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Determination of the LD-50 Level for 2,4-D on Wild Type Culex pipiens Mosquito Larvae in Illinois

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DETERMINATION OF THE LD-50 LEVEL FOR 2,4-D ON WILD TYPE CULEX PIPIENS MOSQUITO LARVAE IN ILLINOIS

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William H. Ettinger

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ABSTRACT

The LD-50 levels for 2,4-D and Ronnel were determined and compared on the larvae of Culex pipiens Linneaus mosquitoes. The World Health Organization technique for the 24 hour bioassay was employed. Ronnel served as an indicator of susceptibility to pesticides and it was found that 2,4-D is toxic to mosquito larvae with an LD-50 level of 1.95 p.p.m. It was also found that a concentration of 20 p.p.m. ethanol or greater causes lethality in the larvae.

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INTRODUCTION

For many years, scientific literature has contained numerous reports on pesticides. These articles have dealt mainly with the effectiveness of particular pesticides in killing target organisms, the buildup of resistance in populations to a wide spectrum of pesticides, and the detrimental effects of these chemicals on non-target organisms. This research has been largely responsible for bringing about a public awareness of the harmful effects caused by many pesticides. Public pressure has caused many governmental agencies to regulate the sale and use of numerous insecticides and herbicides and in some instances to prohibit their use altogether. DDT, for example, has been banned completely from the United States since 1970.

The bioassay is the experimental technique employed in this research. According to Hoskins and Craig (1962), it may be used to determine the "relation between a physiologically active agent and the effect which it produces in a living organism." The bioassay is widely used because it is easy to set up and requires little equipment. Any number of pesticides and organisms, plant or animal, can be used; although, animals and particularly insects are the most common. Most biological assays are conducted over a 24 hour

period and only the percentage dead or moribund are recorded. When these data are transferred to a dosage mortality graph, the LD-50 or median lethal dosage can be readily determined. As in most other techniques, the larger the number of organisms used, the greater the accuracy obtained.

The adult and larval stages of mosquitoes are often used as test organisms because they are easily reared and maintained under laboratory conditions. The larvicides and adulticides are administered either by contact with a treated surface for adults or by adding them to the water of the larvae. Mosquitoes are also used because of their susceptibility to a great many insecticides. From a medical and economic standpoint, mosquitoes are vectors for many diseases that afflict a large part of the world's population, malaria and yellow fever, for example. For these reasons, mosquitoes are a standard test organism for insecticide research.

While the effects of most current insecticides on mosquitoes are known, little investigative work has been done with herbicides since their main function is to kill certain noxious broadleaf plants. It might be possible, however, that a herbicide such as 2,4-D also has an effect upon fauna. If mortality in mosquitoes can be demonstrated at certain critical concentrations of 2,4-D then it might also have deleterious effects upon desirable animals. This is because concentrations of toxic chemicals are often increased in

higher animals due to the biological magnification effect of the food chain (Ehrlich and Ehrlich, 1970).

LITERATURE REVIEW

2,4-D on Plants

Before researching the effect of 2,4-D on mosquitoes, its method of action on plants must first be understood. 2,4-D was first used in the late 1940's and its effects upon the plant cell have been well researched. The primary sites of action are on the fundamental growth processes of the cell: division, elongation, and differentiation. In studies on Allium cepa, Croker (1953) found that 2,4-D caused stickiness, condensation, delayed spindle formation, and breakage in the chromosomes and chromatids. At a concentration of 3500 p.p.m. 2,4-D, mitosis was completely halted.

Meiosis was also affected by applications of 2,4-D. Unrau and Larter (1952) reported aberrations to the microsporocyte and megasporocyte including univalent chromosomes, bridges, fragments, chains and rings, and sticky chromosomes. The authors felt the univalent chromosomes could result in different types of aneuploid progeny while the bridges were thought to have resulted from tardiness of disjunction, stickiness of individual anaphase chromosomes, or dicentric chromosomes. Fragmentation was attributed to sticky chromosomes breaking at anaphase. The formation of rings and chains was

attributed to reciprocal translocations occuring in mitosis preceding reduction division. The chromosome stickiness was attributed to changes in the chemical or electrostatic properties of the nucleic acids.

The movement of ions and minerals is sometimes affected by the presence of auxin herbicides. Wort (1964) found that 2,4-D altered the distribution of minerals within the plant, causing the leaves to be deficient and the stems to be overly supplied. In bean plants, 2,4-D caused a decrease in the absorption of sodium, phosphorus and nitrate (Freiberg and Clark, 1952), but in sugar beet tops, a twenty-fold increase of nitrate was noted (Stahler and Whitehead, 1950). Loustalot and Muzick (1953) found an increase in phosphorus absorption in white bean plants following 2,4-D application but Fang and Butts (1959) and Rebstock, Hamner, and Sell (1954) felt there was a decrease of phosphorus absorption in other bean species.

