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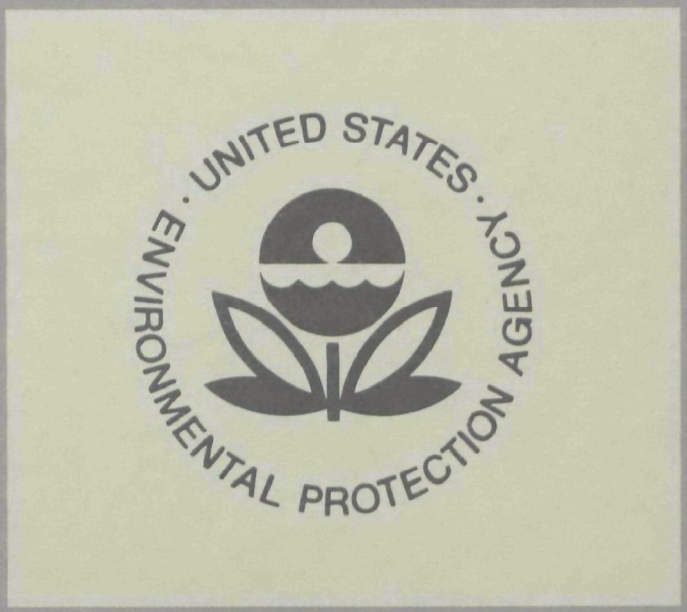
February 1977

Ecological Research Series

ACUTE AND CHRONIC TOXICITY OF CHLORDANE TO FISH AND INVERTEBRATES



Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, Minnesota 55804

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ACUTE AND CHRONIC TOXICITY OF CHLORDANE TO FISH AND INVERTEBRATES

by

Rick D. Cardwell
Dallas G. Foreman
Thomas R. Payne
Doris J. Wilbur
Chemico Process Plants Company - Envirogenics Systems
El Monte, California 91734

Contract No. 68-01-0187

D. T. Allison
Environmental Research Laboratory-Duluth
Duluth, Minnesota 55804

ENVIRONMENTAL RESEARCH LABORATORY - DULUTH
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
DULUTH, MINNESOTA 55804

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FOREWORD

Our nation's freshwaters are vital for all animals and plants, yet our diverse uses of water---for recreation, food, energy, transportation, and industry--physically and chemically alter lakes, rivers, and streams. Such alterations threaten terrestrial organisms, as well as those living in water. The Environmental Research Laboratory in Duluth, Minnesota, develops methods, conducts laboratory and field studies, and extrapolates research findings.

--to determine how physical and chemical pollution affects aquatic life

--to assess the effects of ecosystems on pollutants

--to predict effects of pollutants on large lakes through use of models

--to measure bioaccumulation of pollutants in aquatic organisms that are consumed by other animals, including man

This report describes the acute and chronic effects of the pesticide chlordane on a number of freshwater fishes and invertebrates.

Donald I. Mount, Ph.D.
Director
Environmental Research Laboratory
Duluth, Minnesota

ABSTRACT

The acute and chronic toxicity of technical chlordane to bluegill (Lepomis macrochirus), fathead minnow (Pimephales promelas), brook trout (Salvelinus fontinalis), Daphnia magna, Hyallela azteca, and Chironomus No. 51 were determined with flow-through conditions. The purpose was to estimate concentrations producing acute mortality and those having no effect on the long-term survival, growth, and reproduction of the various species. Whole body residues of technical chlordane components were measured in the three invertebrate species at the end of the chronic exposure tests.

Concentrations of technical chlordane causing 50% mortality in 96 hr were 36.9 µg/l for fathead minnow, 47 µg/l for brook trout, and 59 µg/l for bluegill, while that causing 50% immobilization in the cladoceran, D. magna, was 28.4 µg/l. The amphipod, H. azteca, was only slightly affected at 96 hr by the chlordane concentrations tested, and the 168-hr EC50 was 97.1 µg/l. Acute mortality of midges, Chironomus No. 51, was not successfully evaluated.

With respect to the test conditions employed and life cycle stages evaluated, the lowest concentrations of technical chlordane found to cause major chronic effects were 0.32 µg/l for brook trout, 1.22 µg/l for bluegill, 1.7 µg/l for midges, 11.5 µg/l for amphipods, and 21.6 µg/l for cladocerans.

Technical chlordane accumulation in the invertebrate species varied directly with the aqueous concentration to which the animals were exposed. The component accumulated to the greatest extent was trans-nonachlor, for which whole body residues were up to 145,000-times higher than the aqueous concentration.

This report was submitted in fulfillment of Contract No. 68-01-0187 by the Chemico Process Plants Company-Envirogenics Systems under the sponsorship of the U.S. Environmental Protection Agency. Work was completed as of June 1974.

CONTENTS

TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

LC50	-- median lethal concentration
EC50	-- median effective concentration
MATC	-- maximum acceptable toxicant concentration
A.I.	-- active ingredient
LC100	-- lethal concentration to all test organisms
LC0	-- lethal threshold concentration
LT50	-- median lethal time
C.F.	-- concentration factor

SYMBOLS

$\hat{\sigma}$	-- logarithm of the standard deviation of the population tolerance frequency distribution
S	-- antilogarithm of σ
mg/l	-- milligram per liter
µg/g	-- microgram per gram
ppb	-- parts per billion = microgram per kilogram or microgram per liter

ACKNOWLEDGMENTS

We wish to thank Mr. D. T. Allison, Project Officer, for providing valuable guidance during the course of the project and for critically reviewing the manuscript. Mr. L. H. Mueller, Research Chemist at the Environmental Research Laboratory, Duluth, Minnesota (ERL-D), provided valuable assistance in analytical procedures for measuring chlordane in water and biological tissues. Mr. W. E. Wright and Ms. J. L. Wright conducted all chemical analyses of water quality and assisted in the conduct of the toxicity tests. Mr. R. Stankiewicz contributed to all aspects of computer programming and analysis. Mr. W. Richardson of the California Department of Fish and Game arranged for the acquisition of brook trout. Mr. K. E. Biesinger and Ms. B. J. Halligan, ERL-D, provided helpful advice on culture and testing of daphnids and amphipods, respectively. Dr. M. Mulla, University of California Department of Entomology (Riverside), supplied Chironomus No. 51 and suggested effective culture techniques. We would also like to thank Ms. B. Leistikow, Fisheries Research Institute, University of Washington, for providing positive identification of the amphipod, Hyallela azteca (Saussure). Finally, we extend our appreciation to the Velsicol Chemical Corporation (Chicago, Illinois) for supplying analytical reference technical chlordane and literature on its composition for use in this program.

SECTION I

INTRODUCTION

The organochlorine insecticide, chlordane, is widely used for the control of insect pests, particularly in non-agricultural areas. The insecticide has been extensively used relative to other organochlorine insecticides; in 1971, over 11.4×10^6 kg of chlordane was produced compared to 5×10^4 kg endrin, dieldrin and lindane, 4.5×10^6 kg aldrin, and 21×10^6 kg DDT (1). Because this chemical has a high biological potency and is relatively long-lived in the environment (2), it presents a potential hazard to non-target species (fish and wildlife) and ultimately to public health.

The adverse effects of chlordane on fish and aquatic invertebrates can be examined with acute and chronic toxicity tests or with studies of the accumulation of toxic (to predatory animals including man) residues (3). All of these methods have limitations (4), but the chronic toxicity test, which encompasses all or most of one reproductive cycle, probably represents the most direct method of estimating the concentration of chlordane which is "safe" for long-term survival, growth, and production of a species.

Less than a decade ago, water quality criteria for aquatic organisms were set with application factors ranging from 1/10 to 1/100 or with various equations (5, 6). These factors were multiplied by an acute toxicity test result such as the concentration lethal to 50% of the test specimens (LC50) to estimate environmentally "safe" concentrations. But these application factors were arbitrary and presented the hazard of over or underestimating the "safe" level. Because of the need to adequately protect the aquatic resource with realistic standards, other methods were examined. The chronic toxicity test has been suggested as the most practicable tool for achieving objective evaluations of sublethal, long-term toxicant effects on many species. The concept of the chronic test, its methods, scope, and application, was initially set forth by Mount and Stephan (7) in their studies of malation and the butoxyethanol ester of 2,4-D using fathead minnows (Pimephales promelas Rafinesque). Basically, the chronic test attempts to estimate the toxicant concentration at which effects on survival, growth and reproduction of all life stages become statistically indistinguishable from those for fish held in uncontaminated water (controls). Between the concentration producing some effect on one or more of the above indices and that having no effect is the maximum acceptable toxicant concentration (MATC), the theoretical "just safe" level. By dividing the MATC estimate by the LC50, an applica-

tion factor can be obtained which can be used to estimate "safe" levels for aquatic organisms which are unsuitable for chronic toxicity testing for one reason or another. Several chronic toxicity tests of pesticides have been completed since that of Mount and Stephan (7). These include evaluations of malathion (8), carbaryl (9), and captan (10). Although application factors may remain relatively constant for a particular chemical and taxonomic group of organisms, the constraints on its applicability need to be defined. It has been recently shown, that the MATC and application factors can be satisfactorily estimated for at least one heavy metal through the use of one rather than a multiple generation test (11).

As discussed in the next section, a moderate amount of information is already available on the toxicity of chlordane to aquatic life. However, the majority of the tests have been conducted with static conditions for short periods and without measurement of chlordane concentrations in the diluent water. While these tests suffice to define the approximate order of chlordane toxicity to aquatic biota, their limited experimental scope restrains, in most cases, their use in establishing water quality criteria. To our knowledge, no chronic toxicity, reproductive studies of chlordane have heretofore been reported in the literature, and these studies are generally thought to be necessary for establishing sound standards. Accordingly, this investigation sought to define the acute and chronic toxicity of chlordane to three species of freshwater fish and three invertebrates using flow-through conditions. Furthermore, measurements of chlordane residues in tissues of the chronically exposed invertebrates were incorporated into the experimental design to provide information on the extent of bioaccumulation and on the potential hazard to predator species from consuming animals contaminated with this insecticide.

SECTION II

CONCLUSIONS

1. The acute mortality tests suggested that technical chlordane was a cumulative poison, causing toxicity as a function of concentration and exposure time. Median lethal thresholds were not attained within 96 hr for any species. The fathead minnow was the only species for which a threshold was observed, and it did not occur until approximately 180 hr.

2. Technical chlordane was generally more toxic on a chronic sublethal basis to the three fish species than to the three species of invertebrates.

3. Chronic toxicity test results suggest that technical chlordane concentrations greater than approximately 0.3 µg/l would be deleterious to the production of at least some fish species, and that concentrations greater than 21.6 µg/l would probably be very deleterious to most aquatic animals.

4. Accumulation of technical chlordane in the cladoceran and the amphipod was substantial, but varied with respect to the component. Cis-nonachlor was concentrated to the greatest extent and heptachlor the least. Residues of technical chlordane were not detected in the midge.

5. Measured concentrations of technical chlordane were consistently less than desired, even though low concentrations of the non-ionic surfactant, Triton X-100, and of the solvent, acetone, were employed to aid dissolution, and the toxicant solutions were continuously replenished. This indicates that toxicity tests of this insecticide would not be valid unless based upon measured concentrations of the dissolved compound.

SECTION III

RECOMMENDATIONS

1. Acute toxicity tests of technical chlordane should be continued until a median lethal threshold is observed rather than discontinued at a specified time. This would permit characterization of the toxicity curve particular to each species and life stage, and might prove useful in calculating effects in mixing zones.

2. Additional acute and chronic toxicity tests of technical chlordane using additional species of freshwater fish (e.g. Ictaluridae, Catastomidae), marine fish (e.g. Cyprinodontidae, Clupeidae), freshwater and marine invertebrates, and algae are needed, regardless of whether water quality standards are set for groups of aquatic organisms inhabiting specific ecosystems or on the average MATC of the most sensitive groups. Such information is needed to permit objective decisions on what levels of this insecticide will have no effect on diverse communities of aquatic organisms since there may or may not be important differences between taxonomic groups and between freshwater and marine organisms.

3. The acute and chronic toxicities of technical chlordane in mixtures of other toxicants and with different environmental conditions should be determined to evaluate interactions.

4. A multiple generation chronic toxicity test should be conducted to determine whether this complex insecticide causes teratogenic or mutagenic effects. For freshwater fish a species which completes its life cycle rapidly, such as the flagfish (Jordanella floridae Goode and Bean) or a poeciliid (e.g. the mosquitofish, Gambusia affinis [Baird and Girard]), should be considered since they have relatively short generation times of 3 to 4 months.

5. Studies of technical chlordane contents in fish and invertebrates should incorporate investigations of the uptake, biotransformation, and tissue distribution of each of the major, and perhaps the minor, constituents (e.g. hexachlorocyclopentadiene).

7. The degree and efficiency of transfer of technical chlordane components through several trophic levels should be determined and compared to uptake from the water.

8. The dosage levels of technical chlordane causing lethal and sublethal effects on predators should be determined by feeding them prey species contaminated with known amounts of the insecticide.

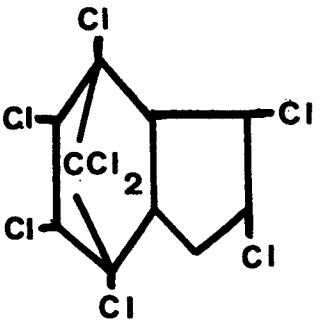
9. Increasing the number of replicates per treatment for the F_0-generation from the presently recommended two to at least three and possibly four would considerably strengthen the value and power of statistical tests.

SECTION IV

LITERATURE REVIEW

CHEMISTRY OF CHLORDANE

Chlordane (1, 2, 4, 5, 6, 7, 8, 8-octachloro-2, 3, 3a, 4, 7, 7a-hexahydro-4, 7-methanoindene) is a chlorinated hydrocarbon insecticide manufactured by the Velsicol Chemical Corporation (Chicago).

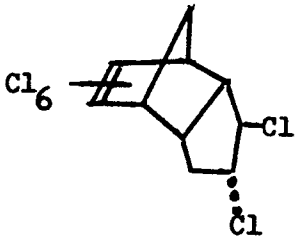


Chlordane

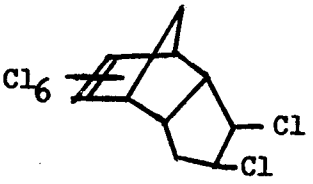
The technical grade used in the present program was supplied by Velsicol and identified as "Analytical Reference Technical Chlordane." It is a complex mixture and has been variously characterized by Velsicol (12), the U.S. Environmental Protection Agency (EPA, 13), and Saha and Lee (14). The predominant constituents are trans-chlordane (24 ± 2%), cis-chlordane (19 ± 3%), heptachlor (10 ± 3%), chlordenes (20.5%), trans-nonachlor (5.1%), and cis-nonachlor (2.8%) (page 7 and Appendix Table 1).

Technical chlordane is a liquid with a molecular weight of approximately 410. Its solubility limit in the laboratory water used in these investigations was of the order of 150 to 220 µg/l at 22°C. Edwards (15) has given its solubility as 100 µg/l at 20 to 30°C. A typical chromatogram of the technical material is shown in Fig. 1. Identification was accomplished with known standards and EPA (13) data.

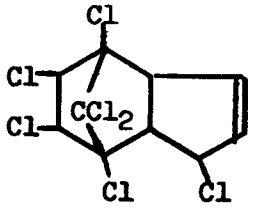
The stability of technical chlordane solutions in distilled water has been evaluated by Bevenue and Yeo (16) for a period of 60 days. The authors



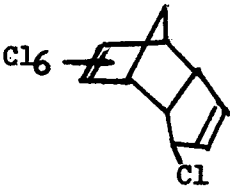
trans-chlordane



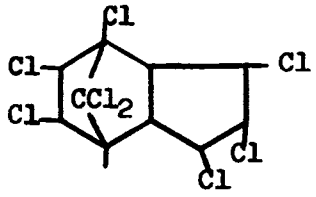
cis-chlordane



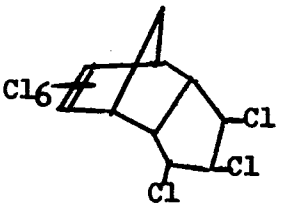
heptachlor



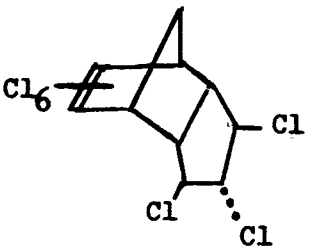
heptachlor



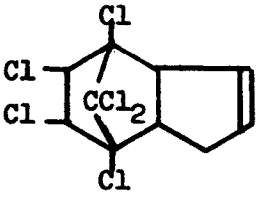
nonachlor



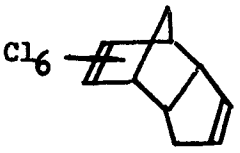
cis-nonachlor



trans-nonachlor



chlordene



chlordene

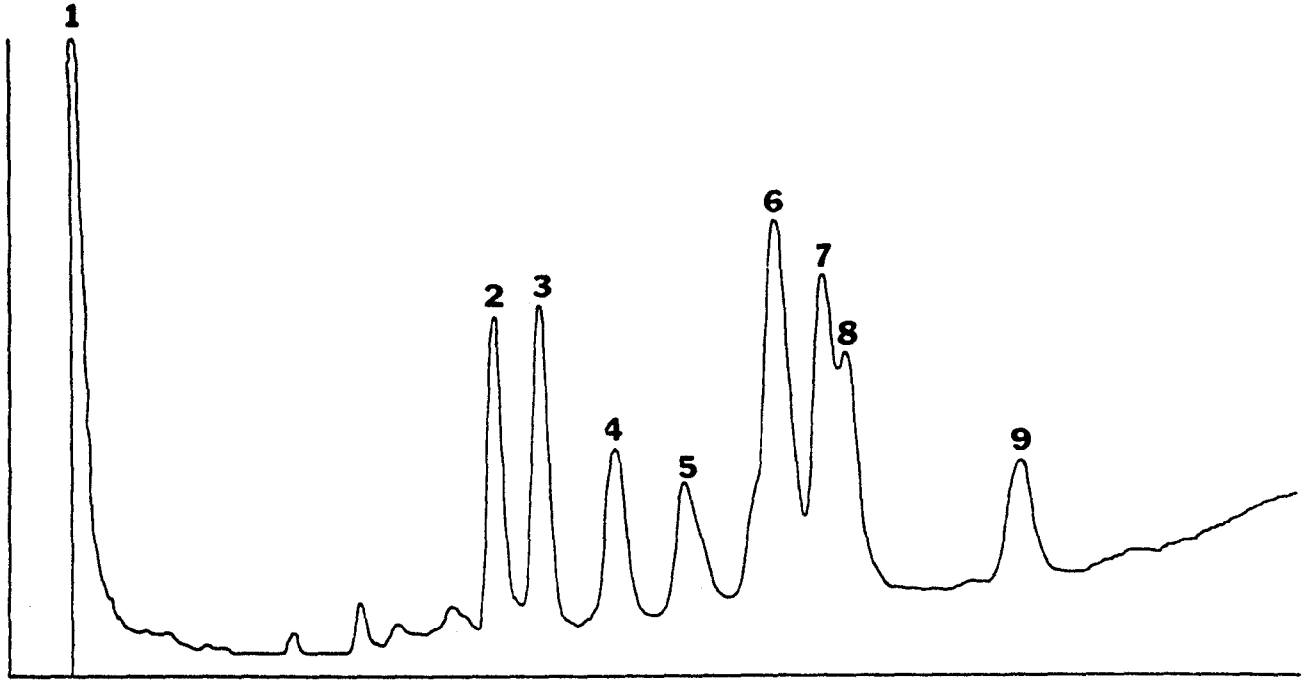


Fig. 1. Chromatogram of technical chlordane standard in hexane run under conditions specified elsewhere: (1) n-hexane; (2) $C_{10}H_6Cl_6$ isomer; (3) heptachlor and chlordene; (4) γ - β and "A"-chlordene; (5) $C_{10}H_7Cl_6$ isomer; (6) trans-chlordane; (7) cis-chlordane; (8) trans-nonachlor; (9) cis-nonachlor.

noted no temporal changes in trans- (γ) chlordane and cis- (α) chlordane, or the nonachlor components, but observed a conversion of heptachlor to 1-hydroxychlordene. There was no generation of heptachlor epoxide, and the occurrence of oxychlordane was not mentioned. Octachlor epoxide, also known as oxychlordane, is formed by the epoxidation of chlordane and is a known metabolite of chlordane in animals (17, 18).

TOXICITY OF CHLORDANE TO AQUATIC ANIMALS

Although chlordane has received far less study than such chlorinated hydrocarbons as DDT and endrin, a substantial volume of literature exists concerning its toxicity to aquatic animals. The majority of the literature concerns the insecticide's efficacy in killing mosquitoes (Diptera: Culicidae).

Fish

Chlordane is generally less acutely toxic to fish than endrin, DDT, dieldrin, and aldrin, and more toxic than lindane and methoxychlor, for example. Much of the available literature on chlordane toxicity to fish is summarized in Table 1. The majority of these toxicity tests were conducted for less than 96 hr and, except for two, employed static test conditions. In preparing Tables 1 and 2, efforts were made to convert test results, where possible, to concentrations equivalent to 100% active ingredient (A.I.) for direct comparison. As can be seen, responses to chlordane ranged from 0.1 µg/l A.I., which significantly increased oxygen consumption in bluegill (19) to 3,050 µg/l A.I., a level lethal to rainbow trout exposed to an emulsion of chlordane in a flow-through system (20).

Comparison of the toxicity test results conducted with methodology similar or identical to the standard procedures set forth by Doudoroff et al. (5) and the American Public Health Association (APHA, 21) lessens the disparity in reported toxic concentrations. Henderson, Pickering and Tarzwell (22) exposed four species of freshwater fish to an enulsifiable concentrate of 75% chlordane and found 96-hr LC50 values which varied from 16.5 µg/l (bluegill) to 142.5 µg/l (guppy, Poecilia reticulata). The concentrate was also more toxic to fathead minnows in soft water than in hard. During the same year, Clemens and Sneed (24) reported that 500 µg/l chlordane produced 50% mortality in channel catfish (Ictalurus punctatus) fingerlings in 96 hr. The later data of Katz (23) for three species of salmonids and the euryhaline stickleback (Gasterosteus aculeatus) and of Macek, Hutchinson, and Cope (25) for bluegill were in closer aggreement with the data of Henderson et al. (22) than with that of Clemens and Sneed (24). Ninety-six hour median lethal concentrations for the three salmonids ranged from 44 to 57 µg/l (23). The study of Macek et al. (25) indicated that chlordane was more toxic to bluegill at higher water temperatures than at lower ones. At higher temperatures successively less chlordane was required to produce 50% mortality in 24 hr. After 96 hr bluegill were killed less rapidly at 12.7°C (LC50 of 85 µg/l) than at 18.3°C (LC50 of 70 µg/l), but they were killed at the same concentration between 18.3°C and 23.8 °C.

TABLE 1. CONCENTRATIONS OF CHLORDANE TOXIC TO FISH

Species name		Response manifest at		Type of response	Reference
Common	Binomial	time, hr	conc.,[a] µg/l		
Chinook salmon	Oncorhynchus tshawytscha	96	57.0	LC50[b]	23
Coho salmon	O. kisutch	96	56.0	LC50	23
Rainbow trout	Salmo gairdneri	96	44.0	LC50	23
Threespine stickleback	Gasterosteus aculeatus	96	90[c]	LC50	23
Threespine stickleback	Gasterosteus aculeatus	96	160[d]	LC50	23
Fathead minnow	Pimephales promelas	96	39[e]	LC50	22
Fathead minnow	Pimephales promelas	96	52[f]	LC50	22
Bluegill	Lepomis macrochirus	96	16.5	LC50	22
Goldfish	Carassius auratus	96	61.5	LC50	22

Continued .....

TABLE 1. CONCENTRATIONS OF CHLORDANE TOXIC TO FISH--continued

Species name		Response manifest at			
Common	Binomial	time, hr	conc.,[a] µg/l	Type of response	Reference
Guppy	Poecilia reticulata	96	142.5	LC50	22
Channel catfish	Ictalurus punctatus	96	500	LC50	24
Bluegill	L. macrochirus	96	85 (12.7 C)	LC50	25
Bluegill	L. macrochirus	96	77 (23.8 C)	LC50	25
Murrel	Channa punctatus fry	115	0.25	LC100[g]	26
Murrel	Channa punctatus fingerling	50	1.25	LC100	26
Murrel	Channa punctatus adult	60	16.0	LC100	26
Rohu	Labeo rohita fingerling	40	0.025	LC100	26
Spiny eel	Mastocembelus pancalus	51	0.4	LC100	26

Continued ....

TABLE 1. CONCENTRATIONS OF CHLORDANE TOXIC TO FISH--continued

Species name		Response manifest at		Type of response	Reference
Common	Binomial	time, hr	conc.,[a] µg/l		
Tengra	Mystus vittatus	60	0.5	LC100	26
Nandus	Nandus nandus	25	0.63	LC100	26
Punti	Puntia sophore	18	1.25	LC100	26
Singhi	Heteropneustes fossilis	51	1.25	LC100	26
Cuchia	Amphipnous cuchia	45	2	LC100	26
Carp	Cyprinus carpio (embryos)	91	3,600	signif. effect on hatching	27
Bluegill	L. macrochirus	30	200	Lethal	28
Largemouth bass	Micropterus salmoides	87	200	Lethal	28
Rainbow trout	S. gairdneri	24	3,050[h]	LC50	20
Bluegill	L. macrochirus	24	218[i]	LC50	29

Continued ....

TABLE 1. CONCENTRATIONS OF CHLORDANE TOXIC TO FISH--continued

Species name		Response manifest at		Type of response	Reference
Common	Binomial	time, hr	conc.,[a] µg/l		
Bluegill	L. macrochirus	24	346[j]	LC50	29
Bluegill	L. macrochirus	?	0.1	Increased O_2 consumption	19
Carp	C. carpio	48	1,160	LC50	30
Rainbow trout	S. gairdneri	24	600	Threshold conc.	30
Rainbow trout	S. gairdneri	48	10	LC50	6
Pike	Esox sp.	24	>5	Threshold conc.	30
White mullet	Mugil curema	24	43	LC50	31
White mullet	Mugil curema	48	5	LC50	31

[a]All chlordane concentrations were adjusted, where possible, to 100% active ingredient.

[b]Median lethal concentration.

[c]Salinity was 5 g/kg.

[d]Salinity was 25 g/kg.

[e]Hardness of 20 mg/l $CaCO_3$.

[f]Hardness of 400 mg/l $CaCO_3$.

[g]Concentration lethal to all test specimens.

[h]LC50 calculated from concentration - % mortality data of authors.

[i]Chlordene (isomers of technical chlordane).

[j]Photochlordene (photolytic degradation product of chlordene).

