These were evaluated and compared to different methods of land and water management for crawfish.

Observations were made on crawfish growth obtained in study cages placed in rice fields at different stocking rates. Three tables on this growth rate are presented.

Soil samples on crawfish and non-crawfish fields were taken, analyzed, and compared.

Production on the fields were determined and recorded as high as 1,000 pounds per acre.

From these trials, observations, and records, field management techniques of a preliminary nature were devised and are presented. These techniques involve water management, vegetative management, field selection, and harvest methods.

THE EFFECT OF FORMULATION DIFFERENCES ON THE TOXICITY OF BENZENE HEXACHLORIDE TO GOLDEN SHINERS

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ABSTRACT

Emulsifiable oil preparations of benzene hexachloride were found to be 25 times more toxic to golden shiners than wettable powder formulations containing the same level of gamma isomer. Tests of the individual components of the oil preparation other than the pesticide indicated that none of these was toxic to fish at the levels normally applied. The addition of a hydrocarbon solvent to a formulation increased the toxicity many times. No difference was noted in the toxicity of the active ingredient used in the various formulations.

Lethal dosages for copepods were not affected by formulation differences. Although aqueous solutions of the oil preparation killed much more quickly than the wettable powder, similar levels of activity produced equivalent results.

INTRODUCTION

Benzene hexachloride, a chlorinated hydrocarbon insecticide, is known to have a relatively low toxicity to warm water fish. This property has provided activity against many arthropods and its use in fish culture for the control of copepod parasites is widely practiced.

Among the copepod parasites of fish, few cause such extensive economic losses to the fish farmer as the "anchor worm," *Lernaea cyprinacea.* Benzene hexachloride has been used with varying degrees of success since Giudice[2] demonstrated its effectiveness as a control for *Lernaea* infestations. Stevenson (1954) found that treatments of 2.0 ppm benzene hexachloride (hereafter referred to as BHC) containing 5% gamma isomer and 7.5% other isomers were effective in reducing the parasite burden on goldfish without significant losses of fish. More recently, Shilo, *et. al.* (1960), Lewis (1961), and McNeil (1961) have discussed their varying degrees of success in using BHC to control anchor parasites.

In the fish farming area of Arkansas, BHC (12% gamma isomer, 34% other isomers) in the form of a wettable powder is used as a standard treatment for *Lernaea* infestations. Five applications at the rate of 1.35 pounds per acre-foot of the above powder applied at five-day intervals are generally effective in controlling the parasites.

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2 Giudice, John. 1950. M.S. Thesis. University of Missouri Library, Columbia, Missouri. (Unpublished)

Commercially produced BHC consists of many isomers. Three of these have been studied for their pesticidal properties. The gamma isomer is most active and exhibits the greatest toxicity to fish and insects. When isolated in a nearly pure form (99%) this isomer is sold under the trade name of Lindane. Surber (1948) found that the pure gamma isomer in acetone solution was toxic to bluegills and trout at 0.05 ppm but that the beta and delta isomers had only temporary non-lethal effects. In his tests, a field formulation (12% gamma in a napthenic solvent and fuel oil) did not kill nine species of warm water fishes including bluegills and golden shiners when applied at the rate of 0.45 ppm active ingredient.

BHC is currently available in several forms. These include wettable powders and emulsifiable oil concentrates of both the purified gamma isomer (Lindane) and of mixtures of all isomers. Applications of various formulations at comparable levels of active ingredient do not give equivalent results. It is the purpose of this report to provide further information concerning these divergent findings.

As stated above, BHC wettable powder (12% gamma) is commonly applied at the rate of 1.35 pounds per acre-foot to control Lernaeid parasites. The recent application of an oil concentrate (13.8% gamma) at an equivalent level resulted in catastrophic losses on a local fish farm. Similar reports from other fish farmers led to the experiments discussed in this report.

MATERIALS and METHODS

Toxicants and emulsifiers used in this study were provided by the Stauffer Chemical Company[1]. Since BHC in the form of wettable powder (12% gamma) is used safely in treating *Lernaea* infestations, this compound was used as reference material. A BHC concentrate (13.8% gamma) sold as Stauffer 12-E; two emulsifiers, Stauffer 14N and 15S; and a solvent, Stauffer Espersol, were obtained for study.

In general, oil concentrates are prepared by the addition of emulsifiers (synthetic detergents) to hydrocarbon solvents. Synergists are sometimes added to formulations to render the product more effective although they themselves may have no inherent toxicity. Solvents, diluents, and other additives may be toxic of themselves or may enhance the toxicity of the pesticide by making the active agent more available.

