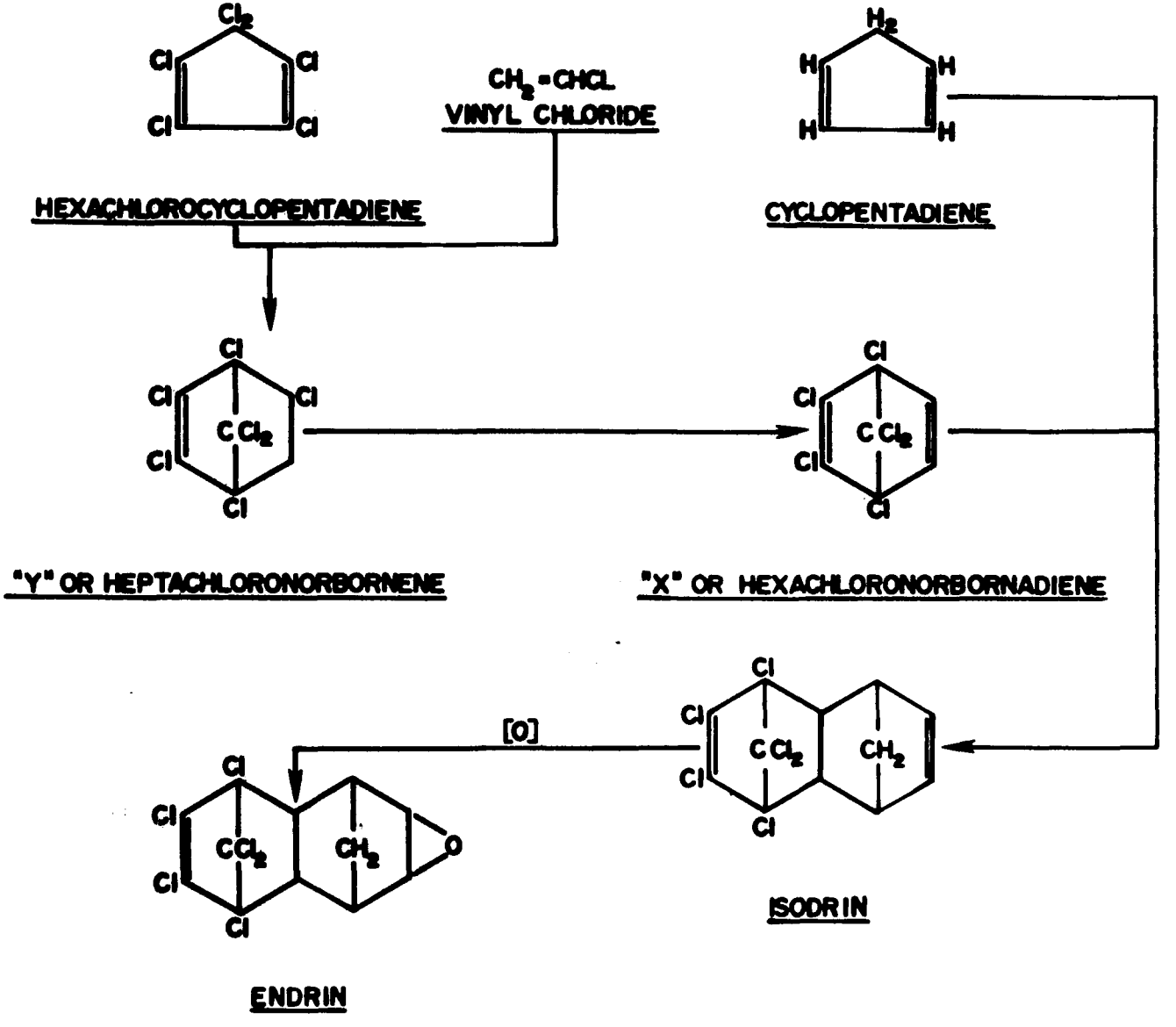

Figure 1. The manufacture of endrin from hexachlorocyclopentadiene ("hex"), reproduced from Barthel et al. (1969).

No specific studies on human toxicity of these three chemical intermediates were shown in the literature. However, several inferences of the effects of "hex" to researchers using these chemicals, such as eye irritation, headaches and skin irritations were made by Ingle (1953), Treon et al. (1955), and Hooker Chemical and Plastics Corporation (1969). Taste and odor threshold concentrations of "hex" in water were reported as 1.4 and 1.6 µg/liter, respectively, (Naishstein and Lisovskaya, 1965).