The presence of phosphorus is very important to the plant because it is involved in a number of metabolic processes. Photosynthesis, nucleotide formation, respiration, and protein synthesis all involve its use. Perhaps the most basic of these processes is that of respiration. If the concentration of phosphorus is affected, phosphorylation and the production of adenosine triphosphate will be affected, causing abnormal levels of energy for all other functions (Rupnow, 1973). For example, Black and Humphreys (1962) found that

corn seedlings treated with 2,4-D showed an increase in the activity of enzymes associated with the pentose phosphate cycle. Ribose-5-phosphate conversion to heptulose and hexose increased as did the oxidation rate of glucose-6-phosphate and 6-phosphogluconate.

The application of 2,4-D results in the depletion of starch and sugar to a wide variety of plants. Six days after treating the red kidney plant with 1000 p.p.m. 2,4-D, Sell et al. (1949) found the stems to be depleted of reducing and nonreducing sugars. They also found a reduction of starch, crude fiber, and hydrolyzable polysaccharides. In the leaves and roots, however, they found a depletion of nonreducing sugars but no change in the other compounds. In the buckwheat plant, Wort (1951) found an increase-decrease pattern of carbohydrate content. An application of 1000 p.p.m. 2,4-D caused an increase the first day but decreased to 48 percent by the eight day.

Rupnow (1973) reported that 2,4-D caused an increase in protein and free amino acids in the stem, and simultaneously, a decrease of these compounds in the leaves and roots. Sell et al. (1949) found that when a 1000 p.p.m. solution of 2,4-D was applied to the primary leaves of beans, the protein content of the stems was double that of the untreated plants. The amounts of arginine, histidine, isoleucine, leucine, phenylalanine, valine, lysine and methionine

were increased. There was evidence, based on the percentages of specific amino acids that the character of the protein was changed. Sell et al. (1949) further observed that sugars, carbohydrate reserves, and acid hydrolyzable polysaccharides decreased, and they concluded that the increase in protein was at the expense of the carbohydrates. Weller, Ball, and Sell (1957) also observed that treatment of bean plants with 2,4-D resulted in a reduction of amino acids and protein in the roots and leaves. They hypothesized that the increase in stem proteins was the result of a transfer of nitrogenous material to the stem from the leaves and roots.

Rupnow (1973) also reported that plant metabolic processes are dependent on enzymes, implying that changes in metabolic processes resulting from application of 2,4-D may be the result of altered enzymatic activity. Wort (1964) has drawn a comparison between sensitivity of castor beans and wheat to 2,4-D, and the effect of this herbicide on the lipase of the two plants. 2,4-D was much more effective as an inhibitor of castor bean lipase than it was on wheat lipase. Wheat is also relatively insensitive to 2,4-D, while the castor bean is very sensitive. Freed, Reithel, and Remmert (1961) have shown that 2,4-D can cause both stimulation and inhibition of an enzyme, depending on the concentration. A marked stimulation of glyceraldehyde-3-phosphate dehydrogenase was obtained at 100 p.p.m. 2,4-D, whereas an inhibition

resulted at 1000 p.p.m. Freed et al. (1961) postulated that the stimulation caused by low concentrations of 2,4-D may result from the participation of the herbicide in a substrate-regulator-enzyme complex. The accumulation of larger amounts of 2,4-D may saturate both the enzyme and substrate separately. Therefore, the possible sites of enzyme-substrate combination are decreased, and the reaction is retarded.

It can easily be seen that 2,4-D affects plants in many ways. Since many of the basic functions such as respiration and growth are common to both flora and fauna, it is very possible that 2,4-D works in much the same way in animals. Although this field of research is relatively new, several investigators have worked in this area and have published their results.

2,4-D on Animals

Most of the work done on 2,4-D and animals has been in the aquatic environment since the herbicide is often used to control aquatic plants and is usually present in natural waters due to agricultural runoff. In summarizing other studies, Sears and Meehan (1971) found the safe upper limits of 2,4-D concentrations to be 1500 p.p.m. for minnows, 500 p.p.m. for sunfish, and approximately 500 p.p.m. for catfish.

They also indicated that concentrations of 5 p.p.m. of a butyl ester of 2,4-D were lethal to rainbow trout and bluegills after 12-hour exposure.

