STUDIES ON FACTORS AFFECTING SUSCEPTIBILITY OF SCHISTOCERCA GREGARIA (~~FORSK~~) TO INSECTICIDES

by

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A thesis submitted in accordance with the regulations governing the award of the degree of Master of Philosophy in the University of London.

May 1973.

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ABSTRACT

The toxicities of the insecticides γ-BHC, Cyolane and dioxacarb to fifth instar hoppers of the desert locust were determined. Insecticides were topically applied by microcapillary, by injection using a microsyringe or as a stomach poison. The standard site of application was the abdomen, between the first and second abdominal sternites, and other sites were tested. The influence of post-dosing temperature on the toxicity of insecticides was examined. The effects of starvation and the type of diet on the resistance of locusts of γ-BHC were investigated.

The movement of γ-BHC in the insect and the amount penetrating in the haemolymph were determined. Penetration and distribution of γ-BHC in fifth instar and adult locusts were briefly investigated. Movement of γ-BHC through the gut and its excretion were studied.

The results are discussed in relation to current knowledge of factors affecting insecticide toxicity.

SUMMARY

The relative susceptibilities of fifth instar hoppers to γ-BHC, Cyolane and dioxacarb in relation to age and method of application were examined and discussed.

Results indicated that hoppers were more resistant in the middle of the instar than at the other ages. γ-BHC was less toxic than either Cyolane or dioxacarb, and insecticides applied orally were less effective than when applied by other methods except for Cyolane.

Changing the site of application of insecticides resulted in different mortality and varied with the insecticide used. γ-BHC and dioxacarb showed large variations in susceptibility, but Cyolane showed a little difference in relation to site of application except when the dose was applied to the neck membrane where it was more toxic.

-BHC and dioxacarb were less effective at a post-dosing temperature of 35°C. than 20°C., but Cyolane showed no significant difference.

Starvation after treatment increased the stomach

toxicity of γ-BHC. Hoppers deprived of food before and after treatment were more susceptible than when deprived of food before or after only.

Results obtained for γ-BHC indicate that resistance is proportional to body weight. No difference in the relative susceptibility between males and females was found when the dose was measured in µg/g of body weight.

Resistance of 4-5 days old fifth instar hoppers and mature adults to γ-BHC was of the same order, but 2-3 days old adults were more susceptible.

The stomach toxicity of γ-BHC was dependent on the amount of food available after treatment. Locusts fed immediately after treatment excreted about 40% of the total dose in 24 hours compared with 21% when not fed.

Locusts reared on an artificial diet were more tolerant to γ-BHC than when reared on fresh grass although the reverse was found with Cyolane and dioxacarb.

The type of solvent used with topically applied γ-BHC did not affect toxicity although γ-BHC was more toxic

when applied by injection in acetone or benzene than in KAB (Kerosene aromatic bottoms).

Most of ^{14}C γ-BHC accumulated in the abdomen after application and the rate of excretion following oral application was about 3 times more than when applied topically or by injection.

INTRODUCTION

The desert locust Schistocerca gregaria (Forsk.) is one of the most dangerous species of locusts and is found across the whole northern and eastern Africa and much of south east Asia. The desert locust, for long considered one of the world's serious agricultural pest, can alone invade a total area of about 11 million square miles which is 20% of the world's land suface (Hemming,1968). Complete figures of the total cost are not available, but the damage done in some countries is estimated by the Food and Agriculture Organisation as about £1,500,00 per year between 1949-57 and in 1955 over £5,000,000. In Morocco in 1954-55 it was over £4,500,000 in a single season. The desert locust is characterised by outstanding mobility (Rainey,1958). Movements of whole groups of swarms over 100 miles are not only common but even regular. Its populations vary in density from several thousands to less than one per square yard. In a typical locust swarm there are usually 35-70 million insects in a single square kilometre, and swarms up to 100 square kilometre may contain 40,000 million locusts. As a locust eats roughly its own weight of fresh vegetation each day, a swarm would have the potential to eat enough food to feed 400,000 people for a year (Haskell,1970).

Unlike other species, its permanent breeding areas are not restricted but it can breed in vast and sometimes inaccessible areas. It can completely or nearly destroy or damage vegetation over a large areas inhabited by million of people dependent on agriculture for their existance. Apart from the damage by feeding, locusts can cause physical damage as a result of breaking branches of trees, and plantation crops. Crops loss estimates (Haskell 1970) due to locusts and grasshoppers are at least 1,000,000 tons annually.

Early control methods for the desert locust were based on defence such as preventing locusts from settling on cultivated areas by lighting fires, banging tins and drums, Other methods are ploughing or flooding fields especially after locusts have laid egges, and directing hoppers into trenches where they were buried or burnt. The earliest chemical was sodium arsenate used extensively as bait against hoppers. It was effective, but unconsumed arsenical bait caused cattle deaths (Hemming,1968).

The practical control methods in use against the desert locusts are based mainly on application of insecticides. γ-BHC is used in baiting and dusting at rates of 0.1-1.0% and in spraying with ultra low

volume techniques in 10-12% in oil solution (MacCuaig,1964). Similar concentrations of DNOC and malathion have been used as well as dieldrin. The toxicity of insecticides is affected by several factors (Busvine,1957). Some of the variations are due to intrinsic factors such as age, instar and weight, and extrinsic factors which are caused by change in the environment e.g. temperature and food.

Insecticides normally enter the body of insects by penetration through the cuticle or by absorption through the gut as in the case of stomach poison and this is influenced by the amount of food ingested and the cumulative effect of insecticide in the insect. Laboratory studies of the relative toxicity of difference insecticides applied by different methods of application are critical factors in large scale control operation. Furthermore, investigations on distribution, metabolism and penetration contribute to better understanding the mode of action and the proper methods of application.

Most of the experiments described have been made using γ-BHC. Whilst it would have been preferable to carry out each experiment also on the other two insecticides (Cyolane and dioxacarb) this has not been possible because of the restricted numbers of locusts available

and the need for individual treatment. For this reason, and because γ-BHC is the most commonly used insecticide in desert locust control (MacCuaig,1964), it was used for preference when it was possible to use only one insecticide.

The work presented in this thesis was carried out to investigate some factors affecting susceptibility of the desert locust Schistocerca gregaria to insecticides.

REVIEW OF THE LITERATURE

A review of the literature indicates that the toxicity of an insecticide applied by different methods is affected by several factors. The following is a short account.

AGE WITHIN THE INSTAR

The influence of age on the resistance to insecticides has been reported by some workers. Collins and King (1952) found that resistance of adult Tribolium confusum to DDT when exposed up to 3 hours did not change significantly with age, but older beetles were found to be more susceptible after longer periods of exposure. Mukerjea (1953) reported changes in resistance of different stages during development of the egg of Diataraxia oleracea, pupae of Diataraxia oleracea and Tenebrio molitor and adult of Tenebrio molitor and Periplaneta americana when sprayed with pyrethrins and DDT. With last instar, Diataraxia oleracea, Way (1954) found that resistance to DDT and γ-BHC as contact insecticides progressively increased up to the 5th or the 6th day and then decreased with the beginning of prepupal formation. Kerr (1954) found that susceptibility of adult male

Drosophila melanogaster to DDT decreased with age up to five days and then increased up to nine days. The susceptibility of adult female decreased rapidly during the first three days, and thereafter changed little. Bass and Rawson (1960) showed that the age of the boll weevil, Anthonomus grandis, was an important factor in susceptibility to insecticides. They found that 9 days old weevils were 7 times more resistant than 2 days old weevils to γ-BHC and only 2 times to Guthion. El Nosa and King (1964) found no significant evidence of an age effect with adult Hylema antiqua tested at 2-5 days, 9-12 days and 10-19 days with dieldrin, but changes in the susceptibility of houseflies with age were found by Sawicki and Green (1964). Adults of both sexes were most susceptible to diazinon soon after they had emerged from the pupae. Their resistance increased for two days after emergence and remained steady for the next three days, the increase being proportionately larger in a resistant strain. Green and Dorough (1968) also studied the relationship between age of houseflies and their response to certain carbamate insecticides. They found that resistance of adult houseflies increased with age up to 5 days and then decreased slowly until 15 days. Insects were most resistant at 5 days old, and susceptibility was greatest in adults at 1 and 15 days old. With honey bees, Apis mellifera, tested

at different ages, Maryland and Burkhardt (1970) found that 3 weeks old bees were less susceptible to insecticides than the newly emerged bees. Mount *et al.*, (1970) found substantial variation in the toxicity of several insecticides against different ages of nymphs of *Amblyomma americana*. They showed that younger nymphs of 1-2 months old were about 9.7 more resistant than the older nymphs of 4-6 months old.

INSTAR SPECIFICITY

It is known, that, within a species, resistance varies with different instars. Mukerjea (1953) using a spraying technique showed the extent of changes in resistance to DDT and pyrethrins of the developmental stages of *Diataraxia oleracea*, *Tenebrio molitor* and *Periplaneta americana*. He found a large differences of resistance with the stage of development of the insect, for example, 5th instar larvae of *Diataraxia oleracea* was about 50 times more resistant to DDT than 1st instar. Sanches and Sherman (1963) compared susceptibility of 1st and 3rd instars of *Cryptophlebia ellepida* to different insecticides. They found no significance difference in susceptibility between first and third instar to dieldrin, aldrin and γ-BHC, but the first instar was significantly more susceptible to DDT and malathion than the third instar. Bacon and Rilley (1964) investigated the susceptibility of the various growth stages of *Lygus hesperus* to DDT, and showed that susceptibility decreased consistently from the third instar to adult. The differences in LD50's between third and fourth instar nymphs were not so great as between fourth and fifth instars. Adults were much more tolerant to DDT than nymphs. Smith (1965) studied the susceptibility of life history stages of *Psylla pyricola* to oil treatment.

He found that adults and nymphs were killed by direct contact with oil emulsions well below the standard concentrations, while eggs was unaffected. Harris and Core (1971) found a remarkable difference between different insecticides against various stages of development of the cutworm, Euxoa messoria. DDT was effective against 1st-3rd instars, less effective against 4th instar and ineffective against 5th-7th instars, eggs, pupae and adults. Dursban was similar in activity to DDT, but Galecron was effective against eggs and ineffective against other stages of development.

Chauthani and Adkisson (1966) studied the relative resistance of different stages of two species of Heliothis. They found that endrin was less toxic to eggs, but it was considerably more toxic to 3rd and 4th instar larvae. Adults of both species were tolerant than were the larvae. Campbell (1926) compared the susceptibility of the last four instars of silkworm Bombyx mori larvae to arsenic given as a stomach poison. He found that the LD50 increased during successive larval instars, and 2nd instar larvae were 3.5 times as susceptible as 5th instar larvae.

SITE OF APPLICATION

The toxicity of insecticides was found to be influenced with the changes of site of application. When a uniform doses of DDT was applied as saturated droplets or crystaline masses to different loci of application on housefly, Fisher (1952) found that the dose produced lower mortality as the locust of application moved distally along the leg and posteriorly from the head to the abdomen. Hoskins et al. (1954) showed that γ-BHC applied to the tarsi of housefly by residual film was more effective than when applied to the ventral suface or the thorax. Hewlett and Gostick (1963) found no significant difference in toxicity of dieldrin when applied to different parts of Alphitobius laevigatus. Gunda and Baidu (1967) found that the head of Dysdercus cingulatus was the most vulnerable site for all insecticides tested followed by the wing base, and least on the abdomen.

SOLVENTS

The nature of the solvent in which the insecticide is dissolved may affect the toxicity. Haffiger (1949) found that DDT emulsion was about 20 times more toxic than wettable powder applied as a stomach poison to Bombyx mori. Roan et al. (1950) applied sub-lethal doses of some organophosrous insecticides in five solvents to Periplaneta americana and found that this had considerable effect on toxicity. Busvine (1951) showed that change in the solvent affected the resistance of houseflies to DDT. He reported that solutions of DDT in mineral oil were more toxic to DDT resistant houseflies than solutions in acetone. When oil was used as a solvent for DDT instead of acetone, Barker and Edmunds (1958) found no significant difference in toxicity of DDT to adult resistant houseflies. Farnham (1965) examined the toxicity of diazinon in different solvents to resistant and susceptible houseflies. He found that toxicity of diazinon in oil decreased compared with other solvents and it did so much more with resistant than with susceptible insects. With injection, diazinon in ethyl cellosove was four times more toxic to susceptible flies than in oil.

TEMPERATURE

Change in post-dosing temperature may also affect the toxicity. Hafliger (1949) examined the influence of post-dosing temperature on DDT, γ-BHC, calcium arsenate and parathion applied as a stomach poisons to the honey bees. He found that DDT was more toxic at the higher temperature, but the toxicity of γ-BHC and parathion was independent of temperature. Cressman et al.(1953) showed that change in temperature had no affect on resistance of California red scale to parathion. Vinson and Kearns (1952) found that when DDT was topically applied to Periplaneta americana placed at 15°C. and 35°C. post-treatment temperature, the resultant and percentage mortality at the lower temperature was much greater at the higher temperature. El Mosa and King (1964) found that the toxicity of dieldrin to Hylema antiqua increased at higher post-treatment temperature compared with a lower temperature. Bacon and Riley (1964) found that the toxicity of DDT to adult Lygus hesperus increased at 70°F. compared with 90°F. Evans and Hansens (1968) applied γ-BHC topically to susceptible and resistant houseflies kept at 15°C. and 30°C. post-treatment temperatures. They found no significant difference in mortality between the two temperatures after 24 hours. Barker (1969)

studied the influence of post-treatment temperature after fumigation against Cryptolestes ferrugineus and Cryptolestes turcicus. He found that Cryptolestes turcicus was more susceptible at the lower temperature, but Cryptolestes ferrugineus was only slightly more tolerant to fumigant at the higher temperature.

El Nosa and King (1964) found no significant difference of the effect of different pre-treatment temperatures on resistance of Hylema antiqua to dieldrin. Gaines and Dean (1949) found that the toxicity of γ-BHC and DDT to Anthonomus grandis was reduced at higher temperatures during treatment but the toxicity of calcium arsenate remained nearly of the same order.

FOOD

Hadaway and Barlow (1956) found that the susceptibility of female Aedes aegypti to DDT fed on human blood was not significantly different from that of female fed on guinea-pig blood. Potter and Gillham (1957) examined the effect of host plant on resistance of aphids, Acyrthosiphon pisum, to rotenone. By means of a spraying technique they found that aphids reared on clover were more resistant than those reared on broad bean. Lofgren and Cutkomp (1956) showed Periplaneta americana fed on a high protein diet was more susceptible to DDT than those fed on low protein diet. Bass Rawson (1960) found that adult food of Anthonomus grandis affected its susceptibility to insecticides. Boll-fed weevils were 25 times more difficult to kill with toxaphene, 12 times with γ-BHC and 3 times with Guthion than bloom-fed weevils.

Rattan and Attair (1967), with Trogoderma granarium reared on different diets, found that insects reared on groundnut or maize were less susceptible to γ-BHC and malathion than when reared on cowpea or wheat. Raccah and Tahori (1970) found that mites reared on beans grown in nutrient solution containing a relatively high phosphorus level were more susceptible to malathion than from plants grown in diet containing

no phosphorus. Nitrogen did not exert a significant influence on mite tolerance to malathion. However, Hennebry (1964) working with the two spotted spider mite, Tetranychus telarius, showed that with phosphorus in the diet higher mortality was obtained with an increase in the nitrogen level of the plant nutrient solution.

WEIGHT

Susceptibility is not always directly proportional to body weight Bliss (1936). Working on data from Campbell (1926) found that for a given dose of arsenic per unit weight of Bombyx mori applied as a stomach poison, the larger larvae died more slowly than the smaller larvae. This applied when the larvae were all of the same instar or belong to different instars. Bliss proposed a 'size factor' which he defined as a function of size (in this case body weight) determining the amount of poison to be given to produce equivalent toxic action (in this case measured by survival time). With last instar larvae of Diataraxia oleracea Way (1954) found that parathion applied as a stomach insecticide was linearly related to body weight. TEPP was slightly less and lead arsenate slightly more toxic to the larger larvae. However, DDT as a stomach or contact poison and Y-BHC as a stomach poison were less toxic to the larger larvae. For example, the LD50 for a doubling in larval body weight increased 11 times for DDT as a stomach poison and 12 times as a contact insecticide. Guthrie (1954) with several insecticides applied topically to different larval sizes of tobacco hornworm Protoparce sexta, found that the toxicity decreased with increasing size of the larvae for all insecticides except for

toxaphene. Gast (1959), with four species of lepidopterous larvae treated topically with several insecticides, found that Prodenia eridania had a nearly constant LD50 value for different weight groups with DDT or Phosdrin as did Protoparce sexta with endrin. With Heliothis zea there was no change in the LD50 for Phosdrin but a large increase with DDT within increase in weight was found. The LD50's of Guthion, Diptrex and malathion against Heliothis virescens increased 2-10 times, but endrin, DDT, and toxaphene increased 20-100 times. However, Phosdrin remained constant for different size groups.

Other factors may affect the toxicity of insecticides such as sex, humidity and light have been reviewed by Potter and Way (1958).

PART 1. VARIATION IN TOXICITY TO FIFTH INSTAR SCHISTOCERCA GREGARIA OF DIFFERENT AGES

1.1 Variation in toxicity according to age and method of application

1.11 Introduction

It is known that the relative susceptibility of insects to insecticides varies according to age and stage of development, but the data are somewhat confusing. In locusts, MacCuaig (1960) with fifth instar Schistocerca gregaria found a wide variation in the LD50's of different insecticides acting by contact. With γ-BHC resistance was significantly greater during the middle of the instar than at other ages but this did not happen with other insecticides. To diazinon, locusts appeared to be slightly more susceptible at the end of the instar, while with DNOC resistance increased with age, and locusts of more than 5 days old were about twice as resistant as those of 1-3 days old. With parathion, heptachlor and dieldrin no considerable change in resistance with age was found. With adult Schistocerca, MacCuaig (1961) found that resistance to diazinon decreased with age and three weeks old locusts were more susceptible than one week old. With γ-BHC, insects showed an increased in resistance about

one week after moulting and remained fairly constant during the next three weeks. Resistance of adult Locusta to diazinon was nearly constant, but with γ-BHC resistance increased with age and one week old adults were more than twice as susceptible as those three weeks old. With first instar Schistocerca (MacCuaig et. al., 1964) showed that younger hoppers of 3-16 hours age were more susceptible than the older ones to γ-BHC, diazinon and parathion but these and 1-2 day old were nearly three times as resistant to DNOC as the older hoppers of 3-4 and 4-5 days old.

Resistance of locusts was found to be affected by the method of application. MacCuaig (1960) found no significant difference in resistance of fifth instar Schistocerca to γ-BHC, diazinon and heptachlor when the insecticide was injected instead of being applied by contact. Yet Ahmed and Gardiner (1968) showed that malathion was more effective against adult Schistocerca when applied by contact than by injection. As regards stomach action, Pearson (1951) reported that the toxicity of dieldrin to fifth instar Schistocerca was found to be dependent on the rate of passage of food

and starvation period. MacCuaig and Watts (1960) found that resistance to Schistocerca increased when insects were fed immediately after treatment.

1.12 Materials and Methods

Throughout these experiments, fifth instar hoppers of the Desert Locust Schistocerca gregaria (Forskal) were used as the experimental insects. The main stock of locusts bred at the Centre for Overseas Pest Research, London was used. These locusts originated from the Somali Republic in 1946 and from Saudi Arabia. The locusts from Saudi Arabia were introduced into the Somali stock in 1969 and the culture has been bred extensively ever since.

The locusts were reared in cages measuring 15 x 15 x 20 inches and initially about 1000 newly hatched hoppers were put in each cage; of these about 300-400 became adult. The food was fresh grass and dry bran. The temperature of rearing and breeding was 28°C. at night increasing to 34°C. in the day time. The relative humidity was about 50-60 per cent at night and 30-50 per cent in the day time. Light and heat were provided by

a lamp placed in the cage which was switched on for 8 hours daily from 9.00 a.m. till 5.00 p.m. (For 3 hours only on Saturdays and not at all on Sundays). The constant temperature room provided another 6 hours light without heat from a fluorescent light after 5.00 p.m. The locusts were, therefore, subjected to 14 hours photoperiod. The colour of insects was black and yellow (phase gregaria). For further details of rearing and breeding procedure see Hunter-Jones (1966).

Insects were collected once they started moulting to fifth instar and all insects which moulted between 15-24 hours previously were considered as one day old. Insects were usually collected from more than one cage to get the required number for each experiment and were randomised between insecticides and again into different dose groups in order to reduce some of the variation between batches of locusts. Insects with legs missing were discarded or, in some cases, used as Control. Both sexes were used and as much as possible were distributed in equal numbers.

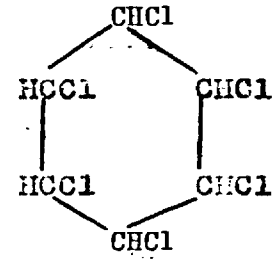
Insecticides used

Three insecticides were used during this investigation, namely benzene hexachloride (organochlorine), Cyolane (organophosphorus) and dioxacarb (carbamate).

Benzene hexachloride (BHC) or more properly hexachlorocyclohexane was used in the gamma isomer form, known as lindane. The formulations were prepared from the technically pure γ-BHC containing 99-100% active ingredient.

Empirical formula: $C_6H_6Cl_6$

Structural formula:

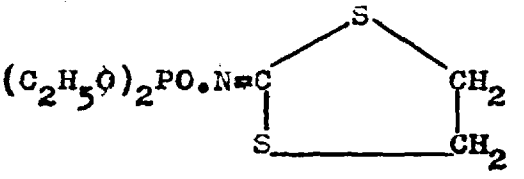


Cyolane

No common name has been approved yet for this compound (Martin 1971), also known as the P,P-diethyl ethylene oster of phosphonodithioimidocabonic acid. The formulations were prepared from stock solution of 25% w/v active ingredient.

Empirical formula: $C_7H_{14}NO_2PS_2$

Structural formula:

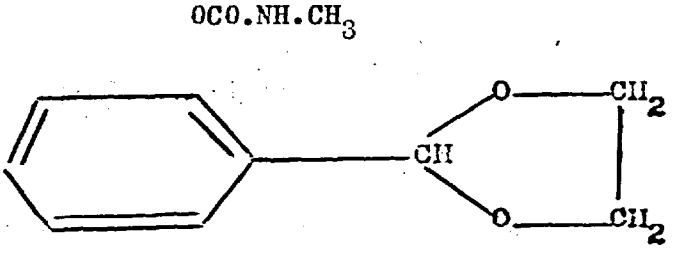


Dioxacarb

Dioxacarb is the common name for 2-(1.3 dioxolane -2-yl) phenyl N-methyl carbamate. The formulations were prepared from stock solution of 20% w/v active ingredient.

Empirical formula: $C_{11}H_{13}NO_4$

Structural formula:



In all cases, solutions of insecticides were made up in kerosene aromatic bottoms (KAB) an aromatic extract of low viscosity often used for insecticide screening tests with locusts e.g. MacCuaig (1958). (For other solvents see 1.9). Different concentrations were made up as required or as dictated by preliminary screening tests. Fresh solutions were prepared at least once every six months.

in case the concentration had changed due to loss of the solvent. The insecticide solutions were stored in the refridgerator except when actually in use.

Application methods

The insecticides were administered to locusts by topical application, by injection or orally. When applied as contact poisons, they were topically applied by a graduated microcapillary pipette developed by MacCuaig and Watts (1968). The microcapillary applicator has also been used in the field in tests of insecticides to locusts, vegetation and armyworm larvae (Watts 1965; Watts and Steel 1965). With this apparatus small accurately measured doses of insecticides can be very easily administered. The accuracy of the technique was checked by using a colorimetric technique in which different concentrations of standard solutions of waxoline red dye in xylene were calibrated using an absorptiometer.