In 1962, Lüdemann and Neumann (30) published extensive data on the toxicity of insecticides to a variety of fish, invertebrates, and a species of toad (Bufo bufo). The chlordane concentrations at which mortality just began to occur (i.e. lethal thresholds) for carp (Cyprinus carpio), rainbow trout (Salmo gairdneri), and pike (Esox sp.) exposed for 24 to 48 hr were 400, 600, and 5 µg/l, respectively. The 48-hr LC50 for carp was 1,160 µg/l. One of the more recent reports on chlordane toxicity dealt with the insecticide's lethality to several species of fish from India. Konar (26) found chlordane to have a uniformly high toxicity, but gave LC50 values without stating the time required for manifestation of the responses. LC100 estimates, concentrations lethal to 100% of the fish, ranged from a 115-hr value of 0.25 µg/l for murrel fry (Channa punctatus) to a 60-hr value of 16.0 µg/l for murrel adults. The 24-hr LC50 value of 10 µg/l chlordane for rainbow trout reported by the National Technical Advisory Committee on Water Quality Criteria (6) was one-sixtieth of the lethal threshold value reported by Lüdemann and Neumann (30) and one-fourth of that reported by Katz (23) for rainbow trout exposed for 96 hr. Although such wide variation among laboratory toxicity test results reinforces the need for standardization of toxicity testing procedures, measurement of the actual concentrations of pesticide to which the organisms were being exposed rather than reliance on the amount added to the tanks would probably have narrowed the range in values.

Little information was found documenting the toxicity of chlordane to marine organisms. Katz (23) exposed stickleback to chlordane at salinities of 5 and 25 g/kg and observed the pesticide to be approximately half as toxic at the higher salinity (96-hr LC50 of 160 µg/l) as at 5 g/kg (96-hr LC50 of 90 µg/l). In one of the few acute toxicity tests conducted with a flow-through rather than a static system, Holden (31) reported 24- and 48-hr LC50 values for juvenile white mullet (Mugil curema) of 43 and 5.5 µg/l, respectively.

The lowest concentration of chlordane (0.1 µg/l) found to elicit a potentially deleterious response (i.e. an increase in oxygen consumption) was observed by Dowden (19) in studies of the effects of various insecticides on bluegill. Augmented metabolic requirements of chlordane-exposed fish were also observed by Malone and Blaylock (27) in evaluations of the toxicity of DDT, chlordane, dieldrin, endrin, diazinon, and O, O, dimethyl-S-(4-oxobenzotriazino-3-methyl) phosphorodithioate to carp embryos. Concentrations of chlordane below 720 µg/l A.I. shortened incubation and stimulated embryonic development. Twenty-three percent of the chlordane-treated embryos hatched after 52.5 hr and 71% after 69.5 hr. None of the control embryos hatched by 52.5 hr and only 54.7% by 69.5 hr. Koch, Cutkomp and Yap (32) reported chlordane inhibition of Mg^{++}-ATPase activity in both mitochondrial and non-mitochondrial preparations of bluegill brain tissue. Since ATPase converts ATP to ADP and inorganic phosphate, inhibition of this enzyme would uncouple oxidative phosphorylation and thereby stimulate metabolism.

Little research has been performed on behavioral responses of fish to chlordane. Summerfelt and Lewis (33) reported that 5, 10 and 20 mg/l concentrations of a 75% emulsifiable concentrate of chlordane repelled green sunfish (Lepomis cyanellus), that 2 mg/l would result in an equivocal

response, and that 1 mg/l would produce no response. Since these levels are considerably greater than lethal levels, it appears that fish encountering an acutely lethal concentration of this insecticide might be unable to detect and avoid it.

There have been several studies of the effects of chlordane introduced into natural watercourses. Using flow-through conditions, Cope, Gjullin and Storm (20) introduced acetone solutions and emulsions of chlordane into troughs and streams containing principally salmonids, caddisflies (Trichoptera) and blackflies (Diptera-Simuliidae) in experiments designed to determine whether concentrations effective in controlling the simuliids would be deleterious to populations of salmonids and their prey. Emulsions of 1,250 µg/l A.I. chlordane immobilized trout in 15 min and caused death within 24 hr in tests conducted in troughs. A median lethal concentration of 3,050 µg/l was calculated from the data of Cope et al. (20) for rainbow trout exposed to chlordane for 15 min and held for 48 hr in uncontaminated water. In stream tests an emulsion of 1,250 µg/l chlordane immobilized the trout in 15 min. In a later study, Mulla (34) assessed the toxicity of various insecticidal preparations, including an emulsifiable concentrate of chlordane, to mosquitofish (Gambusia affinis) and bullfrogs (Rana catesbeiana), both predators of mosquitos. Applied at 0.23 kg/acre (0.5 lb/acre), chlordane was moderately toxic to mosquitofish, but at 0.45 kg/acre (1.0 lb/acre), it was highly toxic. Bullfrog mortality was judged moderate to severe at applications of 0.23 kg/acre of the emulsifiable concentrate. Dosages for insect control are generally recommended to be less than 0.45 kg/acre.

Aquatic Invertebrates

A considerable amount of work has been performed on the toxicity of chlordane to aquatic invertebrates. Owing perhaps to non-standardization of test conditions and use of different response criteria, water quality, and specimens of varying conditions, there is considerable disparity in the test results summarized in Table 2. Toxicities ranged from a 25-hr LC100 of 0.33 µg/l for backswimmers, Notonecta sp. (26), to a 96-hr lethal threshold of 10,000 µg/l for mussels, Dreissena polymorpha (30). Acute sensitivities of invertebrates were generally of the same order as those for fish.

The two most extensive studies on chlordane toxicity to invertebrates were performed by Konar (26) and Lüdemann and Neumann (30). Konar (26) exposed nine species of aquatic insects resident in India to chlordane for up to 168 hr and reported lethal threshold (LC0), LC50, and LC100 concentrations. However, exposure times varied within the 168-hr maximum and were not given for the LC50 values, thus limiting the latter's usefulness. As noted above, Konar (26) found the backswimmer to be the most sensitive species and the water scorpion, Nepa sp., the least sensitive (90-hr LC100 of 78.8 µg/l). Lüdemann and Neumann's (30) work encompassed more taxonomic groups than that of Konar (26). Lethal thresholds encountered with 24- to 96-hr exposure periods ranged from 1 µg/l for the amphipod, Carinogammarus ruesilii, to 10,000 µg/l for D. polymorpha. The lethal thresholds for the

TABLE 2. CONCENTRATIONS OF CHLORDANE TOXIC TO AQUATIC INVERTEBRATES

Species name		Response manifest at			
Common	Binomial	time, hr	conc.,[a] µg/l	Type of response	Reference
Backswimmer	Notonecta sp.	25	0.33	LC100[b]	26
Water stick	Ranatra filiformis	110	1.0	LC100	26
Water scorpion	Nepa sp.	90	78.8	LC100	26
Water bug	Sphaerodema annulatum	68	1.25	LC100	26
Giant water bug	Belostoma indica	88	1.25	LC100	26
Aquatic beetle	Hydrophilus sp.	130	1.58	LC100	26
Aquatic beetle	Dytiscus sp.	90	2.0	LC100	26
Aquatic beetle	Cybister	112	1.25	LC100	26
Dragonfly	Suborder anisoptera	132	1.0	LC100	26
Non-biting midge	Chironomus (larvae)	8	15	LT50[c]	36

Continued ....

TABLE 2. CONCENTRATIONS OF CHLORDANE TOXIC TO AQUATIC INVERTEBRATES--continued

Species name		Response manifest at			
Common	Binomial	time, hr	conc.,[a] µg/l	Type of response	Reference
Brine shrimp	Artemia salina (nauplii)	2-3	10	LT50	37
American oyster	Crassostrea virginica	24	10	Inhibit growth	38
Caddisfly	Hydropsyche sp.	34	1,650[d]	LC50	20
Amphipod (scud)	Gammarus lacustris	96	26	LC50	35
Stonefly	Pteronarcys californica	48	55	LC50	6
Water flea	Simocephalus serrulatus	48	55	EC50[e]	6
Tubificid worm	Tubifex tubifex	96	1,000	Lethal threshold	30
Mussel	Dreissena polymorpha	96	10,000	Lethal threshold	30

Continued ....

TABLE 2. CONCENTRATIONS OF CHLORDANE TOXIC TO AQUATIC INVERTEBRATES--continued

Species name		Response manifest at		Type of response	Reference
Common	Binomial	time, hr	conc.,[a] µg/l		
Amphipod (scud)	Carinogammarus ruesilli	24	1	Lethal threshold	30
Copepod	Cyclops strenuus	24	1,000	Lethal threshold	30
Isopod	Asellus aquaticus	24	50	Lethal threshold	30
Crayfish	Cambarus affinis	24	1,000	Lethal threshold	30
Diptera (Culicidae)	Corethra plumicornis (larvae)	24	100	Lethal threshold	30
Diptera (Chironomidae)	Chironomus (larvae)	24	>5	Lethal threshold	30

[a]All chlordane concentrations were adjusted, where possible, to 100% active ingredient.

[b]Lethal concentration to all specimens.

[c]Median lethal time.

[d]LC50 calculated from concentration - % mortality data of authors.

[e]Median effective concentration (EC50) is the concentration causing immobilization of the test specimens.

amphipod and for larval midges, Chironomus sp. (i.e. 5 µg/l), were similar to results obtained by others for species within the same taxonomic groups. Sanders (35) determined that the 96-hr LC50 for the amphipod, Gammarus lacustris, was 26 µg/l, while Silvey (36) calculated a median lethal time of 8 hr for Chironomus larvae exposed to 15 µg/l. The National Technical Advisory Commission on Water Quality Criteria (6) reported that the 48-hr LC50 and EC50 values for Stoneflies (Pteronarcys californica) and a cladoceran (Simocephalus serrulatus) were 55 and 20 µg/l, respectively.

Very little data have been accumulated on the toxicity of chlordane to marine invertebrates. Michael, Thompson and Abramowitz (37) determined that 10 µg/l would cause 50% mortality in brine shrimp nauplii (Artemia salina) in 2-3 hr. Butler, Wilson and Rick (38) investigated the effects of various insecticides on behavior and growth of Eastern oysters, Crassostrea virginica, and observed inhibition of growth within 24 hr at 10 µg/l chlordane.

Field experiments of chlordane toxicity have dealt primarily with the control of mosquitoes. Although these data will not be discussed herein, selected references are given in the appended Bibliography (Section IX). In an Alaskan field study of the adverse effects of insecticide applications for blackfly eradication on native populations of fish and aquatic insects, Cope et al. (20) found the caddisfly, Hydropsyche sp., to be the most sensitive of three species evaluated. Fifteen minute exposure to 1,250 µg/l chlordane immobilized Hydropsyche sp. Calculation of an LC50 for Hydropsyche sp. using concentration-percent mortality data given by Cope et al. (20) resulted in a value of 1,650 µg/l. The exposure consisted of a 15-min period of insecticide contact followed by 24-hr confinement in flowing freshwater.

Residues of Chlordane in Aquatic Organisms and the Environment

Following its introduction into the environment, chlordane probably shares the same fates as many of the chlorinated hydrocarbon insecticides. Some of the components of the technical material will vaporize and be carried away from the point of application by thermal convection, etc. The vapor pressure of chlordane is 1×10^{-5} mm Hg, more than that of DDT (1.9×10^{-7}) and endrin (2×10^{-7}); hence, its volatilization might be greater than these two compounds (15). Terrestrially, chlordane can be absorbed by organisms, adsorbed to particulate matter (soil), or enter watercourses by dissolving in water or sorbing to suspended particles. Chlordane is a relatively persistent compound in the environment, a characteristic that contributes to its bioactivity. Lichtenstein and Polivka (2) found that 12.4 to 17.8% of the chlordane applied to turf plots remained after 12 yr in undisturbed sandy loam soil. Heptachlor, a constituent of technical chlordane, had disappeared completely after 9 yr in silty clay loam soil. In comparison, Miami silt loam and muck soils treated with 10 and 100 lb/acre DDT retained approximately 22 and 33% of the insecticide after 3.5 yr (39). Thus, chlordane is persistent relative to other organochlorine pesticides in soil.

Stability to chemical, physical, and biological degradation is one of the prerequisites for a chemical to be available for uptake by organisms (bioaccumulation) and accumulated and transferred up food chains (biomagnification). There are several reports in the literature indicating that chlordane is accumulated, but no studies were found documenting biomagnification of this pesticide. Godsil and Johnson (40) found that DDT, chlordane and endrin, when applied seasonally during the summer growing season, would not accumulate in the aquatic food chain over successive years, but rather would decrease during winter to the limits of detection in both water and the biota. During the growing season, however, when the water contained up to 0.100 µg/l chlordane, algae (Cladophora sp.) were found to contain up to 50 ppb chlordane; vascular plants (Myriophyllum sp. and Potamogeton sp.), to 67 ppb; chubs (90% Siphateles bicolor and 10% S. gila), to 24 ppb; and clams (Gonidea sp.), to 12.0 ppb. Four different groups of largemouth bass and two groups of clams were also held for varying periods in cages in the same stream in which the natural populations of plants and animals were sampled. Largemouth bass accumulated from 8 to 43 ppb chlordane in less than 120 days, whereas accumulation by clams was 2 to 25 ppb (40). In a far more extensive pesticide monitoring program, Henderson, Johnson and Inglis (41) collected and analyzed 62 species of fish from the Great Lakes and major U.S. river basins for nine pesticides and their metabolites. Roughly 128 of the 587 composites of fish sampled (21.8%) contained detectable residues of chlordane. Whole body contents ranged to 7.3 ppm. The Gulf Coast fish contained the highest incidence of chlordane (45.8%) and the Columbia River system the least (1.7%). Green et al. (42), in a survey of 109 sites in U.S. rivers, stated that aqueous concentrations of chlordane in situ were only 0.1 µg/l.

Although the oceans can be regarded as an ultimate depository for a proportion of the chlordane applied on the mainland, a recent study by Duke and Wilson (43) on the contents of insecticides in 29 species of marine fish from the West Coast of the United States revealed no detectable residues of chlordane. While this finding might imply that chlordane's stability and susceptibility to biomagnification are less than those of DDT and its metabolites, for which residues up to 1,026 ppb were measured by Duke and Wilson (43), analytical methods for DDT and its metabolites are generally much more sensitive than those for chlordane and may, in fact, obscure chlordane on a chromatogram (personal communication, L. Mueller, EPA, Environmental Research Laboratory--Duluth). Hence, results of pesticide residue surveys in situ may not necessarily reveal the extent of chlordane biomagnification in natural populations of aquatic organisms.

SECTION V

MATERIALS AND METHODS

ACUTE TOXICITY TESTS

Test Species

Fish--The acute lethal toxicity of technical chlordane was examined using brook trout, Salvelinus fontinalis (Mitchill), bluegill, Lepomis macrochirus Rafinesque, and fathead minnow. Yearling brook trout were obtained from the California State Department of Fish and Game, held for 1 year, and tested as adults. Bluegill were obtained as juveniles from a local commercial dealer, while fathead minnow were reared in the laboratory from stock that had been originally obtained from the U.S. Environmental Protection Agency's Environmental Research Laboratory (ERL-D) at Duluth, Minnesota. The ages and sizes of the fish used for testing are given in Table 3.

Invertebrates--The crustacean, Daphnia magna Straus (Branchiopoda, Cladocera), was obtained from ERL-D and cultured in both continuous-flow and static systems. The non-biting midge, Chironomus No. 51[1] (Diptera, Chironomidae), was obtained from the University of California's Department of Entomology at Riverside, and the amphipod or "scud", Hyallela azteca (Saussure), was collected from small, constant temperature (16°C) streams immediately adjacent to the California Department of Fish and Game's Fillmore Hatchery.

Acclimation and Toxicity Testing Conditions

All acute toxicity tests were conducted in accordance with methods recommended by The Committee on Methods for Toxicity Tests with Aquatic Organisms (44) and Sprague (45).

The fish were acclimated to toxicity test conditions for at least 2 months under controlled conditions. Brook trout and bluegill were fed a dry pelleted ration (Moore-Clark Co., Salt Lake City, Utah), the former at 2% of their body weight per day and the latter ad libitum twice daily. Fathead minnow fry were fed a mixture of 50% brine shrimp nauplii, 25% dry trout starter (Moore-Clark), and 25% "TetraMin" (Tetra-Werke, West Germany), ad libitum twice daily. Three days prior to conducting an acute toxicity test, 10 fathead minnow, 10 bluegill, or 5 brook trout were randomly distributed

[1]The species has not been described, but has been classified in the interim as No. 51 by the University.

TABLE 3. CHARACTERISTICS OF FISH EXPOSED TO TECHNICAL CHLORDANE IN ACUTE TOXICITY TESTS

Species	Approximate age at testing, months	Developmental stage	Density in test chamber, g fish/l	Total length mm	Wet body weight g
Brook trout					
Test 1	24	A[a]	32.8	233[b] ±14	131 ±26
Test 2	24	A	43.0	248 ±15	172 ±36
Fathead minnow	3	J[c]	0.11	26.2 ±4.5	0.18 ±0.10
Bluegill	3	J	0.41	50.8 ±6.4	1.85 ±0.75

[a]Adult.

[b]Mean ±1 standard deviation.

[c]Juvenile.

into each of 12 randomly positioned test chambers. The orders of tank placement were established with a random numbers table. The large size of the brook trout necessitated use of smaller sample numbers. Unfortunately, much smaller trout or larger test chambers, though highly desirable, were unavailable at the time these tests had to be carried out. Test chambers were constructed of glass and silicone rubber cement (Dow-Corning). Those utilized for the brook trout and fathead minnow tests measured 30.5 x 30.5 x 30.5 cm, with water depths of 21.5 cm (20 l) and 17.7 cm (16.5 l), respectively. Bluegill were exposed in 91.4 x 30.5 x 30.5 cm glass chambers containing 4.25 l of water at a depth of 15.2 cm. For all three species, 2-l proportional diluters (46) supplied each of the 12 test chambers, which comprised five toxicant concentrations and a control in duplicate. Each diluter supplied sufficient water to replace 10 tank volumes per day, a rate which insured 90% molecular replacement in 6 hr. Toxicant concentrations were successively diluted by a factor of 0.75. A syringe dosing device delivered microliter amounts of the toxicant at each diluter cycle. During the 72-hr acclimation to test conditions and the 96-hr and 192-hr toxicant exposure periods, the fish were not fed. A photoperiod of 16 hr was employed in all experiments. Light intensity from fluorescent lamps (Sylvania "Gro-Lux" and Durotest "Optima") averaged 1010 lux (lu). Black plastic curtains were used to shield the fish from disturbance. Water temperatures in tests with bluegill and fathead minnow were thermostatically regulated at 25°C in air-conditioned rooms. Those with brook trout were similarly controlled at 15°C. Although water flow into the tanks maintained dissolved oxygen concentrations above 70% of air saturation in most tests, artificial aeration was required in those using trout to meet this requirement.

Dead fish were measured for total length to the nearest millimeter and, after excess moisture had been removed with toweling, for wet body weight to the nearest gram or milligram, depending on size. All fish were measured from the six treatments constituting one replicate. The data were later pooled when no size differences between treatments were detected. Length-weight measurements were not taken prior to toxicity testing since it was believed that the stresses associated with handling and anesthesia (47, 48, 49) would have a greater influence on the LC50 than the changes in body weights during the course of the test.

Daphnia magna were cultured with both static and flow-through conditions at room temperature (20° to 21°C). Those held in the static system were fed once daily and those in the flow-through system twice daily. The ration consisted of a blended and sieved (100 mesh) mixture of dried baker's yeast (4.5%), pelleted fish food (91%) and alfalfa (4.5%) in water. Second to third instars were used for testing, and they were not fed during toxicant exposure.

The amphipod, H. azteca, was cultured in a flow-through system at 16°C and fed pre-soaked aspen leaves, supplemented with dry pelleted fish food. Live Myriophyllum sp. was also introduced as a possible food supply and habitat. Juveniles (~ 5 mm in total length) were used for acute toxicity testing and were not fed during the test.

The sensitivity of Chironomus No. 51 to technical chlordane was not evaluated successfully in an acute toxicity test because a satisfactory means of testing was not found. Use of a substrate of fine sand, the preferred habitat of this species in the laboratory, would not permit temporal examination of the status of the test specimens without causing considerable disturbance, whereas preliminary trials indicated that confinement of the larvae in egg incubation cups resulted in a random, anomalous mortality.

Cladocerans and amphipods used for acute toxicity testing were acclimated to test conditions, i.e., photoperiod and temperature, but not to the specific test apparatus prior to introduction into the chambers. Shortly before chlordane exposure, 10 specimens were randomly distributed into each of the randomly positioned test chambers. The test chambers consisted of glass cylinders, 6.5 cm inside diameter (i.d.) and 7.5 cm long, suspended in 30.5 x 30.5 x 30.5 cm glass chambers. Nylon screen ("Nitex", 500 μ openings) was attached to one end of the cylinder with silicone rubber cement (Dow-Corning) to permit circulation and retain the specimens. Each of the large chambers contained two cylinders and was supplied with appropriate test concentrations from a 2-l proportional diluter (46). Chlordane solutions were delivered in microliter amounts to the diluter's mixing (M-1) cell with the syringe dosing device described by Mount and Brungs (46). Toxicant concentrations were successively diluted by 25%. The tests with D. magna lasted 96 hr. The test utilizing H. azteca was extended to 168 hr since mortalities were minimal within 96 hr, even though the highest calculated chlordane concentration tested approximated the solubility limit.

The response criterion of immobilization was satisfied when the specimens lay motionless on the bottom and did not move when gently prodded.

Water Quality

The diluent water for all tests was supplied from local wells and was unchlorinated except for treatment of storage reservoirs for algal control 1 day per week. Total residual chlorine was not detected upon periodic measurement (leucocrystal violet method of APHA [21]), even on the day of chlorination (Friday). As a precautionary measure, however, activated carbon filters were installed on the line supplying the D. magna, Chironomus No. 51, bluegill, and fathead minnow acute and chronic tests. Due to high flow requirements (76,000 l/day) of several brook trout chronics being conducted concurrently, the associated high cost of dechlorination equipment, and the belief that there was a low probability of a chlorine toxicity problem, no activated charcoal filters were installed on this line which supplied all trout as well as the amphipod tests. As will be seen, mortalities believed due to residual chlorine were observed near the end of this research project in approximately 20- to 40-day trout alevins, but not in older or younger trout or in the other species.

Seven water quality variables were monitored routinely during each test: water temperature, dissolved oxygen concentration, pH, total alkalinity, acidity, total hardness, and specific conductance. Except for water temperature, which was recorded continuously, water quality in the test chambers was

measured 24 hr prior to and at least once during chlordane addition for comparison of the effects of the toxicant's presence on water quality, as a check on the water's suitability for uncompromised organism survival, and for estimation of water quality variation. All variables were determined with standard methods (21, 50). Except for rare instances, measurements were made on samples collected less than 4 to 6 hr earlier.

A number of ions were also determined by a commercial laboratory to gain a more complete description of the water's composition. Calcium, magnesium, potassium, sodium, chloride, and sulfate ions were determined every 4 months over 1 yr, ammonia was measured biannually and the other compounds once (Appendix Table 2).

CHRONIC TOXICITY TESTS

Fathead Minnows

The design, apparatus and conditions employed for the chronic toxicity tests using fathead minnows were developed by the U.S. Environmental Protection Agency (51). The basic apparatus consisted of a 2-1 proportional diluter which supplied successively diluted (by a factor of 0.5) concentrations of technical chlordane to twelve 42.5-l (91.4 x 30.5 x 30.5 cm) glass tanks, comprising five toxicant concentrations and a control in duplicate. Daily flow through each chamber averaged six tank volumes, assuring 90% molecular replacement in approximately 9 hr. After spawning commenced, two glass chambers (28.5 x 14 x 15 cm) were placed into one end of each adult tank for rearing the progeny. All three chambers were supplied separately with test water, although effluent from the fry chambers passed directly through screening into the tank containing the adults.

The test was conducted with a photoperiod regulated to produce gonadal recrudescence and senescence at a cycle simulating that existing at Evansville, Indiana. Sunlight was simulated with Durotest "Optima" and Sylvania "Glo-Lux" fluorescent bulbs and the intensity averaged 1010 lu.

The water temperature was 21°C upon initiation of the chronic test, but was raised within the first 8 weeks to 25°C and maintained at that temperature thereafter.

At the beginning of the chronic toxicity test, 5-day-old fathead minnow fry were randomly distributed into one fry chamber (50 specimens each) in each of the 12 adult chambers. The fish were initially fed Oregon Moist Pellet starter mash (Moore-Clark) and "TetraMin" tablets crushed to a fine powder. Since growth and survival were poor, the diet was replaced after 2 months with one of frozen brine shrimp nauplii. As the fish grew, they were fed increasing proportions of frozen adult brine shrimp and dry trout pellet (0.047 mm dia.).

Reduction in the density of f_0-generation test specimens, "thinning", was not undertaken after the recommended 60-days' toxicant exposure because of appreciable mortality in all test chambers. Rather, excess fish were

removed after 5.5-months' chlordane exposure, just prior to anticipated spawning. Concurrently, five spawning substrates, consisting of halved 10 cm i.d., red-clay channel pipe were placed into each adult chamber. Spawning commenced within 24 hr, indicating that the substrates could have been introduced earlier.

From each spawning, all embryos were counted and one to several groups of 50 eggs incubated to determine hatching success. When there were fewer than 50 embryos per spawn or when embryos were spawned on weekends, they were only counted. Incubation cups consisted of polypropylene pipe, 7 cm long and 5 cm i.d., covered at one end with "Nitex" screen (500 μ openings). The cups were oscillated continuously with a rocker-arm assembly (52). At hatching, the numbers of normal, abnormal (i.e. having vertebral abnormalities or otherwise abnormal morphology or behavior), and dead fry were counted. Each rearing chamber was stocked with 50 fry from concurrent hatches, and the f_1-generation progeny reared. After 30 days' growth, fry were captured and photographed using the method of McKim and Benoit (53) for measurement of total length. After 60 days all fry were sacrificed and measured to the nearest millimeter for total length and weighed to the nearest milligram after removal of external moisture with toweling. In the first photographic measurement, 20.3 x 25.4 cm black and white prints were made. Thereafter, slides were made of the negatives and projected onto a screen for measurement of lengths. This was believed to have enhanced measurement accuracy, owing to the larger image, and was less costly.

The chronic toxicity test utilizing fathead minnows was terminated after all adults had completed spawning and all fry had been reared for 60 days. Adults were weighed, measured, sexed, and examined for general condition.

Bluegill

The partial chronic toxicity test utilizing bluegill was conducted with a method recommended by the EPA (54). Since the experiment was not begun with embryos or fry, but with yearlings, the test constituted a partial rather than full chronic because it did not encompass one complete generation. The experimental apparatus consisted of a 2-l proportional diluter and 12, randomly positioned, 91.4 x 61 x 38 cm tanks containing 178 l of water at a depth of 32 cm. The tanks were illuminated at an average intensity of 1010 lu by two fluorescent lamps (Durotest "Optima" and Sylvania "Gro-Lux"). Photoperiod was regulated to simulate that existing at Evansville, Indiana. The proportional diluter delivered 10 tank volumes per day to each chamber, assuring 90% molecular replacement in about 5 hr.