The study herein describes the toxicity and bioaccumulation of the above named chlorinated cyclodiene insecticide intermediates in 30 day tests with early life stages of the fathead minnow. The use of short term toxicity tests involving the early developmental stages of fish to predict chronic toxicity has been proposed by several investigators (Pickering and Thatcher, 1970; Pickering and Gast, 1972; McKim et al., 1975; Eaton et al., 1977; and McKim et al., 1977). An extensive review of the literature (McKim, 1977) on life-cycle toxicity tests with fish 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.

The ability to predict the bioconcentration potential and steady state concentrations of chemicals from relatively short exposure periods was discussed by Blau et al., 1975. Hansen et al. (1971) demonstrated that chlorinated compounds such as PCB's were rapidly stored in fish with maximum residue concentrations being attained in 14 to 28 days. Thereafter, the concentrations in the tissues remained constant with continued exposure. Consequently, the bioconcentration factors reported in this study can be considered estimates of what would be determined from longer exposures.

SECTION 2

CONCLUSIONS

1. Thirty day exposures with larval and early juvenile fathead minnows showed that concentrations of 7.3, 38.4, and 40.0 µg/liter and above of hexachlorocyclopentadiene ("hex"), hexachloronorbornadiene ("X"), and heptachloronorbornene ("Y"), respectively, would be deleterious to this species.

2. Hexachloronorbornadiene and heptachloronorbornene decreased growth at 40 µg/liter, 2 to 3 times lower than concentrations affecting survival.

3. Larval and early juvenile stages of fathead minnows under the present test conditions were more sensitive to all three intermediate compounds than this same species and other species tested for similar time periods in earlier studies.

4. The toxicity curve for "hex" showed that this compound was a non-cumulative poison. A median lethal threshold was attained within 4 days. Toxicity curves for "X" and "Y" indicate that these compounds had a cumulative action.

5. Residue concentrations of "X" and "Y" increased with increased exposure concentrations up to a concentration causing decreases in growth of the fish. The average bioconcentration factors for fish exposed to "X" and "Y" were 6,400 and 11,200, respectively. Residue concentrations of "hex" in fathead minnows were not obtained.

6. A rapid assessment of the toxicity of hexachlorocyclopentadiene, hexachloronorbornadiene, and heptachloronorbornene utilizing 30 day tests with larval and early juvenile fathead minnows indicate that these compounds may present a potential hazard to aquatic systems. The bioaccumulation of hexachloronorbornadiene and heptachloronorbornene in these fish suggest their possible biomagnification in higher food chain organisms.

SECTION 3

RECOMMENDATIONS

1. Concentrations of hexachlorocyclopentadiene, hexachloronorbornadiene, and heptachloronorbornene not exceeding 3.7, 20, and 25.9 µg/liter, respectively, appear "safe" for fathead minnows under the conditions tested and may be used as first approximations of non-toxic concentrations. Concentrations may need to be lowered to comply with residue limits once acceptable residue concentrations are established.

2. An assessment of the toxicity of these compounds to other aquatic life should be completed.

3. Studies involving the toxicity of mixtures of these chemical intermediates along with mixtures of other toxicants, particularly the organochlorine insecticides for which they are used to synthesize, are needed to evaluate interactions.

4. Since these intermediate compounds have been found in edible fish, investigations concerning what concentrations are safe for human consumption should be made.

SECTION 4

MATERIALS AND METHODS

WATER CHARACTERISTICS

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 (in milligrams per liter): 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.

EXPOSURE SYSTEM

The three tests were conducted with intermittent-flow exposure systems consisting of a multi-toxicant injection system (DeFoe, 1975) which delivered five toxicant concentrations with equal amounts of acetone (4 mg/liter) and a control to duplicate exposure chambers. The test chambers were glass aquaria, 45 x 16 x 18 cm, with a water volume of 8.9 liters. 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-hr (Sprague, 1969).

Flourescent bulbs provided a light intensity of 18-28 lumens at the water surface. An automatically controlled 16-hr photoperiod was used.