The acute affects of 2,4-D on aquatic organisms is well covered by Wojtalik, Hall and Hill (1971). After spraying two reservoirs on the Tennessee River with 2,4-D to eliminate an aquatic weed, Myriophyllum spicatum, the authors monitored the ecological conditions for one year. General observations gave no evidence of fish kills or acute toxicity of the herbicide to other aquatic organisms. Most of the 2,4-D was accumulated by plankters which retained it for more than six months. A buildup of 2,4-D in fish was found only in filter-feeding gizzard shad and in one predatory largemouth bass. Even at the height of the fishing season, there were no complaints from fishermen about the quantity or quality of the fish. Mussels taken from commercial colonies downstream from the reservoirs were found to accumulate 2,4-D quite efficiently because they are filter-feeders. During the interval of actual application, however, the highest accumulation of 2,4-D in the mussels amounted to less than 1 mg/kg wet weight and the amounts progressively decreased as they went downstream. Large cladocerans, snails, some diptera, and some lepidoptera larvae were absent two to four weeks after treatment but the authors felt this was mainly due to

the absence of tangled stems and leaf mats which afforded these organisms a place of residence. Scattered individuals could be found, however, by plankton towing or benthic sampling.

The extensive use of this agricultural chemical has led to some concern that low concentrations maintained over a period of time might be deleterious to animal life and cause sub-clinical damage not readily recognized. In an effort to determine the chronic effects of 2,4-D on animals, Cope, Wood, and Wallen (1970) exposed bluegill (Lepomis macrochirus) to different concentrations of 2,4-D and then studied the fish and pond environment for five months. They reported pathologic changes consisting of marked depletion of liver glycogen, the appearance of PAS-positive deposits in the vascular system and vascular stasis of the central nervous system. The PAS-positive material was credited by the authors to cause inhibition of the vascular flow. This blockage was probabaly responsible for the lethargy, ataxia, paralysis, and coma often observed in the fish and helps explain why fish can withstand greater doses of 2,4-D at high oxygen levels. Unless the fish died shortly after developing these lesions, however, recovery was eventually complete.

Research has also been done with honey bees. Morton and Moffett (1972) found that eggs from colonies consuming 2,4-D did not hatch and larvae died when transferred into

colonies consuming this herbicide. However, the effects of phenoxy herbicides on reproduction were temporary, because once the herbicide was removed from the colony, brood development resumed. The effects of 2,4-D on adult bees was minimal. In fact, bees fed 10 and 100 p.p.m. 2,4-D lived significantly longer than the control bees (Morton, Moffett, and MacDonald, 1972). In concluding their study, Morton and Moffett (1972) wrote that herbicides were more likely to affect honey bee colonies by depriving them of their food source than by reducing brood production.

Resistance to Pesticides

Insects have developed resistance to all major classes of insecticides with the exception of oil because of the extensive use of pesticides (Benson, 1971). A prime prerequisite for the development of specific resistance under pressure from an insecticide is the presence in the population of certain individuals carrying in their genetic makeup the factor or factors that cause resistance. Each generation will reach a level of resistance that has been predetermined by the most resistant individuals in that particular population (Hoskins, 1959).

Genetic resistance to insecticides develops slowly at

first and is rather unstable in its early stages. This indicates, according to Georghiou (1969), that the expression of major resistant genes is dependant upon the gradual accumulation of a sufficient background of auxiliary or modifying genes. These resistant genes are usually dominant or semidominant; especially when resistance results from a change in metabolism. Recessive resistant mutations also occur but metabolism is seldom involved (Benson, 1971).

Resistance takes many forms in insects, according to Benson (1971). The rate of penetration of the chemical may be altered or the compound may be partitioned into an area where it does not act. The metabolism of the insect may change so that a toxifying conversion is lost or decreased. Sometimes a special detoxifying reaction develops to convert the pesticide into a harmless derivative. There may be an alteration in the site of action or a change in the rate of excretion. All of these mechanisms are not found in every insect. Some are used singly or in combination and some provide resistance to more than one class of insecticide, all depending upon the species of insect.

Georghiou (1969) has developed terminology for insecticide resistance. If a hybrid is very susceptible to an insecticide, then it is probably of the homozygous recessive phenotype and is termed completely recessive. When a species exhibits significantly more resistance than the homozygous

recessive but less than the intermediate phenotype it is called incompletely recessive. An intermediate hybrid is the logarithmic average of the homozygous recessive and homozygous dominant phenotypes. The term incompletely dominant is given to a hybrid that is significantly more resistant than the intermediate but less than the homozygous dominant. Finally, very resistant species are called completely dominant and are identical with the homozygous dominant phenotype.