At the beginning of the test (5 December 1972), juvenile bluegill, obtained from a commercial fish breeder, were anesthetized with ethyl m-aminobenzoate methanesulfonic acid salt (tricaine methanesulfonate), weighed, measured for total length, and 20 specimens randomly distributed into each of the 12 chambers. They were fed twice daily *ad libitum* a dry pelleted ration (Moore-Clark). After 3, 5, and 9.5 months' toxicant exposure, all surviving fish were captured, anesthetized, weighed, and measured for length to

determine relative growth.

At 5 months the density of fish in each tank was reduced to three males and seven females in anticipation of spawning. Those which appeared to be sexually immature were discarded.

After 6 months' exposure (6 June 1973) bluegill commenced spawning. The initial spawning occurred 1 month after two 30.5 x 30.5 cm gravel-cement spawning substrates had been placed into each of the 12 chambers. Each substrate had a 24-cm oval depression 4 cm in depth. Generally, fish spawned in the depression, but eggs also tended to be deposited in adjacent areas of the tank, due in part to turbulence produced by the spawning activity. All substrates were checked daily for spawns. Embryos were brushed from the substrate and a number taken for determination of percentage hatch and for the growth and survival studies. The remainder were preserved in 5% formalin for later estimation of spawn size. From each spawn, groups of 100 embryos were incubated in a manner similar to that for fathead minnows, except that 10-l glass rearing chambers (30.5 x 30.5 x 15 cm), which were situated downstream from the 178-l tanks and received test water directly from them (six tank turnovers per day), were used for incubating and rearing the progeny. Substantial embryo mortality consistently occurred as a result of the fungus, Saprolegnia sp., spreading from dead to contiguous living eggs. Although efforts were directed toward obtaining only live embryos for incubation, the substantial amounts of organic debris associated with the adhesive embryos made it difficult to obtain only live embryos or separate dead embryos from within masses of living ones. Treatment of the embryos with 4 mg/l zinc-free malachite green, the level prescribed by Smith (55) for treating flagfish (Jordanella floridae) embryos and found by us to be of some value for treating fathead minnows, was abandoned after initial 5 min baths caused substantial mortality.

Upon hatching, the proportions of normal and abnormal fry were determined and all normal fry reared. Abnormal fry were segregated visually on the basis of physical (e.g. vertebral) defects and erratic behavior. Fry were initially fed blended, cooked chicken egg yolk and "green" water (composed of unicellular chlorophyte algae, protozoans, and copepods) until they became large enough to consume frozen brine shrimp nauplii and "Tetra-Min" tablets crushed to a fine powder. Fry larger than approximately 15 mm in length were offered frozen adult brine shrimp. Acceptance of the dry diet may have been limited. At 30 and 60 days, fry were transferred into a 30.5 x 30.5 cm glass chamber filled with 1.3 cm water and photographed (53). Total lengths of the fry were determined with reference to a metered grid from black and white negatives projected from slides. After 90 days, surviving fry were measured manually for total length and for wet body weight. After the adults had completed spawning and the fry reared for 90 days, the chronic toxicity test was terminated. Adults were weighed, measured for total length, sexed, and examined for general condition.

Brook Trout

The partial chronic toxicity test using yearling brook trout was begun on 29 March 1973 using procedures and conditions recommended by the U.S. Environmental Protection Agency (56). Just prior to their introduction, the brook trout were anesthetized, measured for total length and weighed. Twelve fish were placed randomly into each of the randomly positioned chambers.

The toxicity testing apparatus consisted of a 4-l proportional diluter and twelve 91.4 x 61 x 38.1 cm glass chambers containing 178 l of water at a depth of 32 cm. The diluter delivered approximately nine tank volumes per day (1602 l), assuring 90% molecular replacement in 5.5 hr. Technical chlordane concentrations were successively diluted by a factor of 0.5. Each tank was covered with screening to retain the fish, and pairs of tanks were illuminated with fluorescent lamps (Durotest "Optima" and Sylvania "Gro-Lux") at an intensity of 1010 lu. The photoperiod simulated that existing at Evansville, Indiana, and the temperature followed the cycle recommended by EPA (56). When the numbers of trout in each tank were reduced in anticipation of spawning, black plastic was used to cover the sides and tops of the tanks in an effort to minimize antagonistic behavior between males in adjacent tanks and provide a more secluded environment for spawning.

Shortly after the test was begun, antagonistic behavior between the fish was observed. Hierarchies were eventually established, but not without considerable fighting. Consequently, up to two fish in each tank developed Saprolegnia sp. infestations. Initially, all tanks were treated for 1 min with 67 mg/l zinc-free malachite green and the fungoused areas of the fish painted with a 200 mg/l solution of the compound (57). Both methods were unsuccessful, and the fish perished. After testing different concentrations of malachite green, a level of 0.2 mg/l, employed as a "flush" treatment, was found to be successful in preventing outbreaks of the fungus, but not in arresting an advanced infestation. These treatments were employed once daily for 2 weeks after the fish were handled (i.e. after measurement of growth at 3, 6.5, and 12 months) and during spawning. Several fish also developed what appeared to be bacterial hemorrhagic septicemia subsequent to their initial introduction and after assessment of growth at 3 months. This was successfully treated by incorporating oxytetracycline ("TM-50", Pfizer) into the feed to give a concentration of 0.44% active ingredient or 75 mg A.I./kg/fish/day. Use of oxytetracycline at this level cured some fish and prevented further outbreaks of the disease.

During the test, brook trout were fed a dry pelleted ration (Moore-Clark) at a rate of 2% of their body weight per day. At 3, 6.5 and 12 months, the trout were measured for length and weighed to determine relative growth. After 6.5 months, the numbers of fish residing in each tank were reduced to three males and four females in anticipation of spawning. Since only two of the fish held in 5.8 µg/l chlordane remained, excess control specimens were transferred to one of the 5.8 µg/l tanks. Upon reduction in specimen density, two glass spawning chambers, 30.5 x 25.4 x 10.2 cm, were placed into each tank. The chambers, described by Benoit (58), were designed to simulate a redd.

Brook trout began spawning on 28 December 1974, 8 months after introduction and 1.5 months after "thinning". Each day the number of spawns and embryos per spawn were recorded, and embryos from selected spawns placed into an incubation apparatus (52) for determination of hatching success or viability (development of a neural keel after 12 days). Black plastic was used to shield the developing embryos from light. Viability determinations were usually made on every spawn totaling 20 embryos or more. Determinations of hatching success, which utilized 50 embryos collected from a single spawning, were spread essentially equally throughout the spawning period. Up to eight such determinations were made from the spawns from each of the 12 adult tanks (16 determinations per treatment). Data collected in the hatch study included times to hatching of 50% of the alevins, percentages of normal, abnormal and dead alevins, and total lengths and weights of the alevins. Lengths and weights were determined only for the alevins discarded at hatching.

To assess the effects of chlordane on growth and survival of the progeny, groups of 25 alevins each were reared for 90 days in each of the treatments. Up to four groups of alevins were ultimately used per concentration. These studies were conducted in 37.5 x 18 x 13 cm glass chambers (10 cm depth) which were separated from the adult tanks, received water directly from the diluter, and were covered with screening to retain the fish.

Fry were fed Oregon Moist Pellet trout starter (Moore-Clark) until they were old enough to consume adult brine shrimp and dry pellets. After 30 days' growth, fry were measured for total length using the photographic method (53). At 90 days, fry were measured, weighed and killed.

When the adults from all chambers had not spawned for 2 weeks, they were killed, weighed, measured for total length, and examined for general condition.

Hyallela azteca

The chronic toxicity test utilizing H. azteca was conducted according to a procedure of EPA (59) and a system consisting of a 2-l proportional diluter and twelve 17.5 x 20.5 x 25 cm glass tanks. Each tank contained 8.3 l of test solution at a depth of 23 cm and was immersed in a water bath to minimize fluctuations in water temperature. The diluter replaced four tank volumes (i.e., 33 l) in 24 hr, a rate equivalent to 90% molecular replacement in approximately 15 hr.

On 26 March 1974, 25 newly hatched H. azteca were introduced into each of the twelve chambers comprising five chlordane concentrations and a control in duplicate. The photoperiod was held at a constant 16-hr light and the water temperature at 17°C. Aspen leaves (Populus sp.), soaked in water for 30 to 60 days prior to feeding, and live Myriophyllum sp. were introduced as food and habitat. Small (2 mm) pellets of fish food (Moore-Clark) were introduced periodically to supplement their diet. The value of the plant and fish pellet as food for this amphipod species was unknown, but both were included because specimens in stock cultures appeared to consume them. The

aspen leaves were definitely of dietary importance.

Amphipods were reared under the above conditions for the 65 days of toxicant exposure. Due to time constraints, the test could not be extended through reproduction, although copulating pairs and ovigerous females were observed at termination. At the end of the test, the contents of the test chambers were successively passed through 8, 24, and 100 mesh stainless steel screens (W.S. Tyler Co.) to isolate the amphipods. They were then placed on tissue paper to remove excess moisture and individually weighed on an analytical balance. All specimens from each chamber were then dried to constant weight at 50°C, and the dried specimens analyzed for chlordane residues.

Daphnia Magna

The chronic toxicity test utilizing Daphnia magna was conducted according to a procedure recommended by EPA (60). A 500-ml proportional diluter delivered technical chlordane to a duplicated series of five concentrations and a control. Each glass test chamber measured 28 x 13.5 x 15 cm and contained 5.7 l of test solution. The diluter delivered 3 l to each tank daily, equivalent to replacement of 0.5 tank volumes per day or 90% molecular replacement in approximately 100 hr. Higher flows are believed deleterious to this species (personal communication, K.E. Biesinger, EPA, ERL-D). The diluent was tap water that had been aged at least 2 days in sunlight. Aged rather than ambient tap water was instituted to insure removal of any residual chlorine, which is known to produce effects on D. magna at levels as low as 3 µg/l (61).

The 4-week test was begun by introducing 10 first instars, obtained from adults reared in a continuous-flow system, into each of the 12 chambers. The photoperiod was a constant 16-hr light and the water temperature approximately 21°C. The cladocerans were fed daily the blended and sieved mixture of dried yeast, pelleted fish food, and dried alfalfa grass described earlier. Numbers of surviving f_0-generation daphnids and f_1-generation progeny were counted weekly and the progeny removed. At termination, the progeny produced in the fourth week were removed, composited by treatment, dried to constant weight at 50°C, weighed on an analytical balance, and analyzed for chlordane content.

Chironomus No. 51

The conduct of the chronic toxicity test of the midge, Chironomus No. 51, was similar to that recommended for C. plumosus (62). Chlordane was mixed and apportioned in a 2-l proportional diluter operating at a rate sufficient to replace 11.4 tank volumes per day (90% molecular replacement in approximately 5 hr) in 38 x 13 x 18 cm glass chambers containing 4.2 l of water. Each container was covered with screen to contain the adults upon their emergence. The toxicity test was conducted at a water temperature of 25°C and a photoperiod of 16 hr light. The dimming device to simulate dawn and dusk and induce copulation was not used for several reasons. First, it was believed that evaluation of toxicant effects on reproduction had a low probability of success because the helical arrangement of the embryos in

the skeins prevented their segregation and accurate enumeration. Secondly, absence of dawn and dusk periods was intended to temporarily delay oviposition, which was known to occur regardless of the occurrence of copulation, and allow harvest of the gravid adults. The latter was apparently successful since only one to two spawnings were observed during the course of testing. At the beginning of the test, newly hatched larvae were randomly distributed into each of the test chambers. They were fed "TetraMin" flakes twice weekly. Adults were captured, sexed, and composited for total dry weight measurement. After determination of dry weight by drying to contant weight at 50°C, the adults were analyzed for chlordane residues.

Water Quality Analysis

In each chronic toxicity test, six water quality variables, namely dissolved oxygen concentration, pH, total alkalinity, total hardness, and specific conductance, were monitored in the test chambers every week. Replicates were measured on alternate weeks. Water temperatures were continuously recorded and a representative reading taken daily. Measurement of acidity was instituted toward the middle of the project. In the first chronic toxicity tests (fathead minnow and bluegill), measurements were made in each of the six tanks comprising a replicate. Since variation in water quality between treatments was small and was not altered by the presence of chlordane, the carrier, or Triton X-100, water quality analyses in the trout, cladoceran, amphipod, and chironomid tests were confined to the control, mid-range, and high concentrations. Methods of analysis were the same as those described for the acute toxicity tests.

ANALYSIS OF TECHNICAL CHLORDANE

Stock solutions of technical chlordane were prepared by dissolving the insecticide in double distilled, pesticide-free acetone containing a small amount of the nonionic surfactant, Triton X-100 (Rohm and Haas). The surfactant was intended to enhance the rate of chlordane dissolution in the proportional diluter and decrease the difference between desired and measured insecticide concentrations. The expected concentrations of Triton X-100 were half the nominal level of technical chlordane for both acute and chronic experiments. Thus, for example, in the chronic tests with the three fish species, where desired chlordane concentrations were 0.625, 1.25, 2.5, 5.0 and 10.0 µg/l, the nominal concentrations of the surfactant were 0.3, 0.6, 1.3, 2.5 and 5.0 µg/l, respectively. Although solvent controls were not employed in the chronic tests, the acute and chronic effects of Triton X-100 on brook trout and fathead minnow have been examined in a separate program (63), and concentrations having no chronic effect were uniformly above 100 µg/l.

All water and invertebrate tissue samples were analyzed for technical chlordane with a gas chromatograph equipped with a ^{63}Ni electron capture detector (Model 990, Perkin-Elmer Corp.). The 183 cm long, 0.64 cm o.d. glass column was packed with 2% SE-30 on 100 - 120 mesh "Gas Chrom Q". The carrier gas was nitrogen, and the flow rate was 60 ml/min. Oven temperatures varied depending upon the type of sample being analyzed.

For measurement of technical chlordane in water, 10.0 to 20.0 ml water samples were extracted once for 1 min with 2.0 to 5.0 ml portions of pesticide-grade n-hexane. The larger sample volumes were applied to determinations made in the chronic toxicity tests, whereas smaller sample volumes sufficed for measurements made in acute toxicity tests. One to 10 microliters of sample extract were injected into the chromatograph. The chromatograms were measured for peak area with a planimeter and compared to technical chlordane standards. Separation of the chlordane constituents was performed with isothermal conditions (200°C) and detector, manifold, and injector temperatures of 240°C.

The accuracy and reproducibility of the method were checked by spiking technical chlordane into laboratory water. Samples containing technical chlordane concentrations of 10 µg/l were analyzed with a recovery of 100.2 ± 5.2%, while concentrations as low as 0.5 µg/l were recovered to the extent of 87.2 ± 11.0%. The coefficients of variation for the 0.5 and 10.0 µg/l concentrations were 12.6 and 5.2%, respectively.

Analyses of the contents of the various technical chlordane constituents in the three fish species were considered unreliable due to several technical errors which were not identified and corrected in time to have the analyses repeated. These problems were corrected prior to analyses of technical chlordane residues in the three invertebrate species.

Whole body extracts of dried samples of the invertebrates were obtained by grinding the specimens in a tissue grinder with pesticide residue-free petroleum ether (30° to 75°C). One to 10 microliter volumes of the extract, which was used without further manipulation, were separated on the gas chromatograph using a 160° to 240°C temperature program (6°C/min) and the operating conditions described for chlordane analysis in water. No major interferences were detected in the chromatograms; heptachlor, the chlordenes, cis-chlordane, trans-chlordane, cis-nonachlor, and trans-nonachlor were selected for quantitation.

STATISTICAL ANALYSIS

Acute Toxicity Tests

Concentration-percent mortality data were analyzed with logarithmic-probability (log-probit) methods using either the manual procedure of Litchfield and Wilcoxon (64) or the computer program of Dixon (65). Computer processing was accomplished using IBM 360/75 hardware. The log-probit method was selected because it is a more objective approach than the graphical interpolation method, offers a test of the regression line's goodness-of-fit, and provides the statistics necessary for calculating 95% confidence limits for median lethal concentrations (LC50) and for comparing differences between two LC50 values.

For homogeneous data, upper and lower 95% confidence limits for LC50 value were calculated as $(LC50)(f)$ and $LC50/f$, respectively, where f is the antilogarithm of $1.96\ \hat{\sigma}\ (N'/2)^{-1/2}$, $\hat{\sigma}$ is the standard deviation of the

logarithm of the population tolerance frequency distribution, and N' is the number of test animals expected to have perished within the percent mortality interval of 16 to 84% (64). An equivalent equation is $\pm$ 1.96 times the standard error of the log LC50 (66). For heterogeneous data, i.e. where chi-square analysis of the fitted line indicated lack of goodness-of-fit, the following equation was used: $f = (\text{Student's t-value})\ (\sigma)\ (\chi^2/n)^{1/2}\ (N'/2^{-1/2}$, where n is the number of concentrations used in calculating the LC50 (66). The logarithms of the median lethal concentrations were plotted against the logarithms of the exposure times to give toxicity curves (45) for the fish species and D. magna.

The accuracy of the LC50 estimates and their 95% confidence limits, generated by the Litchfield and Wilcoxon (64) and computer program (65) methods, were compared with similar calculations made by eight other aquatic toxicology laboratories using standard concentration-percent response data (Appendix Table 3) supplied by The Committee on Methods for Toxicity Tests with Aquatic Organisms. Our LC50 estimates were in agreement with the average LC50 computed by the other laboratories (Appendix Table 4) using both methods of analysis, but the 95% confidence limits were usually narrower with the computer method than with that of Litchfield and Wilcoxon (64).

Median lethal times (LT50) for measured toxicant concentrations were calculated in some cases. The LT50 is the time required for 50% of the test specimens to die in a given concentration. Data were analyzed in the same manner as for the calculation of LC50 values and were plotted on the same toxicity curve, with the exception that 95% confidence limits were determined for the independent variable, time, rather than the LC50.

Control mortality occurred in less than 5% of the toxicity tests and was less than 10% in all cases. Median response estimates were corrected for any control mortality with the computer program method.

Several statistical comparisons were made to determine the significance of differences between the 96-hr LC50 values of the different species using data generated by probit analysis. The equation, Student's $t = (\hat{\mu}_1 = \hat{\mu}_2)\ (\hat{\sigma}_1^2/N_1' /2 + \hat{\sigma}_2^2/N_2' /2)^{-1/2}$, was used to test significance. The symbol $\hat{\mu}$ denotes an LC50 and subscripts pertain to the particular LC50 values being compared. The degrees of freedom were $n_1 + n_2 - 2$, where n is the total number of test specimens employed to derive an LC50 estimate.

Chronic Toxicity Tests

Analysis of variance with a one-way design was used exclusively in evaluating the significance of differences between treatments. Dunnett's test (67) was used to determine whether controls were different from each of the other treatments. Log-probit analysis was used to determine median emergence times of adult chironomids in the chronic tests of that species.

SECTION VI

RESULTS

ACUTE TOXICITY TESTS

Water Quality and Toxicant Concentrations

Water quality during the various acute toxicity tests is summarized in Table 4. Water temperatures were 25°C in tests of fathead minnow and bluegill, 15°C in the tests of brook trout and H. azteca, and 21°C in that of D. magna. Dissolved oxygen concentrations were greater than 70% in all toxicity tests except the second one employing brook trout where it was 62% of air saturation. The diluent water was moderately alkaline and of intermediate hardness.

Concentrations of technical chlordane were determined from two to five times during each test, depending upon exposure time, and are summarized in Table 5.

Toxicity

The order of decreasing species sensitivity to acutely lethal concentrations of technical chlordane was D. magna, fathead minnow, brook trout, bluegill, and H. azteca. Median effective concentrations ranged from 96-hr values of 28.4 and 35.2 µg/l for D. magna to a 168-hr value of 97.1 µg/l for H. azteca. Too few H. azteca had perished at 96 hr to permit an EC50 estimate for this period of exposure (Appendix Table 5). The 96-hr LC50's were 36.9 µg/l for fathead minnow, 45 µg/l for brook trout, and 59 µg/l for bluegill (Appendix Tables 6, 7, and 8).

Comparisons were made to determine the significance of differences between species in terms of their respective 96-hr EC50 and LC50 values. As shown in Appendix Table 9, the only statistically significant differences were between D. magna and bluegill and brook trout ($p < 0.001$). None of the fish species were significantly different in sensitivity.

Chlordane exposures were sufficiently long (up to 192 hr) in the acute toxicity tests to allow partial delineation of toxicity curves (Fig. 2). Linear curves described the toxicity of chlordane to brook trout, bluegill, and possibly to D. magna, whereas a rectangular hyperbola characterized its toxicity to fathead minnows. The 192-hr LC50 of 32 µg/l may be considered an estimate of the median lethal threshold of technical chlordane for fathead minnow--the concentration at which acute lethality to 50% of the test specimens ceases.

TABLE 4. WATER QUALITY DURING ACUTE TOXICITY TESTS OF TECHNICAL CHLORDANE

Species	Water temperature, °C	Dissolved oxygen, mg/l	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/l $CaCO_3$	Acidity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
Fathead minnow	24.8[a] +0.4 (23)	6.5 +0.3 (33)	77.1 +3.3 (33)	7.70 +0.14 (28)	169 +1 (28)	...[b]	152 +1 (28)	370[c] +24 (5)
Bluegill	25.3 +0.2 (24)	6.4 +0.3 (14)	76.5 +3.7 (14)	7.63 +0.10 (12)	160 +2 (11)	...	161 +1 (11)	393 +4 (2)
Brook trout								
Test 1	14.7 +0.6 (24)	7.5 ±1.5 (9)	73.2 ±14.9 (9)	7.71 ±0.41 (9)	155 +9 (9)	11.6 ±2.8 (9)	149 ±13 (9)	393 ±26 (3)
Test 2	15.2 +0.5 (15)	6.6 +1.5 (10)	61.7 ±16.8 (10)	7.70 +0.14 (10)	155 +4 (10)	6.5 +1.8 (10)	135 +2 (10)	362 +4 (3)
Daphnia magna								
Test 1	20.9 +0.3 (6)	6.8 +0.1 (6)	78.8 +2.3 (6)	8.07 +0.04 (10)	144 +2 (10)	6.7 +1.7 (10)	143 +3 (10)	372 +0 (3)

Continued ....

TABLE 4. WATER QUALITY DURING ACUTE TOXICITY TESTS OF TECHNICAL CHLORDANE--continued

Species	Water temperature, °C	Dissolved oxygen, mg/l	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/l $CaCO_3$	Acidity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
Daphnia magna								
Test 2	20.8 ±0.5 (9)	6.6 ±0.6 (6)	72.9 ±6.6 (6)	8.04 ±0.09 (6)	149 ±3 (6)	5.4 ±2.2 (6)	154 ±8 (6)	394 ±9 (2)
Hyallela azteca	15.5 ±0.5 (8)	7.2 ±0.3 (7)	71.4 ±3.3 (7)	7.85 ±0.18 (13)	156 ±4 (13)	6.0 ±3.0 (13)	144 ±2 (13)	363 ±7 (4)

[a]Means ±1 standard deviation, and number of measurements made per parameter.

[b]No observation.

[c]Specific conductance measurements were composites taken at each sampling time.

TABLE 5. MEASURED CONCENTRATIONS OF TECHNICAL CHLORDANE IN ACUTE TOXICITY TESTS

Test species	No measurements per test	Chlordane concentration, µg/l					
		Tank I	Tank II	Tank III	Tank IV	Tank V	Tank VI
Fathead minnow	3	N.D.[a]	12.3 ±1.1	20.0 ±6.5	28.4 ±14.5	34.1 ±9.4	53.4 ±10.0
Bluegill	4	0.06 ±0.3	12.8 ±3.1	39.2 ±7.2	59.1 ±3.4	81.5 ±0.1	104.3 ±9.8
Brook trout							
Test 1	5	N.D.	12.4 ±3.6	17.8 ±10.2	20.0 ±7.2	34.2 ±8.2	41.0 ±15.2
Test 2	2	N.D.	21.0 ±0.6	37.2 ±3.5	52.9 ±34.8	117 ±63	125 ±104
Daphnia magna							
Test 1	3	N.D.	10.4 ±5.0	16.5 ±2.4	21.8 ±2.6	28.4 ±6.2	33.9 ±3.7
Test 2	3	N.D.	10.4 ±4.2	14.4 ±5.2	20.8 ±7.6	28.3 ±11.5	42.8 ±16.1
Hyallela azteca	4	N.D.	35.3 ±12.3	66.7 ±10.3	83.7 ±11.9	115.2 ±13.5	161.3 ±15.3

[a]No technical chlordane detected.

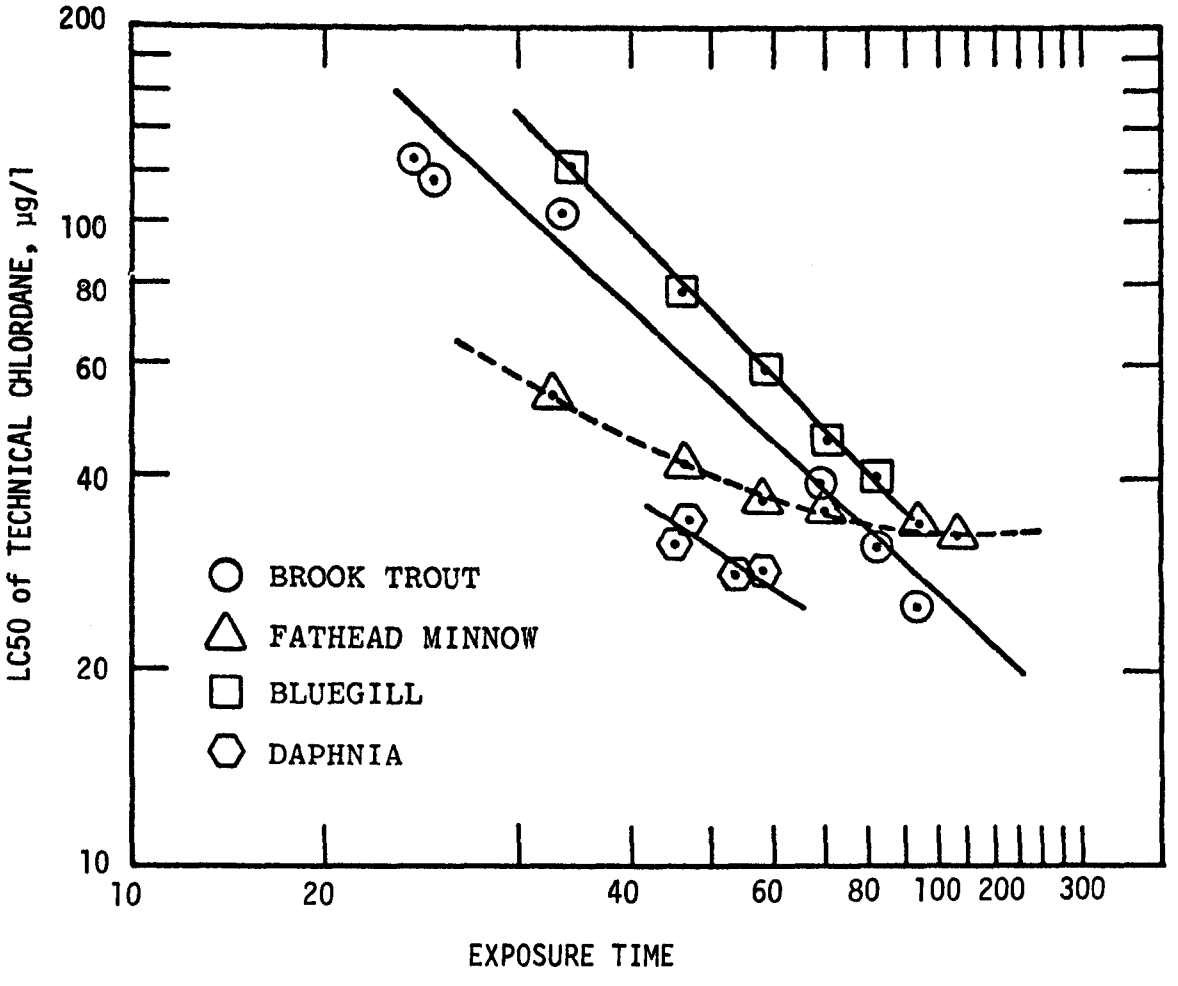


Fig. 2. Toxicity curves for three fish species and Daphnia magna exposed to technical chlordane.