CHEMICAL CONDITIONS

Chemicals used in this study were supplied by Velsicol Chemical Corporation, Memphis, Tennessee. Water samples were collected daily from the test chambers by siphoning each sample through a glass tube directly into volumetric flasks containing hexane. Due to the differences in test concentrations, 500 ml flasks containing 50 ml hexane were used in the test with "hex" and 250 ml flasks containing 50 ml hexane were used in the tests with "X" and "Y". All samples were stirred for 1.5 hr at a speed great enough to cause rigorous vortex mixing. The phases were allowed to separate for 1 hr and an aliquot of hexane was transferred to a vial for gas-liquid chromatograph (GLC) analysis. The samples were 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.

To estimate the precision of the GLC injection procedure, six aliquots of extracts from the next to the lowest concentration in each test were transferred to vials for replicate analysis. The results showed that the GC reproducibility gave relative standard deviations of 9.8% for the hexachlorocyclopentadiene at a concentration of 0.67 µg/liter, 1.5% for hexachloronorbornadiene at 36.7 µg/liter, and 0.6% for heptachloronorbornene at 45 µg/liter. It is generally accepted that GLC analyses should have a precision of approximately 5%. Consequently, the precision of hexachlorocyclopentadiene analysis is slightly less than many pesticide analyses and the precision of the other analyses are well within the anticipated variability. The decrease in precision in the former case is likely due to the much greater volatility of hexachlorocyclopentadiene and the fact that the concentrations were a order of magnitude lower than the other chemicals.

The precision of the sampling and extraction procedure was examined by analyzing six replicate water samples from a single tank in each diluter. The results showed that the measured concentration could be estimated with a relative standard deviation of 10.3%, 1.4%, and 0.9% for "hex", "X", and "Y", respectively. Since the variation of water analyses is typically less than 10%, these measurements are within acceptable limits. Moreover, the variability due to sample collection and extraction does not significantly increase the variability of the overall analysis arising from the GLC injection.

Measured concentrations of each chemical are included in Tables 1, 2, and 3. One tank representing each concentration in each diluter system was sampled 18 times during this 30 day study, except those tanks in which all fish died before the end of the test. On 11 and 12 of these sampling days, the appropriate volume of Lake Superior water from the respective control tanks was spiked to a concentration of 10 µg/liter of each chemical in acetone and the sample was extracted and analyzed to determine the recovery of the extraction procedure. The recovery of hexachlorocyclopentadiene from 12 spiked samples was 93.7 $\pm$ 6.6%, of hexachloronorbornadiene from 11 spiked samples was 102 $\pm$ 4%, and of heptachloronorbornene from 11 spiked samples was 101 $\pm$ 3%.

BIOLOGICAL METHODS

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 failureof the animals to respond to probing.

After the 30 day period all surviving fish were killed in ice water and immediately measured for total length, blotted and weighed. Whole fish were then frozen in stainless steel weighing dishes for residue analysis.

TABLE 1. SURVIVAL AND GROWTH OF FATHEAD MINNOWS EXPOSED TO VARIOUS CONCENTRATIONS OF HEXACHLOROCYCLOPENTADIENE ("HEX"). ASTERISK (*) DENOTES VALUES SIGNIFICANTLY LESS THAN CONTROLS (ANALYSIS OF VARIANCE, DUNNETT'S TEST, P=0.05).

	Measured concentration (µg/liter)											
	9.1[a] ± 1.8		7.3 ± 4.7		3.7 ± 1.2		1.7 ± 0.78		0.78 ± 0.31		<0.04	(control)
Item	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 ("X"). ASTERISK (*) DENOTES VALUES SIGNIFICANTLY LESS THAN CONTROLS (ANALYSIS OF VARIANCE, DUNNETT'S TEST, P=0.05).

Item	Measured concentration (µg/liter)											
	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 ("Y"). ASTERISK (*) DENOTES VALUES SIGNIFICANTLY LESS THAN CONTROLS (ANALYSIS OF VARIANCE, DUNNETT'S TEST, P=0.05).

Item	Measured concentration (µg/liter)											
	180.3[a] ± 14.8		164.9 ± 36.6		83.5 ± 7.1		40.0 ± 10.8		25.9 ± 3.4		<0.04 (control)	
	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.