This study, therefore, began with a study of the toxicity of the individual components of the oil concentrate. Later these ingredients were studied in combination with others and with a mixture of BHC isomers (12% gamma) or with Lindane.

Golden shiners weighing 25 pounds to the thousand served as test animals. Twelve fish were placed in aquaria containing 40 liters of aerated pond water and left for 48 hours before treatment. All tests were conducted over an ensuing 48-hour period at temperatures of 77° F. ± 3°. Physical-chemical data on the pond water are as follows:

Temperature	77° F.
Methyl Orange Alkalinity	474 ppm
Phenolphthalein Alkalinity	0
pH	7.3
CO_2	5.3 ppm
Specific Conductance	1,040 ohms
Dissolved Solids	720 ppm
Suspended Solids	2 ppm

EXPERIMENTAL RESULTS

Threshold limits were used as the basis of comparison for studying the toxicity of the various mixtures. Tests were made at appropriate levels in a range of concentrations that included .031, .062, .125, .25, .50, .75, 1.0, 1.25, 1.5, 1.75, 2.0, and 10.0 ppm active ingredient. The reference compound, BHC Wettable Powder (12% gamma), was tolerated at applications of 1.5 ppm (active ingredient). The suspect material,

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Stauffer 12-E, an emulsifiable oil concentrate (12% gamma), was tolerated at 0.062 ppm (active ingredient). The difference in threshold tolerance represented an approximate 25-fold increase in toxicity.

Fish subjected to applications of over 0.25 ppm of the emulsifiable oil concentrate responded violently. At these levels the fish exhibited severe symptoms of toxicosis within minutes after exposure. Excess mucus production, convulsive swimming, and erratic leaping characterized the frenzied behavior of the fish under treatment. Death followed in short succession. Applications of the insecticide as a dust caused no such reactions.

Tests of the emulsifiers, 14N and 15S, indicated that they were nontoxic at 10 ppm. (See Table 1.) The solvent, Espersol, likewise proved to have no effect on fish at 10 ppm. A mixture of Espersol, plus 14N and 15S was also without effect at this concentration. Each component was then mixed with wettable powder BHC to check if the effectiveness of a water solution of that formulation was increased. As indicated in the table, there was, indeed, an obvious decrease in the threshold tolerance limit. Both emulsifiers increased the toxic effect, although 15S was the more effective of the two. Upon the addition of all three components to the wettable powder, the tolerance limit was reduced to that of the emulsifiable oil concentrate. Fish exposed to the mixture exhibited symptoms of severe toxicosis.

Since Espersol is a crude xylol product, tests were conducted using technical grade xylol as a solvent. BHC wettable powder was mixed with xylol to extract the insecticide from its clay carrier and the supernatant fluid was used with and without the addition of emulsifiers. Tolerance limits obtained from this material were similar to those for the commercial oil emulsion. Tests using Lindane in xylol at equal levels of activity gave essentially identical results.

Further studies were conducted using the BHC powder or Lindane dissolved in acetone and in kerosene. Lindane was not readily soluble in kerosene but dissolution was achieved by allowing the mixture to stand for 48 hours with frequent agitation. Tests conducted with these materials again showed no significant change in the threshold tolerance levels of golden shiners from that of the commercial oil preparation and evoked the same convulsive response from the fish.

To test the response of copepods to the various formulations, culture dishes containing 50 adult *Cyclops* in 200 cc. of filtered pond water (No. 25 mesh plankton net) were prepared. Two dishes, *i.e.*, a total

Table 1. Threshold values for golden shiners of concentrations (ppm) of gamma isomer benzene hexachloride in various solvents.

Solvent (in water)	LD/O of Solvent	LD/O of BHC*	LD/O of Lindane
Water	——	1.5	——
Emulsifier 14N	>10.0	0.5	——
Emulsifier 15S	>10.0	0.25	——
14N + 15S (3:1)	>10.0	0.25	——
Espersol (Crude Xylol)	>10.0	0.125	0.125
Espersol + 14N + 15S (6:3:1)	>10.0	>0.062	>0.062
Xylol	>10.0	0.125	0.125
Xylol + 14N (2.5:1)	>10.0	0.125	>0.062
Xylol + 15S (9:1)	>10.0	0.125	>0.062
Xylol + 14N + 15S (6:3:1)	>10.0	>0.062	0.125
Acetone	>10.0	0.125	>0.062
Acetone + 14N (2.5:1)	>10.0	0.125	0.125
Acetone + 15S (9:1)	>10.0	0.125	0.125
Acetone + 14N + 15S (6:3:1)	>10.0	0.125	0.125
Kerosene	>10.0	0.125	0.125
Kerosene + 14N (2.5:1)	>10.0	0.125	0.125
Kerosene + 15S (9:1)	>10.0	0.250	0.125
Kerosene + 14N + 15S (6:3:1)	>10.0	0.125	0.125
Stauffer 12-E	——	>0.062	0.125

* Mixed Isomers as Wettable Powder

of 100 organisms were used to test each concentration. Various manipulations of the compounds were made in an effort to determine the causes for the differential toxicity to fish. These included:

A—the preparation of aqueous solutions of each formulation for use as a stock solution in the treatments.