CHRONIC TOXICITY TO FATHEAD MINNOWS

Water Quality and Chlordane Concentrations

Water quality during the chronic toxicity test is summarized in Appendix Table 10. Since the concentrations of chlordane, acetone, and Triton X-100 had no apparent influence on the water quality parameters measured, the data for each week were pooled and mean values reported. Water temperatures were approximately 21°C upon initiation of toxicant exposure, but were raised to 25°C during a 6-week interval and maintained within a degree of that temperature for the duration of the experiment. Problems with the temperature control apparatus prevented starting the test at 25°C. Dissolved oxygen concentrations were maintained at greater than 60% of air saturation without artificial aeration. Alkalinity, pH, and hardness levels of the ambient water were very uniform.

Concentrations of technical chlordane, measured in the six tanks comprising each replicate, are summarized in Appendix Table 11. In general, measured concentrations were 55% of desired. Although the reasons for the loss were not ascertained, they could have been due to chlordane adsorption to the surfaces of the test apparatus, to organic material, or because of assimilation by the test organisms and epiphytes. Chlordane analysis of hexane extracts of scrapings of algal-bacterial mats from the walls of the tubes running between the diluter and the test chambers indicated that substantial amounts of the pesticide were associated with these organisms. Measured aqueous concentrations of technical chlordane averaged 0.36 $\pm$ 0.16, 0.75 $\pm$ 0.25, 1.38 $\pm$ 0.57, 2.78 $\pm$ 1.06, and 6.03 $\pm$ 2.25 µg/l. Traces of chlordane were sometimes detected in control chambers.

Chronic Effects of Chlordane on Survival, Growth, and Reproduction

Under the aforementioned experimental conditions, fathead minnows were cultured through one generation. Growth and survival of fathead minnows introduced as 1- to 5-day-old fry (termed F_0-generation) was very poor, apparently because the food ("TetraMin") was not small enough for all the fry and possibly not of adequate nutritive value. Regardless of the quality of the diet, chlordane may have slightly retarded growth of fry exposed for the first 30 days to concentrations at and above 2.78 µg/l (Table 6). Analysis of variance of the growth data did not indicate that there were any statistically significant differences between the controls and the treatment groups at the 95% level of confidence. After 60 days, there were no apparent differences in growth, leading to the conclusion that the insecticide had no significant adverse effect at the concentrations employed.

At the end of the chronic test, minnows reared in 6.03 µg/l chlordane were significantly larger ($p < 0.01$) than controls (Table 7). However, the importance of this difference is suspect because only eight individuals remained in the high concentration at termination relative to 20 to 30 fish in each of the other treatments.

TABLE 6. TOTAL LENGTHS OF FATHEAD MINNOW FRY CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	Total length, mm			
	30 days		60 days	
	F_0-gen.[a]	F_1-gen.[b]	F_0-gen.	F_1-gen.
Control	9.7[c] ±2.6	13.3 ±2.4	12.3 ±2.8	21.4 ±4.5
Control	8.4 ±1.7	12.1 ±1.9	10.5 ±2.8	17.7 ±3.2
0.36	8.0 ±1.2	11.4 ±1.8	12.9 ±2.7	19.1 ±4.3
0.36	8.8 ±2.1	12.5 ±2.4	12.8 ±4.3	19.4 ±4.4
0.75	8.4 ±1.4	11.1 ±1.9	12.2 ±5.2	20.3 ±3.2
0.75	8.5 ±0.8	12.7 ±2.4	12.5 ±3.7	18.7 ±3.9
1.38	8.3 ±2.1	8.6 ±1.5	12.0 ±4.2	18.2 ±3.7
1.38	8.3 ±2.2	10.3 ±1.7	12.0 ±3.6	20.5 ±4.2
2.78	7.4 ±1.2	12.0 ±2.1	10.7 ±3.3	19.1 ±5.0
2.78	7.6 ±1.1	11.8 ±2.6	12.2 ±4.7	19.3 ±4.6
6.03	6.9 ±0.9	11.3 ±2.6	9.5 ±2.0	19.8 ±5.5
6.03	7.4 ±1.5	11.4 ±1.7	12.5 ±3.4	21.4 ±4.4

[a] F_0-generation constitutes 1- to 5-day-old fry from parents having no known previous history of chlordane exposure.

[b] F_1-generation represents progeny spawned by adults having 6 months' chlordane exposure.

[c] Mean ±1 standard deviation.

TABLE 7. LENGTHS AND WEIGHTS OF ADULT FATHEAD MINNOWS AT TERMINATION OF CHRONIC EXPOSURE TO TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	No. fish	Total length, mm	Wet body weight, g
Control	14	46[a] ±5	1.2 ±0.5
Control	15	49 ±5	1.5 ±0.7
0.36	15	47 ±5	1.0 ±0.6
0.36	15	53 ±5	1.7 ±0.6
0.75	13	50 ±8	1.3 ±0.7
0.75	13	53 ±6	1.4 ±0.7
1.38	15	48 ±6	1.1 ±0.5
1.38	17	48 ±7	1.3 ±0.7
2.78	8	54 ±8	1.7 ±0.7
2.78	13	50 ±6	1.3 ±0.7
6.03	3	59 ±6	1.9 ±0.9
6.03	5	56 ±4	1.7 ±0.4

[a]Mean ±1 standard deviation. With the exception of one of the controls, fish which died during spawning are included in the summaries.

Mortality of minnows was extensive during the first month, particularly in the first 2 weeks, and was presumed to be due to an inadequate diet. Although this was later corrected with a better diet, the poor early survival of the f_0-generation fry tended to obscure the relationship between chlordane concentration and survival for the first 8 weeks of the test (Table 8). Subsequent mortality, i.e. from 8 weeks up to the time of spawning at 24 weeks, was negligible, with fewer than three fish dying in any treatment. Mortality during spawning was extensive, particularly in fish exposed to chlordane concentrations greater than 0.75 µg/l. Although variation in mortality between treatments was considerable, it appears that chlordane posed an additional stress on the fish at a time when they were naturally stressed.

Details of spawning activity, embryo production, and hatching of fry are given in Table 9. In general chlordane had no effect on the number of spawnings, embryos produced per female, or the percentages of normal, abnormal, or dead fry observed at hatching. Problems were regularly encountered with fungus on incubating embryos. In the majority of cases, S. parasitica on dead embryos spread to adjacent living ones despite daily immersion for 5 min in a 4 mg/l solution of zinc-free malachite green. This problem occurred in all treatments including controls.

Subsequent growth of the f_1-generation fry is tabulated in Tables 6 and 10. As also observed for f_0-generation fry, the second generation progeny were slighly smaller after 30 days in 6.03 µg/l chlordane than the controls, although differences were not statistically significant. After 60 days, there were no significant differences in size between fish in the five chlordane concentrations and the control.

Mean mortality of the f_1-generation through the first 60 days ranged from 15 to 40% and was not increased by any of the chlordane concentrations used (Table 10).

Statistical analysis of the results indicated that none of the concentrations employed had any significant deleterious effects on any of the life cycle stages of the fathead minnow. On the other hand, one apparent effect from several of the chlordane concentrations was suggested, in that concentrations greater than 0.75 µg/l caused increased mortality of adult minnows during the period of spawning. Although this result is conceivable since adults are already stressed and incur mortality naturally during spawning, its significance can be challenged because of the high fry mortality that occurred in all treatments during the first 2 months of the experiment. Without repeating the test, the question of whether these fish were already in a weakened condition will remain.

TABLE 8. MORTALITY OF F_0-GENERATION FATHEAD MINNOWS DURING CHRONIC EXPOSURE TO CHLORDANE

Meas. chlordane conc., µg/l	Cumulative percent mortality[a]				% mortality during spawning[c]
	4 weeks	8 weeks	12 weeks	24 weeks[b]	
Control	82	86	86	86	6.6
Control	80	82	82	82	0
0.36	72	78	78	78	13.3
0.36	44	50	50	52	26.7
0.75	56	70	72	72	23.1
0.75	70	72	72	72	0
1.38	50	64	68	68	40.0
1.38	56	64	64	66	93.3
2.78	70	80	80	82	75.0
2.78	68	70	74	76	15.4
6.03	78	94	94	94	33.3
6.03	66	90	90	90	20.0

[a]Based on a 50 fish per concentration.

[b]Fish "thinned" at this time, which was just prior to the commencement of spawning. Excess males were removed and minnows of equivalent age from the culture facility were used to bring the densities in the control tanks to 15 fish each.

[c]Based on 15 fish in each of the control and 0.36 µg/l concentrations, and 26 fish (13 and 13) in 0.75 µg/l, 32 fish (15 and 17) in 1.38 µg/l, 21 fish (8 and 13) in 2.78 µg/l, and 8 fish (3 and 5) in 6.03 µg/l chlordane at the beginning of spawning.

TABLE 9. SPAWNING HISTORY OF FATHEAD MINNOWS CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

Parameter	Measured concentration of chlordane, µg/l											
	Control	Control	0.36	0.36	0.75	0.75	1.38	1.38	2.78[a]	2.78	6.03	6.03
No. females	9	7	11	6	8	8	12	7	3	7	1	2
No. of spawnings	30	16	25	12	16	10	23	14	2	19	6	10
Avg. No. spawnings/ female	3.33	2.29	2.27	2.00	2.00	1.25	1.92	2.00	0.67	2.71	6.00	5.00
No. eggs	4,960	1,262	3,788	627	1,949	487	2,970	1,550	20	1,926	527	576
Avg. No. eggs/ female	551	180	344	105	244	61	248	221	20	275	527	288
Avg. No. eggs/ spawning	165	79	152	52	122	49	129	111	10	101	88	58
Percent hatch	37.5	27.3	60.1	29.1	72.5	38.2	55.7	40.0	2.8	46.0	44.5	54.9
Percent abnormal fry	1.7	0.8	3.6	1.4	2.0	0.2	2.3	1.2	0	1.2	0.9	2.9
Percent dead fry	3.7	1.8	4.6	6.1	12.2	0.1	3.7	4.9	0	6.7	3.0	5.2

[a]Due to complete mortality of mature males during spawning, males and females co-existed for only the first 3 weeks of spawning.

TABLE 10. MORTALITY AND RELATIVE SIZE OF F_1-GENERATION FATHEAD MINNOWS CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	After 60 days' exposure	
	Mortality, %	Wet body weight, g
Control	40[a] ±14 (3)	0.113[b] ±0.062 (90)
0.36	23 ±19 (4)	0.094 ±0.071 (156)
0.75	15 ±6 (3)	0.096 ±0.047 (128)
1.38	38 ±16 (3)	0.106 ±0.067 (93)
2.78	28 ±17 (4)	0.095 ±0.064 (145)
6.03	28 ±19 (4)	0.117 ±0.078 (144)

[a]Mean ±1 standard deviation and number of groups of 50 fry tested.

[b]Weighted mean ±1 standard deviation and number of individuals from which estimate made.

CHRONIC TOXICITY TO BLUEGILL

Water Quality and Chlordane Concentrations

The results of the water quality monitoring program for the bluegill chronic are summarized in Appendix Table 12. Water temperatures were gradually adjusted from an initial level of 19°C to a temperature of 28°C, which was required for induction of spawning. Thereafter, they were maintained at 28°C for the duration of the experiment. From the inception of testing, on 5 December 1972, until 31 March 1973, no aeration of the test chambers was necessary. But as of the first of April 1973, the percentage saturation of dissolved oxygen had dropped to below the required minimum of 60%, and artificial aeration with oil-free compressed air was instituted. During the experiment, the other variables remained relatively constant.

Concentrations of technical chlordane were measured in each set of six treatments every other week. Since data collected prior to 26 March 1973 were considered unreliable because of analytical problems, they were not included in the summary in Appendix Table 13. Mean measured concentrations ranged from 40 to 52% of desired and averaged 0.25 $\pm$ 0.12, 0.54 $\pm$ 0.21, 1.22 $\pm$ 0.53, 2.20 $\pm$ 0.56 and 5.17 $\pm$ 1.57 µg/l.

Chronic Effects on Survival, Growth and Reproduction

At the beginning of the chronic test (5 December 1972), 20 yearling bluegill averaging 144 mm in total length and 58 g in wet body weight were introduced into each of the 12 test chambers. During the 9.5 months of continuous toxicant exposure, they grew an average of 19% in total length, to 172 mm, and 78% in wet body weight, to 103 g (Table 11). Chlordane did not have any statistically significant effect on growth of f_0-generation bluegills at either 3, 5, or 9.5 months.

Aside from an anomalous mortality totalling 35% in one of the control replicates, which was tentatively assigned to a brief outbreak of bacterial hemorrhagic septicemia, bluegill mortality was largely confined to the 2.20 and 5.17 µg/l concentrations (Table 12). Bluegill began dying in greater numbers from 12 to 22 weeks in the 5.17 µg/l concentration, but experienced highest mortality during the period of spawning (22 - 37 weeks). Mortality of fish exposed to 2.20 µg/l chlordane was also confined largely to the 15 weeks of spawning activity. None of the fish exposed to toxicant concentrations lower than 2.20 µg/l died. None of those which died between 12 and 22 weeks or during spawning showed evidence of erratic behavior or disease. Moribund fish died passively. Bluegill held in the two highest concentrations had greatly diminished appetites relative to the other groups.

Bluegill began spawning on 8 June 1973, 6 months after the beginning of the chronic test. Embryo production was greatest in the control, 0.25 and 0.54 µg/l concentrations, was substantially reduced in the 1.22 µg/l level, and did not occur in the 2.20 and 5.17 µg/l concentrations (Table 13). Hatching success ranged from 25.0 to 70.5% and did not appear to be

TABLE 11. GROWTH OF F_0-GENERATION BLUEGILL DURING CHRONIC EXPOSURE TO TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	Total length, mm				Wet body weight, g			
	0 months	3 months	5 months	9.5 months	0 months	3 months	5 months	9.5 months
Control	143[a] ±17	155 ±22	161 ±19	167 ±19	58 ±27	81 ±33	99 ±31	98 ±38
Control	143 ±16	158 ±18	163 ±19	172 ±24	58 ±23	81 ±32	101 ±36	104 ±50
0.25	142 ±13	154 ±18	161 ±15	173 ±17	53 ±15	75 ±24	94 ±26	105 ±29
0.25	147 ±19	158 ±21	163 ±17	172 ±19	63 ±27	85 ±32	102 ±33	103 ±38
0.54	143 ±15	150 ±17	157 ±14	174 ±14	58 ±22	70 ±24	87 ±25	107 ±30
0.54	141 ±14	153 ±15	159 ±15	168 ±20	53 ±17	75 ±24	96 ±33	102 ±42
1.22	146 ±21	160 ±23	166 ±20	169 ±27	64 ±28	84 ±34	107 ±39	110 ±58

Continued ....

TABLE 11. GROWTH OF F_0-GENERATION BLUEGILL DURING CHRONIC EXPOSURE TO TECHNICAL CHLORDANE--continued

Meas. chlordane conc., µg/l	Total length, mm				Wet body weight, g			
	0 months	3 months	5 months	9.5 months	0 months	3 months	5 months	9.5 months
1.22	146 ±17	159 ±17	160 ±15	172 ±16	59 ±21	78 ±26	92 ±30	106 ±37
2.20	146 ±18	158 ±21	160 ±16	179 ±13	58 ±13	79 ±22	91 ±28	98 ±20
2.20	145 ±17	156 ±21	162 ±19	168 ±22	58 ±25	78 ±34	100 ±38	97 ±52
5.17	143 ±11	153 ±15	160 ±18	178 ± 9	57 ±19	69 ±27	95 ±35	106 ±24
5.17	147 ±20	164 ±24	166 ±23	...[b]	63 ±30	90 ±34	100 ±42	...[b]

[a]Mean ±1 standard deviation.

[b]No fish remaining.

TABLE 12. MORTALITY OF F_0-GENERATION BLUEGILL DURING CHRONIC EXPOSURE TO TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	Cumulative percent mortality[a] 4 weeks	8 weeks	12 weeks	22 weeks	No. fish remaining after thinning	% mortality during spawning, 22-37 weeks
Control	10.0	20.0	35.0	35.0	8	0.0
Control	0.0	0.0	0.0	0.0	10	0.0
0.25	0.0	0.0	0.0	5.0	10	0.0
0.25	0.0	0.0	0.0	0.0	10	0.0
0.54	0.0	0.0	10.0	10.0	10	0.0
0.54	10.0	10.0	10.0	15.0	9	0.0
1.22	0.0	0.0	0.0	0.0	9	0.0
1.22	0.0	0.0	5.0	5.0	10	0.0
2.20	10.0	10.0	10.0	10.0	10	0.0
2.20	0.0	5.0	5.0	10.0	10	30.0
5.17	0.0	10.0	15.0	25.0	8	12.5
5.17	0.0	15.0	35.0	60.0	7	100.0

[a]Based on 20 fish per chamber. At thinning, numbers of fish were reduced to a maximum of 10 per chamber.

TABLE 13. SPAWNING HISTORY OF BLUEGILL CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	No. females	Mean No. spawnings/ female	Mean No. embryos/ female	Mean No. embryos/ spawn	% hatch	% fry	
						Abnormal	Dead
Control[a]	5	0.20	20	100	65.5	1.0	0
Control	7	0.43	2,252	5,255	63.8	1.7	0.8
0.25	6	0.33	400	1,200	51.8	0.6	0.9
0.27	7	0.57	3,559	6,229	32.5	1.1	0
0.54	6	0.67	3,442	1,561	25.0	0	0.2
0.54	6	0.50	2,074	4,148	25.1	0.6	0.3
1.22	6	0.17	263	1,575	45.5	0.7	0.7
1.22	7	0	0	0	...	...	...
2.20	7	0	0	0	54.5[b]	0.5	0
2.20	7	0	0	0	66.5[b]	1.0	0.5
5.17	7	0	0	0	32.5[b]	7.5	7.5
5.17	3	0	0	0	70.5[b]	1.5	0.5

[a]Testes of male fish appeared to be largely immature.

[b]Control eggs incubated.

influenced by the levels of technical chlordane tested. Embryos spawned by control bluegill and transferred to the 2.20 and 5.17 µg/l concentrations hatched in proportions which overlapped those of embryos spawned by fish in the other treatments. However, there were slightly greater proportions of dead and abnormal fry (i.e. erratic swimming behavior or structural defects) at hatch in the 5.17 µg/l concentration.

When it had been determined that all bluegill had completed spawning, they were killed, weighed, measured for total length, and the condition and size of their gonads assessed and measured (Table 14). Although the majority of both sexes had well developed and essentially unspent gonads, those of males in one of the control replicates appeared to be immature and weighed less than those of males from most of the other treatments. Testes of bluegill exposed to 5.17 µg/l were also generally smaller than those of other male fish. Since these fish did not spawn, the effect of successful spawning in reducing testicular mass was not a factor. In contrast to the differences in gonadal development noted for males, females had well-developed ovaries. The ovaries of fish exposed to 2.20 µg/l were somewhat smaller than other groups and these fish did not spawn. These observations suggest that the test conditions were not conducive to spawning or the fish were not old enough. According to reports by others (D. T. Allison, EPA, ERL-D, personal communication), most of the bluegill may have simply been too young.

Few progeny were available for growth and survival studies, and the results are considered inconclusive. Survival data, detailed in Table 15, did not indicate treatment effects. Growth data suggested that control fish grew less rapidly than those in the intermediate concentrations. Only in the 2.20 µg/l treatment was there a suggestion of diminished growth (Table 16). All of the fry reared in 5.17 µg/l chlordane died within 30 days.

In summary the most consequential effect of technical chlordane on bluegill was inhibition of reproduction at the 2.20 and 5.17 µg/l levels. Apparent but non-significant effects were noted on reproduction in the 1.22 µg/l concentration and on hatching success in the lowest concentration tested, 0.25 µg/l. Bluegill did not spawn in one of the 1.22 µg/l replicates and hatching success of fry in chambers receiving chlordane was no greater than 80% that of controls. Thus, although adverse chronic effects are certainly possible at lower concentrations, the 2.20 µg/l was the lowest level at which definite effects were observed.

CHRONIC TOXICITY TO BROOK TROUT

Water Quality and Chlordane Concentrations

During the 13-month duration of the chronic toxicity test utilizing brook trout, there were only small fluctuations in water quality. Dissolved oxygen concentrations averaged at least 60% of air saturation, although artificial aeration was needed to maintain this level after the test had been in progress for 2.5 months (Appendix Table 14).

TABLE 14. CONDITIONS OF ADULT BLUEGILL AT TERMINATION OF CHRONIC TOXICITY TEST

Meas. chlordane conc., µg/l	Males				Females			
	Total length, mm	Wet weight, g	Testes weight, mg	Testes weight, mg/g	Total length, mm	Wet weight, g	Ovary weight, mg	Ovary weight, mg/g
Control	188[a] ±8	139 ±25	349 ±178	2.4 ±0.9	154 ±10	73 ±15	2538 ±848	34.1 ±6.1
Control	202 ±7	169 ±20	2686 ±297	16.0 ±4.5	159 ±15	76 ±22	3609 ±832	51.6 ±19.5
0.25	183 ±14	125 ±33	439 ±214	3.4 ±0.8	166 ±16	86 ±22	3970 ±1605	46.1 ±11.4
0.25	196 ±3	157 ±5	1331 ±219	8.5 ±1.6	162 ±13	80 ±12	3132 ±1458	41.5 ±23.5
0.54	187 ±11	137 ±27	820 ±640	6.2 ±4.4	165 ±4	88 ±10	3920 ±1700	44.4 ±17.3
0.54	192 ±6	155 ±18	933 ±263	6.2 ±2.3	157 ±9	76 ±16	3515 ±1604	46.3 ±17.6
1.22	195 ±5	174 ±9	1924 ±579	15.4 ±7.9	156 ±23	78 ±40	4282 ±4296	47.9 ±23.0

Continued ....

TABLE 14. CONDITIONS OF ADULT BLUEGILL AT TERMINATION OF CHRONIC TOXICITY TEST--continued

Meas. chlordane conc., µg/l	Males				Females			
	Total length, mm	Wet weight, g	Testes weight, mg	Testes weight, mg/g	Total length, mm	Wet weight, g	Ovary weight, mg	Ovary weight, mg/g
1.22	188 ±7	152 ±24	1607 ±854	10.6 ±5.9	165 ±13	86 ±19	4016 ±2259	46.8 ±25.4
2.20	186 ±8	110 ±12	754 ±748	6.9 ±7.1	176 ±14	93 ±22	3812 ±2399	39.3 ±21.1
2.20	200 ±8	173 ±14	747 ±8	4.4 ±0.4	155 ±4	67 ±6	1373 ±487	20.7 ±7.3
5.17	177 ±9	106 ±24	588 ±446	5.0 ±3.1	...[b]	...	...	...
5.17	...[b]	...	...	...	...	...	...	...

[a]Mean ±1 standard deviation.

[b]All fish had perished.

TABLE 15. SURVIVAL OF F_1-GENERATION BLUEGILL IN CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	Initial No. fry	% survival 30 days	60 days	90 days
Control	42	45.2	31.0	19.0
Control	34	8.8	2.9	2.9
Control[a]	142	16.9	12.8	10.7
0.25	47	6.4	4.3	2.1
0.25	50	2.0	2.0	2.0
0.25	50	0	...	...
0.25	90	3.3	3.3	3.3
0.25	50	4.0	4.0	4.0
0.54[b]	50	0	...	...
0.54[b]	6	33.3	33.3	33.3
0.54	50	12.0	12.0	12.0
0.54	50	0	...	...
0.54	44	0	...	...
1.22	50	0	...	...
1.22	50	4.0	4.0	4.0
2.20[c]	50	30.0	16.0	14.0
2.20[c]	50	28.0	14.0	14.0
2.20[c]	50	2.0	0	...
2.20[c]	50	10.0	2.0	2.0
5.17[c]	50	0	...	...
5.17[c]	50	0	...	...

[a]Fry transferred from 0.25 µg/l concentration.

[b]Fry from eggs incubated in control.

[c]Fry hatched from control eggs.

TABLE 16. GROWTH OF F_1-GENERATION BLUEGILL DURING CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	Growth						
	30 days		60 days		90 days		
	No. fry	Total length, mm	No. fry	Total length, mm	No. fry	Total length, mm	Wet weight, g
Control	23	6.4[a] ±0.6	14	9.9 ±0.9	9	17.3 ±3.9	107 ±35
Control	24	11.1 ±1.8	18	12.1 ±1.7	15	16.9 ±2.0	64 ±28
0.25	8	13.0 ±0.6	8	28.0 ±2.4	7	36.8 ±3.3	752 ±206
0.54	8	14.0 ±2.6	8	24.8 ±2.4	8	32.8 ±4.0	481 ±198
1.22	2	11.5	2	27.5	2	36.0	760
2.20[b]	35	6.7 ±0.6	16	14.9 ±3.5	15	18.7 ±6.5	132 ±88

[a]Mean ±1 standard deviation are given.

[b]Fry hatched from control embryos.

As was observed in the chronic tests of the other fish species, measured chlordane levels were 42 - 58% of desired and ranged from 0.32 ± 0.18 to 5.80 ± 2.15 µg/l (Appendix Table 15).

Chronic Effects on Survival, Growth and Reproduction

Growth of f_0-generation brook trout throughout the chronic test did not vary significantly between treatments in terms of either total length or wet body weight, although fish exposed to 2.21 and 5.80 µg/l chlordane tended to be smaller at 3 months. At the beginning of the test, the trout averaged 188 mm in total length and 70 g in wet weight. After 6.5 months, they had increased 25% in length (to 248 mm) and more than doubled their body weight (188 g). At termination average lengths and weights were 213 mm and 281 g, respectively (Tables 17 and 18).

Prior to and during spawning, mortality was much higher among fish exposed to 2.21 and 5.80 µg/l than among controls or those exposed to the lower (0.32 - 1.29 µg/l) concentrations (Table 19). For trout exposed to 5.8 µg/l chlordane, mortality was 91.7% after 6.5 months' exposure and complete at the conclusion of spawning. Control trout, which had been transferred to one of the 5.8 µg/l tanks at thinning (the sole fish remaining being transferred to the other tank), also died during spawning. Interestingly, about half of the fish which perished had signs characteristic of bacterial hemorrhagic septicemia (e.g. exophthalmia and lesions). Those dying from what was believed to be chlordane poisoning were emaciated and most exhibited impaired equilibrium for up to several weeks before death. Convulsive or other behavior indicative of poisoning by some insecticides was not observed.