RESIDUE ANALYSIS

Whole fish were analyzed on a wet weight basis using the methods described by Veith and Lee (1971). Composite samples of all surviving fish in each duplicate tank were homogenized with anhydrous Na_2SO_4 and exhaustively extracted with a mixture of hexane and methylene chloride in a Soxhlet extractor. The extracts were transferred to a 20 g Florisil column and eluted with 250 ml hexane. The sample was adjusted to the appropriate volume for GLC analysis.

The recovery of the test chemicals was determined by spiking control fathead minnows with 1.0 µg/g of each chemical and analyzing the samples in triplicate. The recoveries of hexachloronorbornadiene and heptachloronorbornene were 98.0 $\pm$ 2.3% and 93.0 $\pm$ 2.9%, respectively. The recovery of hexachlorocyclopentadiene was 20.7 $\pm$ 0.2%. The poor recovery of "hex" was due to losses by vaporization during the extraction step. Consequently, tissue residues for hexachlorocyclopentadiene could not be measured at this time. Additional experiments are underway to determine the bioconcentration factor of this chemical.

STATISTICAL ANALYSIS

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).

SECTION 5

RESULTS

HEXACHLOROCYCLOPENTADIENE ("hex")

Toxicity

Survival of fathead minnow larvae was significantly decreased at 7.3 µg/liter and above after 4 days of exposure (Table 1). A concentration of 9.1 µg/liter killed all but one fish after 4 days and all fish by 30 days. Growth of fish surviving at concentrations of 7.3 µg/liter and below was not significantly decreased from that in the controls (Table 1).

The toxicity curve relating median lethal concentration (LC50) for "hex" to exposure time is illustrated in Figure 2.

HEXACHLORONORBORNADIENE ("X")

Toxicity

Concentrations of 122 and 226 µg/liter of "X" caused significant decreases in survival of fathead minnow larvae after 4 days of exposure (Table 2). By the end of the test (30 days) all fish were dead at 226 µg/liter and the mean survival at 122 µg/liter was 66%. Significant decreases in survival did not occur in concentrations lower than 122 µg/liter. Growth of 30 day-old larvae, however, was significantly decreased at concentrations of 38.4 µg/liter and above.

The toxicity curve relating LC50 for "X" to exposure time is included in Figure 2.

Accumulation

The concentration of "X" accumulated by fathead minnows was directly proportional to the mean exposure concentration up to a concentration of 38.4 µg/liter which decreased the growth of the fish. As illustrated in Figure 3, residues in 30 day-old fish exposed to 20 µg/liter contained an average of 129 µg/g which resulted in a bioconcentration factor of 6450. A similar bioconcentration factor of 6350 was obtained when fish were exposed to 38.4 µg/liter. However, the bioconcentration factor at 56.9 µg/liter decreased to 4500, and further decreased to 4000 at 122 µg/liter. Consequently, the average bioconcentration factor for hexachloronorbornadiene in fish exposed to concentrations less than 38.4 µg/liter was approximately 6400.