Injection methods

In the injection work, Shandon or Hamilton

micro-syringes were used. The volume of the injection was 1.0-3.5 µl according to dose and the weight of insect. Similar volumes of the solvent injected alone caused no death among the control group. Injection was made into the body cavity between the first and second abdominal sternites and care was taken during the injection not to push the needle too far as it may injure the alimentary canal. It was observed, in a few cases, that some of the insecticide solution escaped while injecting; these insects were not used.

Stomach methods

Methods of feeding treated leaf areas to test insects were similar of the sandwich technique described by Campbell and Filmar (1929) and Bulger (1937), in which doses of insecticides were applied to small pieces of grass about 2 inches in length, and folded together to prevent any contact between insects and the toxic material before eating. The dose according to weight was applied to a piece of grass as a single drop, using a micro-pipette, near one end of the leaf portion and allowed

to dry. After the drop had dried the other half of the leaf was folded over, thus containing the drop between two leaf surfaces. Locusts were normally starved for 24 hours before treatment and each locust received a known dose of insecticide sandwiched between two halves of a blade of grass. This was clipped at one end of a cork in a small wire cylindrical cage (4" x 3"). Untreated food was available to the insect after it had eaten the poisoned food. Insects not eating the sandwich within 15-20 minutes were discarded.

Dose of insecticides

The doses used were determined for each insecticide from preliminary experiments using a logarithmic dosage scale covering a range such that at the minimum dose a kill of 0-10 per cent and at the maximum dose 90-100 per cent were expected. Before dosing, each locust was weighed and the quantity of insecticides applied was adjusted to give a preselected amount of active ingredient per g. of body weight. The volume of insecticide was calculated from the concentration (w/v), dose required (µg/g) and the weight of insect (g).

This means, for example, if the locust's weight is 1.6 g and the dose is 12.0 µg/g, the concentration of insecticide is 3% w/v; in a volume of 0.64 µl.

Test technique

Locusts of the required age were collected from the laboratory stock. They were treated individually and kept in groups of 10 insects in wire mesh cage (6.5" x 6.5" x 6") at 25-27°C. temperature and 60-80 per cent relative humidity. Food was supplied after treatment.

Mortality

Counts of insects were made 24 hours after treatment and continued on successive days till there were either no more deaths, or for 6 days, whichever was the shorter. The insects were recorded as slightly affected, badly affected or moribund or dead. Locusts badly affected or moribund were considered dead for statistical analyses. Badly affected insects could not right themselves when turned upside down. No cases of insects recovering after reaching this state were observed.

Control

Control groups were maintained throughout the work and treated in the same way as the test insects except that they were treated with the solvent only. Little mortality was obtained, and this allowed for by using Abbott's (1925) formula to correct for natural mortality.

Results

The LD50 and LD99 levels were usually calculated by probit analysis (Finney,1952) otherwise graphical estimation was made from a provisional probit regression line. In each experiment 100-150 insects were used and each treatment was replicated three times. A total of 650-900 insects were used for each insecticide. Insecticides were applied to the intersegmental membrane between the first and second abdominal sternites, for both topical application and injection. This site was chosen as being convenient and also because insecticides are not easily wiped off the locust after application. It is the standard site used at C.O.P.R. for experimental work. (For other sites of

application see 1.3). Toxicity figures were determined from the mortalities at the completion of the experiments. The LD50's were also obtained from earlier data in order to show the relative speed of action of the different insecticides.

The purpose of the experiments described in this section was to study the relative susceptibility of fifth instar hoppers Schistocerca gregaria of three selected ages to γ-BHC, Cyolane and dioxacarb applied by topical application, by injection or orally. (For details of other ages, see 1.2).

1.13 Toxicity of topically applied insecticides

Insecticides were applied by means of the micro-capillary as described in Materials and Methods. Locusts were used at (15-24 hours) 1 day after moulting, 2-3 and 5-6 days old.

γ-BHC

In this experiment, approximately 160 insects were used at each age group and a total of 510 locusts were used to cover all three age groups.

The first inspection was made 24 hours after application and it was noticed that locusts remained apparantly normal in all doses, the effect of poisoning being gradual. Throughout the experiment, poisoning was for the most part progressive and locusts classified in one category had to be transferred to the next category e.g. normal, slightly affected, badly affected, moribund and dead. The symptoms observed were typical of -BHC poisoning as described by Pasquier (1947). He described the succession of symptoms in Schistocerca as (1) Prodromal phase: the abdomen is raised to a position characteristic of anophelines. (2) Typical phase: the abdomen exhibits telescopic movements, and is rubbed by the hind legs. (3) The insect falls on its side or back, tremors develop in legs and mouthparts. (4) Paralytic phase. At the end of the experiment, all locusts not dead were counted as normal.

The results are shown in Table 1 with the LD50's, 95% confidence limits and the relative resistance. Graph 1 shows the probit regression lines.

Table 1

Effect of differences of age of Schistocerca on the resistance to γ-BHC by topical application

Age (days)	Regression equation	LD50 µg/g	95% confidence limits	Relative resistance
1	6.19x+0.17	6.8	6.4-7.3	1.0
2-3	6.73x-1.37	8.7	7.9-9.3	1.28
5-6	5.90x1.19	11.1	10.4-12.0	1.63

Table 1 indicates that the resistance to γ-BHC is greater at 5-6 days old than at the other ages.

Cyolane

The procedure followed was the same as that adopted for γ-BHC. A total of 110 insects were used at each age and 330 insects were used throughout the experiment to determine the toxicity of Cyolane.

The first inspection was made 24 hours after treatment and it was observed that locusts in most treatments showed signs of poisoning. After treatment, locusts were very active but later on appeared to be badly affected. The full effect of Cyolane could be assessed

within 4 days, after that little change in mortality figures occurred. The symptoms observed were excitability, followed by tremors that are especially noticeable in the extremities, and finally paralysis and death. The criteria used for assessment of the effect were the same as before. At the end of the experiment, all locusts not dead were counted as normal.

The results are shown in Table 2, with the LD50's, 95% confidence limits and the relative resistance. Graph 2 shows the probit regression lines.

Table 2

Effect of differences of age of Schistocerca on the resistance to Cyolane by topical application

Age (days)	Regression equation	LD50 µg/g	95% confidence limits	Relative resistance
1	6.70x-2.17	2.6	2.4-2.9	1.0
2-3	6.41x-2.68	3.1	2.8-3.4	1.19
5-6	5.44x-0.97	2.8	2.6-3.0	1.07

The results in Table 2 indicate that the resistance of different age group of fifth instar hoppers did not differ significantly

at the 95% level so would appear to be similar although there are indications that the 2-3 days old hoppers might be more resistant than the older or younger hoppers.

Dioxacarb

The method of treatment was the same as in the previous experiments. A total of 110 insects were used at each age group and 360 insects were used throughout the experiment to determine the toxicity of dioxacarb.

The first inspection was made 24 hours after treatment and it was observed that locusts in all treatments showed signs of poisoning. Some of the locusts were motionless, others were badly affected. The full effect of dioxacarb could be assessed within 4 days and after that little change in mortality occurred. The symptoms observed were salivation, convulsion and death. At the end of the experiment, all locusts not dead were counted as normal.

The results are shown in Table 3, with the LD50's, 95% confidence limits and the relative resistance. Graph 3 shows the probit regression lines.

Table 3

Effect of differences of age of Schistocerca on the resistance to dioxacarb by topical application

Age (days)	Regression equation	LD50 µg/g	95% confidence limits	Relative resistance
1	4.00x-1.33	2.1	1.6-2.3	1.0
2-3	5.44x-0.79	2.5	2.2-2.8	1.19
5-6	5.30x-1.13	2.8	2.5-3.2	1.33

Table 3 suggests that the resistance of different age groups to dioxacarb increased slightly as the age increased. Although the differences between 1 and 2-3 days and between 2-3 and 5-6 days are not significant at the 95% level, there is a difference between 1 day and 5-6 days old insects at this level.

1.14 Toxicity of insecticides applied by injection

Insecticides were injected into the abdomen by the micro-syringe as described in Materials and Methods.

γ-BHC

The toxicity of γ-BHC applied by injection was determined at different age groups and the experimental condition of treatment were similar to those of the proceeding experiments.

Approximately 270 insects were used in the whole experiment. The first inspection was made 24 hours after treatment and it was observed that the effect of γ-BHC was gradual. The symptoms observed, as poisoning progressed, followed closely those described for topical application, however, the development of poisoning was at a slower rate. The criteria used for assessment of the effect was the same as before. In the final analysis, all locusts not dead were counted as normal.

The results are shown in Table 4, with the LD50's, 95% confidence limits, and the relative resistance. Graph 4 shows the probit regression lines.

Table 4

Effect of differences of age of Schistocerca on the resistance to γ-BHC by injection

Age (days)	Regression equation	LD50 µg/g	95% confidence limits	Relative resistance
1	6.39x-1.92	12.0	11.4-12.7	1.29
2-3	5.77x-1.46	13.1	11.8-14.6	1.40
5-6	6.50x-0.93	9.3	8.3-10.5	1.0

Results in Table 4 indicate that the resistance to γ-BHC is most at 2-3 days old than at other days but it did not differ significantly at the 95% level from 1 day old hoppers. The older hoppers are more susceptible than the younger ones although the reverse was found by topical application.

Cyolane

A total of 240 insects were used throughout the experiment to determine the toxicity of Cyolane.

The first inspection was made 24 hours after treatment and it was observed that locusts in

all treatments showed signs of poisoning. The symptoms observed were the same as of topical application. The criteria used for assessment of the effect were the same as before. In the final analysis, all locusts not dead were counted as normal.

The results are shown in Table 5, with the LD50's, 95% confidence limits and the relative resistance. Graph 5 shows the probit regression lines.

Table 5

Effect of differences of age of Schistocerca on the resistance to Cyolane by injection

Age (days)	Regression equation	LD50 µg/g	95% confidence limits	Relative resistance
1	5.50x-1.68	3.2	2.9-3.6	1.6
2-3	4.7x-1.12	2.0	1.9-2.1	1.0
5-6	5.65x-3.07	2.7	2.6-3.1	1.35

Table 5 indicated that the resistance of different age groups of fifth instar to Cyolane was significantly less in insects 2-3 days old, but 5-6 days old insects did not

differ from that 1 day old.

Dioxacarb

In this experiment, approximately 220 insects were used to determine the toxicity of dioxacarb.

The first inspection was made 24 hours after treatment and it was observed that locusts in all treatments showed signs of poisoning. The symptoms of poisoning were the same as described for topical application, but it developed at a faster rate. The criteria used for assessment of the effect were the same as before. At the end of the experiment, all locusts not dead were counted as normal.

The results are shown in Table 6, with the LD50's, 95% confidence limits and the relative resistance. Graph 6 shows the probit regression lines.

Table 6

Effect of differences of age of Schistocerca on the resistance to dioxacarb by injection

Age (days)	Regression equation	LD50 µg/g	95% confidence limits	Relative resistance
1	5.50x-0.40	1.9	1.7-2.10	1.73
2-3	5.80x-1.31	1.2	1.1-1.40	1.09
5-6	6.13x-3.81	1.1	0.95-1.25	1.0

The results in Table 6 indicate that the resistance of different age groups of fifth instar hoppers to dioxacarb is greater at 1 day old than at other ages, and becoming progressively less as the insects became older.

1.15 Toxicity of insecticides applied orally

Insecticides were ingested through the gut, the technique and details of treatment as described in Materials and Methods. In the following experiments, locusts were treated at 2-3 days only.

γ-BHC

A total of 140 locusts were used throughout the experiment. The first inspection was made 24

hours after ingestion of the poisoned sandwich, and it was observed that in badly affected locusts a small quantity of faecal matter was excreted. The symptoms observed were as described before.

At the end of the experiment, all locusts not dead were counted as normal. The result is shown in Table 7 and Graph 7.

Cyolane

In this experiment, a total of 100 locusts were used. The first inspection was made 24 hours after ingestion of the poisoned sandwich and it was found that badly affected locusts has excreted a smaller quantity of faecal matter, in some cases, producing the faeces as complete rod. The symptoms observed were as described before. At the end of the experiment, all locusts not dead were counted as normal.

The result is shown in Table 7 and Graph 7.

Dioxacarb

A total of 130 locusts were used throughout the experiment. The first inspection was

made 24 hours after ingestion of the poisoned sandwich, and it was observed that badly affected locusts has excreted a smaller quantity of faecal matter, in some cases, as a complete rod. The symptoms observed were as described before, but developed at a slower rate.

The result is shown in Table 7 and Graph 7.

Table 7

Stomach toxicity of insecticides to 2-3 days old Schistocerca

Insecticide	Regression equation	LD50 µg/g	95% confidence limits
γ-BHC	6.32x-2.35	14.6	13.40-15.80
Cyolane	9.86x-0.05	3.3	3.02-3.50
Dioxacarb	8.73x-2.84	8.6	8.10-9.20

Table 7 indicates that 2-3 days old hoppers are more resistant to γ-BHC than other insecticides when these are taken orally. Comparing this result with those found by topical application or by injection it appears that hoppers are more resistant to

insecticides when administered orally than other methods.

1.16 Effect of the stomach toxicity of γ-BHC in relation to age

Materials and Methods

Hoppers were used at 1-2 and 7-8 days after moulting, and they were starved for 24 hours before treatment. γ-BHC was administered orally as described before. A total of 80 locusts of each age were used throughout the experiment.

Results

The first inspection was made 24 hours after ingestion the poisoned sandwich and observations were continued on successive days till there were no more deaths or for 6 days. The results are given in Table 8 and Graph 8.

Table 8

Effect of differences of age of Schistocerca on the resistance to γ-BHC applied orally

Age (days)	Regression equation	LD50 µg/g	95% confidence limits
1-2	5.90x-2.83	10.6	9.40-12.0
2-3	6.23x-2.35	14.6	13.40-15.8
7-8	5.00x-0.85	7.4	6.40-8.6

Table 8 indicates that the resistance of different age groups of fifth instar to γ-BHC is greater at 2-3 days than at other ages, and 7-8 days old hoppers are less resistant than the younger hoppers.

Discussion

Dioxacarb is generally the most effective poison, irrespective of the manner of application or age of the insect. This clearly shown by the LD50's expressed relative to the figures for dioxacarb taken as unity (Table 9). Cyolane is only slightly less effective by injection or topical application, but it is considerably more effective as a stomach poison.

γ-BHC is markedly less effective.

The results show that there are clear differences in the effects of different insecticides depending on the manner of application and the age of the insects. The results are summarised in Tables 10 and 11 which give the calculated LD50's and LD99's values.

When insecticides applied by injection, γ-BHC was less effective than by topical application. MacCuaig (1960) found no significant difference in the toxicity of γ-BHC by topical application or by injection. He injected γ-BHC in propylene glycol as a solvent instead of KAB which was used for topical application. Thus, the change in the solvent is likely to influence the results. In contrast to γ-BHC, dioxacarb was slightly more effective when injected than when applied topically, while the susceptibility to Cyolane was similar irrespective of the method of application. Greater toxicity of insecticides following injection was found with houseflies for dieldrin (Sun et.al.,1967), but topical application was more effective than injection with adult Schistocerca for malathion

(Ahmed and Gardiner 1968).

In all cases oral application was the least effective method of killing the locusts. Similar results were reported by Pearson (1951) using dieldrin against fifth instar Schistocerca.

An examination of the relative susceptibility and age of topically applied insecticides showed that γ-BHC and dioxacarb resistance increased within increase in age, with Cyolane locusts were more resistant at 2-3 days than at the other days. By contrast, susceptibility increased with age following the injection of γ-BHC or dioxacarb.

Insecticide	Treatment	Relative resistance Age (days)		
	Topical application	1	2-3	5-6
γ-BHC		3.23	3.48	4.44
Cyolane		1.23	1.24	0.89
Dioxacarb		1.0	1.0	1.0
	Injection			
γ-BHC		6.3	10.9	8.45
Cyolane		1.68	1.67	2.45
Dioxacarb		1.0	1.0	1.0
	Stomach			
γ-BHC		-	1.69	-
Cyolane		-	0.38	-
Dioxacarb		-	1.0	-

Table 9

Relative resistance of γ-BHC, Cyolane and dioxacarb, based on the LD50's, applied by different methods to different aged 5th instar desert locust hoppers.

Insecticide	Treatment	Age (days)		
		1	2-3	5-6
γ-BHC	Topical application	6.8	8.7	11.1
	Injection	12.0	13.1	9.3
	Stomach	-	14.6	-
Cyolane	Topical application	2.6	3.1	2.5
	Injection	3.2	2.0	2.7
	Stomach	-	3.3	-
Dioxacarb	Topical application	2.1	2.5	2.8
	Injection	1.9	1.2	1.1
	Stomach	-	8.6	-

Table 10

Calculated LD50's (μg/g) for three insecticides applied by different ways to different aged 5th instar desert locust hoppers.

Insecticides	Treatment	Age (days)		
		1	2-3	5-6
γ-BHC	Topical application	16.6	19.2	27.5
	Injection	28.0	33.1	21.2
	Stomach	-	33.9	-
Cyolane	Topical application	5.8	7.2	6.76
	Injection	8.6	6.2	6.9
	Stomach	-	5.6	-
Dioxacarb	Topical application	6.9	6.7	7.8
	Injection	5.1	3.1	2.6
	Stomach	-	14.6	-

Table 11

Calculated LD99's (µg/g) for three insecticides applied by different ways to different aged 5th instar desert locust hoppers.

1.2 Further experiments on age in relation to susceptibility to insecticides

1.21 Introduction

In the previous section it was shown that susceptibility of the Desert Locust to some insecticides changed with the age of the insects, with the insecticides and with the method of application. Thus, with γ-BHC resistance increased with age and older locusts (5-6 days old) were almost as twice as resistant as the younger locusts (1 day old) when the insecticide was applied by topical application. With injection, resistance decreased with age and one day old insects were more resistant than older insects. However, only three age-groups were considered in those experiments, and moreover both males and females were taken together.

The present section, therefore, describes experiments in which only males were used, in order to reduce the variation in weight and sex between individuals, and in which the maximum spreads as regards age within a group was less than 24 hours, also the range considered was broadened to include hoppers that were 9 days old. Insecticides were applied

by topical application or by injection.

1.22 Materials and Methods

Insects used were fifth instar (males) Schistocerca collected at 15-24 hours after moulting from fourth instar, and used during the fifth instar period. The instar, under condition of breeding already described in the previous section, lasted for 10 days.

Change in the weight of fifth instar hoppers with age

To determine the change in the weight of the insects during the fifth instar period, 50 locusts of each age were weighed at the time of treatment throughout the experiment. The mean weight per locust in each age is given in Figure 1. The weight increased progressively with age up to the 7th day and then decreased. Locusts weighed about 0.9-93 g 24 hours after moulting, gained weight with increasing age, and weighed most on the 7th day, but lost weight thereafter; coinciding with the physiological changes before moulting.

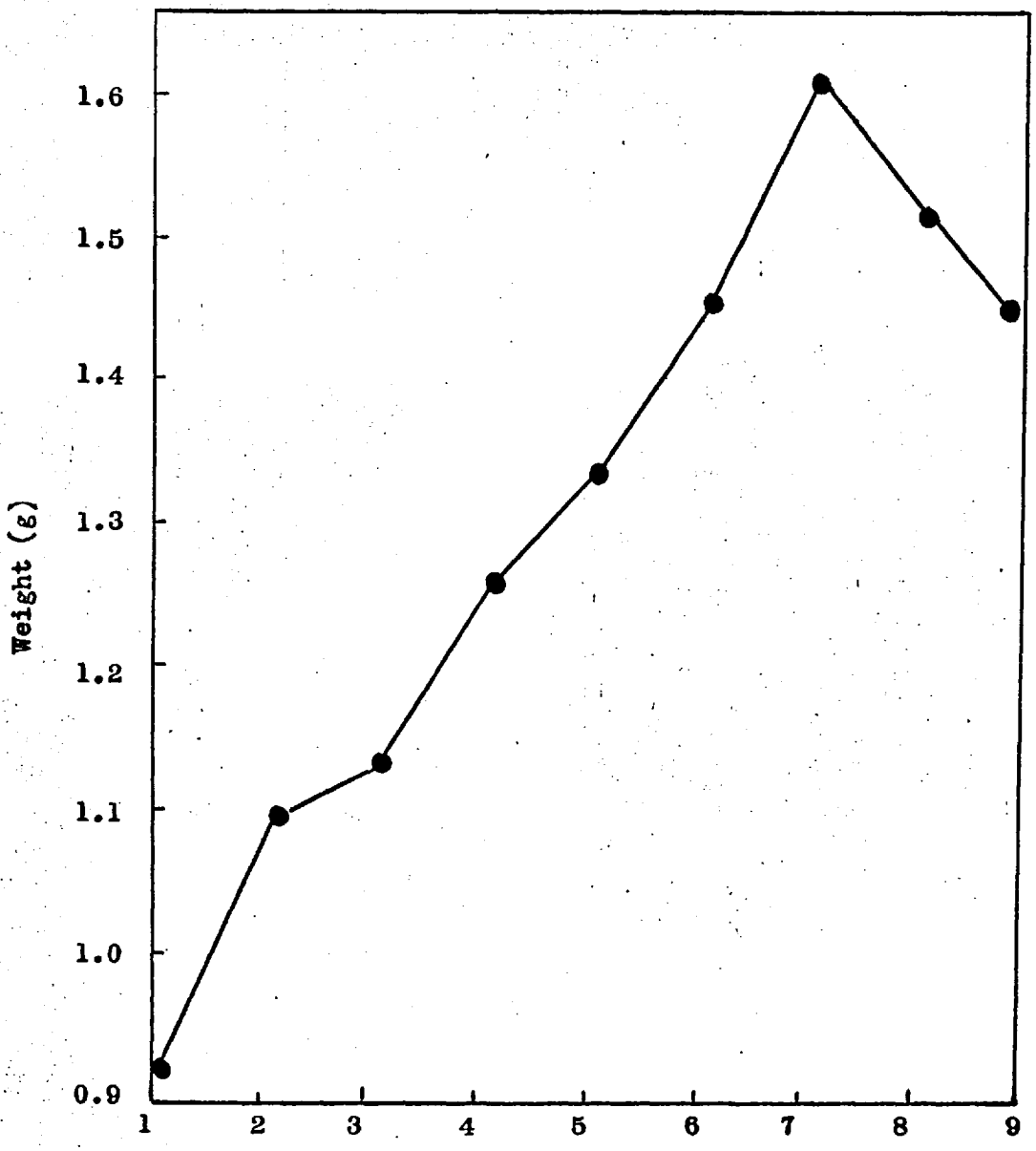


Figure 1

Age from the beginning of fifth instar

The change in body weight during the fifth instar of Schistocerca gregaria.

Most of the toxicity data were obtained from the provisional regression line and details of susceptibility are shown in Appendix. However, groups of insects of different age but approximately similar in resistance, on the LD50's level, to each insecticide were grouped together and the LD50's and LD99's were calculated using the methods given by Finney (1952).

1.23 Toxicity of topical application

γ-BHC

In this experiment, a total of 370 locusts were used. The first inspection was made 24 hours after treatment and observations were continued on successive days till there were no more deaths.

The results are shown in Figure 2, where each point is based on 10 or 20 insects. The figure suggests that the LD50 of 1 day old is lower than subsequently, but that insects 2-7 days are similar. Hence for purposes of analysis the results are combined in Table 12 and Graph 9.