After 8 months' exposure (28 November 1973), trout began spawning. Total embryo production per female ranged from 62 to 400 in the control, 0.32, 0.66, and 1.29 µg/l concentrations, but only from 0 to 47 in the 2.21 and 5.80 µg/l concentrations (Table 20). Trout exposed to the two highest chlordane concentrations also spawned fewer than 100 embryos at any time, whereas spawns greater than 100 embryos each comprised from 9.1 to 26.9% of the total spawns in the lower pesticide concentrations and the controls.

At the time spawnings were checked, i.e. within 24 hr of spawning, the proportions of dead (opaque) embryos were greater at higher pesticide concentrations (Table 20). On the average, only 8.0% of the embryos produced by controls were dead, compared to 14.8, 23.4, and 67.5% in the low, mid-range, and high concentrations, respectively.

Spawns totalling more than 20 embryos were used for determination of viability. Embryo viability was lower at higher technical chlordane concentrations, although embryos produced by one of the control tanks and one of the 0.66 µg/l tanks were nonviable. Average embryo viabilities declined from 65% in the controls to 47% in the 0.32 µg/l concentration to 17% in the 0.66 and 1.29 µg/l concentrations (Table 21). None of the embryos produced in the 2.21 and 5.80 µg/l concentrations were viable. Four lots of 50

TABLE 17. TOTAL LENGTHS OF F_0-GENERATION BROOK TROUT CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	Total length, mm			
	0 month	3 months	6.5 months	12 months
Control	188[a] ±8	211 ±10	250 ±12	285 ±17
Control	185 ±11	208 ±14	243 ±16	281 ±22
0.32	187 ±13	208 ±14	249 ±16	278 ±33
0.32	192 ±9	211 ±10	250 ±10	286 ±19
0.66	188 ±15	210 ±15	246 ±21	286 ±31
0.66	187 ±13	209 ±14	243 ±22	286 ±11
1.29	190 ±15	210 ±13	252 ±16	285 ±11
1.29	195 ±11	213 ±12	254 ±15	288 ±14
2.21	187 ±10	195 ±21	257[b] ...	...[b]
2.21	186 ±13	203 ±17	247 ±21	279 ±34
5.80	188 ±9	209 ±10	241[b] ...	...[b]
5.80	187 ±15	199 ±27	210[b] ...	...[b]

[a]Mean ±1 standard deviation.

[b]One or no fish remaining.

TABLE 18. BODY WEIGHTS OF F_0-GENERATION BROOK TROUT CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	Wet body weight, g			
	0 month	3 months	6.5 months	12 months
Control	69[a] ±12	106 ±18	190 ±33	288 ±65
Control	68 ±12	103 ±13	177 ±34	268 ±94
0.32	68 ±14	107 ±20	192 ±42	301 ±78
0.32	72 ±12	105 ±16	190 ±22	276 ±70
0.66	72 ±18	107 ±21	186 ±53	297 ±149
0.66	67 ±15	104 ±23	181 ±50	278 ±40
1.29	74 ±15	107 ±15	196 ±31	291 ±38
1.29	77 ±13	110 ±16	198 ±36	286 ±59
2.21	71 ±14	90 ±38	210[b] ...	...[b]
2.21	68 ±16	97 ±25	179 ±57	247 ±100
5.80	69 ±10	96 ±17	157[b] ...	...[b]
5.80	70 ±17	96 ±31	107[b] ...	...[b]

[a]Mean ±1 standard deviation.

[b]One or no fish remaining.

TABLE 19. MORTALITY OF F_0-GENERATION BROOK TROUT CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	Cumulative mortality[a], %		Mortality during spawning[b], 6.5 - 12 mo	
	3 mo	6.5 mo (up to thinning)	Males	Females
Control	0	8	1/3	1/4
Control	0	0	0/3	0/4
0.32	8	17	0/3	0/4
0.32	0	17	0/3	0/4
0.66	0	17	0/2	0/5
0.66	0	8	0/3	0/4
1.29	8	17	0/3	0/4
1.29	8	17	0/2	0/5
2.21	67	92	1/3	1/2
2.21	0	17	3/4	1/2
5.80	42	92	3/3[c]	4/4
5.80	83	92	0/0	2/2

[a]Based on 12 fish per chamber.

[b]Numerator and denominator of each expression equivalent to number of fish dying out of total, i.e. 1/3 (male column) indicates one of three males died during spawning.

[c]Represent control fish transferred at thinning. The fish which had survived to thinning in this treatment was transferred to the other replicate.

TABLE 20. SPAWNING SUCCESS OF BROOK TROUT CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	No. females	Embryos/ female	No. embryos/spawn ≥ 1	≥20	≥100	% embryos initially dead
Control	4	290	22	11	4	7.3
Control	4	90	9	3	1	10.1
0.32	4	400	29	14	4	13.9
0.32	4	62	15	4	0	21.1
0.66	5	215	16	12	5	25.3
0.66	4	153	10	6	2	5.1
1.29	4	259	14	5	4	6.6
1.29	5	126	29	8	1	50.9
2.21	2	47	10	1	0	40.9
2.21	2	30	5	1	0	13.3
5.80	4	32	16	1	0	67.5
5.80	2	0	...	...	...	...

TABLE 21. VIABILITY AND HATCH OF EMBRYOS AND CONDITIONS OF F_1-GENERATION BROOK TROUT ALEVINS

Meas. conc., chlordane, µg/l	Viability			Hatching success					
	No. embryos incubated	% viable embryos		No. embryos incubated	% alevins			% hatch	
		tank	conc.		normal	abnormal	dead	tank	conc.
Control	970	82.0	65.4[a]	450	97.3	2.4	0.3	91.8	91.8
Control	247	0		...	...	...	...	...	
0.32	1,256	42.7	46.7	200	92.9	4.7	2.4	42.5	59.7
0.32	154	79.8		100	100.0	0	0	94.0	
0.66	734	0	17.1	0	...	...	...	...	90.0
0.66	567	39.3		50	93.4	4.4	2.2	90.0	
1.29	941	23.1	17.4	50	97.3	2.7	0	74.0	74.0
1.29	309	0.3		0	...	...	...	...	
2.21	0	...	...	...	...	...	...	...	...
2.21	0	...	...	...	...	...	...	...	...
5.80[b]	34[c]	0	0	0	...	...	...	...	...
5.80	...	...		...	...	...	...	...	

[a]Weighted mean.

[b]Control fish transferred at "thinning".

[c]Both fish were females.

embryos were transferred from control chambers to the 2.21 and 5.80 µg/l concentrations, and viabilities for both treatments were as high (80 to 96%) as controls, suggesting that the concentrations employed were not deleterious within the 12-day period following spawning.

Hatching success was largely unaffected by technical chlordane up to concentrations of at least 1.29 µg/l (Table 21). Although too few eggs were spawned by fish reared in the 2.21 and 5.80 µconcentrations to evaluate hatching success, control eggs, incubated in these treatments for the 50 to 55 days (range of median hatch dates for all treatments) required for hatching, survived as well (hatching success of 74 to 98%) as controls. Furthermore, technical chlordane had no effect on the proportions of abnormally developed or dead alevins at hatching (less than 3% in all cases).

Growth of the f_1-generation generation progeny was followed over a 90-day period (Table 22). Upon hatching total lengths of subsamples of alevins from each of the treatments were similar. After 30 days' growth, total lengths of fry reared in 0.66, 1.29, 2.21 and 5.80 µg/l tended to be less than controls, but after 60 and 90 days, all chlordane-exposed fry were larger than controls. Although analysis of variance indicated significant differences after all three growth periods ($p < 0.05$), Dunnett's test indicated that the significant differences were between the treatments and not between the treatments and the control. Data on wet body weights suggested similar relationships to those discussed for the total length data (Table 22).

Survival data for fry were incomplete, owing to high mortality in approximately 20- to 40-day-old alevins which occurred on two successive weekends. The cause was traced to chlorination of the water supply on Fridays for removal of algae in storage reservoirs. The water supply to the brook trout chronic test was not being passed through an activated charcoal filter owing to the high volume flow required for this test. Lack of this protection was responsible for the unanticipated mortalities. After the cause of the mortality was identified, a solution of sodium thiosulfate was pumped into the water supply line at a concentration of 100 µg/l to convert chlorine to chloride ion. This eliminated further mortality.

In summary, chronic exposure of brook trout to technical chlordane appeared to cause detrimental effects on survival, embryo production, spawn size, and the viability and hatch of f_1-generation progeny. However, the importance of these effects can only be speculated since none were statistically significant ($p > 0.05$). Survival, embryo production, and spawn size were substantially lower than controls in insecticide concentrations greater than 1.29 to 2.21 µg/l. Greater proportions of the embryos spawned were found to be dead down to the lowest concentration tested (0.32 µg/l), while 12-day survival of the embryos was 29% lower in this concentration than in the controls. On the other hand, survival to hatching was reduced only above 1.29 µg/l. From the above data it appears that the lowest concentration employed, 0.32 µg/l, would be deleterious to populations of brook trout.

TABLE 22. GROWTH OF F_1-GENERATION BROOK TROUT DURING CHRONIC EXPOSURE TO TECHNICAL CHLORDANE

Meas. conc. chlordane, µg/l	Total length, mm				Wet body weight, g	
	At hatch	30 days	60 days	90 days	At hatch	90 days
Control	14.4[a] ±0.6 (125)	21.4 ±0.8 (89)	28.4 ±3.5 (39)	39.8 ±2.9 (20)	0.050 ±0.005 (110)	0.610 ±0.144 (20)
0.32	14.5 ±0.6 (114)	21.8 ±0.8 (58)	26.5 ±1.8 (36)	44.7 ±3.5 (24)	0.044 ±0.004 (60)	0.910 ±0.201 (24)
0.66	13.8 ±0.4 (25)	17.2 ±0.7 (24)	29.6[b] ±2.1 (25)	43.4[b] ±2.8 (14)	0.041 ±0.003 (19)	0.803[b] ±0.155 (14)
1.29	14.8 ±0.4 (25)	19.4 ±0.6 (25)	29.4[b] ±2.0 (25)	43.8[b] ±3.1 (16)	0.050 ±0.002 (12)	0.847[b] ±0.169 (16)
2.21	14.5[b] ±0 (25)	18.6[b] ±0.9 (21)	...	...	0.047[b] ±0.004 (21)	...
5.80	14.6[b] ±0.6 (75)	17.4[b] ±0.7 (71)	...	...	0.050[b] ±0.006 (57)	...

[a]Means ± 1 standard deviation and sample size are given.

[b]Control eggs that had been transferred to these concentrations.

CHRONIC TOXICITY TO HYALLELA AZTECA

Water Quality and Chlordane Concentrations

Water quality was measured eight times during the 9-week chronic test utilizing H. azteca (Appendix Table 16). Water temperatures averaged 16.7 ± 1.0°C and dissolved oxygen, 7.5 ± 0.4 mg/l (76% of air saturation). As in all toxicity tests, the water was alkaline (pH of 7.86) and of intermediate hardness (148 mg/l). Fluctuations from one week to the next were small.

Measured chlordane concentrations were approximately 50% of desired, averaging 1.41 ± 0.77, 2.64 ± 1.32, 5.32 ± 3.24, 11.53 ± 6.14, and 20.52 ± 9.85 µg/l (Appendix Table 17).

Toxicity

Two of the main indices of chlordane's chronic effects on H. azteca, namely growth and survival, were determined at the end of the experiment. Use of only one point of measurement rather than several was selected because the responses of the animals to handling were unknown. Adult H. azteca are about one-quarter to one-third the size of Gammarus pseudolimnaeus, the species for which this test was patterned; accordingly, it was presumed that much greater care would be required during handling.

Survival of H. azteca was unaffected at technical chlordane concentrations less than 11.5 µg/l, where 92% or more of the specimens survived 9 weeks relative to 88 - 108% of controls (Table 23). However, survival was significantly reduced to 12 - 36% in the 11.5 µg/l concentration and to zero in the 20.5 µg/l level.

Growth of the amphipods was also affected by the presence of chlordane. In both replicates, analysis of variance and Dunnett's test indicated that amphipods exposed to 11.5 µg/l chlordane were significantly smaller ($p < 0.05$) than controls in terms of wet and dry weights (Table 23 and Appendix Table 18).

The chronic toxicity test indicated that growth and survival of H. azteca were significantly reduced between concentrations of 5.3 and 11.5 µg/l. The maximum acceptable toxicant concentration for technical chlordane may exist within this range, but effects of this insecticide on reproduction and on growth and survival of progeny should be examined before arriving at this conclusion.

Accumulation of Chlordane

Contents of heptachlor, β-, γ- and "a"-chlordane, cis- and trans-chlordane, and cis- and trans-nonachlor were determined on a dry weight basis in H. azteca at the conclusion of the chronic test at 65 days. Contents of each constituent increased with aqueous concentration. Concentration factors tended to remain unaffected by the level of treatment for

TABLE 23. RELATIVE SURVIVAL AND GROWTH OF HYALLELA AZTECA EXPOSED TO TECHNICAL CHLORDANE

Parameter	Measured concentration of technical chlordane, µg/l					
	Control	1.4	2.6	5.3	11.5	20.5
Replicate 1						
No. survivors[a]	27	23	23	24	3	0
% survivors	108	92	92	96	12	0
Wet body weight, mg[b]	6.3 ±1.3	6.2 ±1.5	6.4 ±1.2	5.1 ±0.9	3.8 ±0.7	... ...
Dry weight, mg[b]	1.58	1.49	1.57	1.37	0.87	...
Replicate II						
No. survivors	22	25	25	24	9	0
% survivors	88	100	100	96	36	0
Wet body weight, mg[b]	7.5 ±1.3	5.8 ±1.3	5.8 ±1.6	5.5 ±1.6	5.3 ±1.0	... ...
Dry weight, mg[b]	1.92	1.55	1.53	1.35	1.33	...

[a]25 individuals introduced initially per chamber.

[b]Average calculated weight per individual.

heptachlor, the chlordenes, and cis-nonachlor, and increased only slightly for cis-chlordane, trans-chlordane and trans-nonachlor. The highest contents of each constituent were 357 µg/g for heptachlor, 92.3 µg/g for the chlordenes, 260 µg/g for trans-chlordane, 220 µg/g for cis-chlordane, 71.8 µg/g for trans-nonachlor, and 46.4 µg/g for cis-nonachlor (Table 24).

Net concentration of all constituents was very extensive. Even though heptachlor was concentrated to a lesser extent than the other compounds, its concentration factors were still quite high (16,700 to 31,040). Although the cis- and trans-nonachlors comprised only 2.8 and 5.1% of the technical chlordane, their storage was proportionally greater than all other major constituents including the cis- and trans-chlordanes, which comprised 43% of the insecticide. The compound accumulated to the greatest extent was cis-nonachlor, for which concentration factors ranging from 95,030 to 144,100 were calculated. The propensity of the components to be concentrated increased in the following order: heptachlor, the chlordenes, trans-chlordane, cis-chlordane, trans-nonachlor, and cis-nonachlor (Table 24).

The proportions of the chlordane constituents present in the amphipods were different from those characterizing the neat insecticide, suggesting differences in water solubility, uptake, or metabolism. For example, the ratio of cis- trans-nonachlor in the neat technical insecticide was 0.55:1, but the average ratio in the organisms was 0.71:1, indicating an enhanced accumulation of cis-nonachlor relative to the trans isomer. A preferential storage of cis-chlordane relative to that of the trans isomer was also apparent, with the mean ratio of cis- trans-chlordane of 0.85:1 in the tissues being somewhat greater than that (0.79:1) characterizing the neat insecticide. There was also a diminution in heptachlor storage relative to the two chlordanes, for the ratio in the tissues (0.07:1) was only 32% of that in the stock formulation (0.23:1). Considerably more cis- and trans-nonachlor were stored relative to cis- and trans-chlordane. The ratio of chlordanes to nonachlors was 4.1:1 in amphipod tissues and 5.4:1 in the neat insecticide. Although these studies were not intended to elucidate the in vivo fate of the various chlordane components, it appears that the nonachlors may have been particularly susceptible to uptake and deposition or were generated in part through metabolism of such constituents as cis- and trans-chlordane or heptachlor, which were present in diminished proportions in amphipod tissues.

The relative proportions of some of the constituents also appeared to change as a function of aqueous technical chlordane concentration. For example, the heptachlor/cis- and trans-chlordane ratio declined from 0.085:1 in specimens exposed to 1.4 µg/l technical chlordane to 0.063:1 in those exposed to 11.5 µg/l, while the cis-/trans-nonachlor ratio declined from 0.83:1 in amphipods exposed to 1.4 µg/l to 0.59:1 in those exposed to 11.5 µg/l. In contrast, the contents of the chlordanes relative to the nonachlors may have increased since the ratio between them was slightly greater (4.2:1) in the two highest concentrations than in the two lowest (3.76:1 and 3.79:1). The cis-/trans-chlordane ratio was essentially constant between treatments (0.83:1 to 0.87:1). These differences suggest that amphipods which were exposed to the higher technical chlordane concentrations

TABLE 24. CONTENTS AND CONCENTRATION FACTORS (C.F.) OF CHLORDANE CONSTITUENTS IN DRIED *HYALLELA AZTECA* THAT HAD BEEN EXPOSED TO TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	Heptachlor		Chlordenes[a]		Cis-chlordane		Trans-chlordane		Cis-nonachlor		Trans-nonachlor	
	Content, µg/g[b]	C.F.	Content, µg/g	C.F.	Content, µg/g	C.F.	Content, µg/g	C.F.	Content, µg/g	C.F.	Content, µg/g	
Control	0.3	...[c]	1.4	...	1.9	...	2.2	...	0.2	...	0.5	...
Control	0.5	...	1.4	...	2.7	...	2.9	...	0.6	...	0.5	...
1.4	3.4	24,290	10.1	55,500	19.2	72,180	22.1	65,770	5.3	135,200	6.1	85,430
1.4	3.1	22,140	8.2	45,060	16.2	60,900	18.7	55,660	3.8	96,940	4.9	68,630
2.6	5.3	20,390	17.8	52,660	36.9	74,700	42.5	68,110	9.2	126,370	11.8	88,990
2.6	5.8	22,310	17.7	52,370	35.5	71,860	41.3	66,190	9.1	125,000	11.4	85,970
5.3	10.9	20,570	29.6	42,960	73.5	72,990	88.6	69,650	14.9	100,400	23.2	85,830
5.3	11.7	22,080	32.6	47,320	81.7	81,130	97.4	76,572	16.8	113,210	26.1	96,560
11.5	19.2	16,700	60.7	40,600	176.9	80,960	216.0	78,261	30.6	95,030	58.9	100,430
11.5	35.7	31,040	92.3	61,740	220.0	100,690	259.9	94,170	46.4	144,100	71.8	122,420

[a]Consisting of γ - β peaks (13) and "a" peak (12).

[b]µg/g residue per dry body weight.

[c]Concentration factors (C.F.) not calculated for controls since on only one occasion was technical chlordane detected.

may have dealt with the various constituents differently than those exposed to lesser concentrations.

CHRONIC TOXICITY TO DAPHNIA MAGNA

Water Quality and Measured Chlordane Concentrations

The standard battery of water quality parameters was measured four times, just prior to and during the course of the chronic toxicity test using D. magna. The water temperature averaged 20.9 ± 0.5°C and the water quality was very similar to that described earlier for the other chronic toxicity tests (Appendix Table 19).

Desired concentrations of technical chlordane ranged from 6.1 to 96.9 µg/l, excluding the control, and were set high because of excessive loss of the insecticide under the conditions of limited toxicant renewal. The concentrations existing during the test ranged from 1.7 ± 0.1 to 21.6 ± 9.6 µg/l for the five treatments (Appendix Table 20).

Effects on Survival, Growth and Reproduction

Survival of the cladocerans from first instars to adults was poor regardless of treatment. After 1 week, control survival averaged 80%, but declined to 30% in the fourth week (Table 25). Survival of daphnids in chlordane was essentially the same as that of the controls, except for those exposed to 21.6 µg/l chlordane, where only one of the initial 20 specimens survived to the middle of the fourth week.

Growth of the cladocerans was determined only for instars produced in the fourth week. Since dry weights of instars produced in the various chlordane solutions were commensurate with those for controls, there did not appear to be any adverse effects upon growth (Table 26).

Reproduction of Daphnia was highly variable and was apparently unaffected by any of the concentrations of technical chlordane used (Table 25).

On the basis of the above data, which should be considered preliminary pending completion of experiments having good control survival and reproduction, it appears that the only toxicologically effective concentration was 21.6 µg/l, which had killed all first generation daphnids by the end of the fourth week.

Accumulation of Chlordane

Accumulation of the major components of chlordane in D. magna was similar in magnitude to that of H. azteca, even though the cladocerans were exposed for a maximum of 1 week[2] and the amphipods for 2 months. Uptake and storage are evidently very rapid in D. magna.

[2]First instars produced in the last 7 days of the chronic test were used for for residue analysis.

TABLE 25. SURVIVAL AND REPRODUCTION OF DAPHNIA MAGNA IN CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE

Week	Meas. chlordane conc., µg/l	Adult survival,[a] %	Production	
			Total instars	Instars/avg. No. adults
5/8/74	Control	80	0	0
	Control	80	0	0
	1.7	90	0	0
	1.7	80	0	0
	2.5	70	0	0
	2.5	80	0	0
	6.2	60	0	0
	6.2	70	0	0
	12.1	90	0	0
	12.1	90	0	0
	21.6	50	0	0
	21.6	100	0	0
5/15/74	Control	50	25	5
	Control	40	16	4
	1.7	80	13	2
	1.7	70	7	1
	2.5	40	13	3
	2.5	70	34	5
	6.2	20	7	4
	6.2	50	13	3
	12.1	80	39	5
	12.1	80	27	3
	21.6	0	0	0
	21.6	90	36	4
5/23/74	Control	40	12	3
	Control	30	22	7
	1.7	70	176	25
	1.7[b]	40	3	1
	2.5	40	28	7
	2.5	70	334	48
	6.2	20	28	14
	6.2	50	91	18
	12.1	70	74	11
	12.1	80	220	28
	21.6	0	0	0
	21.6	40	15	3

Continued.....

TABLE 25. SURVIVAL AND REPRODUCTION OF DAPHNIA MAGNA IN CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE--continued

Week	Meas. chlordane conc., µg/l	Adult survival,[a] %	Production	
			Total instars	Instars/avg. No. adults
5/30/74	Control	40	115	29
	Control	20	57	29
	1.7	60	804	134
	1.7	0	...	...
	2.5	40	215	54
	2.5	70	619	88
	6.2	20	162	81
	6.2	50	166	33
	12.1	60	143	24
	12.1	70	791	105
	21.6	0	0	0
	21.6	0	85	170

[a]Ten Daphnia initially introduced into each test container. Values represent percentage of specimens remaining at the end of a given week.

[b]Accidental discard of remaining adults in this concentration.

TABLE 26. AVERAGE DRY BODY WEIGHTS OF FIRST INSTAR *DAPHNIA MAGNA* PRODUCED DURING FOURTH WEEK OF CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	No. specimens	Average dry weight/individual, µg
Control	115	49.1
Control	57	27.8
1.7	804	31.2
1.7	0	...
2.5	215	36.7
2.5	619	29.5
6.2	162	28.3
6.2	166	23.4
12.1	143	24.4
12.1	791	27.5
21.6	0	...
21.6	85	43.5

As shown in Table 27, which gives the actual tissue levels of the various components as well as their individual concentration factors, heptachlor residues were lowest (range of 0.9 - 27.9 µg/g) and trans-chlordane residues highest (9.6 - 370 µg/g) of the six components selected for analysis. Daphnids tended to preferentially concentrate more cis-and trans-nonachlor and less heptachlor and chlordenes than cis- or trans-chlordane. Tissue residues of each compound appeared to be directly proportional to the aqueous concentration of technical chlordane to which the animals were exposed. The linearity of uptake and storage was corroborated by the uniform factors for each component, which varied little as a function of treatment, except for the low aqueous concentration, where concentration factors were considerably lower. Concentration factors varied from 5,290 to 12,900 for heptachlor to 32,000-144,850 for trans-nonachlor. The order of increasing bioconcentration was: heptachlor, the chlordenes, trans-chlordane, cis-chlordane, cis-nonachlor, and trans-nonachlor.

As was observed for amphipods, the relative proportions of the chlordane constituents differed from those characterizing the neat insecticide. There was preferential deposition of the cis- and trans-nonachlors and a relative diminution of heptachlor and the cis- and trans-chlordanes.

CHRONIC TOXICITY TO CHIRONOMUS NO. 51

Two partial chronic toxicity tests were conducted with Chironomus No. 51 to delimit "safe" and "unsafe" concentrations. The first test was preliminary, limited to introduction of 25 newly-hatched larvae into each of five insecticide concentrations and a control. The second test utilized 50 newly-hatched larvae and 12 test chambers, comprising five concentrations and a control in duplicate.

Water Quality and Chlordane Concentrations

Levels of each of the water quality parameters comprising the standard battery were essentially the same in both experiments. Concentrations of dissolved oxygen were 80% of air saturation, pH levels averaged 7.94 and 7.90, and total hardness averaged 154 and 150 mg/l $CaCO_3$, respectively (Appendix Table 21).

Measured concentrations of technical chlordane ranged from 1.0 ± 0.1 to 24.9 ± 16.8 µg/l in the first test and from 0.7 ± 0.1 to 15.5 ± 3.7 µg/l in the second (Appendix Table 22).

Toxicity

Adult Chironomus No. 51 emerged in the first test 11 to 15 days after their introduction as newly-hatched larvae. All control larvae and those held in 1.0 µg/l emerged, but none of those reared in 2.4 to 24.9 µg/l did. The time to 50% adult emergence was 12.5 to 13 days for both treatments (Table 28). Males, which constituted only 28-32% of all adults, tended to emerge 1 day earlier (50% emergence in 11 days) than females.