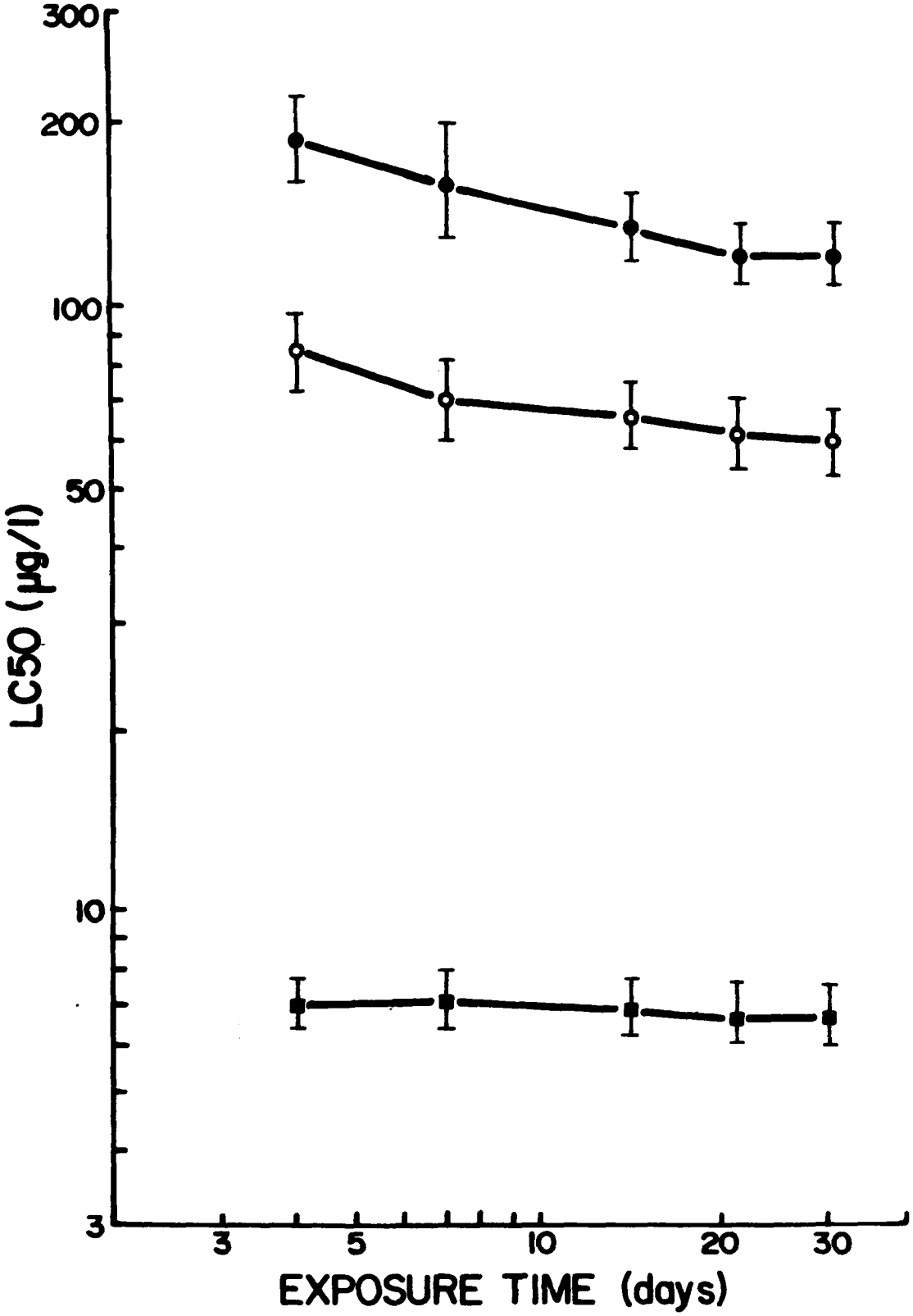

Figure 2. Relationship between LC50 (log scale) of hexachlorocyclopentadiene ("hex"), hexachloronorbornadiene ("X"), and heptachloronorbornene ("Y") to exposure time (log scale) for fathead minnows. Symbol ■ indicate mean value of "hex", • of "X", and o of "Y". Bars indicate 95% confidence limits.