To determine the susceptibility of a population to a particular compound, results of the bioassay are plotted on a dosage mortality graph. A regression or log dosage-probit (ld-p) line is fitted to the graph and from this, population responses can be determined. If a population has a steep ld-p line before application of a pesticide with few individuals lying off the line at high concentrations, then it is safe to conclude that resistance will develop slowly (Hoskins, 1959). If no inflections appear in the line, one can assume that resistance is due to multiple factors (Brown, Lewallen, and Gillies, 1963). When the line becomes rather flat, having a slope of 2, but all individuals seem to be upon the line, the effect of the insecticide will be to raise the LD-50 a few fold only. However, Hoskins (1959) states, when a few individuals lie

to the right of a flat ld-p line, then resistance can be quickly developed, the degree being dependent upon how resistant those aberrant individuals are.

The major disadvantages to heavy use of pesticides are that pest populations rapidly evolve resistance and that environmental side effects occur. Since some species are resistant to several classes of insecticides and a single gene may confer resistance to more than one class of insecticides, total reliance on chemicals writes Benson (1971), may ultimately mean famine because of destruction of crops by resistant insect populations. The best way to combat resistance, the author feels, is to manipulate the gene-pool. He wants to introduce large numbers of susceptible insects into the population. This, he feels, will bring the resistant gene level down and pesticides will again become effective. Unfortunately, this is a continual process with the end coming only after complete irradication of the species, which is not feasible. The author also suggests the sterile male technique be used where possible but it also presents drawbacks. Both techniques release millions of insects into the environment that do just as much damage to the crops as the resistant kind. Both techniques are also very expensive in terms of research and development.

Mosquito Characteristics

The family name of mosquitoes is Culicidae, and belong to the order Diptera, or two-winged flies. Adult mosquitoes can be differentiated from other Dipterans by their elongated proboscis which is many times longer than their head and by their antennae which are much longer than the head and are composed of many small, well-separated segments, each with a ring of hairs. The larval stage of the Culicidae have no legs and possess a large head with a hard covering. The thorax is larger and wider than the abdomen, the respiratory system opens dorsally on the next-to-last segment of the abdomen, and four anal papillae extend posteriorly from the end of the last segment. In many species, the larvae also have a long or stout, usually hard and dark, air tube (Ross and Horsfall, 1965).

Each species of mosquito differs from each other in the habitats it uses and in certain aspects of its life history. All of them, however, do have some traits in common as Ross and Horsfall (1965) summarize. The immature form or larva, often known as a wriggler, is aquatic. This form requires several days and four molts to become a full-grown larva. At this time, it transforms into the next life

history stage, the pupa or tumbler. This stage is also aquatic. The pupal stage, which lasts only a few days, is a transformation stage; within the pupa the tissues of the larva are changed into those of the adult mosquito. When transformation is complete, the pupa floats at the surface of the water, its shell cracks and breaks the surface film, and the winged adult emerges. The adults are entirely aerial and never enter the water. After a period of feeding and mating, the females lay eggs either on the surface of the water or in soil that will be flooded at a later date. Eggs laid on the water hatch in a few days; each small larva emerges directly into the water from the end of the egg that is submersed. Eggs laid in soil hatch when the soil is flooded with deoxygenated water.

Mosquito larvae are primarily filter-feeders although some are predatious and consume other larval species. Usually, however, larvae feed indiscriminately upon the microplankton of their habitat, including algae, rotifers, protozoa, bacteria, fungal spores, and detritus (Clements, 1963). Attached to the normal insect mouthparts (labrum, hypopharynx, mandibles, maxillae, and labium) are several brushes that, when moved rapidly, causes water to flow toward the head. Food particles become trapped in the labral brushes and are then moved inward toward the pharynx. The larvae have such

an efficient food gathering mechanism that they have been known to clear 520 mm^2 of water per minute (Clements, 1963; Snodgrass, 1959).

The feeding organs of the adult mosquito include the proboscis and two sucking pumps. One of the latter is a preoral cibarial pump beneath the clypeus, the other is a pharyngeal pump, being a part of the alimentary canal behind the brain. The female mosquito is usually the biting and bloodsucking member of the species and has fully developed mouth parts, while in the nectar-feeding male some of the parts are reduced or absent (Snodgrass, 1959).

Not only are mosquitoes pests but many are also vectors for parasitic diseases. One of the most widespread diseases in the world, malaria, is carried by the Anopheles mosquito, a genus that is abundant in Illinois. Yellow fever, several types of encephalomyelitis, tularemia, and filariasis are also carried by mosquitoes (Ross and Horsfall, 1965).