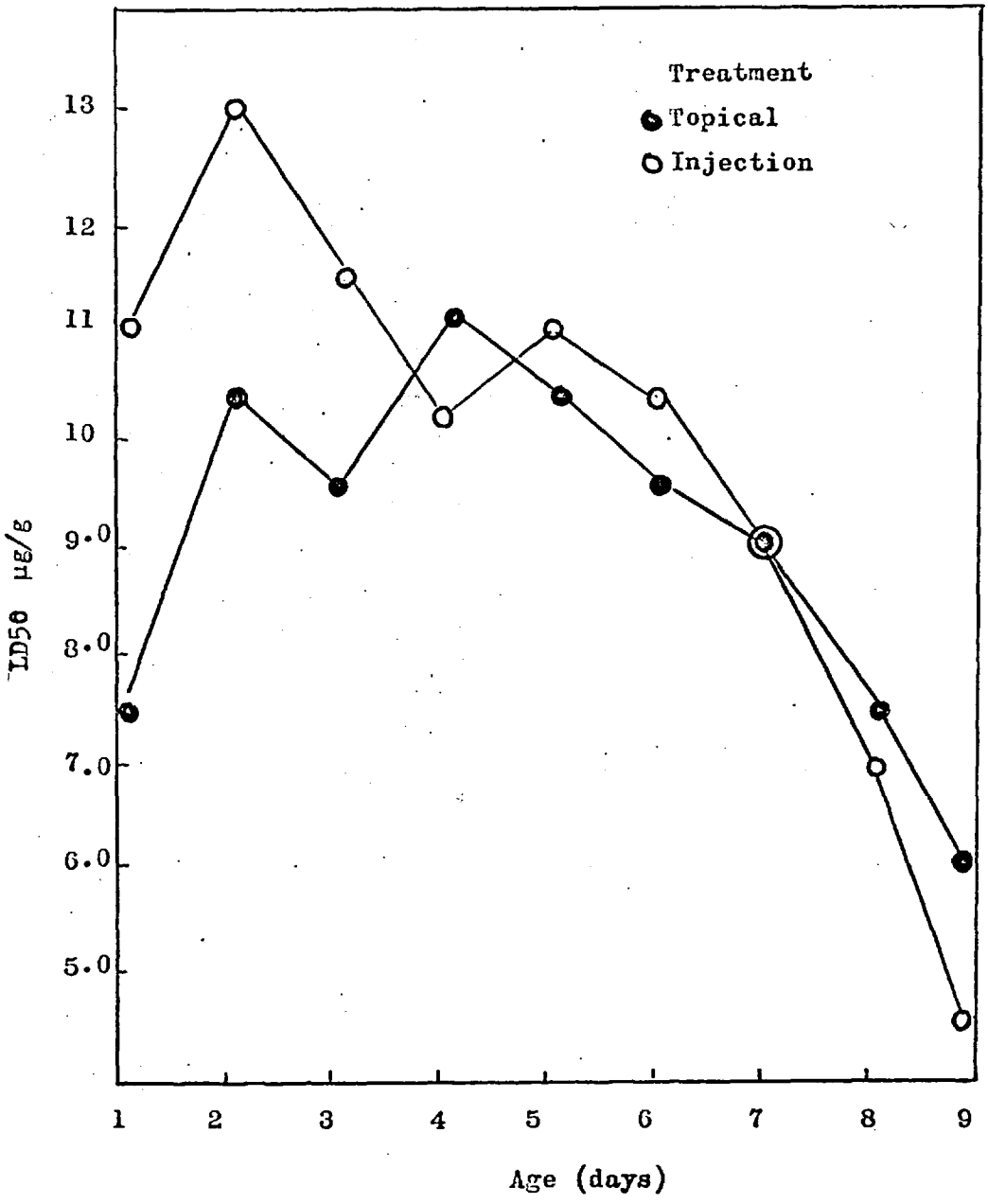


Figure 2

Relation between age of Schistocerca and resistance to γ-BHC.

Table 12

Toxicity of γ-BHC by topical application to different age groups of Schistocerca.

Age (days)	Regression equation	LD50 µg/g	95% confidence limits
1	4.62x-2.19	7.2	6.04-8.37
2-7	7.12x-3.98	9.12	8.70-9.6
8-9	4.91x-2.58	7.0	6.60-736

Table 12 indicated that the resistance to γ-BHC was significantly greater at 2-7 days old, but it was similar at 1 and 8-9 days old.

Cyolane

A total of 350 locusts were used throughout the experiment. The first inspection was amde 24 hours after treatment and observations were continued on successive days till there were no more deaths. The symptoms of poisoning and criteria of effect were similar to those described in 1.13.

The LD50's of 1-9 days, based on 10 or 20 insects, are shown in Figure 3. The results are combined in Table 13 and Graph 10.

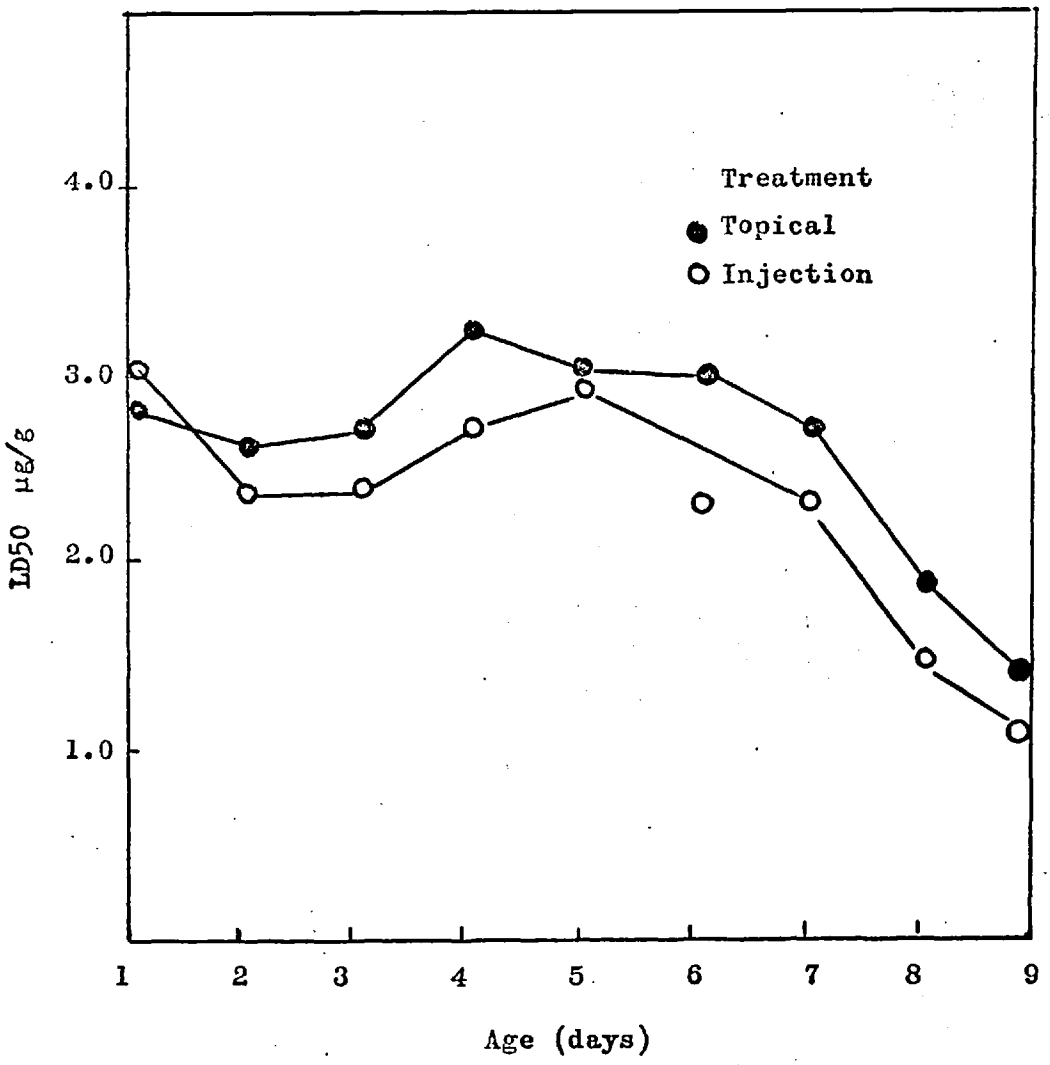


Figure 3

Relation between age of Schistocerca and resistance to Cyolane.

Table 13

Toxicity of Cyolane by topical application to different age groups of Schistocerca.

Age (days)	Regression equation	LD50 µg/g	95% confidence limits
1-7	5.72x-5.59	2.8	2.66-3.0
8-9	4.22x-1.67	1.6	1.40-1.9

Table 13 indicated that 1-7 days old hoppers were significantly more resistant to Cyolane than 8-9 days old hoppers.

Dioxacarb

In this experiment, a total of 440 locusts were used. The first inspection was made 24 hours after treatment and observations were continued on successive days till there were no more deaths. The symptoms of poisoning and criteria of effect were as described in 1.13.

The LD50's of 1-9 days, based on 10 or 20 insects, are shown in Figure 4. The results are combined in Table 14 and Graph 11.

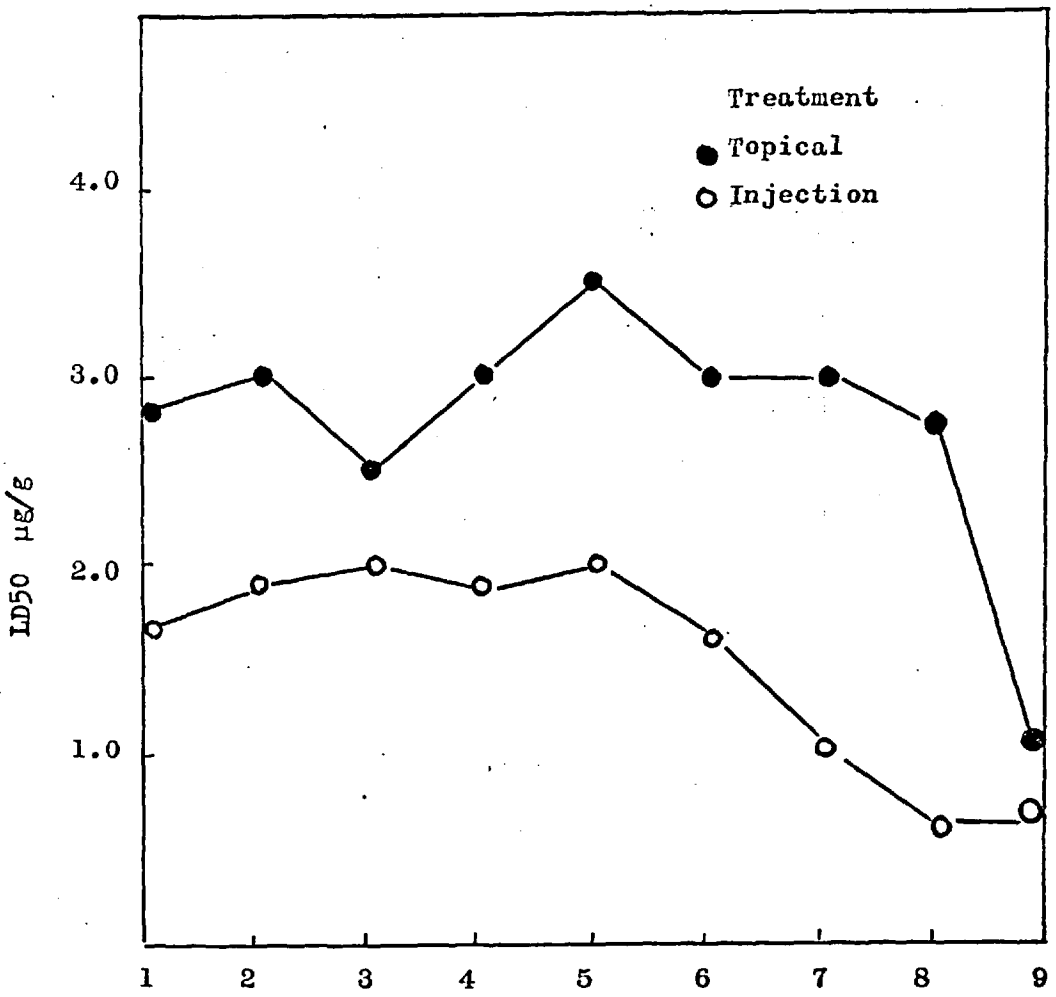


Figure 4

Relation between age of Schistocerca and resistance to dioxacarb.

Table 14

Toxicity of dioxacarb by topical application to different age groups of Schistocerca.

Age (days)	Regression equation	LD50 µg/g	95% confidence limits
1-7	6.64x-7.64	3.2	2.9-3.5
8	4.28x3.0	2.9	2.6-3.4
9	3.99x-0.5	0.95	0.7-1.2

Table 14 indicated that older hoppers of more than 7 days were more susceptible than the younger hoppers, although resistance of 1-7 days old hoppers was of the same order of 8 days old at the 95% level.

1.24 Toxicity by injection

γ-BHC

In this experiment, a total of 420 locusts were used. The first inspection was made 24 hours after treatment and observations were continued for 6 days. The symptoms of poisoning and criteria of effect were as described before.

The LD50's of 1-9 days, based on 10 or 20 insects, are shown in Figure 2. The results are combined in Table 15 and Graph 12.

Table 15

Toxicity of γ-BHC by injection to different age groups of Schistocerca.

Age (days)	Regression equation	LD50 µg/g	95% confidence limits
1-7	5.90x-4.96	9.8	9.2-10.3
8-9	5.18x-2.24	5.0	4.4-5.6

Table 15 indicated that 1-7 days old hoppers were more resistant to γ-BHC than 8-9 days old hoppers.

Cyolane

A total of 460 locusts were used throughout the experiment. The first inspection was made 24 hours after treatment and observations were continued for 6 days. The symptoms of poisoning and criteria of effect were as described before.

Figure 3 shows the LD50's of 1-9 days, where each point is based on 10 or 20 insects. The results are combined in Table 16 and Graph 13.

Table 16

Toxicity of Cyolane by injection to different age groups of Schistocerca.

Age (days)	Regression equation	LD50 µg/g	95% confidence limits
1-7	5.10x-4.39	2.77	2.6-3.0
8-9	3.45x-0.195	1.28	1.3-1.4

Table 16 indicated that 8-9 days old hoppers were less resistant to Cyolane than the younger hoppers of 1-7 days old.

Dioxacarb

In this experiment, a total of 400 locusts were used. The first inspection was made 24 hours after treatment and observations were continued for 6 days. The symptoms of poisoning and criteria of effect were as described before.

Figure 4 shows the LD50's of 1-9 days old, where each point is based on 10 or 20 insects. The results are combined in Table 17 and Graph 14.

Table 17

Toxicity of dioxacarb by injection to different age groups of Schistocerca.

Age (days)	Regression equation	LD50 µg/g	95% confidence limits
1-5	7.26x-6.96	1.80	1.70-1.9
6-7	3.74x-0.10	1.01	0.85-1.2
8-9	8.08x-5.23	0.74	0.73-0.8

Table 17 indicated that the resistance to dioxacarb was greatest at 1-5 days and hoppers of more than 5 days old were less resistant.

Discussion

The toxicity of Cyolane and dioxacarb to fifth instar by topical application was of the same order, but dioxacarb was slightly more effective by injection. Y-BHC was considerably less effective.

Results indicated that the LD50's of insects during the fifth instar period was lower at 1 day after moulting, increased slowly and reached maximum value at 4-5 days, and decreased after the 7th day. Susceptibility of hoppers to insecticides increased at the end of the instar, this may be attributed to changes in the body, part from a

decrease in weight, coinciding with preparation for moulting.

Variation in susceptibility with age has been reported by some workers. Bass and Rawson (1960) found that 9 days old adult Anthonomus grandis were more resistant to γ-BHC than 2 days old weevils. Sawicki and Green (1964) found that adult houseflies were most susceptible to diazinon after they had emerged from the pupae than 5 days old adults. El Mosa and King found little difference of effect of age in resistant of Hylemya antiqua to dieldrin.

1.3 Influence of the site of application

1.31 Introduction

In the earlier experiments, the effect of age and the effect of methods of application was investigated. It has been shown that for some insects the site of topical application can affect susceptibility. For locusts, Kennedy et al. (1948) found that the legs of Locusta was the most susceptible part to DNOC, and application to the wings was less effective. Yet, with the same

insecticide against Schistocerca (MacCuaig, 1965) found that the antenna was the most vulnerable part and application to the legs or the wings was less effective. With malathion, (Ahmed and Gardiner 1968) found a considerable variation in toxicity against Schistocerca. It was lethal on the neck, sub-lethal on the abdomen and less effective on the legs.

A series of experiments, therefore, were set up to investigate the effects of site of application on fifth instar Schistocerca using γ-BHC, Cyolane and dioxacarb.

Materials and Methods

Locusts were used at 2-3 days old and the conditions of breeding were essentially similar to those described in the previous sections. The sites selected were the head, the neck membrane, the wing base, the hind leg and between the 1st and 2nd abdominal sternites (the standard site) at the tip of the abdomen. The choice was made on the basis of difference in cuticle thickness and distance from the head. Sites such as the neck membrane and the wing base have been used by other workers.

Insecticides were applied topically by means of the micro cap and it was noticed that the drop spreads from the position which it was placed. When applied to the head on the frons, the drop was usually seen to spread around the region and sometimes to the mandibular articulations. When applied to the wing base, the drop was applied dorsally in the region of the axillary sclerites. On the femur of the hind leg, the drop was placed on the centre of the large flat lateral area and the locust was held for a few seconds to allow the drop to spread sufficiently. On the neck, the drop was applied dorsally to where the neck membrane. On the abdomen, the drop was ventrally applied to the intersegmental membrane between the 1st and 2nd abdominal sternites.

Toxicity tests were carried out using a single dose applied topically to different parts of the body. After treatment, locusts were held for a few seconds in order to prevent insecticides being wiped off by accidental contact. Locusts were supplied with food and maintained at constant conditions of temperature and humidity as described in 1.12.

1.32 Influence of the site of application on the toxicity of γ-BHC

In this experiment, all six sites were used and a total of 120 locusts was used.

The first inspection was made 6 hours after application, and it was found that locusts treated on the neck were badly affected, those treated on the wing base were slightly affected, and insects treated at other sites were normal. The full effect of -BHC applied to the neck was complete in less than 24 hours, compared with 72 hours on the wing base and up to 6 days on other sites.

The results are shown in Table 18 with numbers of locusts killed at each site of application with a single dose of 12.6 µg/g.

Table 18

Susceptibility of different parts of the body of Schistocerca to γ-BHC

Experiment	No. of locusts per site	Mortalities for different sites					
		Head	Neck Membrane	Wing base	Hind Leg	Abdomen posterior end	Abdomen standard
1	10	5	10	9	1	5	6
2	10	10	10	9	0	3	9
Total killed	20	15 75	20 100	18 90	1 5	8 40	15 75

Table 18 shows a large variation in toxicity of different sites of application to γ-BHC. Thus application on the neck membrane was the most toxic and on the hind leg, least toxic.

1.33 Influence of the site of application on the toxicity of Cyolane

In this experiment, five sites were tested and a total of 100 locusts were used.

The first inspection was made 6 hours after treatment, and it was observed that locusts treated on the neck or the wing base were badly affected, while after application to other sites they were only slightly affected. All locusts treated on the neck were dead before 24 hours, on the wing base in 48-72 hours, while insects treated on other sites lasted for 6 days.

The results are given in Table 19 with numbers of locusts killed following topical application to each site of single dose of 4.0 µg/g.

Table 19

Susceptibility of different parts of the body of Schistocerca to Cyolane

		Mortalities for diffement sites				
Experiment	No. of locusts per site	Head	Neck Membrane	Wing base	Hind leg	Abdomen standard
1	10	8	10	10	7	8
2	10	8	10	9	6	6
Total % killed	20	16 80	20 100	19 95	13 65	14 70

Table 19 shows a little variation in toxicity of Cyolane between different sites of application. Cyolane was toxic on the neck membrane or the wing base and application to the hind leg was as effective as to the abdomen.

1.34 Influence of the site of application on the toxicity of dioxacarb

In this experiment, five sites were tested and a total of 100 locusts were used.

The first inspection was made 6 hours after application, and it was observed that locusts treated on the neck or on the wing base were badly affected; other sites were only slightly affected. All locusts treated on the neck were dead before 24 hours, on the wing base in 48-72 hours, while after treatment of other sites they lasted for 6 days.

The results are given in Table 20 with the numbers of locusts killed following topical application to each site of a single dose of 3.5 µg/g.

Table 20

Susceptibility of different parts of the body of Schistocerca to dioxacarb

Experiment	No. of locusts per site	Mortalities for different sites: Head	Neck Membrane	Wing base	Hind leg	Abdomen standard
1	10	2	10	8	0	9
2	10	7	10	8	0	7
Total % killed	20	9 45	20 100	16 80	0 0	16 80

Table 20 shows a wide variation in toxicity of dioxacarb between different sites of application. Thus application on the neck membrane was lethal, on the abdomen or the wing base was effective, while on the head or the leg was least.

1.35 Discussion

It can be seen that with γ-BHC very large differences in effectiveness were observed between sites of application. Mortality ranged from 100% to 5%.

The most sensitive part was the neck membrane followed by the wing base, abdomen (intersegmental membrane), posterior end of the abdomen and the hind leg.

With Cyolane, not much difference between different sites of application was found. Table 19 shows that the neck membrane and the wing base are the most susceptible part followed by the head, the abdomen and the hind leg. The toxicity of Cyolane on the leg is about the same as on the abdomen.

With dioxacarb, a wide variation was found depending on sites of application. This ranged from 100% killed to non-killed. The most susceptible part was the neck membrane, followed by the wing base and the abdomen, the head and the leg.

The results show that the toxicity of an insecticide is affected by site of application where higher mortality is obtained from application to the neck membrane and lower mortality from the hind leg as shown with γ-BHC and dioxacarb. It indicates also that the relative effectiveness of different insecticies at any given site depends on their pysco-chemical properties. For example, application to the hind leg, Cyolane is effective as on the head

(65% mortality), γ-BHC less effective (5% mortality) and dioxacarb is least (non-killed).

Results showed that the neck membrane was the most susceptible part to the insecticides tested. This may be attributed to the fact that the neck is near the central nervous system which is considered to be the site of action of most insecticides (O'Brien, 1967). However, application to the head is of intermediate toxicity for γ-BHC and Cyolane and leas effective for dioxacarb. To test the possibility of the cuticle role in the toxic action of γ-BHC, another set of experiment was carried out using a fixed dose of γ-BHC applied topically or by injection to the neck membrane.

In this experiment, a total of 80 locusts were used. A fixed dose of 10 µg/g of γ-BHC was applied topically or by injection. The results are given in Table 21.

Table 21

Toxicity of γ-BHC applied topically or by injection into the neck membrane

	No. of locusts killed		
Experiment	No. of locusts per site	Topical application	Injection
1	10	10	2
2	10	10	6
3	10	10	4
4	10	9	6
Total	40	39	18
% killed		97	45

The results show that γ-BHC is less toxic when injected than when applied topically to the neck membrane. This difference in toxicity may be due to the spread of topically applied γ-BHC through the cuticle so that acts as a reservoir and maintains an effective concentration of the insecticide at site of action. With injection, γ-BHC may be transferred to less sensitive areas possible by the haemolymph as suggested

by Le Roux and Morrison (1954) and Burt (1970). Gerolt (1969&1972) suggested that the transport of dieldrin and oxime carbamtes applied topically to houseflies does not involve the haemolymph.

When the three insecticides are compared a similarity can be observed. On the abdomen 70-80% were always killed, on the neck the figure is 100% and on the hind leg always least. The speed of reaction following application to the neck membrane was quickest because of the sensitivity of this part to topical application. Application to the intersegmental membrane between the first and second abdominal sternites is of intermediate, and so suitable for general testing purposes.