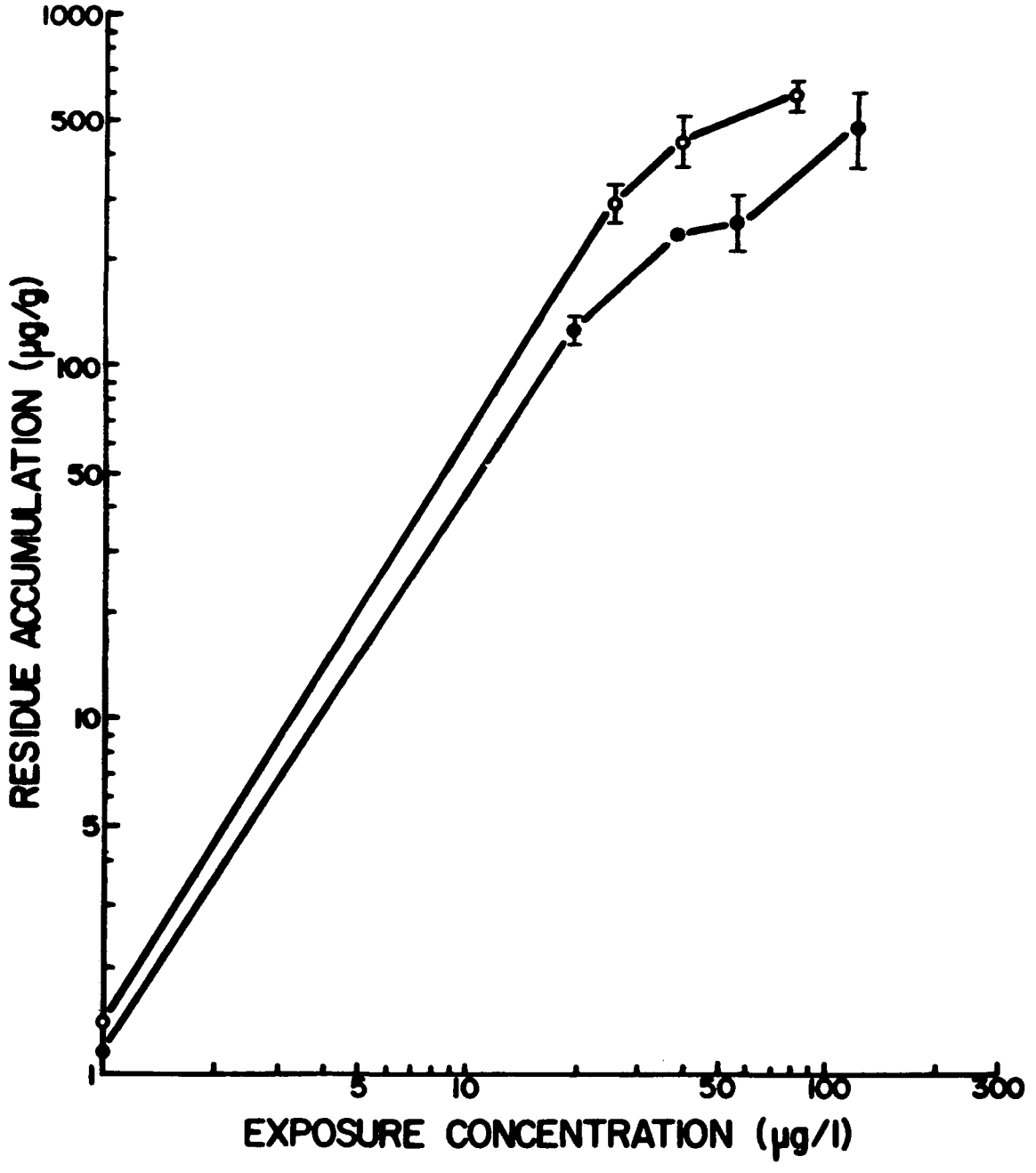

Figure 3. Residue accumulation (log scale) of "X" (•) and "Y" (o) measured in fathead minnows exposed for 30 days. Symbols and bars indicate the mean of duplicate pooled samples and S.D., respectively. Symbol with no bar indicates one pooled sample.

HEPTACHLORONORBORNENE ("Y")

Toxicity

Survival of fathead minnow larvae exposed to "Y" was significantly decreased at 83.5 µg/liter and above after 4 days. Higher concentrations of 164.9 and 180.3 µg/liter, killed all the fish within this period. Additional mortality occurred at 83.5 µg/liter after 30 days resulting in 22% survival. Decreases in survival were not observed at the two lower concentrations of 40 and 25.9 µg/liter. Lengths of fish, however, were significantly reduced at 40 µg/liter and above after 30 days of exposure.

The toxicity curve relating LC50 for "Y" to exposure time is included in Figure 2.

Accumulation

The concentration "Y" accumulated by fathead minnows was directly proportional to the mean exposure concentration up to the "effect" concentration of 40 µg/liter (Figure 3). Fish exposed to 25.9 µg/liter "Y" accumulated an average of 296 µg/g which resulted in a bioconcentration factor of 11,400, Fish exposed to 40.0 µg/liter contained residues of 438 µg/g, which resulted in a bioconcentration factor of approximately 11,000. However, at 83.5 µg/liter, 588 µg/g were accumulated and the bioconcentration factor was reduced to 7000. Consequently, the average bioconcentration factor for fish exposed to "Y" at less than 40 µg/liter was 11,200.

SECTION 6

DISCUSSION

Hexachlorocyclopentadiene ("hex") was more toxic to fathead minnows than hexachloronorbornadiene ("X") or heptachloronorbornene ("Y"). A concentration of "hex" causing adverse effects was approximately 5 times lower than concentrations of "X" and "Y" causing adverse effects to fathead minnows after the same time period. Hexachloronorbornadiene and heptachloronorbornene significantly decreased growth at approximately the same concentrations. Concentrations that decreased growth were 2 to 3 times lower than those affecting survival. This effect was different from that caused by "hex" since this compound caused significant decreases in survival but not in growth.