MATERIALS AND METHODS

The Culex pipiens larvae used in this study were collected from a septic tank drainage ditch on the Paul Bunting farm, 2.5 miles northwest of Crossville, Illinois. According to the owner, no insecticides or herbicides have been used on the farm for several years. The larvae were gathered in lots of 50 to 100 by a fine, wire mesh tea strainer. After collection, the larvae were kept in 10 x 16 inch white enamel pans containing aged tap water and were fed daily a mixture of yeast, wheat germ, and whole wheat. When more larvae were needed, they were collected from the same place. The density of larvae in each pan varied between 150-200.

All glass-ware used in the bioassay was washed thoroughly with detergent and water and then rinsed in chromic acid to eliminate all pesticide residues. Pipettes were rinsed in 95 percent ethanol (World Health Organization, 1960; Davidson, 1958).

A 100 p.p.m. standard solution of technical or purified Ronnel (0-0-dimethyl-0-2,4,5-trichlorophenyl phosphorothioate) was prepared by adding 10 mg of the larvicide to 100 ml of 95 percent ethanol. The insecticide was measured on a Mettler, model H-8, analytical balance and mixed with

the alcohol in a volumetric flask. After preliminary tests showed the standard solution to be too concentrated, it was diluted to 0.01 p.p.m. Similarly, a 500 p.p.m. standard solution of technical 2,4-D (2,4-dichlorophenoxyacetic acid) was prepared by adding 50 mg of the herbicide to 100 ml of 95 percent ethanol. Preliminary tests showed that this standard solution did not need dilution. Both standard solutions were stored in tightly stoppered, brown glass bottles and refrigerated to avoid evaporation of the alcohol and decomposition of the chemicals.

All bioassay tests were conducted in 6 glass culture dishes approximately 10 centimeters in diameter. Five of these dishes contained varying concentrations of the pesticide being tested while the sixth served as the control. Preliminary tests were conducted with both compounds to determine the concentrations necessary to produce a good range of mortality. After this range was found, precise quantities of the standard solution were pipetted into each of the 5 culture dishes containing 200 ml of aged tap water. The control contained the same amount of ethanol, without the pesticide, as the highest test concentration. Approximately 30 minutes after the addition of the pesticide, lots of 20 fourth instar larvae were transferred to each of the test vessels by a tea strainer (World Health Organization,

1960). The larvae were left in these dishes for 24 hours at room temperature and without food(Jones, 1967). At the end of the test, the number of dead or moribund larvae were counted and recorded as a percentage. Larvae were considered moribund when they responded with gross body tremors or by undulating, snakelike, in-place movements after being excited (French and Kitzmiller, 1965). If more than 10 percent of the larvae in a test concentration pupated, the test was rejected and rerun. If more than 20 percent of the control larvae pupated, it also was rejected and run again. Four replicates of each pesticide concentration were run and all larvae were discarded at the end of a test (World Health Organization, 1960).

All data were recorded on a specimen report form (Figure 1) suggested by the World Health Organization (1960). The results of the four replicates were averaged and plotted on a dosage mortality graph with the percentage mortalities plotted on the probability scale against concentration of the actual toxicant on the abscissa (Klassen, Keppler, and Kitzmiller, 1964). A regression line was fitted to the graph and from it was determined the LD-50 value.

Date: _____

Insecticide: _____ Species: _____

Investigator: _____

Locality: _____

History of insecticide treatment (including agriculture): _____

Condition of larvae: instar: _____ reared/collected/other: _____

Tests	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Totals
Date of test					(for comparable
Temp. during test					tests only)
Insecticide conc. (p.p.m.)	M&D Total Mort. (%)	M&D Total Mort. (%)	M&D Total Mort. (%)	M&D Total Mort. (%)	M&D Total Mort. (%)
Control					

Remarks:

Signature of investigator:

Fig. 1. Report form for mosquito larvae bioassay.

RESULTS

A search for mosquito larvae in White County, Illinois, revealed only Culex pipiens Linnaeus. However, other species undoubtedly exist. Careless overflow from many of the oil-fields in the county has made much of the natural waters uninhabitable by mosquito larvae. The population used for this study came from a septic tank drainage ditch on a farm near Crossville, Illinois, and was found in great numbers until the first week in November when the population finally began to taper off. A small number of larvae were collected on 13 November, after the temperature had dropped to 28°F the previous night.