TABLE 27. CONTENTS AND CONCENTRATION FACTORS (C.F.) OF CHLORDANE CONSTITUENTS IN DRIED *DAPHNIA MAGNA* THAT HAD BEEN EXPOSED TO TECHNICAL CHLORDANE

Meas. chlordane conc., µg/l	Heptachlor		Chlordenes[a]		Cis-chlordane		Trans-chlordane		Cis-nonachlor		Trans-nonachlor	
	Content, µg/g[b]	C.F.	Content, µg/g	C.F.	Content, µg/g	C.F.	Content, µg/g	C.F.	Content, µg/g	C.F.	Content, µg/g	C.F.
Control	0.5	...[c]	5.7	...	6.8	...	6.7	...	1.5	...	1.6	...
Control	0.5	...	4.3	...	6.1	...	6.0	...	1.4	...	1.5	...
1.7	0.9	5,290	4.8	21,720	8.9	27,550	9.6	23,530	2.2	46,220	2.8	32,300
1.7[d]	...	...	...	...	...	...	...	...	...	...	...	...
2.5	2.3	9,200	8.5	26,150	27.7	58,320	29.5	49,170	5.4	77,140	9.7	76,080
2.5	1.9	7,600	9.2	28,310	26.6	56,000	29.8	49,670	5.9	84,290	7.7	60,390
6.2	3.0	4,840	11.8	14,640	71.8	60,950	77.0	51,750	16.7	96,200	27.8	87,920
6.2	9.8	15,810	36.9	45,780	126.0	106,960	135.6	91,129	30.4	175,120	45.8	144,850
12.1	7.4	6,120	26.1	16,590	124.3	54,070	137.5	47,350	28.4	83,830	44.2	71,630
12.1	9.8	8,100	31.6	20,089	152.0	66,120	168.0	57,850	29.6	87,370	62.7	101,600
21.6	27.9	12,920	72.5	25,820	333.8	81,340	369.8	71,340	52.2	86,310	125.5	113,930
21.6[d]	...	...	...	...	...	...	...	...	...	...	...	...

[a]Consisting of γ - β peaks (13) and "a" peak (12).

[b]µg/g dry body weight.

[c]Concentration factors not calculated for controls since technical chlordane was undetected.

[d]No instars produced in fourth week.

TABLE 28. CHRONIC EFFECTS OF TECHNICAL CHLORDANE ON CHIRONOMUS NO. 51

Parameter	Test 1			Test 2					
	Control	1.0 µg/l	2.4 µg/l	Control	Control	0.7 µg/l	0.7 µg/l	1.7 µg/l	1.7 µg/l
Total adult emergence	25	25	0	11	24	10	11	0	0
% emergence	100	100	0	22	48	20	22	0	0
% males	33	28	...	63	63	44	36	...	...
% females	67	72	...	37	37	56	64	...	...
Median emergence time, days	13	12.5	...	13	16	17	15	...	...

In the second experiment, adult midges emerged only from the control and low concentration (0.7 µg/l) and were not observed in the 1.7 to 15.5 µg/l treatments (Table 28). Although adults were observed on the same day (day 11) as in the first test, emergence was inexplicably spread over a longer period (11 to 25 days) and median times to emergence were longer. In the two control chambers, 50% of the adults had emerged 12 and 16 days, respectively after their introduction as larvae. Fifty percent of the midges exposed to 0.7 µg/l chlordane emerged after 17 and 15 days.

Survival to emergence was much poorer in the second experiment than in the first. Of the 50 larvae originally introduced into each chamber, only 22-48% of the controls and 22-24% of those exposed to 0.7 µg/l chlordane survived. Larvae were observed 4 days after introduction in the 1.7 µg/l concentration, but not thereafter. There was no evidence that chironomids survived for even a short time in the 3.3 to 15.5 µg/l concentrations.

In contrast to the first experiment where females predominated, more males (60 to 63%) emerged in the controls than in the 0.7 µg/l concentration (36 to 44%) in the second test. Males tended to emerge earlier in the control chambers than females, but in the 0.7 µg/l concentration, they emerged at the same rate. Of the first 50% of the control midges to emerge, 80 to 92% were males.

On the basis of the results of the two experiments, technical chlordane concentrations above 0.7 to 1.0 µg/l, i.e. 1.7 µg/l, were clearly unsafe because they were lethal to the developing larvae. Complete life cycle tests encompassing reproductive and hatching success, etc, would be needed to ascertain whether lower levels are deleterious.

Accumulation of Chlordane

No residues of technical chlordane were detected in extracts of dried adult Chironomus No. 51 and there was no evidence of the presence of oxychlordane.

SECTION VII

DISCUSSION

ACUTE TOXICITY TESTS

Chlordane appears to be generally less toxic in the short-term to freshwater fish and invertebrates than endrin, dieldrin, aldrin, and DDT, but more toxic than methoxychlor, lindane, benzene hexachloride (BHC) and Guthion®. In the present study continuously-renewed solutions of technical chlordane were lethal in 96 hr to three fish species between 37 and 59 µg/l. These values agree well with the data of Katz (23), Henderson et al. (22), and of Macek et al. (25), but are consistently higher than values reported by Konar (26) and lower than those given by Lüdemann and Neumann (30). For example, Katz (23) reported 96-hr LC50 estimates of 44 to 57 µg/l for three species of salmonids. Our estimate of 47 µg/l for brook trout was within this range. The 96-hr LC50 of 77 µg/l at 23.8°C for bluegill reported by Macek et al (25) was somewhat higher than we found (59 µg/l) for the same species, but both of these estimates were above the 16.5 µg/l LC50 estimate given by Henderson et al (22). In general brief exposure to chlordane would appear to be lethal to many fish species within the concentration range of 1 to 100 µg/l. More than half of the toxic responses of fish to chlordane reported in the literature (Table 1) and determined in this project were within this range.

Acute toxicity tests conducted with continuous toxicant renewal would in many cases be expected to result in lower lethal limits than tests conducted without renewal (e.g. static conditions) because toxicant concentrations would not decline due to assimilation by the test organisms or by sorption to debris or to the walls of the test vessel. Use of measured rather than expected insecticide concentrations in estimating median response limits should also improve their validity. While these arguments are valid, the LC50 values obtained by us were not demonstrably lower than those reported by others, as indicated above. Differences between static and flow-through test results for chlordane may come to light when exposures are extended beyond approximately 96 hr. For example, the data of Henderson et al. (22) and of Katz (23) indicate that median lethal thresholds, the concentration at which lethality to 50% of the specimens ceases, were approached or reached within 96 hr for fathead minnow, goldfish (Carassius auratus), rainbow trout, and chinook salmon (Oncorhynchus tshawytscha). In our studies a median lethal threshold was attained only with fathead minnows, but only after 168 hr; toxicity curves for brook trout and bluegill were linear. The absence of median lethal thresholds for exposures less than 96 hr would

be expected if the poison had a cumulative action--which chlordane apparently does--and if toxicant concentrations remained relatively constant for the duration of the test.

Comparison of the toxicity test results for D. magna and H. azteca with those reported in the literature for similar species indicates relatively good agreement. The two 96-hr LC50 values for D. magna of 28 and 35 µg/l were only slightly higher than the 48-hr LC50 of 20 µg/l for the cladoceran Simocephalus serrulatus (6). Hyallela azteca are decidedly less sensitive to chlordane than Gammarus lacustris. The 96-hr LC50 of 26 µg/l for the latter species (35) was only 25% that for H. azteca, which were exposed for a longer (168 hr) period. Although there is a great range in the levels of chlordane reported to be toxic to aquatic invertebrates, their sensitivity to this insecticide appears to be of the same order as that for fish (i.e. acute lethal range of 1 to 100 µg/l).

CHRONIC TOXICITY TESTS

The lowest aqueous concentration of technical chlordane which we found to have marked deleterious chronic effects was 0.32 µg/l, which lowered brook trout embryo viability. This apparent "unsafe" level for chronic exposure was less than 1% of the 96-hr LC50 for this species. Prominent chronic effects were observed for bluegill and the chironomid at concentrations around 2 µg/l. High mortality prior to and during the spawning period and failure to spawn were the salient responses of bluegill that had been exposed to 2.2 and 5.2 µg/l chlordane. Larval mortality, which accounted for the failure of adult emergence, was the main effect of 1.7 µg/l chlordane on Chironomus No. 51. Of lesser apparent sensitivity were fathead minnows, daphnids, and the amphipod. These species were unaffected by chlordane concentrations lower than about 5 to 10 µg/l.

Although the chronic toxicity tests we conducted produced much needed information on the effect of this insecticide on growth, survival, and reproduction of several fish and invertebrate species, they failed in every case to produce hard, unequivocal data on what concentrations were detrimental and which were not for all major life stages of each species. Consequently, technical chlordane may ultimately be found to be more toxic to these species than reported here. For example, the brook trout and bluegill experiments were partial rather than full life cycle tests because they were begun with yearlings instead of fry or embryos. It is quite conceivable, accepting that chlordane's toxicity is cumulative, that greater effects on the f_0-generation would have been observed had younger specimens been used. Further, neither the trout nor bluegill tests fully evaluated effects on f_1-generation progeny. In both experiments, poor survival compromised interpretation of results. The most notable liability of the fathead minnow chronic was the poor early survival of the fry. As stated earlier, the question of whether the surviving fish were in a weakened condition will remain, even though there is the possibility that the survivors represented the most fit individuals because the weaker fish were selected out. Similarly, poor survival--and hence reproduction--of f_0-generation daphnids made

conclusions tenuous on the extent of effects. Finally, while the chironomid and amphipod tests went well, they did not evaluate toxicant effects on the f_1-generation.

Chronic toxicity, life cycle tests represent an important advance in the sophistication and sensitivity of aquatic toxicity testing. Though costly and time-consuming, they are probably the best means for directly estimating cumulative, long-term effects on most developmental stages of an organism. Because emphasis is placed, when possible, on the results of such tests in setting water quality standards, it is important that the tests be standardized to some extent to insure maximum utility and validity of results. This standardization could include improvements in test conditions to achieve better and more uniform control of specimen quality, identification of key response parameters, and recommendations as to acceptable statistical analyses. For the full potential of the statistics to be realized, several of the chronic tests require better design. Most notable is the need for additional replicates for assessing the various responses of the f_0-generation. Increasing the replicates from two to perhaps four, for example, would increase the within-treatment degrees of freedom from 1 to 3 (for a simple one-way analysis of variance). With the present design, differences often have to be rather astounding to be significant because there is considerable within-treatment variation. Also, it would be desirable to stipulate minimum replication for the viability-hatching success determinations since there is the possibility that more data will be accumulated than necessary. Since few laboratories have resident biometricians, information could be wasted if the experimental design were left solely to the discretion of the investigator. This would be particularly true for the first few tests conducted.

In addition to the fundamental changes suggested above, there are specific changes in methodology which should be considered for each of the organisms we tested. The apparent lack of fertilization of spawned eggs in the trout tests was anomalous and should be studied further. It was also encountered in three other brook trout tests we conducted concurrently for a separate project (63). Possible reasons for the infertility, which appeared to be random, include physical inability to spawn because the substrates were too small or behavioral changes caused by fish density or the nature of the heirarchal relationships. It is also unknown whether all trout from this stock reach reproductive maturity in 2 years.

In the bluegill test, the fish were probably too young to spawn extensively. Better results might be achieved by beginning the test with 2-yr-old specimens. Improved techniques for incubating embryos and rearing fry should be developed which include verified methods of disease control and proven diets. Until the fry can consume brine shrimp nauplii, it would be advisable to feed them laboratory cultures of rotifers, for example, instead of "green" water because the latter might contain parasites and pathogens. Also, maintenance of high food densities, such as cultures of rotifers in phytoplankton, might cause a significant proportion of the toxicant to be sorbed to or assimilated by the food. This could alter the mode of intoxication if not the toxicant concentration. Finally, some consideration

should should be directed to the adequacy of the bluegill spawning substrates and the validity of spawn size estimates, for embryos are often spread about the adult tank, bound to debris, and eaten prior to being checked by the investigator. While the substrates were acceptable to the adults, it was difficult to remove the embryos with a fine brush. To our knowledge, no one has carefully evaluated different methods of embryo removal with reference to the injury they cause.

The conduct of chronic tests with fathead minnow is fairly routine with proper experience, and the only improvement which is recommended is to gain a better fix on the type and concentration of chemicals used for controlling fungus during embryo incubation.

In the test using cladocerans, it might be better to separately assess growth because there are not enough f_0-generation specimens available in the recommended procedure (60) and f_1-generation instars produced with a given week will of course differ in age.

In retrospect, Hyallela azteca and Chironomus No. 51 are not the most desirable species for chronic tests. Newly-hatched H. azteca are really too small for rapid enumeration or capture and would hamper a hatching success determination. A species such as Gammarus lacustris would be more desirable because it is larger (around 20 mm in adults) and quite widespread throughout the United States. Chironomus No. 51, in addition to the liability that it has not been taxonomically described, has the embryos helically arranged within the skein, which makes it very time-consuming to accurately count and separate embryos for a hatching success determination.

ACCUMULATION OF CHLORDANE

Technical chlordane was accumulated extensively in H. azteca and D. magna, but not at all in the winged adults of Chironomus No. 51. For both amphipods and daphnids, tissue concentrations of a particular component were fairly proportional to aqueous concentration. However, both species tended to concentrate the various compounds to different extents. Concentration of the components was comparable between amphipods and daphnids, except that amphipods concentrated at least 2-times more of the chlordenes and heptachlor than daphnids.

The absence of notable residues of technical chlordane in adult chironomids exposed to both 0.7 and 1.0 µg/l chlordane in separate experiments is difficult to interpret. Recent studies of DDE uptake by fourth instar C. tentans by Derr and Zabik (68) indicate a passive mode of uptake and an essentially linear relationship between aqueous and tissue DDE concentration. Similar findings have been reported earlier by Kerr and Vass (69). Although Chironomus No. 51 may possess efficient mechanisms for metabolizing or excreting technical chlordane components at all developmental stages or during the transition from larva to adult, additional experiments designed to monitor the water-tissue concentration relationships for all developmental stages and several toxicant concentrations are needed to clarify the

somewhat anomalous results we obtained for this species.

For both amphipods and daphnids, comparisons of the proportions of the chlordane components in the tissues with those in the neat material indicated that most were stored in different proportions. Cis- and trans-nonachlor were stored to a greater extent in both species than the other components. Similar findings have been made for fish collected from Lakes Superior and Huron (personal communication, L. Mueller, EPA, ERL-D). Since there appeared to be little if any compositional change relative to the neat material when technical chlordane was measured in aqueous solution, these components were either taken up preferentially, were metabolites of other constituents, or were particularly refractory to metabolism. The lower tissue ratio of heptachlor to cis- and trans-chlordane and that of the chlordane isomers to the nonachlor isomers suggests that these compounds may have been either taken up less efficiently by the two invertebrates, converted to nonachlors, or preferentially metabolized and excreted. The relationships may also be altered to some extent by the aqueous concentration since there were notable diminutions in the ratios of cis-/trans-nonachlor and to a much lesser extent for cis-/trans-chlordane at the highest insecticide levels. It can be speculated that if metabolic processes had a role in altering the proportions of the constituents in the tissues, uptake may have been sufficient in the brief period of exposure to temporarily supercede metabolic processes.

Obviously, these observations raise basic questions as to the relative uptake, metabolism, and excretion of these constituents, questions which can only be resolved by additional study. Such investigations are beneficial since the stored compounds may have different toxicological properties which would underlie any potential effects on predators. Laboratory studies of bioaccumulation should also be integrated with controlled experiments in semi-natural environments and with sampling of aquatic organisms in natural environments in order to verify the laboratory results.

LITERATURE CITED

1. Johnson, O. Pesticides '72. Chem. Week, 110:3366, 1972.

2. Lichtenstein, E. P. and J. B. Polivka. Persistance of Some Chlorinated Hydrocarbon Insecticides in Turf Soils. J. Econ. Entomol., 52:289-293, 1959.

3. Stephan, C. E. and D. I. Mount. Use of Toxicity Tests with Fish in Water Pollution Control. Amer. Soc. Test. Mater. Spec. Pub., No. 528:164-177, 1973.

4. Lloyd, R. Problems in Determining Water Quality Criteria for Freshwater Fisheries. Proc. R. Soc. Lond. B. Biol. Sci., 180:439-449, 1972.

5. Doudoroff, P., B. G. Anderson, G. E. Burdick, P. S. Galtsoff, W. B. Hart, R. Patrick, E. R. Strong, E. W. Surber, and W. M. VanHorn. Bio-Assay Methods for the Evaluation of Acute Toxicity of Industrial Wastes to Fish. Sewage Ind. Wastes, 23:1380-1397, 1951.

6. National Technical Advisory Committee on Water Quality Criteria. Water Quality Criteria. Washington, D.C., U.S. Federal Water Pollution Control Administration, 1968. 234p.

7. Mount, D. I. and C. E. Stephan. A Method for Establishing Acceptable Toxicant Limits for Fish--Malathion and the Butoxyethanol Ester of 2,4-D. Trans. Am. Fish. Soc., 96:185-193, 1967.

8. Eaton, J. G. Chronic Malathion Toxicity to the Bluegill (Lepomis macrochirus Rafinesque). Water Res. (Oxford), 4:673-684, 1970.

9. Carlson, A. R. Effects of Long-Term Exposure to Carbaryl (Sevin) on Survival, Growth, and Reproduction of the Fathead Minnow (Pimephales promelas). J. Fish. Res. Board Can. (Ottawa), 29:583-587, 1972.

10. Hermanutz, R. O., L. H. Mueller, and K. D. Kempfert. Captan Toxicity to Fathead Minnows (Pimephales promelas), Bluegills (Lepomis macrochirus), and Brook Trout (Salvelinus fontinalis). J. Fish. Res. Board Can., 30 (12): 1811-1817, 1973.

11. McKim, J. M. and D. A. Benoit. Duration of Toxicity Tests for Establishing "No Effect" Concentrations for Copper with Brook Trout (Salvelinus fontinalis). J. Fish. Res. Board Can. (Ottawa), 31: 449-452, 1974.

12. Velsicol Chemical Corporation. Standard for Technical Chlordane. Unpublished Manuscript, Velsicol Chemical Corporation, Chicago, Illinois, August, 1971.

13. U.S. Environmental Protection Agency. Technical Material Data Sheet, EPA/FDA No. 20. Environmental Protection Agency Pesticides Reference Standards Section, Chemistry Branch, Office of Pesticides Programs, Washington, D.C., 1972.

14. Saha, J. G. and Y. W. Lee. Isolation and Identification of the Components of a Commercial Chlordane Formulation. Bull. Environ. Contam. Toxicol., 4:285-296, 1969.

15. Edwards, C. A. Pesticide Residues in Soil and Water. In: Environmental Pollution by Pesticides (C.A. Edwards, editor), pp. 409-458. London, Plenum Press, 1973.

16. Bevenue, A. and C. Y. Yeo. Gas Chromatographic Characteristics of Chlordane II. Observed Compositional Changes of the Pesticide in Aqueous and Non-Aqueous Environments. J. Chromatogr. 42:45-52, 1969.

17. Schwemmer, B., W. P. Cochrane, and P. B. Polen. Oxychlordane, Animal Metabolite of Chlordane: Isolation and Synthesis. Science, 169:1087, 1970.

18. Polen, P. B., M. Hester, and J. Benziger. Characterization of Oxychlordane, Animal Metabolite of Chlordane. Bull. Environ. Contam. Toxicol., 5:521-528, 1970.

19. Dowden, B. F. Effects of Five Insecticides on the Oxygen Consumption of the Bluegill Sunfish, Lepomis macrochirus. Ph.D. Thesis, Louisiana State University, 1966. 113p. (Read Abstract Only)

20. Cope, O. B., C. M. Gjullin, and A. Storm. Effects of Some Insecticides on Trout and Salmon in Alaska, With Reference to Blackfly Control. Trans. Am. Fish. Soc., 77:160-177, 1949.

21. American Public Health Association. Standard Methods for the Examination of Water and Wastewater. 13th Edition, New York, 1971. 874p.

22. Henderson, C., Q. H. Pickering, and C. M. Tarzwell. Relative Toxicity of Ten Chlorinated Hydrocarbon Insecticides to Four Species of Fish. Trans. Am. Fish. Soc., 88:23-32, 1959.

23. Katz, M. Acute Toxicity of Some Organic Insecticides to Three Species of Salmonids and to the Threespine Stickleback. Trans. Am. Fish. Soc., 90:264-268, 1961.

24. Clemens, H. P. and K. E. Sneed. Lethal Doses of Several Commercial Chemicals for Fingerling Channel Catfish. U.S. Fish. Wildl. Serv. Spec. Sci. Rep. Fish. No. 316, 1959. 10p.

25. Macek, K. J., C. Hutchinson, and O. B. Cope. The Effects of Temperature on the Susceptibility of Bluegills and Rainbow Trout to Selected Pesticides. Bull. Environ. Contam. Toxicol., 4:174-183, 1969.

26. Konar, S. K. Experimental Use of Chlordane in Fishery Management. Prog. Fish. Cult., 30:96-99, 1968.

27. Malone, C.R. and B.G. Blaylock. Toxicity of Insecticide Formulations to Carp Embryos Reared In Vitro. J. Wildl. Manage., 34:460-463, 1970.

28. Lawrence, J. M. Toxicity of Some New Insecticides to Several Species of Pondfish. Prog. Fish. Cult., 12:141-146, 1950.

29. Khan, M. A. Q., R. H. Stanton, D. J. Sutherland, J. D. Rosen, and N. Maitra. Toxicity-Metabolism Relationship of the Photoisomers of Certain Chlorinated Cyclodiene Insecticide Chemicals. Archiv. Environ. Contam. Toxicol., 1:159-169, 1973.

30. Lüdemann, V. D. and H. Neumann. Über die Wirkung der Neuzeitlichen Kontaktinsektizide auf die Tiere des Süsswassers. Anz. Schaedlingskd. Plfanzen.-Umweltschutz. (Berlin), 35:5-9, 1962.

31. Holden, A. V. Effects of Pesticides on Fish. In: Environmental Pollution by Pesticides, (C.A. Edwards, editor), pp. 213-253. London, Plenum Press, 1973.

32. Koch, R. B., L. K. Cutkomp, and H. H. Yap. Inhibition of Oligomycin Sensitive and Insensitive Fish Adenosine Triphosphatase Activity by Chlorinated Hydrocarbon Insecticides. Biochem. Pharmacol. (Oxford), 20:3243-3245, 1971.

33. Summerfelt, R. C. and W. M. Lewis. Repulsion of Green Sunfish by Certain Chemicals. J. Water Pollut. Control Fed., 39:2030-2038, 1967.

34. Mulla, M. Toxicity of Organochlorine Insecticides to the Mosquito Fish Gambusia affinis and the Bullfrog Rana catesbeiana. Mosq. News, 23:299-303, 1963.

35. Sanders, H. O. Toxicity of Pesticides to the Crustacean Gammarus lacustris. U.S. Bur. Sport Fish. Wildl. Tech. Pap., No. 25, 1969. 18p.

36. Silvey, J. K. G. Bloodworms in Distribution Systems. J. Water Works Assoc., 48:275-280, 1956.

37. Michael, A. S., C. G. Thompson, and M. Abramowitz. Artemia salina as a Test Organism for Bioassay (of Insecticides). Science, 123: 464, 1956.

38. Butler, P. A., A. J. Wilson, Jr., and A. J. Rick. Effect of Pesticides on Oysters. Proc. Natl. Shellfish. Assoc., Aug. 1960, 51:23-32, 1962.

39. Lichtenstein, E. P. and K. R. Schulz. Persistence of Some Chlorinated Hydrocarbon Insecticides as Influenced by Soil Types. Rate of Application and Temperature. J. Econ. Entomol., 52:124-131, 1959.

40. Godsil, P. J. and W. C. Johnson. Pesticide Monitoring of the Aquatic Biota in the Tule Lake National Wildlife Refuge. Pest. Monit. J., 1:21-26, 1968.

41. Henderson, C., W. L. Johnson, and A. Inglis. Organochlorine Insecticide Residues in Fish (National Pesticide Monitoring Program). Pest. Monit. J., 3:145-171, 1969.

42. Green, R. S., C. G. Gunnerson, J. J. Lichtenberg, and N. C. Brady (ed.). Agriculture and the Quality of our Environment. Am. Assoc. Adv. Sci. Publ., No. 85, 1967. 137p.

43. Duke, T. W. and A. J. Wilson, Jr. Chlorinated Hydrocarbons in Livers of Fishes From the Northeastern Pacific Ocean. Pest. Monit. J., 5:228-232, 1971.

44. The Committee on Methods for Toxicity Tests with Aquatic Organisms. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. U.S. Environ. Prot. Agency Ecol. Res. Ser., No. EPA-660/3-75-009, 1975. 61p.

45. Sprague, J. B. Measurement of Pollutant Toxicity to Fish I. Bioassay Methods for Acute Toxicity. Water Res. (Oxford), 3:793-821, 1969.

46. Mount, D. I. and W. A. Brungs. A Simplified Dosing Apparatus for Fish Toxicology Studies. Water Res. (Oxford), 1:21-29, 1967.

47. Houston, A. H., J. A. Madden, R. J. Woods, and H. M. Miles. Some Physiological Effects of Handling and Tricaine Methanesulphonate Anesthetization upon the Brook Trout, Salvelinus fontinalis. J. Fish. Res. Board Can. (Ottawa), 28:625-633, 1971.

48. Houston, A. H., J. A. Madden, R. J. Woods, and H. M. Miles. Variations in the Blood and Tissue Chemistry of Brook Trout, Salvelinus fontinalis, Subsequent to Handling, Anesthesia, and Surgery. J. Fish. Res. Board Can. (Ottawa), 28:635-642, 1971.

49. Wedemeyer, G. Physiological Consequences of Handling Stress in the Juvenile Coho Salmon (Oncorhynchus kisutch) and Steelhead Trout (Salmo gairdneri). J. Fish. Res. Board Can. (Ottawa), 29:1780-1783, 1972.

50. U.S. Environmental Protection Agency. Methods for Chemical Analysis of Water and Wastes. Analytical Quality Control Laboratory, Cincinnati, Ohio. 1971. 312p.

51. U.S. Environmental Protection Agency. Recommended Bioassay Procedure for Fathead Minnow Pimephales promelas (Rafinesque) Chronic Tests. Unpublished Manuscript, Environmental Research Laboratory (Formerly National Water Quality Laboratory), Duluth, Minnesota. 1972. 13p.

52. Mount, D. I. Chronic Toxicity of Copper to Fathead Minnows (Pimephales promelas, Rafinesque). Water Res. (Oxford), 2:215-223, 1968.

53. McKim, J. M. and D. A. Benoit. Effect of Long-Term Exposures to Copper on Survival, Reproduction, and Growth of Brook Trout Salvelinus fontinalis (Mitchill). J. Fish. Res. Board Can. (Ottawa), 28:655-662, 1971.

54. U.S. Environmental Protection Agency. Recommended Biosasay Procedure for Bluegill Lepomis macrochirus (Rafinesque) Partial Chronic Tests. Unpublished Manuscript. Environmental Research Laboratory (formerly National Water Quality Laboratory), Duluth, Minnesota. 1972. 11p.

55. Smith, W. A Cyprinodontid Fish, Jordanella floridae, as a Reference Animal for Rapid Chronic Bioassays. J. Fish. Res. Board Can. (Ottawa), 30:329-330, 1973.

56. U.S. Environmental Protection Agency. Recommended Bioassay Procedure for Brook Trout Salvelinus fontinalis (Mitchill) Partial Chronic Tests. Unpublished Manuscript. Environmental Research Laboratory (formerly National Water Quality Laboratory), Duluth, Minnesota. 1972. 12p.

57. Hoffman, G. L. Parasites of Freshwater Fish I. Fungi 1. Fungi (Saprolegnia and Relatives) of Fish and Fish Eggs. U.S. Bur. Sport Fish Wild. Fish Disease Leaflet, No. 21, 1969. 6p.

58. Benoit, D. A. Artificial Laboratory Spawning Substrate for Brook Trout (Salvelinus fontinalis, Mitchill). Trans. Am. Fish. Soc., 103:144-145, 1974.