1.4 Effect of post-dosing temperature on the toxicity of insecticides

1.41 Introduction

Environmental conditions such as temperature can affect the toxicity of insecticides by their action before treatment, during treatment, and after treatment as has been reviewed for different insects. For locusts, MacCuaig (1955) found no difference in toxicity of γ-BHC against hoppers Schistocerca

kept at 19.5,24, and 38.5°C.after treatment. With DNOC, MacCuaig (1958) found that adult locusts kept at higher temperature (35°C) died more rapidly than at lower temperature (25°C), but the final kills were identical. With several insecticides against first instar Schistocerca kept at 26 and 31°C.after treatment (MacCuaig et. al., 1964) found that dieldrin was more toxic at the higher temperature. Other insecticides, including γ-BHC, showed no significant change.

These experiments were designed to determine the toxicity of γ-BHC, Cyolane and dioxacarb to fifth instar Schistocerca at two different temperatures after dosing.

1.42 Materials and Methods

In order to make a quantative determination of variation in toxicity during the post-dosing period, it was necessary to apply the dose to locusts as quickly as possible, and transfer the treated insects immediately to the required condition. Before treatment, locusts were kept in the dosing laboratory, for a short period, in order to minimise the influence of temperature before and during treatment. Two groups of fifth instars 3-4 day old

were treated at each of four dose levels for determination of the LD50's and LD99's. All applications were made topically, as described in 1.12. Food was available after dosing. The cages of treated locusts were after dosing immediately placed in a cabinet under controlled temperature at 25°C. or 35°C. with variation of 2°C for 6 days. Humidity was not controlled but remained at a fairly constant level of about 60-80%.

1.43 Toxicity of γ-BHC at different post-dosing temperatures

In this experiment, a total of 200 locusts were used to determine the toxicity of γ-BHC at 25°C. and 35°C. post-dosing temperatures.

The first inspection was made 6 hours after treatment and it was observed that locusts kept at 25°C remained normal while those kept at 35°C showed signs of poisoning. The toxic action of γ-BHC was rapid at 35°C and most kill took place between 24-48 hours and thereafter changed little. At 25°C the effect of γ-BHC was gradual, and observations were continued for 6 days. The symptoms of toxicity and criteria of effect

were as described in 1.13. At the end of the experiment, all locusts not dead were counted as normal.

The results are shown in Table 22 with the LD50's, 95% confidence limits and the relative resistance at different temperatures. Graph 15 shows the probit regression lines.

Table 22

Effect of post-dosing temperatures on the toxicity of γ-BHC

Post-dosing temperature	Regression equation	LD50 ug/g	95% confidence limits	Relative resistance
35°C.	7.20x-4.03	34.9	33.3-36.6	3.79
25°C.	4.79x-0.95	9.2*	8.1-10.4	1.0

*Difference significant at the 5% level.

Table 22 indicates that at post-dosing temperature of 35°C. the relative resistance to γ-BHC increased as compared with post-dosing temperature of 25°C.

1.44 Toxicity of Cyolane at different post-dosing temperatures

A total of 210 locusts were used throughout the experiment to determine the toxicity of Cyolane at 25° and 35°C. post-dosing temperatures.

The first inspection was made 6 hours after application, and it was found that locusts held at 35°C were slightly affected than those held at 25°C. Throughout the experiment, toxicity developed faster at the higher temperature and the full effect of Cyolane was complete in about 24-48 hours. At 25°C, toxicity developed at slower rate. The symptoms of toxicity and criteria of effect were similar to those described in 1.13. At the end of the experiment, all locust not dead were counted as normal.

The results are given in Table 2 with LD50's, 95% confidence limits and the relative resistance at different post-dosing temperatures. Graph 16 shows the probit regression lines.

Table 23

Effect of post-dosing temperatures on the toxicity of Cyolane

Post-dosing temperature	Regression equation	LD50 µg/g	95% confidence limits	Relative resistance
35°C.	8.90x-0.10	3.7	3.5-4.04	1.27
25°C.	5.16x-4.58	2.9*	2.5-3.3	1.0

*Difference significant at the 5% level

Table 23 indicates that change in post-dosing temperatures has a little effect on the toxicity of Cyolane, although there is a suggestion of increased resistance at the higher temperature.

1.45 **Toxicity of dioxacarb at different post-dosing temperatures**

In this experiment, a total of 220 locusts were used to determine the toxicity of dioxacarb at 25° and 35°C post-dosing temperatures.

The first insepction was made 6 hours after treatment and it was found that locusts kept at 35°C were slightly affected than those held at 25°C. Throughout the experiment, poisoning developed rapidly at higher temperature and at slower rate

at lower temperature. The symptoms of toxicity and criteria of effect were similar to those described in 1.13. At the end of the experiment, all locusts not dead were counted as normal.

The results are shown in Table 24 with the LD50's, 95% confidence limits and the relative resistance. Graph 17 shows the probit regression lines.

Table 24

Effect of post-dosing temperatures on the toxicity of dioxacarb

Post-dosing temperature	Regression equation	LD50 ug/g	95% confidence limits	Relative resistance
35°C.	5.8x-0.03	7.24	6.6-8.0	2.78
25°C.	5.0x-4.11	2.60*	2.3-3.0	1.0

*Difference significant at the 5% level.

Table 24 indicates that at post-dosing temperature of 35°C, the relative resistance to dioxacarb increased as compared with post-dosing temperature of 25°C.

Results indicated that the toxicity of γ-BHC was greatly affected by post-dosing temperature, -BHC was about 3.5 times more toxic at lower

temperature. With Cyolane, when locusts were treated with the LD99, as in other insecticides, 100% kill was obtained at 35°C in contrast to γ-BHC and dioxacarb, and another range of doses was used. Dioxacarb had an intermediate position and was about 2.5 times more toxic at the lower temperature.

1.46 Discussion

It can be seen that the influence of post-dosing on toxicity varies with different insecticides. For example, a considerable change was found with -BHC, while post-dosing temperature had no very marked effect on the toxicity of Cyolane. At a given dose of γ-BHC, kills of locusts decreased as the post-dosing temperature increased from 25° to 35°C. However, temperature had less effect on the action of dioxacarb than it had on that of γ-BHC.

The effect of temperature on the toxicity of γ-BHC has been investigated by several workers who have obtained anomalous results. γ-BHC was more toxic at lower temperature, as reported in this work, against Blatella germania (Guthrie, 1950), less

toxic with housefly (Hoffman et al.,1949) and (Hoffman and Lindquist 1949); not affected by the post-dosing temperature with Schistocerca (MacCuaig,1955). Hadaway and Barlow (1957) found that γ-BHC was slightly more toxic to houseflies at higher temperature, but its toxicity to Aedes aegypti and Anopheles stephensi was not affected by the temperature.

There is no information available on the action of Cyolane and dioxacarb at different temperatures. The effect of post-dosing temperature on other insecticides has been reviewed.

1.5 Effect of prior starvation on the toxicity of γ-BHC

1.51 Introduction

Food is an important factor for growth and development, and lack of food may affect the tolerance of insects to insecticides. Pearson (1957) found that the toxicity of dieldrin applied as a stomach poison to fifth instar Schistocerca was increased by a period of starvation after treatment. MacCuaig and Watts (1960) showed that the resistance of fifth instar Schistocerca

to dieldrin increased when locusts were fed immediately after treatment. MacCuaig (1960) found that lack of food before treatment had no effect on the toxicity of γ-BHC by topical application to fifth instar Schistocerca, but with diazinon, DNOC and parathion the resistance decreased.

This work was designed to study the effect of prior starvation on the toxicity of γ-BHC applied topically or injected into fifth instar Schistocerca. The influence of starvation before treatment, and after treatment on the stomach toxicity of γ-BHC was also investigated.

1.52 Materials and Methods

Fifth instar Schistocerca 3-4 days old were used throughout this experiment, and were deprived of food and water for different periods before treatment.

1.53 Effect of prior starvation on the toxicity of γ-BHC applied topically or by injection

(1) Topical application

In this experiment, a total of 290 locusts were

randomly divided into 3 groups and each group was tested at 3-4 doses of γ-BHC applied by the micro cap as described in 1.12.

Starvation periods before treatment

The first group of 90 locusts was starved for 24 hours, the second group of 110 locusts for 48 hours and the third group of 90 locusts for 72 hours.

After treatment, locusts were supplied with food and kept under constant condition of temperature and humidity as described in 1.12.

The first inspection was made 24 hours after treatment, and it was observed that locusts starved for 72 hours were slightly affected, other group showed signs of γ-BHC poisoning. The symptoms observed and criteria of effect were as those described in 1.13. At the end of the experiment, all locusts not dead were counted as normal.

The results are presented in Table 1 with the LD50's, 95% confidence limit and the relative resistance. Graph 18 shows the probit regression lines.

Table 25

Effect of starvation on the toxicity of γ-BHC applied topically to fifth instar Schistocerca

Starvation periods hours	Regression equation	LD50 µg/g	95% confidence limits	Relative resistance
24	6.38x-2.68	8.0	6.6-10	1.33
48	6.29x-2.29	7.1	6.3-8.3	1.18
72	5.80x-1.25	6.0	4.8-7.4	1.0

Table 25 suggests a decrease in resistance with increase in starvation period after treatment although the difference between 24 hours and 48 hours is not significant at the 95% level.

(2) Injection

Approximately 290 locusts were randomly divided into 3 groups and each group was tested at 3-4 doses of γ-BHC injected into the abdomen as described in 1.12. The first group of 90 locusts were starved for 24 hours, the second group of 110 locusts for 48 hours and a third group of 90 for 72 hours. The condition of insects after treatment was essentially similar to those of the preceding experiment.

The first inspection was made 24 hours after treatment

and it was found that locusts starved for 72 hours were slightly affected; other groups showed signs of poisoning. The symptoms observed and criteria of effect were as those described in 1.13. At the end of the experiment, all locusts not dead were counted as normal.

The results are given in Table 26 with the LD50's, 95% confidence limits and relative resistance. Graph 19 shows the probit regression lines.

Table 26

Effect of starvation on the toxicity of γ-BHC applied by injection to fifth instar Schistocerca

Starvation periods hours	Regression equation	LD50 µg/g	95% confidence limits	Relative resistance
24	8.28x-5.37	8.9	8.3-9.6	1.39
48	6.76x-2.8	7.1	6.5-7.8	1.10
72	7.60x-3.4	6.4	4.6-7.4	1.0

The results in Table 26 indicate that a decrease in resistance with increase in starvation periods after treatment although the difference between 48 hours and 72 hours is not significant at the 95% level.

1.54 Effect of prior starvation on the stomach toxicity of γ-BHC

In this experiment, γ-BHC was applied as stomach poison, using the method of application as described in 1.12. Food was available after treatment and insects were kept at 25°C temperature and 60-80% relative humidity.

Approximately 340 locusts were randomly divided into 3 groups according to treatment. The first group of 110 locusts were starved for 24 hours prior to treatment and fed after, and the second group of 120 locusts were fed before and starved after treatment for 24 hours. The third group of 110 locusts were starved for 24 hours before and after treatment.

The first inspection was made 24 hours after treatment and it was found locusts starved for 24 hours before and after treatment were slightly affected, while locusts starved after treatment showed signs of poisoning, the other group was normal. It was also observed that badly affected locusts excreted less faecal matter. The symptoms of poisoning and criteria of effect were similar to those described in 1.13. At the end of the experiment,

all locusts not dead were counted as normal.

The results are shown in Table 3 with the LD50's, 95% confidence limits and relative resistance. Graph 20 shows the probit regression lines.

Table 27

Effect of starvation on the stomach toxicity of γ-BHC against fifth instar Schistocerca

Treatment groups	Regression equation	LD50 µg/g	95% confidence limits	Relative resistance
1	5.80x-3.39	12.9	12.3-14.3	1.76
2	7.64x-2.86	10.7	9.7-11.6	1.46
3	7.90x-1.82	7.3	6.8-7.8	1.0

Table 27 indicates that hoppers starved after treatment are more susceptible to γ-BHC than when starved before treatment. Locusts are much more susceptible when starved before and after treatment.

The results in Tables 25 and 26 indicated that starvation prior to treatment has increased the toxicity of γ-BHC applied by topically or by injection. Starvation has increased the stomach toxicity of γ-BHC and locusts starved before and

after treatment were more susceptible than those starved before or after.

Discussion

From the results, it can be seen that the resistance of fifth instar hoppers to γ-BHC was affected by the starvation periods. Hoppers starved from 72 hours were more susceptible than when starved for 24-48 hours by topical application or by injection.

When hoppers were fed immediately after treatment they were more resistance than those not fed by factor of 1.2. This finding agree with Pearson (1957) and MacCuaig and Watts (1960) who found that the resistance of Schistocerca to dieldrin as a stomach poison was increased when hoppers were fed after treatment. It is probable, therefore, that when the stomach dose is followed immediately by further food some of the ingested dose may be excreted. While in case when hoppers were not fed, insecticides may retain in the gut for much longer time which allows for a great amount to be absorbed.

1.6 Effect of body weight on the toxicity of γ-BHC

1.61 Introduction

It is generally assumed in biological assay tests that an organism responds to insecticides on a weight basis. In proportion to its body weight a larger insect requires a larger dose to kill than smaller insect of the same species. However, susceptibility may not always be directly proportional to body weight as has been reviewed with arsenic against Bombyx mori (Bliss,1936), last instar Diataraxia oleracea for DDT and γ-BHC (May,1954) and several insecticides against four species of lepidopterous larvae (Gast,1959).

For locusts, Hopf (1951) found that the kill of Locusta by a number of insecticides including γ-BHC correlated better with the dose expressed as µg per locust instead per unit of body weight. Stringer (1956) found similar results with some organophosphours insecticides against Locusta when the dose was measured in µg per locust. MacCuaig (1956) with DNOC applied topically to adult Schistocerca and Locusta found that the resistance was directly related to the body weight when the dose was measured

in µg of body weight of locust. With other insecticides, MacCuaig (1961) found that resistannce to γ-BHC was proportional to weight within any one age but resistance to diazinon decreased within increase in age. With Locusta, resistance to γ-BHC was dependent on weight but the resistance to diazinon was found to be independent of weight.

1.62 Materials and Methods

Since the weight of insects change during growth and varies between different groups within one instar of the same age and between sexes therefore, this experiment was designed to study the toxicity of γ-BHC to different weight groups and sexes of fifth instar Schistocerca 5-6 days old. Locusts were not randomised but were divided according to weight into light and heavy groups and the sexes were treated separately. The mean weight of hoppers used was as follows:

Sex	Heavy	Light
Males	1.50	1.16
Females	1.75	1.35

γ-BHC was applied so that different groups of locusts received similar doses regardless of

of individual variations of weight. The weight of each locust was recorded so that the dose in µg/g body weight could be calculated. Treated locusts were supplied with food and maintained at similar condition as described in 1.12.

Results

In this experiment, a total of 440 insects were used through the experiment. Doses of similar size, measured in ug per locust, were applied topically to all locusts irrespective of their size.

The first inspection was made 24 hours after treatment and it was observed that the lighter locusts treated with higher doses showed signs of toxicity but none of the heavier insects did so. The symptoms of poisoning and criteria of effect were as described in 1.13. At the end of the experiment, all locusts not dead were counted as normal.

The results are shown in Table 28 with the LD50's and 95% confidence limits. Graph 21 shows the probit regression lines.

Table 28

Resistance of Schistocerca of different weight Groups of γ-BHC applied topically

Sex	Weight	LD50 µg/locusts	95% confidence limits	LD50 µg/g	95% confidence limits
Males	1.16	15.5	14.5-16.6	13.4	12.0-14.7
	1.50	22.7	20.0-25.0	14.2	12.3-16.0
Feamles	1.35	18.4	17.1-19.7	14.4	13.2-15.7
	1.75	25.0	22.8-27.4	15.1	13.8-16.5

Table 28 indicated that the lighter locusts were more susceptible than the heavier locusts, and the LD50 for the females were higher than for the males when the dose was measured per locust. When the dose was applied in µg/g the difference between the groups was not significant.

1.64 Discussion

The results indicated that resistance to fifth instar to γ-BHC is proportional to weight. MacCuaig (1961) recorded similar results with adult Schistocerca when the dose was measured in µg/g of body weight.

1.7 Toxicity of -BHC to different instars

1.71 Introduction

It is known that within a insect species, resistance to insecticides varies with different instars and stage of development as reviewed. With successive instars of Schistocerca Pradham and Bindra (1958) showed that resistance to γ-BHC suspension applied by contact increased with instar, and older instar was about 4 times as resistant as the younger instar. MacCuaig (1960 and 1961) found that resistance to γ-BHC applied topically to hoppers and adult of Schistocerca was least a few days before moulting and a few days after. MacCuaig et. al. (1964) found that first instar Schistocerca were much more susceptible than fifth instar, by factor of 2.0 for dieldrin and by about 8 times for γ-BHC.

This experiment was designed to investigate resistance of fifth instar hoppers and adult of Schistocerca to γ-BHC applied topically or by injection.

1.72 Materials and Methods

Test insects

Fifth instar hoppers 4-5 days old, immature adults 2-3 days old and mature adults 2 weeks old of both sexes were used. γ-BHC was applied by topical application and by injection into the abdomen. The method of application and condition of insects after treatment were as described in 1.12.

Approximately 190 locusts of each age group were randomly divided into two groups. The first group of 80 insects were used to determine the toxicity by topical application and the second group of 110 insects by injection.

1.73 Results

The first inspection was made 24 hours after treatment and it was observed that most insects were normal. The symptoms of poisoning and criteria of effect were as those described in 1.13. Observations were counted till there were no more deaths or for 6 days.

At the end of the experiment, all locusts not dead

were counted as normal. The results are shown in Table 29 with the LD50's, confidence limits, Graphs 22 and 23 show the probit regression lines.

Table 29

Toxicity of γ-BHC to Schistocerca by topical application and injection

Age	Treatment	Regression equation	LD50 µg/g	95% confidence limits
	Topical			
Fifth instar		7.97x-5.35	9.9	9.2-10.8
Immature adults		8.50x-3.96	5.7	5.2-6.1
Mature adults		5.60x-2.19	9.5	8.5-10.7
	Injection			
Fifth instar		6.74x-3.92	10.5	8.6-12.9
Immature adults		4.96x-6.56	6.7	4.4-8.7
Mature adults		5.00x-0.03	10.4	9.6-12.4

Results indicate that immature adults are more susceptible to γ-BHC than fifth instar or mature adults and the resistance of fifth instar is similar to those of mature adults. Locusts appear to be more resistant to injection than to topical application but the difference is not significant at the 95% confidence level.

1.74 Discussion

The results indicated that resistance of hoppers and adults of Schistocerca was of the same order although 2-3 days old immature adults were more susceptible. The toxicity of γ-BHC by applied topically to hoppers and adults of Schistocerca has been determined by MacCuaig (1960 and 1961). He found that the LD50's of 4-5 days old hoppers was about 10.1 µg/g, decreased to 5.3 µg/g for 3 days after moulting and the increased to an average value of 8.9 for 2-4 weeks old adults. These values are similar to those found in this experiment.

When insects of different instars were used, older insects were more resistant than younger ones (MacCuaig et. al., 1964, Sanches and Sherman 1963) and these changes were found to be influenced by insecticides and stage of development (Harris and Core 1971).

1.8 Effect of food on the toxicity of γ-BHC

1.81 Introduction

The quantity and quality of food are the two principal ways in which food may influence the

resistance of insects to insecticides. A change in the type of diet on which the insects were reared may affect their tolerance (Busvine,1957).

The following experiments were, therefore, designed to investigate the effect of lack of food and reduced feeding on the toxicity of γ-BHC. Also the effect of change of type of diet on the toxicity of γ-BHC, Cyolane and dioxacarb.

1.82 Effect of lack of food

Materials and Methods

Only 3-4 days old fifth instar hoppers of Schistocerca were used in this experiment. γ-BHC was applied topically or by injection, and the conditions of rearing were similar as described in 1.12.

Locusts deprived of food before treatment

A total of 220 locusts were used. The first group of 110 insects was used to determine the toxicity by topical application and the second group of 110 insects by injection. Treated locusts were

supplied with food.

Locusts deprived of food after treatment

A total of 180 locusts were used. The first group of 90 insects was used to determine the toxicity by topical application and the second group of 90 insects by injection. They had ample food available before treatment.

Control group

In this experiment, a total of 240 locusts was used and they were supplied with food before and after treatment. Approximately, 210 insects were used to determine the toxicity of γ-BHC by topical application and 120 insects by injection.

Treated locusts were kept at constant condition of temperature and humidity as mentioned in 1.11.

Results

The first inspection was made 24 hours after treatment abd observation were continued for 6 days. The symptoms of toxicity and criteria of effect were similar to those described in 1.13. At the end

of the experiment, all locusts not dead were counted as normal. The results are shown in Table 30 with the LD50's and 95% confidence limits. Graphs 24 and 25 show the probit regression lines.

Table 30

Effect of lack of food on the toxicity of -BHC

Method of application	Treatment	Regression equation	LD50 µg/g	95% confidence limits
Topical	Control	6.30x-3.22	10.1	9.5-11.4
	No food before	8.52x-6.15	10.2	9.7-10.6
	No food after	5.26x-1.03	6.4	5.6-7.2
Injection	Control	7.12x-4.77	11.8	10.8-12.9
	No food before	8.30x-6.04	10.7	9.6-11.4
	No food after	5.76x-1.81	7.6	7.0-8.3

Table 30 indicates that locusts not fed after treatment for 24 hours were more susceptible to γ-BHC by topical application or injection although the resistance of locusts not fed before did not differ significantly at 95% confidence limits from the control group.

1.83 Effect of reduced feeding

In this experiment, a group of locusts was fed on small grass meals for a period of time while the other group was fully fed. They were used to determine the effect of quantity of food on the toxicity of γ-BHC.

Materials and Methods

A total of 230 locusts were collected from mid fourth instar and were used at 5-6 days old throughout this experiment. They divided into two groups according to treatment. The first group of 110 locusts (fully fed) was supplied with three meals of grass per day in addition to a small amount of grass standing in water in a small jar. Before introducing new food the old grass was removed from the cage so that grass was always in the normal condition. The second group (less fed) of 120 locusts was given a grass meal once a day and this grass was not changed till the next morning; by that time it has lost some of its water contents. Both groups moulted to the fifth instar at the same time. γ-BHC was applied topically as described in 1.12.

Results

The first inspection was made 24 hours after treatment, and observation were continued for 6 days. The symptoms of toxicity and criteria of effect were similar to those described in 1.13. At the end of the experiment, all locusts not dead were counted as normal. The results are given in Table 31 with the LD50's and 95% confidence limits. Graph 26 shows the probit regression lines.

Table 31

Effect of reduced feeding on the toxicity of γ-BHC

Treatment	Regression equation	LD50 µg/g	95% confidence limits
Fully fed	6.42x-3.97	12.5	11.3-13.8
Less fed	8.30x-5.68	9.7	9.0-10.4

Table 31 indicates that locusts fed on relatively poor food for about 7-8 days were more susceptiblle than those given ample fresh food.

1.84 Effect of type of diet

This experiment was carried out to investigate the

effects of feeding locusts on artificial diet, dry food and very dry grass on the toxicity of γ-BHC, Cyolane and dioxacarb.

Locusts fed on artificial diet

Hoppers were fed on the artificial diet from the first instar and were used at 5-6 days old fifth instar throughout this experiment. To determine the change in weight of hoppers fed on artificial diet, 20 hoppers were weighed at intervals during the experiment and the mean weight, is shown in Figure 51. It was observed that in hoppers fed on artificial diet the cuticle pigments were absent, although where numbers of hoppers were supplied with small portion of fresh grass in the diet, hoppers developed the colour. The absence of the colour was due to the lack of carotene in the diet, but this was found not to be essential for growth (Dadd 1957, & 1960). The artificial diet was supplied in small petri dishes and water was available. The composition of the diet as described McKinley (1970).