Preliminary tests were conducted with both pesticides to determine the concentrations needed to provide a good range of mortality. The 100 p.p.m. standard solution of Ronnel produced complete mortality; whereas, the 1 p.p.m. and 0.01 p.p.m. standard solutions produced the desired range. Both standard solutions were utilized because it was found that if more than 4 ml of 95 percent ethanol was used, partial larval mortality resulted. For the three highest concentrations of Ronnel, 0.06, 0.05, and 0.04 ml of the 1 p.p.m. standard solution were used. Below 0.04 ml,

it was thought that the degree of accuracy would be reduced. Therefore, 2 and 3 ml of the 0.01 p.p.m. standard solution were used for the two lowest concentrations. The inflection in the Ronnel dosage mortality graph (Figure 2) indicates the interval between the 3 ml of the 0.01 p.p.m. standard solution and the 0.04 ml of the 1 p.p.m. standard solution. The third replicate in the bioassay displayed much lower mortality values than did the other runs. The Ronnel LD-50 level for this population of Culex pipiens larvae was approximately 0.0002 p.p.m.

The preliminary tests with 2,4-D showed that the 500 p.p.m. standard solution was sufficient to produce the desired mortality range. All concentrations for the actual bioassay were prepared with the 1 ml pipette. There are no inflections in the 2,4-D dosage mortality graph (Figure 3) as there were with the Ronnel. The 2,4-D LD-50 level for this population of Culex pipiens larvae was 1.95 p.p.m.

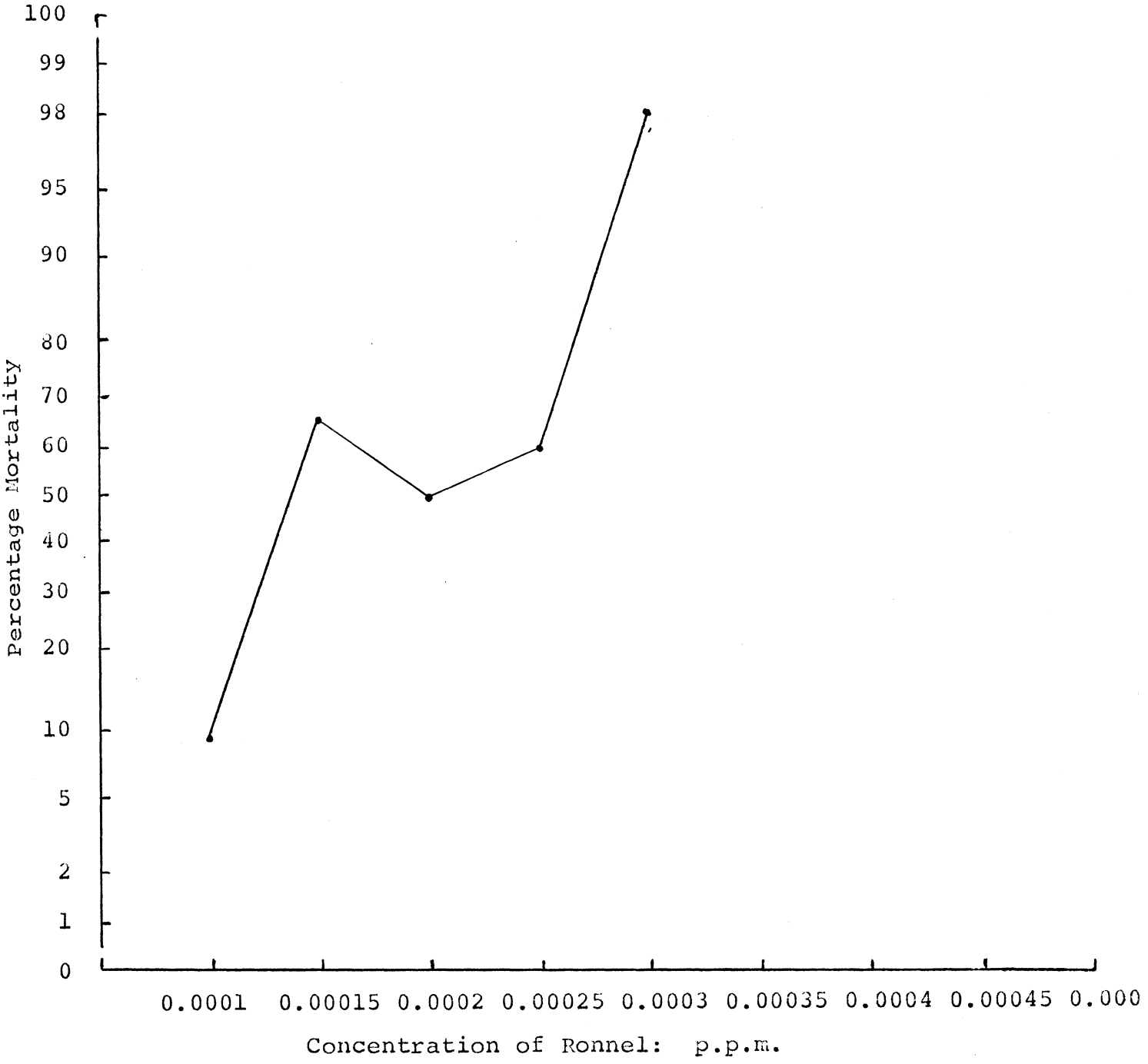

Fig. 2. Dosage mortality relationship to Ronnel for fourth instar wild type Culex pipiens mosquito larvae.