59. U.S. Environmental Protection Agency. Tentative Bioassay Procedure for the Amphipod, Gammarus pseudolimnaeus Bousfield. Unpublished Manuscript. Environmental Research Laboratory (formerly National Water Quality Laboratory), Duluth, Minnesota. 1971. 1p.

60. U.S. Environmental Protection Agency. Recommended Bioassay Procedure for Daphnia magna Chronic Tests in a Flowing System. Unpublished Manuscript. Environmental Research Laboratory (formerly National Water Quality Laboratory), Duluth, Minnesota. 1971. 3p.

61. Brungs, W. A. Effects of Residual Chlorine on Aquatic Life. J. Water Pollut. Control Fed., 45:2180-2193, 1973.

62. U.S. Environmental Protection Agency. Proposed Midge Bioassay Procedure - Chironomus plumosus. Unpublished Manuscript. Environmental Research Laboratory (formerly National Water Quality Laboratory), Duluth, Minnesota. 1971. 4p.

63. Cardwell, R. D., D. G. Foreman, T. R. Payne, and D. J. Wilbur. Acute and Chronic Toxicity of Four Organic Chemicals to Fish. U.S. Environ. Prot. Agency Ecol. Res. Ser., (in preparation).

64. Litchfield, J. T., Jr. and F. Wilcoxon. A Simplified Method of Evaluating Dose-Effect Experiments. J. Pharmacol. Exp. Ther., 96:99-113, 1949.

65. Dixon, W. J. (ed.). BMD Biomedical Computer Programs. Berkeley, University of California Press, 1973. 773p.

66. Bliss, C. I. Confidence Limits for Biological Assays. Biometrics Bull., 1:57-65, 1945.

67. Steel, R. G. D. and J. H. Torrie. Principles and Procedures of Statistics. New York. McGraw-Hill Book Company, Inc. 481 p.

68. Derr, S. K. and M. J. Zabik. Bioactive Compounds in the Aquatic Environment: Studies on the Mode of Uptake of DDE by the Aquatic Midge, Chironomus tentans (Diptera:Chironomidae). Archiv. Environ. Contam. Toxicol., 2:152-164, 1974.

69. Kerr, S. R. and W. P. Vass. Pesticide Residues in Aquatic Invertebrates. In: Environmental Pollution by Pesticides (C. A. Edwards, editor), p. 134-180. London, Plenum Press. 1973.

BIBLIOGRAPHY[a]

Ali, M., R. H. Sawy, and M. M. Bishara. Toxicity of Insecticides to Aquatic Fauna. Agr. Res. Rev. (Cairo) 36:159-165, 1958.

Baker, W. C. and H. F. Schoof. Temporary Control of Adult Mosquitos at Outdoor Places of Public Assembly. Mosquito News 15:32-34, 1955.

Belois, G. D. and G. Familiares. Resistance of Anopheles Larvae to Chlordan and Dieldrin. Field and Laboratory Tests of Anopheles sacharoni. Bull. World Health Organization 15:415-423, 1956.

Bowman, M. C., F. Acree, Jr., C. S. Lofgren, and M. Beroza. Chlorinated Insecticides' Fate in Aqueous Suspensions Containing Mosquito Larvae. Science 146:1480-1481, 1964.

Burchfield, H. P., J. D. Hilchey, and E. E. Storrs. An Objective Method for Insecticide to Bioassay Based on Photomigration of Mosquito Larvae. Contrib. Boyce Thompson Institute 17:57-86, 1952.

Chapman, H. C., J. C. Keller, and G. C. Labrecque. Relative Effectiveness of Several Insecticides as Sprays and as Fogs Against Salt-Marsh Mosquito Adults. Mosquito News 14:1-5, 1954

Coluzzi, A. and G. Raffaele. Residual Action of DDT and Chlordan on Houseflies and mosquitoes. Riv. Malariol. 30:113-136, 1951.

Davidson, G. Insecticide Resistance in Anopheles gambiae. Nature (London) 178:705-706, 1956.

Ferrigno, F. and T. F. Bast. Chemical Mosquito Control Evaluations on Salt-Hay Marshes. Proc. New Jersey Mosquito Exterm. Assoc., 49th Annual Meeting, p. 97-111. 1962.

Gentry, J. W. and A. A. Hubert. Resistance of Culex quinquefasciatus to Chlorinated Hydrocarbons on Okinawa. Mosquito News 17:92-93, 1957.

[a]References read only in abstract. Most of the references to fish and aquatic invertebrates other than mosquitoes were not used either because they were unavailable or not pertinent.

Ginsburg, J. M. Tests with New Toxicants in Comparison with DDT on Mosquito Larvae and Fish. Proc. New Jersey Mosquito Exterm. Assoc., 34th Annual Meeting, p. 132-135. 1947.

Hadaway, A. B. and F. Barlow. Aqueous Suspension of Insecticides. The Behavior of Mosquitoes in Contact with Insecticidal Deposits. Bull. Entomol. Res. 44:255-271, 1953.

Hocking, B., C. R. Twinn, and W. C. McDuffie. Preliminary Evaluations of Some Insecticides Against Immature Stages of Blackflies (Diptera: Simuliidae). Sci. Agr. 29:69-80, 1949.

Hoffman, R. A. Results of 1953-54 Field Tests with Insecticides for Control of Mosquitoes in Oregon. Proc. Papers Ann. Conf. Calif. Mosquito Control Assoc., p. 80-82. 1955.

Jammback, H. and W. Wall. Control of Salt Marsh Tabanus Larvae with Granulated Insecticides. J. Econ. Entomol. 50:379-382, 1957.

Keller, J. C., H. C. Chapman, and G. Labrecque. Tests with Granulated Insecticides with Control of Salt-Marsh Mosquito Larvae. Mosquito News 14:5-9, 1954.

Keller, J. C., G. C. Labrecque, and H. C. Chapman. Seasonal Variations in Susceptibility of Salt-Marsh Mosquito Larvae to Insecticides. Mosquito News 16:20-21, 1956.

Labrecque, G. C., J. R. Noe, and J. B. Gahan. Effectiveness of Insecticides on Granular Clay Carriers Against Mosquito Larvae. Mosquito News 16:1-3, 1956.

Lividas, G. Resistance of Anophelines to Chlorinated Insecticides in Greece. Mosquito News 15:67-71, 1955.

Ludvik, G. F. Topical Application of Insecticide Solution to Anopheles quadrimaculatus. J. Econ. Entomol. 46:364-365, 1953.

Mehrle, P. M., W. W. Johnson, and F. L. Mayer, Jr. Nutritional Effects on Chlordane Toxicity in Rainbow Trout. Bull. Environ. Toxicol. 12:513-517, 1974.

Minchew, C. D. and D. E. Ferguson. Toxicities of 6 Insecticides to Resistant and Susceptible Green Sunfish and Golden Shiners in Static Bioassays. J. Miss. Acad. Sci. 15:29-32, 1970.

Misra, J. N., S. L. Perti, and R. K. Paul. Residual Effects of Insecticides Applied on Mud Surface. Indian J. Malariol. 17:107-111, 1963.

Moretti, G. P. Chlorinated Insecticides and Their Toxicity to Certain Aquatic Arthropods and Vertebrates. Att. Soc. Ital. Sci. Nat. Museo Civico Storia Nat. (Milano) 87:5-39, 1948.

Mulla, M. S. Frog and Food Control With Insecticides. Pest Control 30: 64, 1964.

Nagasawa, S. Comparison of the Toxicities of γ - Benzene Hexachloride, Chlordan, and p, p'-DDT to the Pupa of the Common House Mosquito (Culex pipiens). Botyu Kagaku, No. 11, p. 20-23, 1949.

Naqvi, S. M. and D. E. Ferguson. Pesticide Tolerance of Selected Freshwater Invertebrates. J. Miss. Acad. Sci. 14:121-127, 1969.

Pal, R., M. I. D. Sharma, and B. S. Krishnamurthy. Laboratory and Field Studies on Residual Toxicity of Chlordan, Aldrin, and Dieldrin Against Mosquitoes. Indian J. Malariol. 5:559-568, 1951.

Reid, J. A. Laboratory Method for Testing Residual Insecticides Against Anopheline Mosquitoes. Bull. Entomol. Res. 41:761-777, 1951.

Travis, B. V. and W. C. McDuffie. Chemicals for Use in Mosquito Control. Proc. New Jersey Mosquito Exterm. Assoc. 37:96-100, 1950.

APPENDIX TABLES

APPENDIX TABLE 1. APPROXIMATE COMPOSITION OF TECHNICAL CHLORDANE AS REFLECTED IN "NORMALIZED" CHROMATOGRAM OF SIGNIFICANT PEAKS[a]

Constituent		Percentage
$C_{10}H_7Cl_5$ - Diels-Alder Adduct (DAA): Pentachlorocyclopentadiene and cyclopentadiene (C_5H_6; "Cyclo")		2 ± 1%
$C_{10}H_6Cl_6$ - Isomers in order of GLC retention time		
(1) Isomer-1, chlordane - DAA; hexachlorocyclopentadiene (Hex) and "Cyclo"		1 ± 1%
(2) Isomer-2		7.5 ± 2%
(3) Isomers-3, 4 (combined)		13 ± 2%
$C_{10}H_5Cl_7$ - Heptachlor		10 ± 3%
$C_{10}H_6Cl_8$ - Chlordane isomers		
(1) cis-chlordane		19 ± 3%
(2) trans-chlordane		24 ± 2%
$C_{10}H_5Cl_9$ - Nonachlor		7 ± 3%
Other constituents:		
Hex (C_5Cl_6)	Maximum	1%
Octachlorocyclopentene		1 ± 1%
$C_{10}H_{7-8}Cl_{6-7}$		8.5 ± 2%
Constituents of lower GLC retention time than C_5Cl_8, including Hex		2 ± 2%
Constituents of higher GLC retention time than nonachlor		4 ± 3%

[a]The foregoing approximations are based upon unadjusted values derived from moderate resolution gas-liquid chromatography. Apparent values obtained are typically influenced by conditions of analysis and the chromatographic systems employed, and the relative response sensitivity of the components. Under standardized conditions, these profiles are useful in comparing Technical Chlordane samples with Reference Technical Chlordane (taken from Velsicol Chemical Corp. [12]).

APPENDIX TABLE 2. REPRESENTATIVE QUALITY OF LABORATORY WATER

Variable	Unit	Mean concentration	Variable	Unit	Mean concentration
Calcium	mg/l	31.1	Cyanide	mg/l	0.0005
Magnesium	mg/l	13.1	Iron	mg/l	0.001
Potassium	mg/l	2.0	Copper	mg/l	0.005
Sodium	mg/l	15.4	Zinc	mg/l	0.001
Chloride	mg/l	11.3	Cadmium	mg/l	0.010
Sulfate	mg/l	8.6	Chromium	mg/l	0.025
Sulfide	mg/l	<0.002	pH		7.70
Nitrate	mg/l	4.65	Alkalinity	mg/l $CaCO_3$	166
Nitrite	mg/l	0.005	Acidity	mg/l $CaCO_3$	6
Ammonia	mg/l NH_3-N	0.16	Total hardness	mg/l $CaCO_3$	156
Phenol	mg/l	0.001	Specific conductance	µmhos/cm	376
Fluoride	mg/l	0.96			

APPENDIX TABLE 3. STANDARD CONCENTRATION-PERCENT MORTALITY DATA SUPPLIED BY COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS[a]

	Percent mortality						
	Toxicant concentration, µg/l						
Data set	Control	7.8	13	22	36	60	100
A	0	0	0	10	100	100	100
B	0	0	0	70	100	100	100
C	0	0	0	10	40	100	100
D	0	0	0	20	70	100	100
E	0	0	0	20	30	100	100

[a]Data used to check the validity of statistical analyses of acute toxicity test results. Ten specimens/treatment.

APPENDIX TABLE 4. COMPARISON OF MEDIAN LETHAL CONCENTRATIONS AND 95% CONFIDENCE LIMITS WITH OTHER AQUATIC TOXICOLOGY LABORATORIES[a]

	Our laboratory					
	Litchfield and Wilcoxon (64)		Computer program		Average of eight other laboratories[b]	
Data set	LC50, µg/l	95% confidence limits for LC50	LC50, µg/l	95% confidence limits for LC50	LC50,[c] µg/l	95% confidence limits for LC50
A	25.4	22.1 - 29.2	...[d]	...	25.3 ±1.9	20.4 - 32.0 ±5.1 ±4.8
B	21.2	18.8 - 23.9	...	...	20.4 ±0.9	14.8 - 49.3 ±4.0 ±57.0
C	35.5	28.2 - 44.7	35.6	30.3 - 41.7	35.3 ±2.4	27.5 - 45.1 ±3.3 ±3.6
D	29.8	23.9 - 37.2	29.5	25.0 - 34.7	29.4 ±0.8	23.5 - 36.9 ±1.9 ±1.2
E	35.5	27.1 - 46.9	35.5	29.1 - 43.3	36.5 ±3.4	28.2 - 46.8 ±3.5 ±4.3

[a]All calculations performed on standard data given in Appendix Table 3.

[b]Results obtained through use of both manual and computer methods.

[c]Means ± 1 standard deviation are given for concentrations in µg/l.

[d]Calculations not made since computer was not programmed to process data having only one partial kill.

APPENDIX TABLE 5. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR *DAPHNIA MAGNA* AND *HYALLELA AZTECA* EXPOSED TO TECHNICAL CHLORDANE

Exposure time, hr	LC50, µg/l	95% confidence limits for LC50, µg/l	$\hat{\sigma}$[a]	Log-probit regression equation[b]
D. magna - Test 1				
70	31.1	27.1 - 35.7	0.1594	$-4.37+6.28 (\log x_i)$
96	28.4	25.3 - 31.9	0.1329	$-5.94+7.53 (\log x_i)$
D. magna - Test 2				
66[c]	42.2	39.0 - 45.7	1.1121	...
74[c]	37.5	34.0 - 41.3	1.1726	...
94[c]	35.2	30.1 - 41.2	1.2910	...
Hyallela azteca				
168	97.1	70.9 - 133.0	0.4056	$0.10+2.47 (\log x_i)$

[a]Logarithm of the standard deviation of the population tolerance frequency distribution.

[b]Probit $\hat{y}_i = a + b (\log x_i)$.

[c]Calculated according to the method of Litchfield and Wilcoxon (64). The slope function S replaces $\hat{\sigma}$, and is the antilogarithm of $\hat{\sigma}$.

APPENDIX TABLE 6. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR FATHEAD MINNOW JUVENILES EXPOSED TO TECHNICAL CHLORDANE

Exposure time, hr	LC50, µg/l	95% confidence limits for LC50, µg/l	$\hat{\sigma}$[a]	Log-probit regression equation[b]
45[c]	53.4	...	...	...
72	41.7	38.3 - 45.5	0.1022	$-10.86+9.79 (\log x_i)$
96	36.9	33.0 - 41.3	0.1301	$- 7.27+7.69 (\log x_i)$
120	35.9	32.6 - 39.5	0.112	$- 9.11+9.08 (\log x_i)$
168	33.9	30.5 - 37.6	0.1175	$- 8.03+8.51 (\log x_i)$
192	32.1	29.5 - 35.0	0.0956	$-10.76+10.46 (\log x_i)$

[a]Logarithm of the standard deviation of the population tolerance frequency distribution.

[b]Probit $\hat{y}_i = a + b (\log x_i)$.

[c]Median lethal time.

APPENDIX TABLE 7. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR BROOK TROUT EXPOSED TO TECHNICAL CHLORDANE

Exposure time, hr	LC50, µg/l	95% confidence limits for LC50, µg/l	a $\hat{\sigma}$	Log-probit regression equation[b]
27.2[c]	125	...	...	...
28.9[c]	117	...	...	...
46	102	93 - 112	0.0681	$-24.49+14.68 (\log x_i)$
96[d]	47	...	...	...
118	39	34 - 44	0.0450	$-30.30+22.21 (\log x_i)$
142	31	26 - 38	0.1494	$15,05+6.69 (\log x_i)$
166	25	21 - 29	0.1192	$18.49+8.39 (\log x_i)$

[a]Logarithm of the standard deviation of the population tolerance frequency distribution.

[b]Probit $\hat{y}_i = a + b (\log x_i)$.

[c]Median lethal time.

[d]Interpolated from regression equation; (LC50) calculated from known LC50 values and exposure times.

APPENDIX TABLE 8. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR BLUEGILL EXPOSED TO TECHNICAL CHLORDANE

Exposure time, hr	LC50, µg/l	95% confidence limits for LC50, µg/l	$\hat{\sigma}$[a]	Log-probit regression equation[b]
48	121	98 - 149	0.2051	$-5.15+4.88 (\log x_i)$
72	77	68 - 87	0.1397	$-8.50+7.16 (\log x_i)$
96	59	50 - 71	0.2057	$-3.62+4.86 (\log x_i)$
120	46	39 - 54	0.1759	$-4.40+5.65 (\log x_i)$
144	40	35 - 45	0.1233	$-7.99+8.11 (\log x_i)$

[a]Logarithm of the standard deviation of the population tolerance frequency distribution.

[b]Probit $\hat{y}_i = a + b (\log x_i)$.

APPENDIX TABLE 9. SIGNIFICANCE OF DIFFERENCES IN 96-HR LC50 BETWEEN DAPHNIA MAGNA, FATHEAD MINNOWS, BROOK TROUT, AND BLUEGILL

Comparison	t-value	Significance
D. magna vs fathead minnow	-1.754	N.S.
Fathead minnow vs brook trout	-1.232	N.S.
Brook trout vs bluegill	-1.094	N.S.
D. magna vs brook trout	-3.520	$p<0.001$
D. magna vs bluegill	-2.989	$p<0.001$
Fathead minnow vs bluegill	-1.651	N.S.

APPENDIX TABLE 10. WATER QUALITY DURING EXPOSURE OF FATHEAD MINNOWS TO CHLORDANE

Date	Water temperature, °C	Dissolved oxygen, mg/l	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
12/11/72	21.5 ± 0.6	9.0	102.1	7.81	162	163	...[a]
12/18/72	22.5 ± 0.9	8.2	93.0	7.80	158	...	...
12/25/72	23.8 ± 0.9	8.7	99.2	8.18	164	168	...
1/1/73	23.2 ± 0.5	8.6	101.5	7.94	166	157	...
1/8/73	24.0 ± 1.9	8.5	95.9	7.91	165	155	...
1/15/73	24.1 ± 2.3	7.6	92.3	7.93	165	159	...
1/22/73	23.4 ± 1.2	7.5	88.4	7.79	168	173	...
1/29/73	24.7 ± 0.4	7.8	92.8	7.86	166	160	...
2/5/73	24.5 ± 0.7	7.5	88.0	7.88	163	161	...
2/12/73	24.9 ± 0.2	7.7	91.1	7.79	163	158	...
2/19/73	25.0 ± 0.3	7.4	87.8	7.80	157	155	...
2/26/73	25.0 ± 0.4	...	...	7.68	162	163	368
3/5/73	25.3 ± 0.2	7.4	88.6	7.67	168	172	337

Continued ....

APPENDIX TABLE 10. WATER QUALITY DURING EXPOSURE OF FATHEAD MINNOWS TO CHLORDANE--continued

Date	Water temperature, °C	Dissolved oxygen, mg/l	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
3/12/73	25.0 ± 0.3	6.6	77.5	7.70	163	158	360
3/19/73	24.8 ± 0.8	6.4	75.7	7.58	163	164	377
3/26/73	24.4 ± 1.4	6.8	80.4	7.60	163	167	327
4/2/73	25.1 ± 0.2	6.8	81.2	7.62	167	146	383
4/9/73	25.2 ± 0.3	6.9	81.9	7.50	161	142	360
4/16/73	25.3 ± 0.2	6.2	74.1	7.47	166	158	385
4/23/73	25.1 ± 0.7	6.4	77.8	7.50	160	148	380
4/30/73	25.3 ± 0.3	6.2	74.1	7.60	159	143	360
5/7/73	25.3 ± 0.2	6.8	81.4	7.70	162	145	...
5/14/73	25.1 ± 0.4	5.7	68.1	7.45	166	161	410
5/21/73	24.9 ± 0.2	6.1	72.0	7.52	162	152	385
5/28/73	25.6 ± 0.1	6.0	72.8	7.73	160	160	400
6/4/73	25.3 ± 0.2	6.1	73.5	7.48	158	147	370

Continued ....

APPENDIX TABLE 10. WATER QUALITY DURING EXPOSURE OF FATHEAD MINNOWS TO CHLORDANE--continued

Date	Water temperature, °C	Dissolved oxygen, mg/l	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
6/11/73	24.9 ± 0.1	5.9	82.0	7.55	...	162	400
6/18/73	24.8 ± 0.1	6.0	71.1	7.73	169	153	388
6/25/73	24.8 ± 0.2	5.8	69.9	7.74	165	137	337
7/2/73	24.7 ± 0.4	6.5	73.0	7.60	169	152	351
7/9/73	24.4 ± 0.5	6.6	77.7	7.72	168	147	354
7/16/73	24.4 ± 0.3	6.4	75.7	7.64	172	154	375
7/23/73	24.9 ± 0.4	6.5	77.6	7.57	172	160	398
7/30/73	25.2 ± 0.1	6.6	78.1	7.62	170	152	379
8/6/73	24.9 ± 0.2	6.6	78.6	7.57	176	162	398
8/13/73	24.9 ± 0.1	6.6	79.9	7.65	174	159	408
8/20/73	24.9 ± 0.1	6.7	79.4	7.67	172	157	339
8/27/73	24.5 ± 0.1	7.3	86.9	7.73	169	155	376
9/3/73	24.3 ± 0.1	7.4	88.1	7.65	173	162	404

Continued ....

APPENDIX TABLE 10. WATER QUALITY DURING EXPOSURE OF FATHEAD MINNOWS TO CHLORDANE--continued

Date	Water temperature, °C	Dissolved oxygen, mg/l	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
9/10/73	24.6 ± 0.3	6.9	87.3	7.67	168	160	375
9/24/73	24.3 ± 0.1	7.0	82.9	7.88	165	145	365
10/1/73	24.6 ± 0.3	6.9	82.2	7.70	170	166	405
10/8/73	24.3 ± 0.1	...	...	7.89	163	148	389
Mean	...	6.9	82.5	7.70	166	156	376
Standard deviation	...	0.8	8.7	0.15	5	8	23
No. of observations[b]	...	237	237	180	180	178	61

[a]No observation. Majority of later conductance readings were composites collected from control, mid-range, and high chlordane concentrations.

[b]Monitored continuously.

APPENDIX TABLE 11. MEASURED TECHNICAL CHLORDANE CONCENTRATIONS DURING CHRONIC TOXICITY TEST USING FATHEAD MINNOWS

Sample date	Desired concentration of chlordane, µg/l					
	Control	0.625	1.25	2.50	5.0	10.0
3/22/73	0.010	0.661	1.050	1.520	3.150	6.830
4/6/73	0.120	0.490	0.530	1.820	2.730	6.460
4/11/73	0.010	0.246	0.678	0.930	2.960	4.840
4/16/73	0.066	0.260	0.512	0.794	1.840	3.490
4/24/73	Tr[a]	0.341	0.646	1.720	3.280	6.970
4/30/73	Tr	0.238	0.585	0.860	1.750	3.450
5/7/73	0.082	0.190	0.678	0.214	0.358	0.888
5/18/73	0.039	0.397	0.755	1.530	3.520	5.790
5/24/73	Tr	0.226	0.684	1.290	3.460	7.070
5/30/73	0.140	0.552	0.716	1.660	3.590	2.920
6/8/73	Tr	0.362	0.728	2.340	3.950	7.710
6/13/73	Tr	0.338	0.429	1.240	2.380	4.500
6/20/73	0.080	0.198	1.500	0.264	0.322	...[b]
7/3/73	Tr	0.444	0.906	1.750	3.300	7.420
7/11/73	Tr	0.557	0.836	1.910	3.830	6.380
7/18/73	Tr	0.526	0.960	1.800	3.880	7.690
7/26/73	Tr	0.514	0.854	1.590	3.060	7.000
8/16/73	Tr	0.072	0.437	1.510	2.620	6.440
8/30/73	Tr	0.136	...[c]	...[c]	...[c]	5.430
9/6/73	Tr	0.246	...	...	2.840	...[b]
9/13/73	Tr	0.311	...	...	...	...[c]
9/17/73	Tr	0.614	...	...	...	8.580
9/27/73	Tr	0.437	...	...	4.360	11.420
10/4/73	Tr	0.290	...	...	...	...[c]
10/9/73	Tr	...[b]	...	...	2.620	5.280
Mean	Tr	0.360	0.749	1.375	2.780	6.027
Standard deviation	...	0.159	0.254	0.570	1.058	2.248
No. of observations	24	24	18	18	21	21

[a]Trace amounts of less than 5 ng/l chlordane detected.

[b]No observation.

[c]No measurements made because of no fish in test container.

APPENDIX TABLE 12. WATER QUALITY DURING CHRONIC EXPOSURE OF BLUEGILL TO TECHNICAL CHLORDANE

Date	Water temperature, °C	Dissolved oxygen, mg/l	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
12/12/72[a]	26.5 ± 0.4[b]	5.0	61.2	7.65	164	154	...[c]
12/20/72	19.4 ± 0.5	6.2	67.7	7.46	155	173	...
12/27/72	19.5 ± 0.7	5.5	59.9	7.54	167	171	...
1/3/73	19.1 ± 0.5	6.5	70.2	7.58	164	157	...
1/10/73	19.2 ± 0.9	5.6	61.1	7.55	170	157	...
1/19/73	19.3 ± 0.5	6.3	60.4	7.60	166	157	...
1/24/73	18.8 ± 0.6	5.6	60.4	7.50	166	161	...
1/31/73	20.4 ± 0.1	5.9	63.0	7.53	165	159	...
2/7/73	20.2 ± 0.	4.9	53.2	7.48	165	164	...
2/14/73	20.1 ± 0.1	5.5	60.0	7.54	157	156	...
2/21/73	20.2 ± 0.1	5.6	52.6	7.49	167	167	...
2/28/73	20.1 ± 0.1	5.2	56.7	7.40	160	...	...
3/7/73[d]	20.6 ± 0.	4.8	51.8	7.32	168	166	380

Continued ....

APPENDIX TABLE 12. WATER QUALITY DURING CHRONIC EXPOSURE OF BLUEGILL TO TECHNICAL CHLORDANE
continued

Date	Water temperature, °C	Dissolved oxygen, mg/l	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
3/14/73	20.4 ± 0.2	5.3	57.5	7.43	163	166	353
3/19/73	20.3 ± 0.2	5.0	54.7	...	...	...	...
3/21/73	20.1 ± 0.1	4.7	50.6	7.26	163	172	353
3/28/73	20.1 ± 0.1	4.3	46.9	7.32	164	171	377
3/31/73[e]	...	7.0	81.5	...	...	...	...
4/4/73	21.1 ± 1.2	6.3	68.6	7.37	168	155	380
4/11/73	23.9 ± 0.5	6.3	85.2	7.52	160	143	362
4/18/73	23.8 ± 1.6	5.8	67.8	7.50	166	158	360
4/25/73[f]	25.0 ± 0.5	4.0	48.5	...	...	...	...
4/26/73[f]	...	4.6	54.4	7.32	161	146	370
4/30/73	26.7 ± 1.1	6.3	78.9	...	...	...	...
5/2/73	...	5.5	69.2	7.62	163	159	...
5/9/73	27.6 ± 0.3	5.8	69.6	7.73	162	146	393

Continued ....