Bemax	24g
Yeast	22g
Casein	4g
Sucrose	4g
Methyl parahydroxybenzoate	1.2g

Cholesterol	0.1g
Ascorbic acid	2.5g
Choline chloride	0.2g
Glaxo salts	2.0g
Linolic acid	2 drops
Methyl linolenate	2 drops
Vanderzant vitamin mixture	3.5ml

This diet was originally prepared for rearing the cotton leafworm Spodoptera littoralis.

Materials and Methods

5-6 days old fifth instar were used in this experiment. A total of 330 locusts were randomised between γ-BHC, Cyolane and dioxacarb and tested at 4 doses to determine the toxicity by topical application. Treated locusts were supplied with artificial diet and kept at constant condition of temperature and humidity as mentioned in 1.12.

The first inspection was made 24 hours after treatment and observations were continued for 6 days. At the end of the experiment, all locusts not dead were counted as normal.

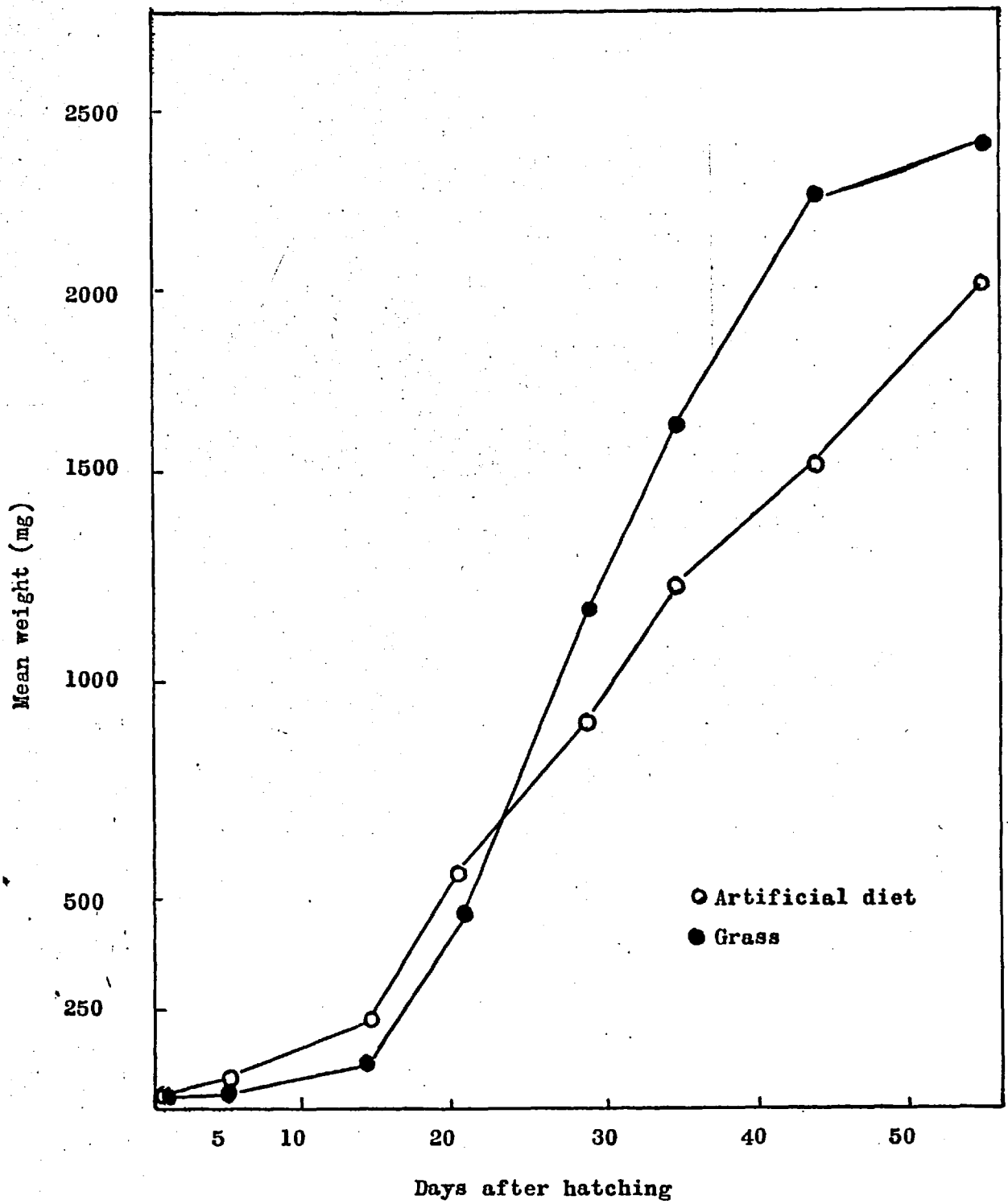


Figure 5

Mean growth curves for Schistocerca hatchlings reared on an artificial diet and grass.

Results

The results are given in Table 32 and Graph 27.

Table 32

Toxicity of topically applied insecticides to Schistocerca fed on artificial diet

Insecticide	Type of diet	Regression equation	LD50 µg/g	95% confidence limits
γ-BHC	artificial	4.36x-1.48	15.0	13.0-17.2
	fresh grass	5.90x-1.19	11.1	10.4-12.0
Cyolane	artificial	6.34x-1.3	1.96	1.8-2.1
	fresh grass	5.44x-0.97	2.80	2.6-3.0
Dioxacarb	artificial	4.46x-2.0	1.5	1.3-1.7
	fresh grass	5.30x-1.13	2.8	2.5-3.2

Table 32 indicates that locusts fed on the artificial diet are more resistant to γ-BHC than other insecticides but locusts fed on fresh grass are more tolerant to Cyolane and dioxacarb than on artificial diet. It appears that locusts fed on artificial diet are twice as susceptible to Cyolane and dioxacarb than when fed on fresh grass.

Effect of feeding locusts on dry food

This experiment were designed to study the toxicity of γ-BHC against locusts fed on dry food.

Locusts fed on bemax (wheat germ)

Materials and Methods

Hoppers were fed on bemax from mid fourth instar and they were used at 5-6 days old fifth instar. Water was provided during the experiment. A total of 90 locusts were used to determine the toxicity of γ-BHC by topical application. Locusts were supplied by fresh grass after treatment.

Results

The results are shown in Table 33 and Graph 28.

Table 33

Toxicity of γ-BHC to Schistocerca fed on bemax

Type of diet	Regression equation	LD50 µg/g	95% confidence limits
Bemax	7.90x-1.47	7.9	7.3-11.7
Fresh grass	5.90x-1.9	11.1	10.4-12.0

Table 33 suggests that resistance of locusts fed on bemax to γ-BHC is similar to those fed on fresh grass.

Locusts fed on very dry grass

Materials and Methods

Hoppers were fed on very dry grass for 2,3 and 4 days from 24 hours after moulting to the fifth instar. Water was not supplied during the experiment. A total of 290 locusts were used to determine the toxicity of γ-BHC by topical application and 270 by injection. Treated locusts were supplied with fresh grass.

The first inspection was made 24 hours after treatment and observations were continued on successive days till there were no more deaths. At the end of the test, all locusts not dead were counted as normal.

Results

The results are given in Table 34 and Graphs 29 and 30.

Table 34

Toxicity of γ-BHC to Schistocerca fed on very dry grass

Age (days)	Treatment	Period of feeding (days)	Type of food	LD50 µg/g	95% confidence limits
3	Topical	2	very dry	7.3	6.7-8.2
			fresh grass	8.7	7.9-9.3
4		3	very dry	6.5	5.6-7.3
5		4	very dry	5.3	4.7-5.8
			fresh grass	11.1	10.4-12.0
3	Injection	2	very dry	10.6	9.2-12.1
			fresh grass	13.1	11.4-12.7
4		3	very dry	9.3	8.4-10.3
5		4	very dry	4.2	3.7-4.6
			fresh grass	9.3	8.3-10.5

Table 34 indicates that locusts fed on very dry grass for 4 days are much more susceptible than those fed for the same period on fresh food, although resistance of locusts fed for 2 and 3 days did not differ significantly at the 95% level.

1.85 Discussion

In routine experimental work, the locusts were usually deprived of food for a short period before treatment to ensure greater uniformity, but food

was always was available after treatment in sufficient quantity. Results in Table 30 indicate that lack of food for 24 hours after treatment results in increases sensitivity to γ-BHC, but deprivation before had no effect. When results of locusts fed on fresh grass (section 1.3) are compared with those fed on artificial diet of the same age, indicate that the quantity of fresh food affected the tolerance of locusts to γ-BHC where as locusts fed on an artificial diet were more resistant to γ-BHC than to Cyolane or dioxacarb. Locusts fed on bemax were as resistant as those fed on fresh grass but locusts fed on very dry grass for 4 days were as twice susceptible as on fresh grass. The effect of changing the type of diet on the toxicity of insecticides to some insects has been reported by some workers. Hadaway and Barlow (1956) found no significant effect on susceptibility of female Aedes aegypti to DDT fed on human blood or guinea-pig blood. Potter and Gillham (1957) found that aphid Acrythosiphon pisum reared on clover was more resistant than when reared on broad bean. Lofgren and Cutkomp (1954) showed that Periplaneta americana fed on high protein diet was more susceptible to

DDT than when fed on low protein diet. Locusts fed on very dry grass for more than 3 days were more susceptible than after shorter periods although there were no significant changes in resistance of locusts fed on very dry grass for 2 or 3 days. Feeding locusts beyond 4 days induced cannabalism. The weight of locusts at the 4th day was less than those fed on fresh grass and bran. This decrease in weight may be related to the insufficient nutritional value of dried grass for development. Lee (1961) examined Schistocerca fed on grass which varied in water content; the blood volume per unit weight of Schistocerca was least when they fed on the driest grass.

1.9 Effect of solvent on the toxicity of γ-BHC

1.91 Introduction

It is recognised that solvents and carriers facilitate the penetration of insecticides through the cuticle of insects anddifferent solvents affect the resistance of insects to insecticides. MacCuaig (1956) found that dieldrin applied topically to fifth instar Schistocerca was about one quarter

as toxic when applied in emulsion as when applied in oil solution. Insecticides dissolved in xylene were found to be less toxic, when applied topically, than in KAB (oil solvent) (MacCuaig, 1960).

This experiment was carried out to determine the toxicity of γ-BHC in three solvents applied topically or by injection.

1.93 Materials and Methods

Fifth instar Schistocerca were used at 3-4 days old. The solvents used were KAB (Kerosene aromatic bottoms), acetone and benzene. γ-BHC in KAB was applied topically by microcapillary or injected using microsyringe, the method of application being as described in 1.12. γ-BHC in acetone or benzene solution was applied topically by microsyring. This change in method of application was found to be necessary because of the difficulty of applying small volume of volatile solvents by micro-capillary. Similar volumes of the solvents applied topically or injected caused no significant deaths among the control group.

In this experiment, a total of 580 locusts were used: approximately 90 locusts were used with each poison

to determine the toxicity of topical application and 110 locusts for the toxicity by injection in KAB and benzene, but only 90 locusts in acetone.

The first inspection was made 24 hours after treatment and observations continued on successive days till there were no more deaths. At the end of the test, all locusts not dead were counted as normal.

1.93 Results

The results are shown in Table 35 with the LD50's and 95% confidence limits. Graphs 31 and 32 show the probit regression lines.

Table 35

Effect of solvent on the toxicity of γ-BHC

Solvent	Treatment	Regression equation	LD50 µg/g	95% confidence limits
KAB	Topical	8.37x-6.17	10.79	10.8-12.3
Acetone		9.93x-5.77	12.1	11.4-13.0
Benzene		9.90x-5.98	12.9	12.0-13.7
KAB	Injection	7.63x-4.75	9.46	8.7-10.3
Acetone		6.42x-6.9	2.87	2.5-3.2
Benzene		4.88x-1.22	3.8	3.3-4.3

Table 35 indicated that resistance to γ-BHC applied topically in different solvents did not differ significantly at the 95% level, but γ-BHC was much more effective when injected in acetone or benzene than in KAB (oil solvent).

1.94 Discussion

The results indicated that change in the solvent did not significantly affect the toxicity of γ-BHC when this was applied topically. MacCuaig (1960) compared the toxicity of γ-BHC by topical application to fifth instar Schistocerca applied in xylene solution with those in oil solution. He found that γ-BHC was as toxic in xylene as in oil. Other authors have observed difference in toxicity of insecticides applied topically in oil solvents. This was shown with houseflies for DDT (Busvine,1951) and for diazinon (Farnham,1965). With fifth instar Schistocerca MacCuaig (1956) found that dieldrin in oil solution was more toxic than when applied in emulsion.

The greater toxicity of γ-BHC when applied by injection in acetone or benzene than in KAB is similar to results with diazinon in different solvents against diazinon susceptible houseflies (Farnham,1965).

PART 2 Investigation of the movement of γ-BHC in the insect

2.1 Introduction

The results of the bioassay were based on observation of mortality among test insects as a result of applying known doses of insecticides. Different results were obtained depending on the intrinsic and extrinsic factors. The experiments described in this part were designed to investigate the movement of γ-BHC applied topically to fifth instar Schistocerca by gas liquid chromatography, with the aim of finding out if difference in the movement of poison could account for the observed difference in toxicity.

2.2 Materials and Methods

Chromatographic technique

Chromatography has been defined as the technique for the separation of a mixture of solutes in which separation is brought about by the differential movement of the individual solutes through a porous medium under the influence of a moving solvent (Abbott and Andrews 1970).

There are different types of chromatography such as adsorption column chromatography, partition column chromatography; paper chromatography; thin layer chromatography and gas chromatography (Kaiser,1963). The basis of gas chromatography separation is the distribution of a sample between two phases. One of these phases is a stationary bed of large surface area and the other phase is a gas which percolates through the stationary bed. There are two types of gas chromatography, gas solid chromatography (GSC) where the stationary phase is a solid, and gas liquid chromatography (GLC) where the stationary phase is a liquid spread as a thin film over an inert solid. Gas liquid chromatography is fully described below since it was the method used for

-EHC determinations in the following experiments.

For ease of description a gas liquid chromatography can be divided into three stages: injector, column and detector. Throughout the work injection of solutions onto the column was made using the flash vaporisation technique. With this technique microlitre aliquots of solution are introduced into the glass-lined heated injector block where they are immediately volatalised and swept by oxygen free nitrogen (carrier gas) onto the column). The column consists of 5' long stainless steel tube of 1/8th inch overall diameter packed with a powdered inert solid (support phase) upon which is absorbed a high boiling liquid (stationary phase). Separation of the components of the solutions is achieved by their differential partitioning between the carrier gas as it passes through the column and the stationary phase. Thus the components of the mixture are eluted from the column individually. Thecolumn effluent is analysed using an electron capture detector.

The detector consists of a positive (anode) and negative (cathode) electrode and a radioactive source (a tritiated

foil). The later ionizes the gas to form slow moving electrons and a potential difference (90v) across the electrodes results in these electrons being collected at the anode. This ion current (the standing current) is effectively reduced when electron absorbing molecules pass through the detector. γ-BHC is such an electron absorbing molecule. This signal is then converted to a voltage, amplified, and a permanenet record made on a 1 mv strip chart recorder. Due to the sensitivity of the detector anomalous results can occur from fluctuations in the gas flow. Therefore by means of pressure and flow regulators and ensuring that all joints in the system are gas tight, the flow is kept constant. Partition is dependent on the solutes remaining in the gaseous phase; the column is therefore maintained at a constant elevated temperature (180°C). In order to ensure immediate volatilisation of the solutes the injector block is kept at a constant temperature higher than that of the column (200°C). To prevent condensation of the column effluent, the detector is maintained at a constant temperature higher than that of the column (200°C). The recorded signal is directly proportional to the amount of electron absorbing

material passing through the detector. Therefore the area under the chromatogram peak gives a quantitative measure of the amount of material injected onto the column. The degree of partitioning that occurs between the gas and the stationary phase is dependent on the chemical properties of the compound. Therefore the length of the time taken by the compound to pass through the column, its retention time, can be used to identify the compound. The retention time of a compound will be constant for any one compound on any one stationary phase under constant temperature and carrier gas flow conditions.

The distance between the hexane peak and the γ-BHC peak was measured on the chromatogram giving a γ-BHC retention time relative to hexane. The distance between a tangent drawn to the base line and the apex of the peak was used to measure the peak height. This measurement was more accurate than using a height x width measurement due to the sharpness of the peak. During each analysis a standard graph was prepared from injections of solutions of known concentration by plotting known weights (ng) of γ-BHC against peak height (mm). Peak with the same retention time as that of γ-BHC were quantified using this standard graph. The presence of γ-BHC was verified using a

column packed with 5% Dow 11 on chromosorb W 60/80 mesh (AW DMCS) and a chemical test (Hamence et al., 1965). The latter is based on a dehydrochlorination reaction with alcoholic potassium hydroxide in which γ-BHC is converted to a product having a retention time indistinguishable from the solvent (hexane).

Instrumentation

Two chromatographs were used. In some experiment, a varian Aerograph model 204A equipped with an electron capture detector (H^3 foil) and 1 mv recorder was used. This was fitted with a 5 feet x 1/8th inch stainless steel column, packed with 5% Dow 11 silicone grease on chromosorb W 60/80 mesh (DMCS treated and acid washed). For other work, a varian Aerograph model 1400 equipped with an electron detector (H^3 foil) and 1 mv recorder was used. This was fitted with a 5 feet x 1/8th inch stainless column, packed with 2% QF-1 silicone grease on chromosorb W 60/80, (DMCS treated and acid washed).

The carrier gas was oxygen free nitrogen, with a flow rate of 50 ml per minute. The temperature of the column was 180°C and of the detector and the injector was 200°C. The attenuation was varied

according to the detector response to the concentration of the sample injected.

Preparation of standard solution

Different concentrations of standard solutions of γ-BHC were made up in redistilled hexane in ng/µl quantities. These solutions were always stored under refrigeration and fresh solutions were made several times during the experiment.

Injection technique

A 10 µl, 2.5 inch fixed needled syringe was used throughout this work. The sample was withdrawn into the syringe before and after injection in order to measure accurately the volume injected.

Insects used and application of insecticide

Fifth instar Schistocerca were used throughout this work. γ-BHC was applied with a micro cap for topical application or injected using a microsyringe. The solvent used was kerosene aromatic bottoms (KAB). The site of application was between the first and second abdominal sternites. After treatment, insects were kept at a constant condition of temperature

(25°C) and 60-80% relative humidity.

Extraction

At known times after treatment the locusts were placed in glass stoppered 30 ml capacity test tubes and rinsed with 5 ml of GLC pure hexane for 2-5 minutes. The solution was then passed through an hydrous sodium suphate in a sintered glass funnel under vacuum. The filtrate was collected in a 25 ml quickfit tube with side arm. This process was then repeated with a further 5 ml portion of hexane and the two portions were combined for analysis. With this method, most of γ-BHC was recovered by the first wash and little by the second wash; the total amount of γ-BHC recovered at 0 time, immediately after application was about 90% of the original dose.

2.3 Effect of temperature on disappearance of γ-BHC from the cuticle

This experiment was designed to determine the amount of γ-BHC lost from the surface of fifth instar Schistocerca due to change in post-dosing temperature.

Materials and Methods

A total of 24 fifth instar hoppers were used at 3-4 days old. γ-BHC was applied by topical application. The first group of 12 hoppers was dosed with the LD50 of the higher temperature which is 35µg/g and they were kept at 35°C. A second group of 12 hoppers was dosed with the LD50 of the lower temperature which is 10 µg/g and they were kept at 25°C. Treated hoppers were supplied with food.

Results

Table 36 indicates that the percentage of γ-BHC recovered from the surface decreased rapidly at the higher temperature. At 35°C, 50% was recovered after 1 hour and it decreased to 14.6% after 3 hours. No γ-BHC was recovered after 6 hours. At 25°C, 78% was recovered after 1 hour and 46.5% after 3 hours. A total of 36% was recovered after 6 hours. This indicates that raising the temperature by 10°C resulted in rapid disappearance of γ-BHC from the cuticle at higher temperature.

Table 36

Effect of temperature on disappearance of γ-BHC from the cuticle

Temperature	Percentage of γ-BHC recovered from the surface Time (hours)			
	0	1	3	6
35°C.	mean 90 range (86-92)	50 (47.5-55.0)	13.6 (12.5-14.3)	0 -
25°C.	mean 92 range (88-95)	78 (74-85)	46.5 (43-52)	36 (30-42)

*Each mean represents three insects.

2.4 Effect of age on disappearance of γ-BHC from the cuticle

This experiment was designed to determine the rate of disappearance of γ-BHC from the cuticle of fifth instar hoppers and adults Schistocerca.

Materials and Methods

Fifth instar 4-5 days old, adults 2-3 days and 2 weeks old were used throughout this experiment.

A total of 36 insects were used where 12 insects represented each age group. A dose of 12 µg/g of γ-BHC was applied topically.

Results

Table 37 indicates that the percetage of γ-BHC recovered from the cuticle 1 hour after treatment was similar in all stages with 65-80% of the γ-BHC recovered. Subsequently the rate of loss was greater from the fifth instar hoppers and young adults than from the older adults. Thus in the former two groups only 14-30% of the poison was recovered after 6 hours, compared with 36-44% in the two weeks old adults.

2.5 Effect of the solvent on disappearance of γ-BHC from the cuticle

This experiment was designed to study the effect of solvent on disappearance of γ-BHC from the cuticle.

Materials and Methods

A total of 36 fifth instar hoppers were used at 3-4 days old in this experiment. The solvents

Table 37

Effect of age on disappearance of γ-BHC from the cuticle

Insects	Age		Percentage of γ-BHC recovered from the surface Time (hours) 0	1	3	6
Adults	2 weeks	mean range	90 (87.5-92)	79 (73-80)	65.5 (70-59.3)	40 (36.8-44.1)
Adults	2-3 days	mean range	89 (87-90)	66 (62-70)	36.5 (31.3-40.7)	17.8 (14.0-21.3)
Fifth instar	4-5 days	mean range	89 (87.3-90)	70 (65-76)	40 (30-48)	23.5 (16.9-30)

*Each mean represents three insects.

used were KAB, acetone and benzene. A dose of 15 µg/g was applied by topical application.

Results

It is impossible to draw positive conclusion from the small numbers of insects used and the differences

between solvents were small (Table 38). However, it is true that consistently more poison was recovered from the locusts treated with acetone than from those treated with either of the other solvents.

2.6 Appearance of γ-BHC in the haemolymph

It is generally assumed that insecticide, after penetration, passes into the haemolymph to other organs. Burt (1970) reported that the haemolymph is usually considered the main vehicle for transferring the insecticide from the innermost layers of the cuticle to the nervous system which is the site of the lethal action. This concept of movement has recently been examined by Gerolt (1969 and 1972). He suggested that the transport of contact insecticides does not involve the haemolymph, but the poison reaches the site of action via the integument of the tracheal system. This has been shown with dieldrin and oxime carbamates.

This experiment was designed to determine the amount of γ-BHC appeared in the haemolymph samples collected from poisoned fifth instar Schistocerca after they have been treated by topical application.

Table 38

Effect of solvents on disappearance of γ-BHC from the surface

Solvents	Percentage of γ-BHC recovered from the surface			
	Time (hours)			
	0	1	3	6
Acetone	mean 88 range (86-90)	81 (78-84)	71 (66-77)	40 (37-45)
Benzene	mean 81 range (77-83)	70 (64.5-75.4)	58 (53-63)	30 (27-32)
KAB	mean 82 range (80-83)	65 (73-61)	48 (41.6-53.5	27 (22-30)

*Each mean represents three insects.

Materials and Methods

A total of 18 fifth instar hoppers were used at 4-5 days old in this experiment. A dose of 15 μg/g of γ-BHC was applied by topical application. The site of application was between the third and fourth abdominal sternites.