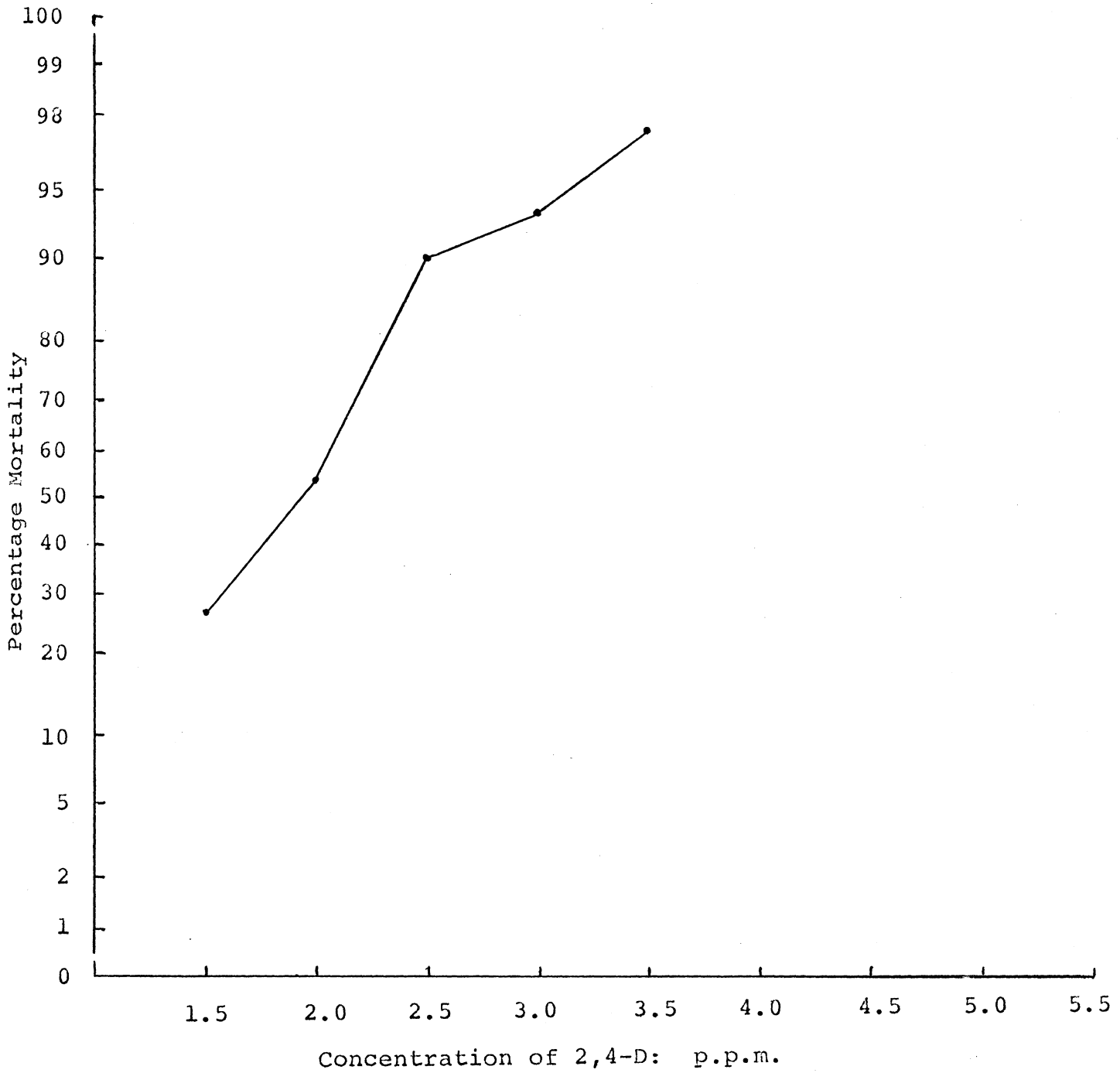

Fig. 3. Dosage mortality relationship to 2,4-D for fourth instar wild type Culex pipiens mosquito larvae.