APPENDIX TABLE 12. WATER QUALITY DURING CHRONIC EXPOSURE OF BLUEGILL TO TECHNICAL CHLORDANE

continued

Date	Water temperature, °C	Dissolved oxygen, mg/l	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
5/17/73	28.0 ± 0.2	6.0	76.0	7.73	164	155	420
5/23/73	28.1 ± 0.3	5.7	72.6	7.53	161	143	387
5/31/73	28.2 ± 0.2	6.5	81.6	7.91	155	155	...
6/6/73	28.2 ± 0.3	5.1	64.7	7.54	160	146	385
6/13/73	28.0 ± 0.1	6.2	78.1	7.74	161	147	370
6/20/73	27.9 ± 0.1	5.9	74.1	7.82	164	153	390
6/27/73	28.0 ± 0.1	6.5	82.1	8.02	175	159	395
7/3/73	27.9 ± 0.2	5.8	73.8	7.73	167	145	375
7/10/73	27.8 ± 0.1	6.6	83.3	7.85	167	148	370
7/17/73	27.7 ± 0.1	6.1	76.6	7.78	166	141	375
7/24/73	27.9 ± 0.1	6.7	85.0	7.94	167	146	380
7/31/73	28.0 ± 0.1	6.4	80.5	7.70	170	152	400
8/6/73	27.7 ± 0.1	6.8	84.0	7.84	173	153	390

Continued ....

APPENDIX TABLE 12. WATER QUALITY DURING CHRONIC EXPOSURE OF BLUEGILL TO TECHNICAL CHLORDANE
continued

Date	Water temperature, °C	Dissolved oxygen, mg/l	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
8/13/73	27.9 ± 0.2	6.1	77.2	7.74	173	159	395
8/20/73	28.0 ± 0.1	5.6	70.9	7.71	173	157	385
8/27/73	27.7 ± 0.1	6.1	77.2	7.69	169	154	385
9/4/73	27.7 ± 0.1	6.4	80.6	7.79	173	159	394
9/11/73	27.9 ± 0.2	5.8	69.1	7.72	169	158	371
9/18/73	27.8 ± 0.1	...	...	...	...	...	...
9/25/73	27.4 ± 0.2	6.6	82.8	7.91	165	144	368
10/2/73[g]	27.6 ± 0.2	7.0	88.3	7.93	169	162	390
10/9/73	27.4 ± 0.1	6.9	87.5	7.98	161	145	386
10/16/73	27.9 ± 0.1	6.3	80.1	7.80	166	157	386
11/23/73	27.9 ± 0.1	6.9	87.4	7.92	159	146	368

Continued ....

APPENDIX TABLE 12. WATER QUALITY DURING CHRONIC EXPOSURE OF BLUEGILL TO TECHNICAL CHLORDANE
continued

Date	Water temperature, °C	Dissolved oxygen, mg/l	Dissolved oxygen, % saturation	pH	Total alkalinity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
Mean	24.7 ± 3.8	5.9	69.9	7.65	165	156	380
Standard deviation	...	0.7	11.8	0.20	5	9	14
No. of observations	267	279	279	184	184	184	31

[a]Dissolved oxygen determined with Yellow Springs Instrument Company oxygen meter (Model No. 54).
[b]Values given are means, except for water temperature where mean ± 1 standard deviation is given.
[c]No observation.
[d]Dissolved oxygen determined with azide modification of Winker method (21).
[e]Artificial aeration instituted.
[f]Malfunction of aeration equipment.
[g]Mean acidity from 10/2/73 to 11/23/73 was 4.42 mg/l $CaCO_3$ with a standard deviation of 2.44.

APPENDIX TABLE 13. TECHNICAL CHLORDANE CONCENTRATIONS DURING CHRONIC TOXICITY TEST USING BLUEGILL

Sample date	Nominal concentration of chlordane, µg/l					
	Control	0.625	1.25	2.5	5	10
3/26/73[a]	0.08	0.06	0.93	0.72	1.95	2.52
3/20/73	0.06	0.26	0.87	0.94	2.10	5.84
4/5/73	0.05	0.05	0.22	0.36	1.00	2.58
4/10/73	0.00	0.25	0.23	1.07	2.86	3.75
4/16/73	0.07	0.26	0.46	1.02	2.34	4.16
4/25/73	0.01	0.19	0.46	1.16	2.38	5.24
4/30/73	0.00	0.24	0.26	0.85	1.43	3.56
5/7/73	0.00	0.29	0.53	1.98	2.12	4.76
5/18/73	0.09	0.30	0.61	1.70	2.71	5.98
5/29/73	0.02	0.28	0.53	1.34	2.51	6.32
6/4/73	0.02	0.38	0.34	0.80	1.98	4.49
6/6/73	0.00	0.00	0.17	1.16	1.95	4.86
6/13/73	0.00	0.17	0.56	1.30	2.81	6.64
6/19/73	0.00	0.34	0.52	1.29	2.44	6.69
7/2/73	0.00	0.32	0.73	1.23	2.27	4.83
7/11/73	0.00	0.33	0.74	1.43	2.78	7.42
7/18/73	0.00	...[b]	...[b]	0.39	...[b]	...[b]
7/27/73	0.00	0.33	0.78	1.43	2.90	7.77
8/1/73	0.00	...	0.35	1.22	2.19	4.37
8/17/73	0.00	0.11	0.43	1.55	2.00	5.36
8/30/73	0.00	0.19	...[b]	0.66	0.65	3.44
9/6/73	0.00	0.21	0.48	0.42	1.73	4.18
9/14/73	0.00	0.51	0.64	1.20	2.28	8.88
9/21/73	0.00	0.31	0.42	1.25	2.88	6.15
9/27/73	0.00	0.37	0.72	2.90	...[b]	...[b]
10/4/73	0.00	0.44	0.83	1.85	2.36	5.32
10/9/73	0.00	0.15	0.57	1.49	...[b]	...[b]
10/19/73	0.00	0.26	0.60	1.47	2.48	4.11
Mean	0.01	0.25	0.54	1.22	2.20	5.17
Standard deviation	0.03	0.12	0.21	0.53	0.56	1.57

[a]Because of analytical problems, data prior to this date were not considered reliable, and consequently not entered.

[b]Other hexane-soluble substances encountered in water samples which interferred with chlordane measurement.

APPENDIX TABLE 14. WATER QUALITY DURING CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE UTILIZING BROOK TROUT

Date	Water temperature, °C	Dissolved oxygen		pH	Total alkalinity,	Acidity,	Total hardness,	Specific conductance,
		mg/l	% saturation		mg/l $CaCO_3$			µmhos/cm
3/30/73[a]	10.8 ± 0.5[b]	7.6	68.3	7.37	164	...	143	...
4/3/73	11.6 ± 0.5	7.6	68.5	7.10	163	...	164	370
4/10/73	10.2 ± 1.5	7.8	67.1	7.10	162	...	154	375
4/16/73	11.3 ± 1.5	7.9	69.9	7.25	164	...	157	368
4/23/73	12.1 ± 0.8	8.7	83.4	7.50	160	...	148	370
4/30/73	12.9 ± 0.7	8.9	82.5	7.48	165	...	158	370
5/8/73	12.8 ± 0.3	9.0	85.1	7.60	162	...	148	400
5/14/73	13.2 ± 0.5	8.9	84.2	7.40	164	...	160	395
5/21/73	14.1 ± 0.6	7.0	66.8	7.37	162	...	149	390
5/29/73	14.3 ± 0.2	6.3	60.9	7.69	159	...	160	405
6/5/73	14.8 ± 0.3	6.4	62.3	7.32	158	...	148	375
6/11/73[c]	14.8 ± 0.1	8.0	78.6	7.52	...	...	160	390
6/18/73[d]	14.6 ± 0.2	7.2	70.2	7.61	166	...	151	385
6/25/73	14.2 ± 0.1	6.9	67.3	7.71	164	...	141	360

Continued

APPENDIX TABLE 14. WATER QUALITY DURING CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE UTILIZING BROOK TROUT--continued

Date	Water temperature, °C	Dissolved oxygen		pH	Total alkalinity, mg/l $CaCO_3$	Acidity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
		mg/l	% saturation					
7/2/73	14.0 ± 0.2	7.5	73.3	7.47	168	...	153	380
7/9/73	14.5 ± 0.4	7.4	71.4	7.50	170	...	154	375
7/16/73	14.4 ± 0.1	8.0	77.1	7.68	171	...	154	385
7/24/73	14.3 ± 0.6	8.1	79.6	7.71	168	...	148	380
7/30/73	14.5 ± 0.8	8.0	75.8	7.65	170	...	152	390
8/6/73	14.4 ± 0.1	8.5	82.5	7.66	175	...	162	400
8/15/73	14.8 ± 0.1	8.3	81.7	7.70	165	...	145	385
8/21/73	15.1 ± 0.7	8.0	78.8	7.71	172	...	158	390
8/27/73	15.0 ± 0.4	8.7	85.7	7.70	171	...	156	380
9/4/73	13.7 ± 1.0	8.1	77.6	7.64	173	...	162	402
9/10/73	13.1 ± 0.3	8.5	79.5	7.73	167	...	156	373
9/18/73	11.8 ± 0.4	9.0	82.9	7.69	165	11.0	150	379
9/25/73	12.0 ± 0.3	8.4	78.2	7.84	166	6.8	144	372

Continued ....

APPENDIX TABLE 14. WATER QUALITY DURING CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE UTILIZING BROOK TROUT--continued

Date	Water temperature, °C	Dissolved oxygen		pH	Total alkalinity,	Acidity,	Total hardness,	Specific conductance,
		mg/l	% saturation		mg/l $CaCO_3$			µmhos/cm
10/3/73	10.8 ± 0.7	9.5	83.8	7.87	168	...	164	405
10/11/73	12.0 ± 2.6	9.0	83.3	7.70	159	10.3	143	386
10/18/73	10.4 ± 0.6	9.4	83.6	7.63	165	6.8	160	397
10/23/73	10.6 ± 0.3	9.0	81.5	7.70	166	6.3	157	396
10/30/73	9.4 ± 1.3	9.5	82.2	7.76	159	4.5	145	379
11/5/73	10.0 ± 0.8	10.0	87.7	7.80	163	6.0	152	387
11/12/73	10.2 ± 0.4	8.8	78.9	7.88	166	11.1	159	411
11/19/73	10.2 ± 0.5	9.2	82.2	7.66	143	4.5	140	...
11/26/73	10.7 ± 1.3	8.6	75.2	7.82	145	14.3	141	374
12/3/73	10.2 ± 0.3	9.8	87.1	8.15	153	4.4	160	...
12/10/73	10.2 ± 0.2	9.6	85.7	7.82	143	8.0	143	377
12/17/73	10.1 ± 0.1	9.6	85.0	8.04	145	7.1	133	357
12/24/73	10.2 ± 0.2	9.9	87.8	8.53	158	...	139	375

Continued ....

APPENDIX TABLE 14. WATER QUALITY DURING CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE UTILIZING BROOK TROUT --continued

Date	Water temperature, °C	Dissolved oxygen		pH	Total alkalinity,	Acidity,	Total hardness,	Specific conductance,
		mg/l	% saturation		mg/l $CaCO_3$			µmhos/cm
12/31/73	9.6 ± 0.4	9.4	83.0	7.83	155	11.8	142	394
1/7/74	9.7 ± 0.2	10.1	88.7	8.00	158	11.5	144	387
1/14/74	9.4 ± 0.4	10.0	86.2	7.96	146	8.6	135	365
1/21/74	9.6 ± 0.5	9.4	81.7	7.83	140	7.7	136	376
1/28/74	9.8 ± 0.6	9.7	84.4	7.82	144	6.3	145	380
2/4/74	8.9 ± 0.3	9.6	84.7	7.87	155	10.7	142	369
2/11/74	9.2 ± 0.4	9.8	86.7	7.83	155	6.3	136	367
2/18/74	9.7 ± 0.4	9.5	84.5	7.79	156	5.8	148	380
2/25/74	10.3 ± 0.2	8.5	76.2	7.86	151	6.2	139	361
3/4/74	10.1 ± 0.4	9.6	85.4	7.92	153	4.2	142	367
3/11/74	10.5 ± 0.2	8.2	72.9	7.83	158	6.0	147	374
3/18/74	10.3 ± 0.2	6.8	59.7	7.87	158	4.8	135	356
3/25/74	10.2 ± 0.2	6.8	60.1	7.73	156	4.3	136	376

Continued ....

APPENDIX TABLE 14. WATER QUALITY DURING CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE UTILIZING BROOK TROUT--continued

Date	Water temperature, °C	Dissolved oxygen		pH	Total alkalinity,	Acidity,	Total hardness,	Specific conductance,
		mg/l	% saturation		mg/l $CaCO_3$			µmhos/cm
4/1/74	10.0 ± 0.3	6.6	59.1	7.82	149	3.5	134	358
4/8/74	9.9 ± 0.1	7.3	71.1	7.77	163	5.4	162	421
4/15/74	10.2 ± 0.4	7.1	63.3	7.62	160	7.3	160	413
4/22/74	10.8 ± 0.6	6.7	62.9	7.62	160	6.8	157	417
Mean	...	8.3	75.7	7.68	160	7.3	150	380
Standard deviation	...	1.2	9.9	0.26	8	2.7	10	23
No. of observations	419	247	247	174	174	79	174	73

[a]Dissolved oxygen determined with azide modification of Winkler method (21).

[b]Values are means. Standard deviations given for temperature data.

[c]Artificial aeration instituted.

[d]Dissolved oxygen determined with Yellow Springs Instrument Company Oxygen Meter after this date.

APPENDIX TABLE 15. MEASURED CONCENTRATIONS OF TECHNICAL CHLORDANE IN CHRONIC TOXICITY TEST UTILIZING BROOK TROUT

Date	Nominal chlordane concentration, µg/l[a]				
	0.625	1.25	2.5	5.0	10.0
4/9/73	0.43	0.77	1.84	2.84	7.14
4/11/73	0.42	0.64	1.84	3.18	6.44
4/16/73	0.34	0.92	2.36	4.01	7.93
4/24/73	0.35	0.49	1.68	1.99	6.80
5/1/73	0.03	0.06	0.16	0.23	0.41
5/7/73	0.36	0.76	1.27	2.28	5.79
5/18/73	0.34	0.66	1.32	1.97	7.07
5/24/73	0.28	0.59	1.34	2.14	8.42
6/4/73	0.22	0.97	1.72	3.28	2.02
6/6/73	0.22	0.55	1.57	2.48	7.86
6/13/73	0.24	0.66	1.59	3.44	6.29
6/19/73	0.42	0.57	1.28	2.05	9.24
7/3/73	...[b]	1.28	1.49	3.14	...
7/11/73	0.31	1.24	1.14	1.57	9.71
7/18/73	0.18	0.87	...	1.39	6.45
7/26/73	0.33	0.63	1.14	3.54	8.43
8/1/73	0.10	0.92	1.22	3.11	4.02
8/16/73	0.09	0.17	0.50	1.44	6.46
8/30/73	0.23	0.67	0.96	1.28	6.58
9/6/73	0.14	0.49	0.90	2.78	7.25
9/13/73	0.41	0.35	0.36	0.90	3.49
9/21/73	0.24	0.42	0.72	1.48	6.36
9/27/73	0.31	...	1.55	3.96	...
10/4/73	0.37	0.49	1.11	1.25	5.30
10/9/73	0.41	0.43	1.23	1.11	6.79
10/19/73	0.25	0.30	0.90	0.15	0.37
10/26/73	0.37	0.45	0.84	1.86	8.01
11/16/73	0.45	0.94	1.39	2.24	3.70
11/23/73	0.28	0.65	1.58	2.35	5.35
11/30/73	0.32	0.76	1.27	1.82	6.97
12/14/73	0.21	0.56	0.96	1.83	...
12/21/73	0.20	0.31	0.59	1.03	3.18
12/28/73	0.25	0.48	0.99	1.75	4.53
1/2/74	0.28	0.55	0.90	1.72	7.63
1/8/74	0.40	0.63	1.18	2.14	5.74
1/15/74	0.30	0.63	1.34	2.42	5.24
1/25/74	0.32	0.69	0.91	2.71	4.98

Continued ....

APPENDIX TABLE 15. MEASURED CONCENTRATIONS OF TECHNICAL CHLORDANE IN CHRONIC TOXICITY TEST UTILIZING BROOK TROUT--continued

Date	Nominal chlordane concentration, µg/l[a]				
	0.625	1.25	2.5	5.0	10.0
2/5/74	0.39	0.74	1.52	1.98	...
2/14/74	0.10	0.84	1.10	2.34	4.31
2/21/74	0.42	0.69	1.39	2.45	...
2/27/74	0.65	0.73	1.23	2.10	4.79
3/6/74	...	...	1.95	2.56	...
3/13/74	0.30	0.52	0.91	1.42	2.71
3/20/74	1.16	1.16	2.62	2.88	...
3/28/74	0.22	0.40	0.90	1.64	3.38
4/10/74	0.29	0.74	1.58	2.23	4.74
4/16/74	0.41	0.79	1.70	2.80	6.29
4/23/74	0.37	0.78	1.61	2.52	4.51
5/2/74	0.65	1.11	2.06	4.37	7.86
Mean	0.32	0.66	1.29	2.21	5.80
Standard deviation	0.18	0.25	0.48	0.89	2.15
No. of observations	47	47	48	49	42

[a]Less than 0.11 µg/l chlordane measured in control at any time during test.

[b]No observation.

APPENDIX TABLE 16. WATER QUALITY DURING CHRONIC EXPOSURE OF HYALLELA AZTECA TO TECHNICAL CHLORDANE

Date	Water temperature, °C	Dissolved oxygen		pH	Total alkalinity,	Acidity,	Total hardness,	Specific conductance,
		mg/l	% saturation		mg/l $CaCO_3$			µmhos/cm
4/2/74	15.4 ± 0.6	7.6	74.8	7.97	151	3.7	135	359
4/10/74	15.3 ± 1.4	8.0	82.8	8.03	154	3.5	142	376
4/17/74	17.5 ± 0.3	7.7	77.8	7.63	162	7.7	161	419
4/24/74	17.5 ± 0.3	7.7	78.5	7.86	151	5.1	149	393
4/30/74	17.5 ± 1.4	7.6	78.7	7.80	146	6.9	151	385
5/6/74	16.5 ± 0.4	7.3	73.3	7.90	150	4.3	148	385
5/13/74	16.9 ± 0.1	7.3	72.7	7.83	152	5.5	150	401
5/20/74	16.7 ± 0.2	6.9	69.7	7.80	152	5.5	150	395
Mean	16.7	7.5	76.1	7.86	152	5.3	148	388
Standard deviation	±1.0	±0.4	±4.3	±0.13	±5	±1.5	±7	±18
No. of measurements	40	24	24	24	24	24	24	8

APPENDIX TABLE 17. MEASURED CONCENTRATIONS OF TECHNICAL CHLORDANE IN CHRONIC TOXICITY TEST UTILIZING HYLALLELA AZTECA

Date	Nominal chlordane concentration, µg/l[a]				
	2.5	5	10	20	40
3/28/74	0.24	0.72	1.08	3.32	5.77
4/5/74	0.52	0.83	1.72	3.06	6.46
4/10/74	0.82	1.69	3.71	6.47	13.99
4/16/74	1.93	4.25	3.16	14.50	19.20
4/23/74	1.82	3.31	9.65	16.40	33.50
5/3/74	1.28	2.91	5.05	13.70	26.40
5/7/74	2.68	4.31	9.92	21.40	28.80
5/17/74	1.85	2.95	7.57	12.60	25.45
5/23/74	1.56	2.75	6.00	12.30	25.10
Mean	1.41	2.64	5.32	11.53	20.52
Standard deviation	0.77	1.32	3.24	6.14	9.85

[a]No chlordane detected in control chambers, except on 4/10/74, when 0.21 µg/l was measured.

APPENDIX TABLE 18. ANALYSIS OF VARIANCE OF WET BODY WEIGHTS, DRY BODY WEIGHTS AND CIS-CHLORDANE CONTENTS OF HYALLELA AZTECA EXPOSED TO DIFFERENT CONCENTRATIONS OF TECHNICAL CHLORDANE

Source of error	Degrees of freedom	Sum of squares	Mean square	F
Wet body weights - replicate I				
Among treatments	4	39.62	9.90	6.71[a]
Within treatments	95	140.13	1.47	
Total	99	179.75		
Wet body weights - replicate II				
Among treatments	4	61.55	15.38	7.39[a]
Within treatments	100	201.54	2.01	
Total	104	263.09		
Dry body weights - both replicates				
Among treatments	4	0.47	0.11	3.53[b]
Within treatments	5	0.16	0.03	
Total	9	0.63		

[a]Significant at $p < 0.005$.

[b]Significant at $p < 0.001$.

APPENDIX TABLE 19. WATER QUALITY DURING CHRONIC EXPOSURE OF *DAPHNIA MAGNA* TO TECHNICAL CHLORDANE

Date	Water temperature, °C	Dissolved oxygen		pH	Total alkalinity,	Acidity,	Total hardness,	Specific conductance,
		mg/l	% saturation		mg/l $CaCO_3$			µmhos/cm
5/6/74	20.9	5.8	64.7	8.03	160	3.3	162	416
5/13/74	20.3	6.0	66.0	8.03	162	4.2	167	440
5/20/74	21.0	5.0	55.7	8.00	165	4.2	169	440
5/28/74	21.4	...	...	8.10	160	2.4	161	413
Mean	20.9	5.6	62.0	8.03	162	3.5	165	428
Standard deviation	0.5	0.5	5.1	0.06	3	0.8	4	15
No. of observations	4	9	9	12	12	12	12	4

APPENDIX TABLE 20. MEASURED CONCENTRATIONS OF TECHNICAL CHLORDANE IN CHRONIC TOXICITY TEST USING *DAPHNIA MAGNA*

	Nominal concentration of technical chlordane, µg/l					
Date	Control	6.1	12.1	24.2	48.5	96.9
5/7/74	0[a]	1.7	1.5	5.5	8.1	13.5
5/17/74	0	1.7	3.1	5.9	12.1	19.0
5/23/74	0	1.8	2.8	7.3	15.0	32.2
Mean	0	1.7	2.5	6.2	12.1	21.6
Standard deviation	0	0.1	0.9	1.0	3.6	9.6

[a]No chlordane detected.

APPENDIX TABLE 21. WATER QUALITY DURING CHRONIC TOXICITY TESTS UTILIZING *CHIRONOMUS* NO. 51

Date	Water temperature, °C	Dissolved oxygen mg/l	Dissolved oxygen % saturation	pH	Total alkalinity, mg/l $CaCO_3$	Acidity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
Test 1								
5/6/74	24.0	6.8	80.3	7.93	155	4.0	154	393
5/13/74	24.0	7.1	82.3	7.97	154	4.7	154	406
5/20/74	23.6	6.3	75.0	7.93	153	4.5	153	401
5/28/74	23.1	...[a]	...	7.83	156	3.3	155	392
Mean	23.8	6.7	79.2	7.94	154	4.2	154	398
Standard deviation	0.3	0.4	3.6	0.05	2	0.7	2	7
No. of observations	9	9	9	12	12	12	11	4
Test 2								
6/6/74	24.7	7.1	84.3	7.90	158	4.5	155	398
6/11/74	24.6	7.0	82.2	7.99	147	2.8	141	351

Continued

APPENDIX TABLE 21. WATER QUALITY DURING CHRONIC TOXICITY TESTS UTILIZING *CHIRONOMUS* NO. 51--continued

Date	Water temperature, °C	Dissolved oxygen		pH	Total alkalinity, mg/l $CaCO_3$	Acidity, mg/l $CaCO_3$	Total hardness, mg/l $CaCO_3$	Specific conductance, µmhos/cm
		mg/l	% saturation					
6/21/74	24.4	6.9	80.3	7.77	160	5.4	160	394
6/24/74	24.2	...	...	7.87	155	4.8	151	380
Mean	24.4	7.0	82.3	7.90	153	4.2	150	380
Standard deviation	0.3	0.1	2.0	0.10	5	1.1	8	21
No. of observations	22	9	9	18	18	18	18	4

[a]No observation.

APPENDIX TABLE 22. MEASURED CONCENTRATIONS OF TECHNICAL CHLORDANE IN CHRONIC TOXICITY TEST UTILIZING *CHIRONOMUS* NO. 51

Date	Measured chlordane concentration, µg/l					
	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6
Test 1						
5/3/74	0[a]	1.1	3.4	...	25.9	48.0
5/7/74	0	0.9	1.8	4.6	9.8	15.2
5/17/74	0	1.0	2.0	3.6	7.1	10.2
Mean	0	1.0	2.4	4.1	14.3	24.9
Standard deviation	...	0.1	0.9	0.7	10.2	16.8
Test 2						
6/6/74	0	0.5	0.9	2.9	5.6	11.8
6/11/74	0	0.8	2.2	3.3	6.9	15.6
6/24/74	0	0.8	2.0	3.6	9.5	19.2
Mean	0	0.7	1.7	3.3	7.3	15.5
Standard deviation	...	0.1	0.7	0.4	2.0	3.7

[a]No technical chlordane detected.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/3-77-019		

4. TITLE AND SUBTITLE	5. REPORT DATE
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	6. PERFORMING ORGANIZATION CODE
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16. ABSTRACT The acute and chronic toxicity of technical chlordane to bluegill (Lepomis macrochirus), fathead minnow (Pimephales promelas), brook trout (Salvelinus fontinalis), Daphnia magna, Hyallela azteca, and Chironomus No. 51 were determined with flow-through conditions. The purpose was to estimate concentrations producing acute mortality and those having no effect on the long-term survival, growth, and reproduction of the various species. Whole body residues of technical chlordane components were measured in the three invertebrate species at the end of the chronic exposure tests.

Concentrations of technical chlordane causing 50% mortality in 96 hr were 36.9 µg/l for fathead minnow, 47 µg/l for brook trout, and 59 µg/l for bluegill, while that causing 50% immobilization in the cladoceran, D. magna, was 28.4 µg/l. The amphipod, H. azteca, was only slightly affected at 96 hr by the chlordane concentrations tested, and the 168-hr EC50 was 97.1 µg/l. Acute mortality of midges, Chironomus No. 51, was not successfully evaluated.

With respect to the test conditions employed and life cycle stages evaluated, the lowest concentrations of technical chlordane found to cause major chronic effects were 0.32 µg/l for brook trout, 1.22 µg/l for bluegill, 1.7 µg/l for midges, 11.5 µg/l for amphipods, and 21.6 µg/l for cladocerans

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Freshwater Fishes Trout Minnows Toxicity Invertebrates Daphnia Chlordane Insecticide Residues	Bluegill Amphipod Midge Chronic Acute	06F, T, C

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