Haemolymph samples

The haemolymph samples were drawn from the thorax, not close to the site of application in order to minimise the chance of the contamination from the cuticle. Volumes of the haemolymph were collected through a small incision in the doral tergites of the thorax by the micro cap. The volumes collected ranged from 10-30 µl; in badly affected and hydrated locusts small volumes of about 5-10 µl were taken.

Analysis of the haemolymph

Methods developed for determination of chlorinated insecticides in the blood of fish, Mount et. al., (1966) and in humans and animals, Crosby and Archer (1966) were not suitable because these involve using KOH which is known to decompose γ-BHC. A simple technique was used where samples of haemolymph were diluted in distilled water, warmed and γ-BHC extracted with redistilled hexane, as follows:

Aliquots of haemolymph volumes were diluted in 1 ml distilled water, warmed in hot water bath at 70°C for 2-3 minutes. 0.5 ml of redistilled hexane was added and shaken or sonicated for 15 minutes.

γ-BHC was extracted from the hexane layer and injected into the chromatograph.

Results

Table 39 indicates that the concentration of γ-BHC in the haemolymph varied according to the time after application. The total concentration increased up to 18 hours and then decreased. Within 1 hour after treatment, the amount of γ-BHC found in the haemolymph was 24 ng/µl, and after 18 hours it had increased to 66 ng/µl. After 24 hours the level had fallen to 30 ng/µl.

Discussion

The results indicated that after different time intervals different amounts of γ-BHC was recoverable from outside. This is affected by temperature, age of locusts and solvents used. At the higher temperature less γ-BHC was recovered than at the lower temperature. When γ-BHC was applied topically in acetone, benzene or KAB not much difference in rate of disappearance from the surface was found although more γ-BHC was recovered from acetone than from KAB. There was no significant difference in the rate of

Table 39

The appearance of γ-BHC in the haemolymph

	Amount of γ-BHC in the haemolymph in ng/ul Time (hours)					
	0	1.0	3.0	6.0	18	24
mean	0	10	24	30	66	30
range	0	(8-12)	(21-29)	(32-40)	(53-78)	(27-33)

* Each mean represents three insects.

disappearance of γ-BHC hoppers or young adults.

In the previous sections, the amount of γ-BHC remaining on the surface or penetrated into the haemolymph was determined. As the amount on the outside of the cuticle declined, the amount in the haemolymph increased. If the blood volume of the insect is taken as about 200 µl, the total amount of γ-BHC in the blood will have been approximately those given in Table 40. Hence the amount of γ-BHC disappeared from the surface is found in the haemolymph, however, the total recovered was less than the amount of γ-BHC applied; perhaps due to poison being absorbed into other organs of the body. This is investigated in the next section.

Table 40

Dose applied		Total of γ-BHC recovered µg/g Time (hours) 1.0	3.0	6.0	18	24
15 µg/g	Total in blood	2.0	4.8	7.2	13.2	6.0
	% of the total					
15 µg/g	Amount on the cuticle	9.7	7.2	4.0	-	-
	% of the total					
	Total recovered	11.7	12.0	11.2		
	% of the total applied					

PART 3

Distribution, metabolism and excretion of γ-BHC

The variation in toxicity of γ-BHC in relation to different internal and external factors has been investigated and the amount of γ-BHC lost from the surface has been determined. These experiments were designed to study the distribution, metabolism and excretion of γ-BHC following different methods of application in order to determine if those processes played any significant role in the toxicity of γ-BHC to Schistocerca.

Internal γ-BHC was determined after the unabsorbed γ-BHC had been removed from the cuticle using ^{14}C γ-BHC . The radioactivity of extracts from the cuticle, internal body parts and locusts faeces was quantitatively determined using liquid scintillation technique.

Materials and Methods

Liquid scintillation counting owes its origin to the discovery by Reynolds and Kallman (1950) that certain organic solutions fluoresce noticeably when bombarded with high-energy radiation (Wang and Willis 1965). The principle of liquid scintillation counting is the conversion of the energy of disintegration of radioactive atom into light by the use of fluorescent organic molecules. The light emitted by each disintegration is detected by photomuliplier tube.

In liquid scintillation, the scintillator is dissolved in a solvent and the sample is dissolved in the test solution. Scintillation solutions used in this work were:

(i) primary, which produce light photons, PPO i.e. (2,5 diphenylexazole),

(ii) secondary, which introduce another stage of energy transfer and act as a wavelength shifter, POPOP i.e. 1,4-bis-2 (5 phenyloxazolyl).

The advantages of liquid scintillation are simplicity of sample preparation, and high sensitivity of the detector which allows low energy beta emissions to

be assayed with considerable efficiency. Amongst the disadvantages is the lowering of efficiency caused by phosphorescence quenching.

Determination of counting efficiency

The efficiency of the method was determined by internal standardisation methods (Wang and Willis 1965). It consists of adding a precisely known amount of the same istotope (an internal standard) in non-quenching form to a previously counted sample.

The standard unit of radioactivity is the curie, which is 'the disintegration occurring in 1 g of pure radium per second'. 1 curie (ci) = 3.7×10^{10} disintegration per second (dps). The counting efficiency of the quenched sample was determined as follows:

$$\text{Efficiency (expressed as a fraction of unity)} = \frac{\text{Net count rate (in cpm) (int. std. + sample)} \quad \text{net count rate (in cpm) (sample)}}{\text{Distintegration rate (int dpm) (int. std.)}}$$

$$\frac{63,910-31,844}{55,500} = 59.4\%$$

γ-BHC (U-^{14}C γ-BHC) in bezene solution was purchased from the Radiochemical centre, Amersham, Bucks, England.

Specific activity: 45 mCi/mmol (155 uCi/mg).
Radiochemical purity by thin layer chromatography on silica gel using (i) petroleum ether (80-100°) : 98%
(ii) cyclohexane/Chloroform (80:20) : 98%

Three concentrations of ^{14}C γ-BHC mixed with unlabelled γ-BHC in benzene were made up:

(i) 1% w/v contains 10 ug/ul (1 µl gives 33,300 dpm)
(ii) 0,5 w/v contains 5 ug/ul (1 µl gives 16,620 dpm)
(iii) 0,2 w/v contains 2 ug/ul (1 µl gives 6,900 dpm)

The sintillation counter used was model SC/LP 2 Panax Equipment Ltd.

Application methods

^{14}C γ-BHC was topically applied by means of the micro pipette (1 µl) and by injection using a Shandon syringe. The site of application was the intersegmental membrane between 1st and 2nd abdominal sternites. It was also administered by ingestion in which the dose was applied to a small piece of grass

of about 2" in length and fed to starved locusts. After treatment, locusts were supplied with food and kept individually in small wire mesh cylindrical cages (4" height x 3" width) at 25°C and 60-80% relative humidity.

Extraction

^{14}C γ-BHC was recovered from the external surface of each locust by rinsing in two 5 ml redistilled hexane, combined together and 0.5 ml of the washings was added to the counting vial contains 3 ml of scintillation solution. ^{14}C γ-BHC was recovered from the internal tissue by two methods. Solubilising the tissue using Hyamine X10 (2-methyl-4-(1:1:3:3-tetramethylbutyl) phenoxyethoxyethyldimethylbenzylammonium chloride monohydrate) (Brown and Badman 1961) resulted in very poor recovery because the tissue was not completely digested. Digestion with hot nitric acid and counting ^{14}C γ-BHC in the acid extract (O'Brien 1964) gave slightly better results. When the ^{14}C γ-BHC was extracted from the acid by shaking and sonicating with equal volume of redistilled hexane the efficiency increased to about 60%.

All or parts of the locust were digested in 1 ml of nitric acid by heating over a water bath at 70°C for about 7-10 m. 9 ml of distilled water and 1 ml of 1.5 M tris (hydroxymethyl) methylamine (tris buffer) was added to neutralise the acid. 10 ml of redistilled hexane was then mixed in and after shaking or sonicating, a 0.5 ml aliquot was taken for counting.

Calibration curves were plotted to determine the discriminator bias setting on the scintillation counter, and the volume of scintillation mixture. Correction were also made for background counts.

Results

3.3 Distribution of γ-BHC in the body

An investigation of the distribution of γ-BHC in the body of the desert locust was carried out using ^{14}C γ-BHC applied topically. Fifth instar locusts 4-5 days old, and adults 2-3 days and 2 weeks old were used. Locusts were treated with 10 ug per insect by topical application on the abdomen as described in 2.2. To recover insecticide remaining on the body surface, each locust was rinsed in two

lots of redistilled hexane, and the radioactivity in the combined washings was measured. To recover insecticide from within the body, the locusts were next divided into head, thorax and abdomen and each part was digested in hot nitric acid. Recovery of insecticide was made at 1,3,6 and 24 hours after application.

Results and Discussion

Results are given in Table 41.

Fifth instar

At 1 hour the external rinse contained 90% of the applied dose. After 3 hours the dose was 73% and about 25% was found in the abdomen. After 6 hours 60% was recovered from the cuticle and 27% from the abdomen. The amount of ^{14}C γ-BHC penetrating the abdomen also increased with time. After 24 hours, the total recovery was 23% in the external rinse to 19% in the thorax, 44% in the abdomen and 8% in the faeces.

Adult (2-3) days old

At 1 hour 70% was recovered in the external rinse and 25% was in the abdomen. After 3 hours, the

external recovery decreased to 51%, while the amount penetrated into the abdomen was increased to 44%. After 24 hours, 18% was recovered in the external rinse, 50% in the abdomen to 5% in the faeces.

Adult (2 week old)

At 1 hour after application 95% was recovered in the external rinse and no change in the amount recovered was observed up to 3 hours. After 6 hours, 65% was found in the external rinse and 30% in the abdomen. After 24 hours, the total amount of ^{14}C γ-BHC recovered from the surface decreased to about one third. The percentage of ^{14}C γ-BHC penetrated into the abdomen increased to 40% with 15% in the thorax and 12% in the faeces.

The distribution of ^{14}C γ-BHC in various parts of the body (head, thorax and abdomen) was estimated. It can be seen that, although the number of insects used was small, the external recovery of ^{14}C γ-BHC was least from 2-3 days old adults than from fifth instar or 2 week old adults. The rapid rate of penetration of ^{14}C γ-BHC through the cuticle of young adults results in a higher amount absorbed in the abdomen. Up to 48% was found after 6 hours

while only 27% in fifth instar and 30% in older adults. Most of ^{14}C γ-BHC penetrated into the body after 24 hours was found in the abdomen, a small amount in the thorax and only a trace of radioactivity was detected in the head. The percentage of ^{14}C γ-BHC excreted in the faeces of 2 weeks old adults was greater than that of 2-3 day old adults and of fifth instar hoppers. It was observed that after 6 hours 2-3 day old adults were badly affected while fifth instar hoppers was only slightly affected. The older adults survived later than 24 hours. It is possible to postulate from the results that the faster rate of penetration of ^{14}C γ-BHC found in 2-3 days old adults may account for their relative susceptibility.

3.4 Penetration through the gut

The passage of food through the alimentary canal was investigated in the desert locust and the migratory locust by means of X-ray technique, (Goodhue,1958). It was found that food passes through the oesophagus into the foregut and enters the anterior part of the midgut and the posterier of lobes of the gastric caeca.

Table 41

Distribution of ^{14}C γ-BHC in different parts of the body of Schistocerca

Age	Time (hours)	Percentage radioactivity recovered: External	Head	Thorax	Abdomen	Faeces	Total recovery
Fifth instar	1	90 (88-90)	-	-	-	-	90
	3	73 (70-75.9)			25 (23-28)		98
	6	60.5 (56-65)		Trace	27.5 (25-31)	-	88
	24	23 (20-26)	Trace	19 (17-21)	44 (38.6-49	8 (7-9)	94
Adults 2-3 days	1	70 (68-74)	-	-	25 (21-29)		95
	3	51 (46.5-55)	-	-	44 (40-48)		95
	6	44 (38-48)	-	-	43.5 (39-44)		87.5
	24	18 (17.1-20)	Trace	20 (15.5-25)	50 (46-54)	5 (4-6)	93
Adults 2 week	1	95 (93-97.4)	-	-	-	-	95
	3	95 (92.4-97)	-	-	-	-	95
	6	65 (63-67.3)	Trace	Trace	30	-	95
	24	30.5 (27.8-32)	Trace	14 (12-16)	42 (38-48)	12 (11-14)	98

Each mean represents three insects.

γ-BHC was used as poison bait in desert locust control, (Rainey 1958, MacCuaig 1964), and this experiment were therefore designed to investigate the absorption and excretion of ^{14}C γ-BHC through the gut of fifth instar Schistocerca.

Materials and Methods

Two groups of 26 3-4 days old fifth instar hoppers were deprived of food for 24 hours and then dosed with 10 ug of ^{14}C γ-BHC applied as stomach poison. One group was then kept supplied with food, the other was not fed. Locusts were maintained at a constant temperature of 25°C and relative humidity of 60-80%, and they were killed at 1,6 and 24 hours and dissected. The alimentary canal was examined to estimate the rate of absorption from the gut into the body and the faeces were collected to estimate the amount of ^{14}C γ-BHC excreted. Extraction and counting ^{14}C was done as in the Materials and Methods.

Results and Discussion

Table 42 shows the rate of absorption of ^{14}C γ-BHC from the alimentary canal into the body and how this is influenced by food.

First group (insects fed after treatment)

One hour after ingestion, 26% had been absorbed. After 6 hours the amount has increased to 36% but 51% of the total dose still remain in the gut. After 24 hours, the total amount of ^{14}C γ-BHC recoverable had declined in the alimentary canal to 17% and 28.5% in the body but about 40% was found in the faeces.

Second group (insects not fed after treatment)

The rate of absorption from the alimentary canal is at first slightly slower than in the first group, but is is doubtful if the difference is really significant. The absence of food after treatment for 24 hours increased the percentage of ^{14}C absorbed in the body to 38% and 21% was found in the faeces. The passage of food can be affected by stomach poison. MacCuaig and Watts (1960) found that when Schistocerca was fed on a small quantity of fluorescent powder mixed with grass and supplied immediately with abundant fresh grass, 80% of the fluorescence was lost in the faeces in less than 2 hours compared with 7 hours when locusts were starved after treatment. In investigations of the

rate of excretion of some organophosphorus insecticides in Periplaneta americana following topical application, Roan et al.,(1950) found that the radioactive materials were rapidly concentrated in the foregut with relatively lower concentration in the rest of the body. The roaches were able to secrete the toxicent into the lumen of the gut to prevent its accumulation in the blood and other tissues and showed no symptoms of poisoning. Certain species of insects may be able quickly to excrete the stomach poison, thus Brown (1951) showed that between 35 and 40% of the ingested arsenic from a mixture of sodium arsenite and Paris green was found in the body tissues of Pieris brasica and Locusta and only 20% in Euxoa segetum.

3.5 Metobolism of ^{14}C -BHC

3.51 Introduction

The metabolism of -BHC isomers by resistant and susceptible houseflies has been the subject of considerable investigation. Sternburg and Kearns (1956) showed that the flies could dehydrochlorinate γ-BHC to give the intermediate metabolite pentachlorocyclohexane using colourimetric

Table 42

Distribution of radioactivity in the body of Schistocerca after ingestion of ^{14}C γ-BHC

Treatment Locusts fed after treatment	Time (hours)	Percentage radioactivity recovered			
		Alimentary canal	Abdomen Thorax	Faeces	Total recovery
	1	62 (58-65)	26 (25-28)	-	88
	6	51 (48-54)	36 (34-38)	-	87
	24	17 (15-18)	28.5 (26.5-30)	39.6 (36-45)	85.1
Locusts not fed after treatment	1	70 (68-75)	19.6 (18-21)	-	89.6
	6	58 (56-60)	28 (26-30)	-	86
	24	27 (26-29)	38 (36-40)	21 (19-22)	86

Each mean represents three insects

methods. Bradbury and Staden (1959) found that less than 3% of the total applied dose of ^{14}C γ-BHC was converted to pentachlorocyclohexane by the flies. By using ^{14}C and ^{36}Cl labelled lindane and isomer Bradbury (1957) found that most of the lindane or any other -BHC isomers absorbed and metobolised by susceptible and resistant houseflies to a water-soluble metabolite. Analysis of the extract by two dimensional paper chromatography showed that at least 11 different metabolites were obtained from both γ and α-BHC, six or seven of these appeared to be common to both -BHC isomers. Bradbury and Staden (1959) showed that alkaline hydrolysis of the water-soluble metabolites from houseflies treated with γ and α-BHC produced, in the presence of reduced gluthathion, diclorothiophenols. These amounted to 68% from α-BHC and 61% γ-BHC. They concluded that the metabolism of -BHC to water-soluble compounds involved the formation of a C-S bond. The nature of the initial product (HSR) was not known.

$$C_6H_6Cl_6 + HSR \longrightarrow C_6H_6Cl_5 \cdot SR \longrightarrow C_6H_3Cl_2SH + ROH + 3HCl$$

Ishida and Daham (1965) isolated an enzyme from the houseflies capable of metabolising certain -BHC

isomers to water-soluble metabolites. The enzyme specifically required reduced glutathione, and it was found that this enzyme metabolised γ and ∝ pentachlorocyclohexane isomers. A study of the distribution of the enzyme among 10 species of insects showed that only the housefly was capable of metabolising γ-BHC and pentachlorocyclohexane isomers. Clark et al., (1966) showed that the major metabolic product from cattle ticks, Locusta and housefly dosed with γ-BHC (and in the enzyme extract fortified with glutathione) was S-(2,4 - diochlorophenyl) glutathione. This results is in agreement with the findings of (Bradbury, 1957) although no other dichlorophenyl isomers were identified.

A recent review by Menzie (1972) showed that there were five isomers whose retention times on gas liquid chromatography corresponded to pentachlorocyclohexane, 1,2,3 and 1,2,4 trichlorobenzene and 1,2,3,4 and 1,2,4,5 tetrachlorobenzene. Only 1,2,4 trichlorobenzene and 1,2,4,5 tetrachlorobenzene were reported after application of γ-pentachlorocyclohexane.

3.52 Materials and Methods

3-4 day old fifth instar Schistocerca was used.

A dose of 10 ug per locust was topically applied to the intersegmental membrane between first and second abdominal sternites, the thorax and to the second spiracles on the thorax (following the suggestion by Gerolt 1969, that contact insecticides reach the site of action via the tracheal system). γ-BHC was applied by injection into the abdomen and the thorax. In injection, a dose of 5 ug per locust was used, as γ-BHC in benzene was found (in 1.9) to be twice as toxic by injection than when topically applied. After treatment, the insects were kept under constant conditions of temperature and humidity and the amount of ^{14}C γ-BHC was estimated externally or internally after extraction with redistilled hexane as described in 3.32. Faeces were collected at the end of the experiment and analysed in the same way.

With topical application, γ-BHC has to penetrate through the cuticle to reach the site of action while with injection, the insecticide is introduced directly into the body and the factor of the cuticle has been eleiminated. These experiments were designed to investigate the metabolism of ^{14}C γ-BHC applied by different methods to fifth instar desert locusts.

3.53 Results and Discussion

Table 43 shows the distribution of ^{14}C γ-BHC in the body of Schistocerca after topical application to the abdomen, thorax and the spiracles.

Topical application to the abdomen

One hour after application, 90% was recovered in the external rinse. After 3 hours, it decreased to 72.6% and only 20% was found in the abdomen. After 6 hours, the total percentage of ^{14}C γ-BHC in the abdomen increased to 27% and 60% was recovered from the surface.

Topical application to the thorax

The rate of penetration was faster at the beginning the decreased slowly. After 1 hour, 69.9% was recovered in the external rinse and 21.5% was found in the thorax. After 3 hours, the percentage of ^{14}C γ-BHC in the thorax increased to 29%. After 6 hours, ^{14}C γ-BHC had moved from the site of application (thorax) to the abdomen and 11% was recovered in the abdomen to 27% in the thorax and 55% from the external rinse.

Topical application to the spiracles

One hour after application, 72.5% was recovered in the external rinse and 13.5% from the abdomen. After 3 hours, the radioactivity in the abdomen increased to 17%. After 6 hours, 12% was found in the thorax to 31.6% in the abdomen and 48% from the external rinse.

There were no marked difference in recoveries or rates of penetration following application at different sites, although the numbers used were too small to be certain. It is, however, clear that application to the spiracles did not induce markedly more rapid penetration.

Injection of ^{14}C γ-BHC

Table 44 shows the distribution of ^{14}C γ-BHC after injection into the abdomen and the thorax. After 1-3 hours, no change in the percentage of ^{14}C γ-BHC injected into the thorax or the abdomen was found. After 6 hours, 30% of the total dose injected into the thorax was recovered from the abdomen and 25% of the dose injected into the abdomen had moved to the thorax.

Analysis of the faeces collected at the end of the experiment showed that about 11% was excreted, following topical application or injection, over 24 hours. By contrast, about 40% of the total dose ingested was excreted in the same period and this could explain the greater resistance of ingested γ-BHC (see 1.15).

Hadaway (1956) showed that sub-lethal doses of γ-BHC had little cumulative effect when applied topically over a period of time to houseflies. Bradbury (1957) in comparative experiments on the absorption and metabolism of BHC with a variety of insects, including locusts, found (Table 45) that both resistant and susceptible houseflies were unique with regard of their ability to metabolise BHC. Locusts metabolised only 4% of the total dose over 24 hours. MacCuaig (1957) found that γ-BHC was fully cumulative when applied topically in small doses to adult Schistocerca and, is not therefore, detoxicied. A small fraction is metabolised, the rest of the γ-BHC is stored unchanged in the fat body within the abdomen.

It is possible that excretion of γ-BHC is the only method to reduce toxicity effects. Cohen and Smith

Table 43

Distribution of ^{14}C γ-BHC applied topically to Schistocerca

Treatment	Time (hours)	Percentage of radioactivity recovered				
		External	Internal			
			Head	Thorax	Abdomen	Total recovery
Thorax	1	69.6 (65-73)	0	21.6 (19-24)	0	91.2
	3	60.6 (57-64)	0	29 (28-31)	0	89
	6	55.8 (51-59.5)	0	27 (20-34)	11 (9-13)	93.8
Abdomen	1	90 (89-92)	0	0	0	90
	3	72 (70-77)	0	0	20 (19-23)	92.6
	6	60.3 (58-63.1)	0	0	27.5 (25-31)	87.8
Spiracles	1	72.5 (69.5-77)	0	0	13.5 (11.5-15)	86
	3	68 (65-73)	0	0	17.3 (16.5-18.4)	85.3
	6	48 (44-54)	0	12 (10-14)	31.3 (29.3-34)	91.6

Each mean represents three insects

Table 44

Distribution of ^{14}C γ-BHC applied by injection into Schistocerca

Treatment	Time (hours)	Percentage of radioactivity recovered			
		Head	Thorax	Abdomen	Total recovery
Abdomen	1	0	0	83.6 (80-86)	83.6
	3	0	0	89 (87.5-90)	89
	6	0	25 (22-28)	61.5 (60-65)	89
Thorax	1	0	90 (87-93)	0	90
	3	0	89 (86-91)	0	89
	6	0	63 (61.5-65)	30.3 (28-33)	93.8

Each mean represents three insects.

(1961) found that if dieldrin was injected into fifth instar Schistocerca, it was slowly excreted unchanged. Kurihara et al.,(1970) using an autoradiographic technique with ^{14}C γ and α-BHC, found that the recovery of radioactivity from outside the body of Periplaneta americana was 80-85% after one hour decreased to about 12% after 24 hours for γ-BHC. Penetration and distribution of γ-BHC was much faster than of the α isomer. γ-BHC reached the central nervous system rather rapidly, but did not penetrate into the system for a long time after treatment.