DISCUSSION

The main purpose of this investigation was to determine if 2,4-D is toxic to mosquito larvae. Since wild type specimens were used for the study, it was impossible to determine the phenotype of the Culex pipiens larvae prior to the bioassay. Therefore, in order to find the susceptibility of this population, Ronnel, an organophosphate larvicide, was first used. It was assumed that if the larvae were very susceptible to the insecticide they would also be very susceptible to 2,4-D.

Even with the inflection between the 0.00015 p.p.m. and 0.0002 p.p.m. concentrations (Figure 2), the ld-p line is steep which, according to Hoskins (1959), indicates high susceptibility to Ronnel. The LD-50 is low, 0.0002 p.p.m., also indicating the high susceptibility of this population to Ronnel. The lowest LD-50 for Ronnel in the literature was 0.019 p.p.m. for a strain of Anopheles quadrimaculatus reported by Klassen et al. (1964).

During the preliminary tests with Ronnel, it was discovered that some mosquito larvae could not tolerate a concentration of 95 percent ethanol higher than 20 p.p.m. or 4 ml of ethanol in 200 ml of water. Therefore, it became

necessary to add no more than 3 ml of ethanol or 15 p.p.m. to the test solutions. Unfortunately, the range of lethality was such that if the 0.01 p.p.m. standard solution was used, more than 3 ml of ethanol would be added. Conversely, if the 1 p.p.m. standard solution was used, it became necessary to add concentrations of Ronnel in one-hundredths of a milliliter to the test solutions and the accuracy of the test would be in doubt. Therefore, both standard solutions were used. For the three highest concentrations, 0.06, 0.05, and 0.04 ml of the 1 p.p.m. standard solution were used. For the two lowest concentrations of Ronnel, 3 and 2 ml of the 0.01 p.p.m. standard solution were utilized. This also presented problems because the 15 p.p.m. ethanol, while not toxic by itself, did cause the mortalities to be higher than expected when combined with Ronnel. The inflection of the graph in Figure 2 results from the use of these disproportionate concentrations of ethanol. Evidently the Ronnel and ethanol act as synergists.

The third replicate of the Ronnel bioassay showed much lower mortality readings than did the other replicates. A slight mortality (7.5 percent over four replicates) was also exhibited. The only explanation for these two events is that the larvae were sometimes crowded in the rearing pans and this possibly affected their vigor tolerance. It

is also possible that rough handling could have injured the larvae also reducing their vigor tolerance.

Cope et al. (1970) has shown that if 2,4-D is given to fish in sufficient concentrations, it will cause mortality. Unfortunately, no research has been done on the method of death but there is little reason to believe that its effect on animals is any different than on plants. The susceptibility of Culex pipiens mosquito larvae to 2,4-D has been shown by the LD-50 value of 1.95 p.p.m. obtained from the bioassay. It is possible to find this value in naturally occuring waters (Wojtalik et al., 1971) so it is probable that 2,4-D might have an effect on wild type mosquito populations. This value may also be taken as a baseline of susceptibility of this population for 2,4-D since it has shown itself to be very sensitive to the organophosphate insecticide, Ronnel.

It is doubtful that 2,4-D is involved in a biological amplification process. Wojtalik et al. (1971) have found that an application of 2,4-D to an aquatic environment is taken up by the plankton and held there for up to six months. However, this does not seem to affect the fish since little 2,4-D was found in their flesh, even the filter-feeding gizzard shad. Cope et al. (1970) found that some fish died from acute 2,4-D poisoning but that all chronic effects

eventually disappeared after a period of time. In honey bee larvae, Morton and Moffett (1972) found that 2,4-D did kill the eggs and larvae but did little harm to the adults. After the herbicide was removed, the colony seemed to begin brood production again without any ill effects. It is doubtful, therefore, that man will ever suffer from the acute or chronic effects of 2,4-D since it is biodegraded quickly, in some reports as soon as one month (McGlamery and Knake, 1973), and does not seem to be biologically amplified.

CONCLUSIONS

1. A concentration of more than 20 p.p.m. ethanol is toxic to some Culex pipiens larvae.

2. When 15 p.p.m. ethanol is combined with Ronnel, the percentage mortality is higher than expected, indicating a synergistic effect between the ethanol and Ronnel.

3. Culex pipiens larvae are highly susceptible to the organophosphate insecticide, Ronnel, with an LD-50 value of 0.0002 p.p.m.

4. Culex pipiens larvae are susceptible to the auxin herbicide, 2,4-D, with an LD-50 value of 1.95 p.p.m.

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