B—the preparation of aqueous solutions and allowing the solutions to stand overnight prior to filtration through Whatman No. 4 filter paper to increase the level of dissolved pesticide and also remove undissolved materials.

C—the preparation of aqueous solutions and filtering them immediately before a test was begun.

See *A*, *B*, and *C* in Table 2 for a summary of the results of these experiments. The data from these studies indicate that there is little difference in toxicity between stock suspensions allowed to soak overnight before filtration and those prepared and filtered immediately prior to application. The emulsions seemingly were unaffected by the filtration process since the milky color and foamy action were retained in the filtrate. The clay carrier was removed with ease.

A comparison of the results obtained from the application of unfiltered suspensions indicates that dust formulations require longer periods of time to produce the same levels of kill obtained from oil preparations at the same level of active ingredient.

DISCUSSION

Benzene hexachloride (mixed isomers) and Lindane are relatively insoluble in water but are soluble in aromatic hydrocarbons. In distilled water, saturation levels of gamma isomer BHC are reached at 10 ppm at 20° C. (Slade, 1945). The solubility of the gamma isomer (Lindane) in various solvents is as follows: acetone—43.5 gms/100 gms.; kerosene (ordinary)—3.2 gms/100 gms.; and xylol—24.7 gms/100 gms. (Shepard, 1951). These solubility data indicate that none of the mixtures used in the experiments produced saturation and it is assumed that, in all cases, the pesticide had the opportunity to go completely into solution.

A complete explanation for the differential toxicity between the wettable powder and emulsifiable oil concentrate of BHC cannot be

TABLE 2. Percentage survival of *Cyclops* sp. exposed to various BHC formulations.

		Time After Treatment							
Concentration in ppm.		24 hours Dust	24 hours Oil	48 hours Dust	48 hours Oil	64 hours Dust	64 hours Oil	136 hours Dust	136 hours Oil
	A*	69	0	33	0	3	0	0	0
0.5	B	62	57	5	0	—	—	—	—
	C	87	40	47	3	—	—	—	—
	A	78	16	61	0	23	0	0	0
0.25	B	87	76	40	15	—	—	—	—
	C	85	77	64	18	—	—	—	—
	A	93	48	78	3	53	0	18	0
0.125	B	90	78	65	53	—	—	—	—
	C	76	82	66	31	—	—	—	—
	A	93	63	89	44	68	5	51	1
0.062	B	95	98	95	98	—	—	—	—
	C	81	87	75	82	—	—	—	—
Controls		99	99	99	99	99	99	99	99

* A—Unfiltered aqueous suspensions used for applications.
B—Aqueous suspensions of formulations soaked overnight and filtered before application.
C—Aqueous suspensions prepared and filtered just before application.

given on the basis of the above experiments. However, an examination of the data provided in tables 1 and 2 indicates several relevant facts. There is no difference in the toxicity of the BHC material used in the preparation of the wettable powder or emulsifiable oil concentrates. The individual components, other than the gamma isomer are non-toxic to fish at levels greater than those regularly applied. The addition of a hydrocarbon solvent increases the toxicity of the formulation twelve times. While emulsifiers will enhance the activity of the mixtures, they have less effect than the addition of a hydrocarbon solvent.

Oil preparations produce lethal results much more quickly than the wettable powder. (See material *A* in Table 2.) For example, 0.5 ppm of the oil produced a complete kill in 24 hours whereas 136 hours were required by the wettable powder.

Differences in the rate of release of the BHC from the carriers may provide a possible explanation. Oil solutions of the insecticide would make it more readily and more completely available for dissolution. The rate of desorption should be faster and more complete from the oil than from the dust due to the low energy required for such a transfer. In addition, some of the pesticide in wettable powder formulations may never become available in water due to its slow release since the clay particles have a tendency to settle to the bottom of the pond. Such a phenomenon has been encountered in the use of herbicides (R. E. Wilkinson, personal communication[1]).

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