Table 45

Absorption and metabolism of -BHC in various insects (after Bradbury, 1957)

Insects	Dose applied ug/g	-BHC recovered in µg/g External	Internal	Water soluble metabolites	Residue
Cockroach	42	10	19	7	8
Bean weevil	111	7	94	8	7
Grain weevil	34	14	13	1	6
Locusts	100	16	83	4	9
Mosquito	213	17	141	10	3
Khapra beetle	100	21	45	11	5
Housefly (resistant)	230	13	21	174	10
Housefly (susceptible)	195	21	54	90	17

GENERAL DISCUSSION

Introduction

The relative toxicity of insecticides varies according to several different inter-actions between insects and insecticides. Some insecticide may evaporate from the surface, the rest penetrate through the cuticle, in the case of topical application, or through the gut in the case of stomach poison. It is assumed that the ultimate toxicity of an insecticide is the direct result of accumulation of the toxic dose at the site of action. The present work was designed to study some factors affecting susceptibility of fifth instar Schistocerca gregaria to insecticides. Of the insecticides tested, one γBHC, has been used for locust control; the others Cyolane and dioxacarb, are new compounds.

The importance of standardisation of the insect, the insecticides and the technique in toxicological work has been emphasised by Busvine (1957). He grouped sources of variability in insects under two headings: (1) intrinsic differences between insects of a species due to different instars, weight and sex; (2) fluctuation in susceptibility of a given type of insect caused by extrinsic changes in the environment such as temperature and food.

Intrinsic differences

The intrinsic differences investigated in this study were: differences between instars, changes within the instar, differences between the sexes and differences due to size. The effects were most fully studied using γ-BHC by topical application and these results will be discussed first.

Experiments were carried out to investigate the changes in susceptibility of the fifth instar hoppers of Schistocerca gregaria to γ-BHC, Cyolane and dioxacarb applied topically by injection or as a stomach poison.

As regards γ-BHC, the resistance to this insecticide increased with increasing age of the insect when the poison was topically applied. In contrast with injection, increasing age was associated with a decrease in resistance. For Cyolane and dioxacarb, the pattern of change in the LD50's with age was small and non-significant.

For topical application to be effective the insecticide must penetrate the cuticle. With injection, however, the insectidide dose not need

to penetrate the cuticle and one would expect it to be more effective than in the case of topical application. This was found with dioxacarb and to some extent with Cyolane. γ-BHC was relatively less toxic when injected, so factors other than cuticle penetration must be involved with this insecticide.

Chan es of weight with age may affect toxicity, thus, MacCuaig (1960) with fifth instar Schistocerca, found that the LD50 of -BHC was greater at 2.5 and 4.5 days than that at older ages. In the present work, resistance to -BHC was related to weight up to 5-6 days, but at 7-8 days, although the insects were nearly the same weight, their resistance decreased. Weight is not always important, thus Sawicki and Green (1964) found no relation between changes in weight and changes in resistance with age of houseflies to diazinon. It appears that physiological changes occurring within the instar may have an effect on the susceptibility of the insect irrespective of weight. Thus pre-moulting physiological changes may make the insect more susceptible.

Another important intrinsic factor affecting

toxicity of insecticides is difference between instars. Pradhan and Bindra (1956), using a spraying technique, showed that first instar hoppers of Schistocerca were more susceptible to γ-BHC than fifth instar hoppers, but the exact doses were not determined. MacCuaig (1960 and 1961) found that adult Schistocerca were slightly less resistant to γ-BHC than 4-5 days old fifth instar hoppers. It was found in the present work (Section 1.7) that the susceptibility of mid fifth instar hoppers and mature adult locusts to γ-BHC was very similar, about 10 µg/g, but the younger insects in both developmental stages were more susceptible. These differences may reflect differences in the rates of penetration of the insecticide through the cuticle since it was found (Section 3.3) that ^{14}C γ-BHC penetrated through the cuticle of young adults more quickly than that of fifth instar or mature adults. Since γ-BHC is found to be cumulative in locusts (MacCuaig, 1957) and little is metabolised (Bradbury, 1957) it is likely that susceptibility of young adults is due to rapidity of penetration. The effect of penetration on resistance to insecticides has been investigated by some workers. Gast (1961) found

that change in susceptibility between early and later instar of Heliothis zea to DDT was due to differences in rates of penetration. Vinson and Brazzel (1966) found that DDT did not effectively penetrate the cuticle of the older fifth instar larvae of Heliothis virescens. Yet, Brook (1960) reported that varying resistance of adult houseflies to aldrin, dieldrin and isodrin did not appear to be due to differences in penetration of insecticides.

In general, younger stages are more susceptible and this may reflect differences in size since it is known that susceptibility is not always directly proportional to body weight. This has been shown by Bliss (1936) for arsenic as a stomach poison on Bombyx mori; and by Way (1954) for DDT as a stomach or contact poison and γ-BHC as a stomach poison on last instar larvae Diataraxia oleracea. Hopf (1951) and Stringer (1953) reported that the mortality of adult Locusta by a number of insecticides correlated best with the dose measured as ug per locusts, but MacCuaig (1956 and 1961) found that resistance of adult Schistocerca and Locusta to γ-BHC was proportional to weight when the dose was measured in µg/g of body weight. It was found

(Section 1.6) that there were no marked differences between sexes but the larger females were relatively more resistant than males when the dose was measured per locust. In all other experiments the LD50 was calculated in terms of µg/g irrespective of the total weight of the insect. MacCuaig (1961) found that sexes of Schistocerca were equally tolerant to γ-BHC, on this basis. On the hand, sex differences in resistance have been reported by other authors. Results indicate that the male is more susceptible than the female. This has been shown by Menusan (1948) for several insecticides on Blatella germanica, by Hadaway and Barlow (1956) for dieldrin on Anopheles stephensi, and by El Nosa and King (1964) for dieldrin on Hylemya antiqua. Since the female is larger than the male, it is possible that this difference can sometimes be due to difference in body weight, and it is likely to disappear when the dose is calculated on a µg/g basis.

Extrinsic differences

Of the extrinsic factors affecting toxicity, the type of the insecticide was, as expected, extremely important. It will be seen from Table 9 that

γ-BHC is muchless toxic on a w/w basis than either Cyolane or dioxacarb. The nature of the solvent used with topically applied γ-BHC did not affect toxicity in this investigation although γ-BHC was more toxic when applied by injection in acetone or benzene than in KAB (Kerosene aromatic bottoms).

The site of application had very profound effects. Toxicity was much higher following application to the neck membrane that to other parts of the body. It is of interest to compare the results in Section 1.3 with those of previous investigations with Schistocerca. MacCuaig (1956) found that DNOC was markedly less effective when applied to the leg or the wing base. Ahmed and Gardiner (1968) found that malathion was more toxic when applied to the neck membrane and less effective on the abdomen. This might be attributed to differences in the nature of the cuticle, but Lewis (1956) found that sclerotization of the cuticle of Phormia terraenovae exerted little or no influence on absorption of DDT. There is some evidence that effectiveness of applications is greatest at loci near the site of action (the head or CNS). This has been shown with DDT to houseflies (Fisher,1952),

with a number of insecticides to Dysdercus cingulatus (Reddy and Naidu 1962) and with dieldrin to houseflies (Gerolt, 1969). Kennedy et al.,(1948) found that the leg of Locusta was the most susceptible part of the body to DNOC. Hewlett and Gostick (1963) found no difference in toxicity of dieldrin when applied to different parts of Alphitobius laevigatus.

The data indicate no general agreement on the causes of the differences that occur, and it is possible that the relative effectiveness of different insecticides depend on the site of application, the insecticide and the solvent used.

Relative toxicity was affected by method of application. γ-BHC was less effective by oral application than by other method (Section 1.1). This difference may be because a large percentage of the ingested dose of γ-BHC was excreted. The stomach toxicity of γ-BHC was found to be dependent on the amount of food available after treatment. When locusts were fed immediately after dosing about 40% of ^{14}C γ-BHC was excreted after 24 hours compared with only 21% when they were not fed (Section 3.5). The fact that the toxicity of γ-BHC was reduced when the stomach dose was followed immediately by

fresh food might prove significant in the field. One of the established control methods is applying γ-BHC as a poison bait, and, where abundant plant food material is available, a smaller percentage of kill than that obtained after baiting in an area of sparse vegetation might be expected. Hoppers 1-2 days old, in the feeding stage, were more resistant to γ-BHC by stomach action than were 7-8 days old hoppers before moulting, when feeding is ceasing (Section 1.16).

It was observed that the older hoppers did not accept the poisoned sandwich as easily as did the younger hoppers. This may explain unreliable results of γ-BHC when used as bait against the desert locust in the field. Rainey (1958) reported that locusts at all stages refused at times to feed on a bait for a dya or two during each of their five moults. Thus, it seems although older hoppers were more susceptible, baiting locusts at the time of the moulting is likely to be relatively ineffective.

Changing the post-dosing temperature affected the toxicity of γ-BHC and dioxacarb. It was found (Section 1.4) that γ-BHC and dioxacarb were more toxic at the lower temperature than at the higher

temperature but change in temperature had no effect on toxicity of Cyolane. More γ-BHC was recovered at the lower temperature than at the higher temperature (Section 2.1). The effect of temperature on the action of insecticides has been investigated by some workers. Vinson and Hearns (1952) found that about twice as much DDT penetrated into Periplaneta americana at 35°C. than at 15°C. Tahori and Hoskins (1953) found that about 4 times as much DDT was absorbed into houseflies at 35°C. than at 13.5°C. A negative temperature coefficient of action of γ-BHC and DDT has been shown with Blatella germanica (Guthrie, 1950), but the reverse was true with aldrin and dieldrin.

The increased rate of penetration observed at the higher temperature does not explain the enhancement of toxicity of γ-BHC at the lower temperature. It is possible that after penetration, internal movement of the insecticide will probably be more rapid at the higher temperature, so more γ-BHC is transferred to less sensitive area e.g. to the fat body. Potter and May (1958) concluded that the process of both poisoning and recovery are speeded up at the higher

temperature irrespective of poison, method of application and test insects. However, the extent of each varies with different poisons and with different formulations of the same poison.

Resistance to γ-BHC was affected by deprivation of food. Starvation before dosing increased the susceptibility of hoppers to γ-BHC and hoppers starved over 48 hours were more susceptible than those starved for 24 hours. Further, hoppers deprived of food before and after treatment were less resistant than hoppers deprived of food before or after only. MacCuaig (1960) found little difference between the two treatments. The utilisation of reserves during starvation in Locusta has been investigated by Hill and Goldsworthy (1970). They found that the haemolymph and water content of the tissues were changed and the total weight decreased due to decrease in reserves of fat body. O'Brien (1967) suggested that after starvation the increase in susceptibility is presumably because depletion of the fat body releases more insecticide into circulation.

The quality of food may also be important as it was found (Section 1.8) that hoppers fed on an

artificial diet were more resistant to γ-BHC but less resistant to Cyolane and dioxacarb than hoppers fed on fresh grass. In other experiment hoppers fed on very dry grass for 4 days were much more susceptible to γ-BHC than when fed on fresh grass. It is possible that changes in quality of food may affect toxicity through altering the weight of the insects (May,1949) and due to differences in tolerance through varying nutrition (Potter and Gillham 1957).

Penetration through the cuticle

The agreement obtained between different methods of analysis showed that the rates of disappearance of γ-BHC from the surface of the cuticle after topical application to fifth instar hoppers varied somewhat according to method used.

It can be seen from Table 46 that scintillation counting suggested a lower rate of entry into the body than did GLC estimation. The reasons for this are not clear, but they show that the results must be interpreted with care. Nevertheless comparison of Tables 37 and 38 (GLC) and 41 (scintillation) show that much of the insecticide

which is neither recovered from the surface or penetrated in the haemolymph is in the tissues of the part of the body to which application was made.

Both methods suggested, although, they did not prove, that insecticide disappeared faster from the surface of young adults than from fifth instar hoppers or adults. This is associated with a faster accumulation of γ-BHC in the tissues of the young adults and possibly reflects the early state of the young adult cuticle at this time.

Conclusions

The changes in susceptibility observed in this study indicate the desirability of standardising the age of the insect and the site of application used in biological assay. The results suggest that environmental factors such as temperature and food may account for some variations in the effectiveness of the insecticide. In almost all experiments the number of locusts used was small and more replication is needed before firm conclusions can be made.

Table 46

Percentage of γ-BHC recovered from the cuticle of fifth instar Schistocerca by two methods of analysis

Method of analysis	Solvent	Percentage of γ-BHC recovered from the surface: Time (hours) 1	3	6	24
GLC (Table 37)	KAB	70	40	23.5	-
GLC (Table 38)	KAB	65	48	27	-
(Table 38)	Benzene	70	58	30	-
Scintillation (Table 41)	Benzene	90	73	50.5	23

Acknowledgements

I would like to express my thanks to my Director of Studies, Professor M.J. Way for his helpful advice and constructive criticism and to my Supervisor, Dr. R.F. Chapman for useful discussion and constant encouragement.

Thanks are also due to Dr. P. Hunter-Jones and Mr. R.D. MacCuaig for their help and interest in this work. I would like to thank Dr. D. Bernays and Mr. A. Davies for their help and advice.

My thanks go to Dr. P.T. Haskell for research facilities. I am grateful to the Food and Agriculture Organisation of the United Nations for the award of a Research Fellowship and to the Somali Government for permission to take up the fellowship.

I would also like to thank Miss Paula Fanco for typing this thesis.

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APPENDIX

1.13 Toxicity of γ-BHC by topical application

Age (days)	Dose (µg/g)	0 (Controls)	4.0	7.0	10	15
1	(n) No. in group	30	40	40	60	20
	(r) No. responding (deaths)	1	7	28	54	20
	% mortality (corrected)	3	11	69	90	100
2-3	Dose (µg/g)	0	4.5	7.0	10	15
	n	20	30	60	80	20
	r	0	1	19	50	20
	%	0	3	32	63	100
5-6	Dose (µg/g)	0	7.0	10	15	33
	n	30	30	60	50	20
	r	1	5	21	39	20
	% (corrected)	3	14	33	77	100

Toxicity of Cyolane by topical application

Age (days)	Dose (µg/g)	0 (Controls)	2.0	3.0	3.5	4.5
1	(n) No. in group	20	30	30	30	20
	(r) No. responding (deaths)	0	6	21	23	19
	% mortality	0	20	70	77	95
2-3	Dose (µg/g)	0	2.5	3.0	4.0	4.5
	n	30	30	30	30	20
	r	0	6	11	25	19
	%	0	20	37	83	95
5-6	Dose (µg/g)	0	2.0	3.0	4.0	5.0
	n	30	30	30	30	20
	r	1	9	16	25	19
	% (corrected)	3	28	52	82	95

1.13 Toxicity of dioxacarb by topical application

Age (days)	Dose (µg/g)	0 (Controls)	1.3	2.0	2.5	3.5
1	(n) No. in group	30	30	30	30	20
	(r) No. responding (deaths)	0	8	16	19	19
	% mortality	0	27	53	63	85
2-3	Dose (µg/g)	0	2.0	2.5	3.0	4.5
	n	30	30	30	30	20
	r	1	7	15	20	18
	% (corrected)	3	21	49	66	90
5-6	Dose (µg/g)	0	1.3	3.0	4.5	7.0	10
	n	30	30	30	30	30	20
	r	0	10	19	22	26	20
	%	0	33	63	73	87	100

1.14 Toxicity of γ-BHC by injection

Age (days)	Dose (µg/g)	0 (Controls)	10	13	15	20	
1	n	10	20	20	20	20	
	r	0	6	11	13	19	
	%	0	30	55	65	95	
2-3	Dose (µg/g)	0	7.5	10	13	20	
	n	10	20	20	20	20	
	r	0	3	5	10	17	
	%	0	15	25	50	85	
5-6	Dose (µg/g)	0	5.0	7.5	10	13	20
	n	20	20	20	30	30	10
	r	0	5	9	19	25	10
	%	0	25	45	63	83	100

1.14 Toxicity of Cyolane by injection

Age (days)	Dose (µg/g)	0 (Controls)	2.0	3.0	4.0	5.0
1	(n) No. in group	20	20	20	20	20
	(r) No. responding (deaths)	0	4	8	13	18
	% mortality	0	20	40	65	90
2-3	Dose (µg/g)	0	1.3	1.5	2.5	3.5
	n	20	20	20	20	20
	r	0	2	4	11	18
	%	0	10	40	55	90
5-6	Dose (µg/g)	0	1.5	2.5	3.5	4.0
	n	20	20	20	20	20
	r	0	2	6	16	20
	%	0	10	30	80	100

Toxicity of dioxacarb by injection

Age (days)	Dose (µg/g)	0	1.0	2.0	3.0	5.0
1	n	20	20	20	20	20
	r	0	2	10	17	20
	%	0	10	50	85	100
2-3	Dose (µg/g)	0	1.0	1.5	2.0	
	n	20	20	20	20	
	r	0	6	14	18	
	%	0	30	70	90	
5-6	Dose (µg/g)	0	0.75	1.5	2.0	3.0
	n	20	20	20	20	20
	r	0	3	15	19	20
	%	0	15	75	95	100

1.15 Toxicity by stomach action

Insecticide	Age (days)	Dose (µg/g)	0 (Controls)	11	14	17	20	25
γ-BHC	2-3	n	20	30	30	30	30	30
		r	0	7	14	20	24	20
		%	0	23	47	67	80	100

Cyolane	Dose (µg/g)	0	1.5	2.5	3.5	4.5	
	n	20	20	30	30	20	
	r	0	0	4	19	18	
	%	0	0	13	63	90	
Dioxacarb	Dose (µg/g)	0	4.5	7.0	9.0	11	12
	n	20	20	30	30	30	20
	r	0	0	9	22	26	20
	%	0	0	30	73	87	100

1.16 Effect of the stomach toxicity of γ-BHC in relation to age

Age (days)	Dose (µg/g)	0 (Controls)	8.0	11	14	17
1-2	n	20	20	20	20	20
	r	0	5	10	15	19
	%	0	25	50	75	95

7-8	Dose (µg/g)	0	5.0	8.5	11	14
	n	20	20	20	20	20
	r	0	4	11	16	20
	%	0	20	55	80	100

1.23 Variation in susceptibility of fifth instar Schistocerca to γ-BHC by topical application.

Age (days)	Number of insects	Dose (µg/g) (r) Total dead % Kill	3.5	5.0	7.5	10.5	15	LD50 (provisional)
1	20	r	4		10	16		
		%	20		50	80		7.2
2	10	r			1	5	10	
		%			10	50	100	10.4
3	10	r			4	7	10	
		%			40	70	100	9.5
4	10	r			1	4	9	
		%			10	40	90	11.0
5	10	r			2	7	8	
		%			20	70	80	10.2
6	10	r			3	8	9	
		%			30	80	90	9.5
7	20	r			8	14	20	
		%			40	70	100	9.0
8	20	r		6	10	20		
		%		30	50	100		7.5
9	10	r	1	4	6	10		
		%	10	40	60	100		6.0

1.23 Variation in susceptibility of fifth instar Schistocerca to Cyolane by topical application.

Age (days)	Number of insects	Dose (ug/g)	0.75	1.0	1.5	2.5	3.5	5.0	LD50
		(r) Total dead							
		% Kill							
1	10	r			1	4	8		
		%			10	40	80		2.8
2	20	r			2	4	18		
		%			10	20	90		2.6
3	10	r			1	3	9		
		%			10	30	90		2.7
4	20	r			2	4	12	18	
		%			10	20	60	90	3.2
5	10	r			1	3	10		
		%			10	30	100		3.0
6	10	r			1	3	10		
		%			10	30	100		3.0
7	20	r			2	5	18		
		%			10	25	90		2.7
8	10	r		1	4	6	9		
		%		10	40	60	90		1.9
9	20	r	2		10	18			
		%	10		50	90			1.4

1.23 Variation in susceptibility of fifth instar Schistocerca to dioxacarb by topical application.

Age (days)	Number of insects	Dose (µg/g) (r) Total dead % Kill	0.5	1.0	1.5	2.0	3.0	4.5	LD50
1	10	r				3	4	9	
		%				30	40	90	2.8
2	20	r				6	8	16	
		%				30	40	80	3.0
3	20	r			0	8	11	18	
		%			0	40	55	90	2.5
4	10	r				3	3	8	
		%				30	30	80	3.0
5	20	r				5	8	16	
		%				25	40	80	3.5
6	10	r				3	4	8	
		%				30	40	80	3.0
7	10	r				3	4	8	
		%				30	40	80	3.0
8	20	r				7	10	16	
		%				35	50	80	2.8
9	20	r	3	11		18			
		%	15	55		90			1.0

1.24 Variation in susceptibility of fifth instar Schistocerca to γ-BHC by injection.

Age (days)	Number of insects	Dose (µg/g) (r) Total dead % Kill	3.5	5.0	7.5	10.5	15	LD50
1	10	r			2	4	10	
		%			20	40	100	11.0
2	10	r			1	2	10	
		%			10	20	100	13.0
3	20	r			4	6	18	
		%			20	30	80	11.4
4	10	r			4	6	9	
		%			40	60	90	10.1
5	20	r			6	7	17	
		%			30	35	85	11.0
6	20	r			4	16	19	
		%			20	80	95	10.2
7	10	r		1	4	7	10	
		%		10	40	70	100	9.0
8	10	r	1	3	8			
		%	10	30	80			6.9
9	20	r	6	14	19			
		%	30	70	95			4.5

1.24 Variation in susceptibility of fifth instar Schistocerca to Cyolane by injection.

Age (days)	Number of insects	Dose (µg/g) (r) Total dead % Kill	0.75	1.0	1.5	2.5	3.5	5.0	LD50
1	20	r			3	8	12	18	
		%			15	40	60	90	3.0
2	10	r			1	5	9		
		%			10	50	90		2.4
3	10	r			1	5	10		
		%			10	50	100		2.4
4	10	r			1	3	10		
		%			10	30	100		2.7
5	20	r			2	7	20		
		%			20	35	100		2.9
6	10	r			2	5	10		
		%			20	50	100		2.3
7	20	r			4	8	18		
		%			20	40	90		2.3
8	20	r		6	10	17			
		%		30	50	85			1.5
9	20	r	4	9	12	18			
		%	20	45	60	90			1.1

1.24 Variation in susceptibility of fifth instar Schistocerca to dioxacarb by injection.

Age (days)	Number of insects	Dose (µg/g) (r) Total dead % Kill	0.5	0.75	1.0	1.5	2.0	3.0	LD50
1	10	r	1			4	7	9	
		%	10			40	70	90	1.7
2	10	r				2	6	9	
		%				20	60	90	1.9
3	10	r				2	5	9	
		%				20	50	90	2.0
4	20	r	2			8	12	18	
		%	10			40	60	90	1.9
5	20	r				6	10	18	
		%				30	50	90	2.0
6	10	r	1			5	8		
		%	10			50	80		1.6
7	20	r	3		10	14	18		
		%	15		50	70	90		1.0
8	10	r	4	6	8				
		%	40	60	80				0.6
9	20	r	6	11	18				
		%	30	55	90				0.65

1.4 Effect of temperature on the toxicity of insecticides

Temperature 25 C.

Insecticide	Dose (µg/g)	0(Controls)	5.0	7.0	10	13
α-BHC	(n) No. in group	20	30	30	30	20
	(r) No. responding (deaths)	0	3	9	17	20
	% mortality	0	10	30	57	100
Cyolane	Dose (µg/g)	0	1.0	2.0	3.0	5.0
	n	20	20	30	30	20
	r	0	2	6	15	16
	%	0	10	20	50	80
Dioxacarb	Dose (µg/g)	0	1.0	2.0	3.0	5.0
	n	20	20	30	30	20
	r	0	2	7	18	18
	%	0	10	23	60	90

Temperature 35 C.