Comparison of the results of this test to earlier studies show that all three 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-hr LC50 value of "hex" reported for fathead minnows in this test was 8 times lower than the value reported for this species by the U.S. Department of Health, Education and Welfare (1956). The 96-hr LC50 values of "hex" in static bioassays reported for bluegills and bass (Davis and Hardcastle, 1959) were approximately 2800 to 3600 times higher than 96-hr values reported in this test. Mount and Putnicki (1966) indicated that compounds of "X" and "Y" would be 1,000 to 10,000 times less toxic than endrin to guppies. An earlier study by Mount (1962) showed that 0.4 to 0.5 µg/liter of endrin caused mortality to guppies and bluntnose minnows and that little mortality was due to endrin at 0.25 µg/liter. These results indicate that "X" and "Y" would be toxic to guppies at concentrations of 500 µg/liter and above and that little effect would occur at concentrations below this. However, in the present test, approximately 40 µg/liter of "X" and "Y" caused significant reductions in growth and 122 and 83.5 µg/liter, respectively, caused significant decreases in survival of fathead minnows. 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).

The toxicity curve 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 non-cumulative. Toxicity curves for hexachloronorbornadiene and heptachloronorbornene showed that these compounds may have had a cumulative action. This is also suggested because adverse effects on growth were observed at concentrations much lower than those which decreased survival and by their high bioaccumulation in whole body tissue.

The accumulation of hexachloronorbornadiene and heptachloronorbornene in fathead minnows was substantial at low exposure concentrations after 30 days. 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 "X" and "Y" have been found in edible fish such as catfish, carp, and others (U.S. Food and Drug Administration, 1976).

Residue concentrations of "hex" have not been found in edible fish and its accumulation in fathead minnows was not demonstratable in this test because of losses by vaporization during extraction. Additional studies are needed to determine the bioconcentration factor of "hex" since it is found in water bodies together with sewage water from manufacturers of poisonous chemicals and plastics (Naishstein and Lisovskaya, 1965).

No information was found in the literature on the toxicity of hexachloronorbornadiene and heptachloronorbornene to mammals or humans. Studies by Ingle (1953), Treon et al. (1955), and Hooker Chemicals and Plastics Corporation (1969), however, have shown that hexachlorocyclopentadiene is toxic to mammals (guinea pigs, rats, mice and rabbits) via inhalation, ingestion or dermal exposure. Other effects caused by "hex" on mammals include degenerative changes in several body organs, pulmonary edema, bronchitis, pneumonia, tremors, irritation of mucous and respiratory membranes, increased breathing and others. All three studies indicated that this compound can cause noxious effects to humans such as skin burns and discomfort, eye irritation and headaches.

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16. ABSTRACT

A rapid assessment study to determine the toxicity and bioaccumulation of three chlorinated cyclodiene insecticide intermediates; hexachlorocyclopentadiene, hexachloronorbornadiene, and heptachloronorbornene to fathead minnow larvae and early juveniles was conducted for 30 days under flow-through conditions.

A concentration of 7.3 µg/liter of hexachlorocyclopentadiene caused significant decreases in survival after 4 days. Growth of fish exposed for 30 days was not significantly decreased at any of the concentrations tested. The highest concentration of hexachlorocyclopentadiene having no adverse effect was 3.7 µg/liter.

Concentrations of 122 and 226 µg/liter of hexachloronorbornadiene caused significant decreases in survival after 4 days. Growth of 30 day-old larvae was significantly decreased at 38.4 µg/liter and was the most sensitive indicator of toxicity. The highest concentration having no adverse effect was 20.0 µg/liter. The average bioconcentration factor for fish exposed to less than 38.4 µg/liter of this compound was 6400.

Survival of fathead minnows exposed to heptachloronorbornene was significantly decreased at 83.5 µg/liter after 4 days. Growth was significantly reduced at 40 µg/liter after 30 days and was the most sensitive indicator of toxicity. The highest concentration having no adverse effect was 25.9 µg/liter. The average bioconcentration factor for fish exposed to less than 40 µg/liter of this compound in water was 11,200.

17. KEY WORDS AND DOCUMENT ANALYSIS

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