Insecticide	Dose (µg/g)	0	26	32	40	52
γ-BHC	n	20	10	30	30	20
	r	0	1	12	19	18
	%	0	10	40	63	90

Cyolane	Dose (µg/g)	0	3.0	3.5	4.0	4.5	5.0
	n	20	10	30	20	30	20
	r	0	0	14	13	24	19
	%	0	0	47	65	80	95

Dioxacarb	Dose (µg/g)	0	3.5	5.0	7.0	9.0	12
	n	10	10	30	30	30	20
	r	0	0	5	13	22	17
	%	0	0	17	43	73	85

1.5 Effect of prior starvation on the toxicity of γ-BHC

Topical application

Starvation period (hrs)	Dose (µg/g)	0(Controls)	6.5	8.5	10.5	
24	n	20	30	30	30	
	r	0	6	16	25	
	%	0	20	53	83	
48	Dose (µg/g)	0	5.0	6.5	8.5	10.5
	n	20	20	30	30	30
	r	0	0	11	20	27
	%	0	0	37	67	90
72	Dose (µg/g)	0	5.0	7.0,	8.5	
	n	20	30	30	30	
	r	0	6	20	27	
	%	0	20	67	90	

Injection

Starvation period (hrs)	Dose (µg/g)	0	6.5	8.5	10.5	
24	n	10	30	30	30	
	r	0	3	14	24	
	%	0	10	47	80	
48	Dose (µg/g)	0	5.0	7.0	8.5	10.5
	n	20	30	30	30	20
	r	0	3	15	22	18
	%	0	10	50	73	90
72	Dose (µg/g)	0	5.0	7.0	8.5	
	n	20	30	30	30	
	r	0	6	18	26	
	%	0	20	60	87	

1.5 Effect of starvation on the stomach toxicity of γ-BHC

Treatment	Dose (µg/g)	0 (Controls)	8.5	11	14	18
Starved before						
	n	20	30	30	30	20
	r	0	3	9	17	16
	%	0	10	30	57	80

Starved after						
	Dose (µg/g)	0	5.0	8.5	11	14	17
	n	20	10	30	30	40	10
	r	0	0	9	15	35	10
	%	0	0	30	50	88	100

Starved before and after						
	Dose (µg/g)	0	5.0	6.5	8.5	10
	n	20	20	30	30	30
	r	0	2	9	21	27
	%	0	10	30	70	90

1.6 Effect of weight on the toxicity of γ-BHC

Weight	Sex	Dose (µg/locust)	0 (Controls)	14	18	23	30
1.16	Males	n	20	30	30	30	20
		r	0	12	21	27	20
		%	0	40	70	90	100
1.50		Dose (µg/locust)	0	14	18	23	30
		n	10	30	30	30	20
		r	0	3	8	15	16
		%	0	10	27	50	80
1.35	Females	Dose (µg/locust)	0	16	21	27	35
		n	20	30	30	30	20
		r	0	8	23	27	20
		%	0	27	80	90	100
1.75		Dose (µg/locust)	0	16	21	27	35
		n	20	30	30	30	20
		r	0	5	8	17	18
		%	0	17	27	57	90

1.7 Toxicity of γ-BHC to different instars of Schistocerca

Topical application

Age	Dose (µg/g)	0 (Control)	7.5	10.5	13.5
Fifth instar	n	20	30	30	20
(4-5 days)	r	0	5	18	17
	%	0	17	60	85
Adults	Dose (µg/g)	0	5.0	7.0	10.0
(2-3 days)	n	20	30	30	20
	r	0	12	24	18
	%	0	40	80	90
Adults	Dose (µg/g)	0	7.0	10.0	15.0
(2 weeks)	n	30	30	30	20
	r	1	8	15	18
	% (corrected)	3	27	50	90

Injection

Age	Dose (µg/g)	0	7.5	10.5	13.5	15
Fifth instar	n	20	30	30	20	20
(4-5 days)	r	0	6	14	16	20
	%	0	20	47	80	100
Adults	Dose (µg/g)	0	5.0	7.0	10	13
(2-3 days)	n	30	30	30	20	20
	r	1	12	17	16	20
	% (corrected)	3	40	57	79	100
Adults	Dose (µg/g)	0	7.0	10	15	20
(2weeks)	n	30	30	30	30	20
	r	0	6	15	24	20
	%	0	20	50	80	100

1.8 Effect of lack of food on the toxicity of γ-BHC

Topical application

Treatment	Dose (µg/g)	0 (Controls)	7.5	10.5	13.5	18
Control	n	0	30	30	30	30
	r		6	16	24	30
	%		20	53	80	100

No food before	Dose (µg/g)	0	7.0	10	12.5	15
	n	0	30	30	30	20
	r		3	12	27	20
	%		10	40	90	100

No food after	Dose (µg/g)	0	5.0	8.5	10.5
	n	0	30	30	30
	r		3	21	27
	%		10	70	90

Injection

Treatment	Dose (µg/g)	0	7.5	10.5	13.5	18
Control	n	0	30	30	30	30
	r		3	9	21	27
	%		10	30	70	90

No food before	Dose (µg/g)	0	7.0	10	12.5	15
	n	0	30	30	30	20
	r		2	9	24	20
	%		6	30	80	100

No food after	Dose (µg/g)	0	5.0	8.5	10.5
	n	0	30	30	30
	r		5	18	24
	%		17	60	80

1.8 Effect of reduced feeding on the toxicity of γ-BHC

Treatment	Dose (µg/g)	0(Controls)	7.0	10.5	12	15.5
Fully fed	n	0	20	30	30	30
	r	0	0	7	14	24
	%		0	23	47	80
Less fed	Dose (µg/g)	0	7.0	10.5	12	15.5
	n	30	30	30	30	30
	r	1	6	18	23	27
	% (corrected)	3	18	59	76	90

Effect of diet on the resistance to insecticides applied topically

Artificial diet

Insecticide	Dose (µg/g)	0 (Controls)	7.0	10	15	22
γ-BHC	n	10	30	30	30	30
	r	0	3	7	15	27
	%	0	10	23	50	90
Cyolane	Dose (µg/g)	0	1.0	2.0	3.0	4.5
	n	10	30	30	30	30
	r	0	7	17	23	28
	%	0	23	57	77	93
Dioxacarb	Dose (µg/g)	0	1.0	2.0	3.0	
	n	10	30	30	30	
	r	0	6	18	28	
	%	0	20	60	93	

Benax

γ-BHC	Dose (µg/g)	0	5.0	7.5	10.5
	n	0	30	30	30
	r	0	3	12	26
	%		10	40	87

1.8 Toxicity of γ-BHC to Schistocerca fed on very dry grass

Topical application

Feeding period (days)	Dose (µg/g)	0 (Controls)	5.0	7.0	10
2	n	20	30	30	30
	r	0	4	13	24
	%	0	13	43	80
3	Dose (µg/g)	0	5.0	7.0	10
	n	20	30	30	30
	r	0	6	18	27
	%	0	20	60	90
4	Dose (µg/g)	0	3.0	5.0	7.0
	n	10	30	30	30
	r	0	3	9	24
	%	0	10	30	80

Injection

Feeding period (days)	Dose (µg/g)	0		5.0	10	15
2	n	10		30	30	30
	r	0		3	12	27
	%	0		10	40	90
3	Dose (µg/g)	0		5.0	10	12.5
	n	20		30	30	30
	r	0		3	17	24
	%	0		10	57	80
4	Dose (µg/g)	0	3.0	5.0	7.0	10
	n	20	30	30	30	30
	r	0	6	17	27	30
	%	0	20	57	90	100

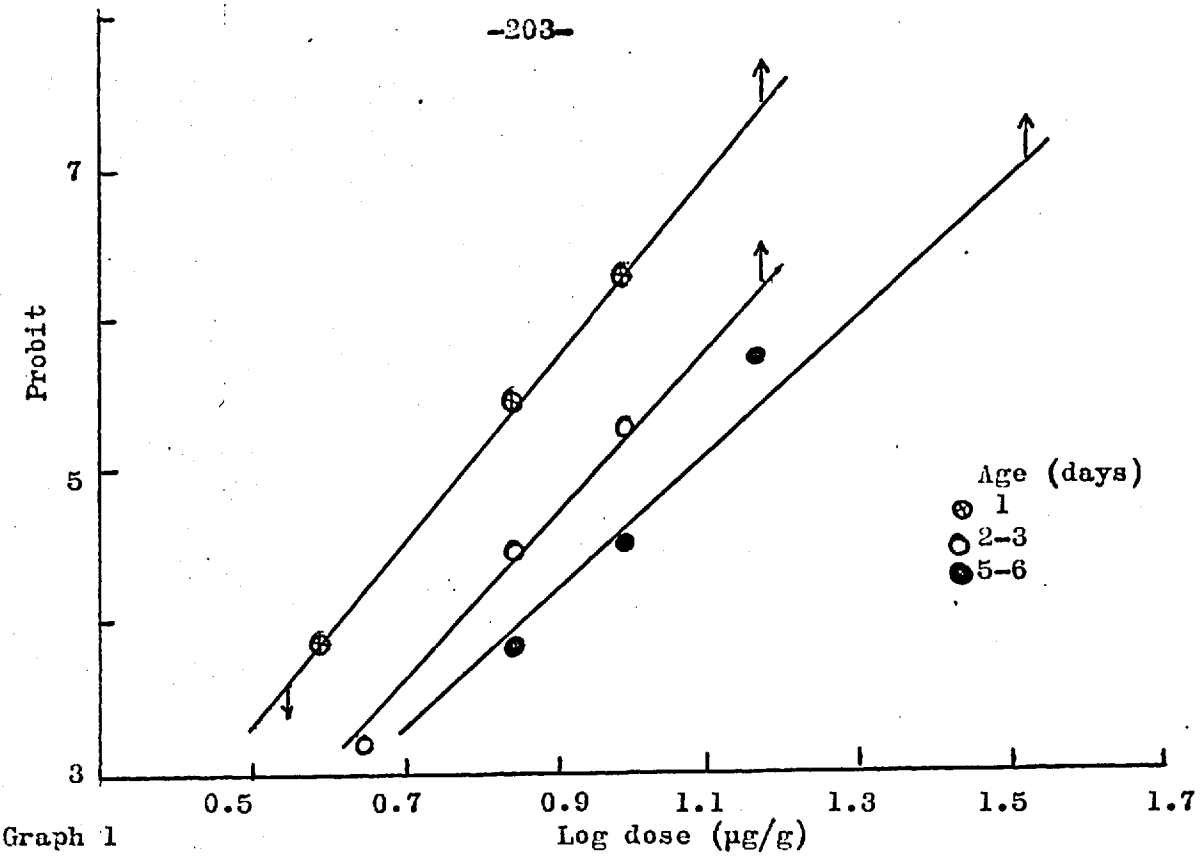
1.9, Effect of solvents on the toxicity of γ-BHC

Topical application

Solvents	Dose (µg/g)	0 (Controls)	7.5	10.5	13.5	17
KAB	n	10	30	30	30	30
	r	0	3	14	24	30
	%	0	10	47	80	100
Acetone	Dose (µg/g)	0	10	13	17	
	n	20	30	30	30	
	r	0	5	21	27	
	%	0	17	70	90	
Benzene	Dose (µg/g)	0	10	13	17	
	n	10	30	30	30	
	r	0	4	14	27	
	%	0	13	47	90	

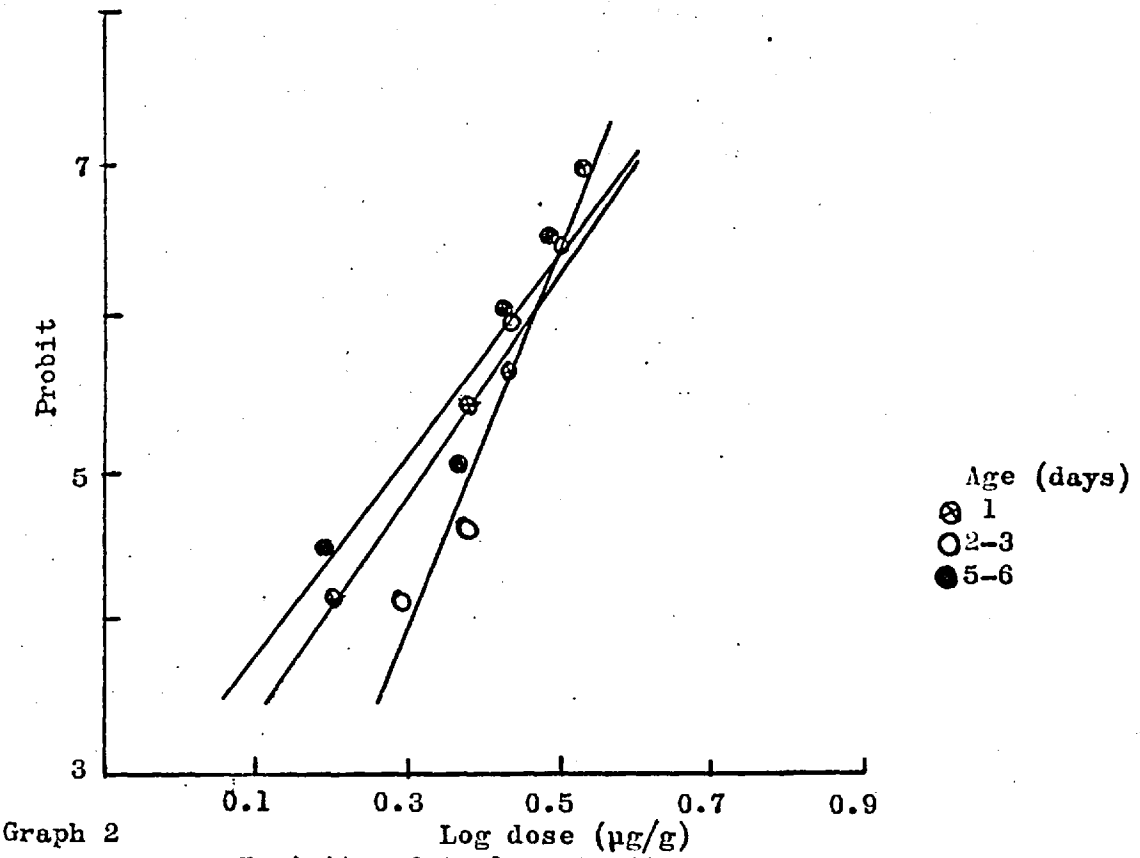
Injection

Solvents	Dose (µg/g)	0	5.0	7.5	10.5	13.5
KAB	n	20	20	30	30	30
	r	0	0	12	18	26
	%	0	0	40	60	87
Acetone	Dose (µg/g)	0	1.5	3.0	5.0	
	n	20	30	30	30	
	r	0	3	18	27	
	%	0	10	60	90	
Benzene	Dose (µg/g)	0	2.0	3.0	5.0	7.5
	n	20	30	30	30	30
	r	0	3	11	18	28
	%	0	10	37	60	93



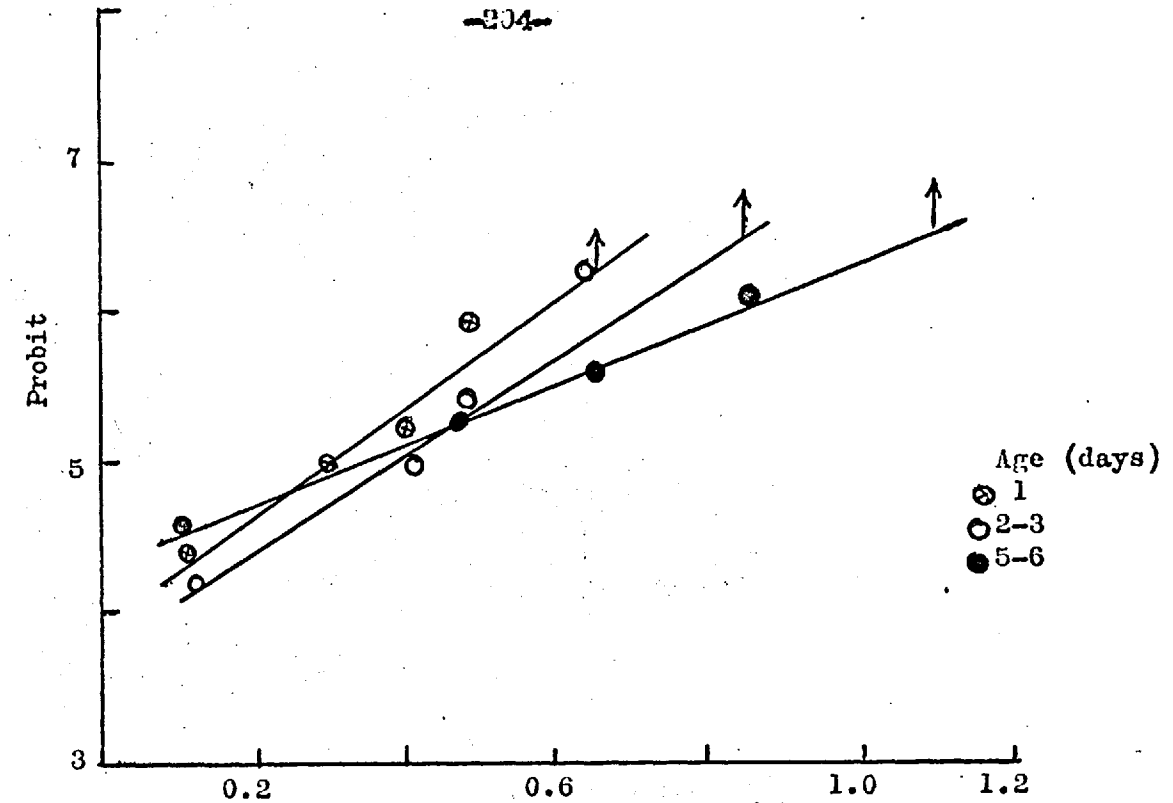
Graph 1

Toxicity of γ-BHC to fifth instar Schistocerca of different ages applied topically.



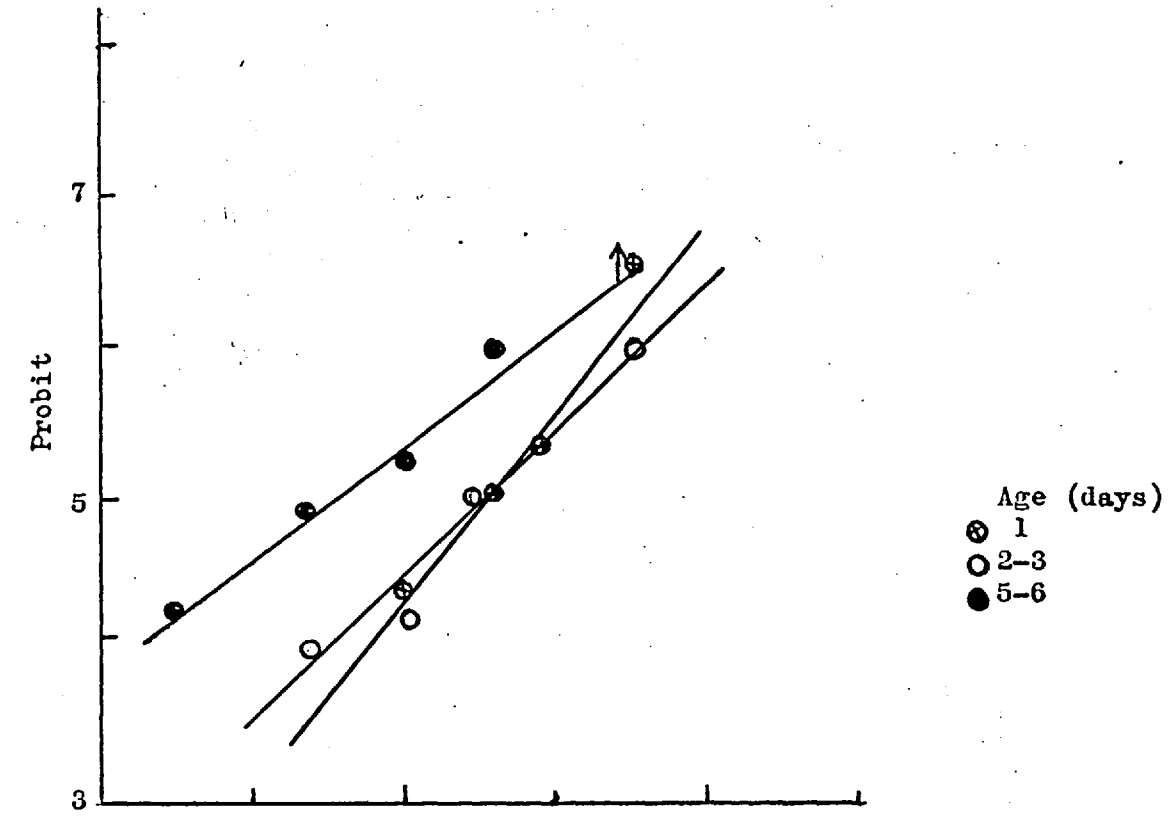
Graph 2

Toxicity of Cyolane to fifth instar Schistocerca of different ages applied topically.



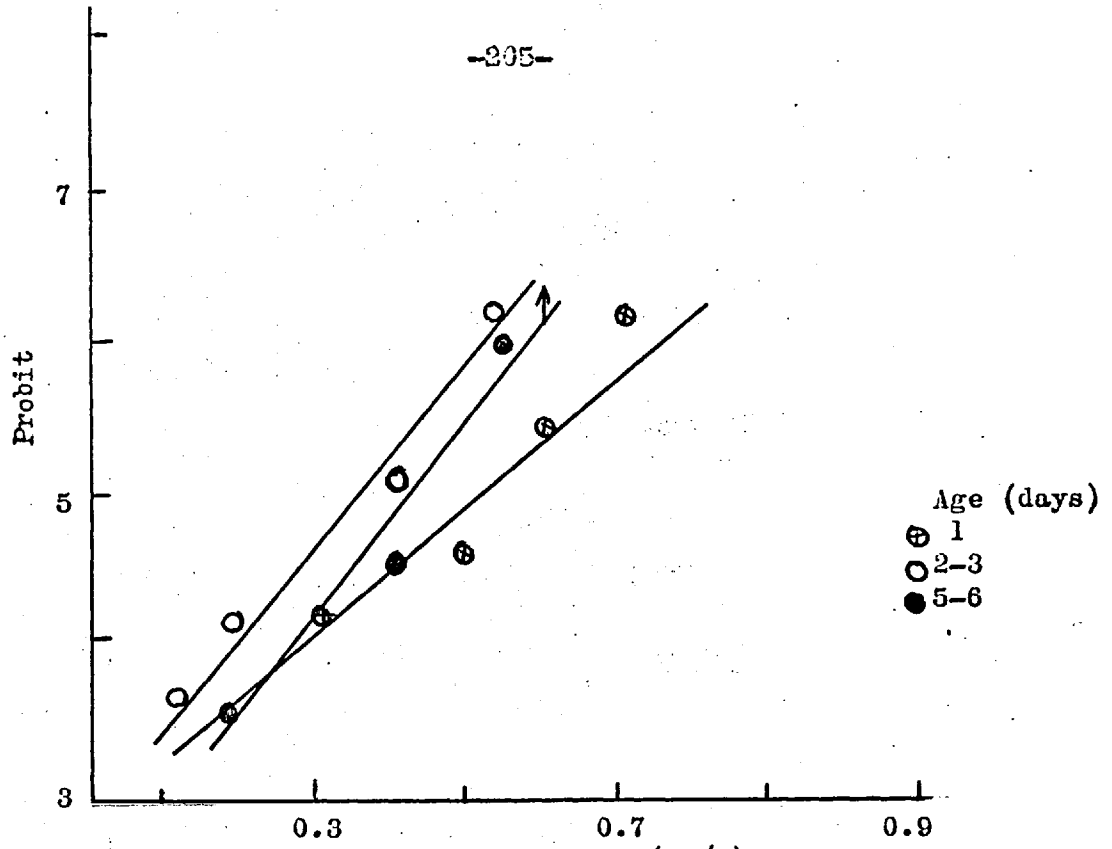
Graph 3

Toxicity of dioxacarb to fifth instar Schistocerca of different ages applied topically.



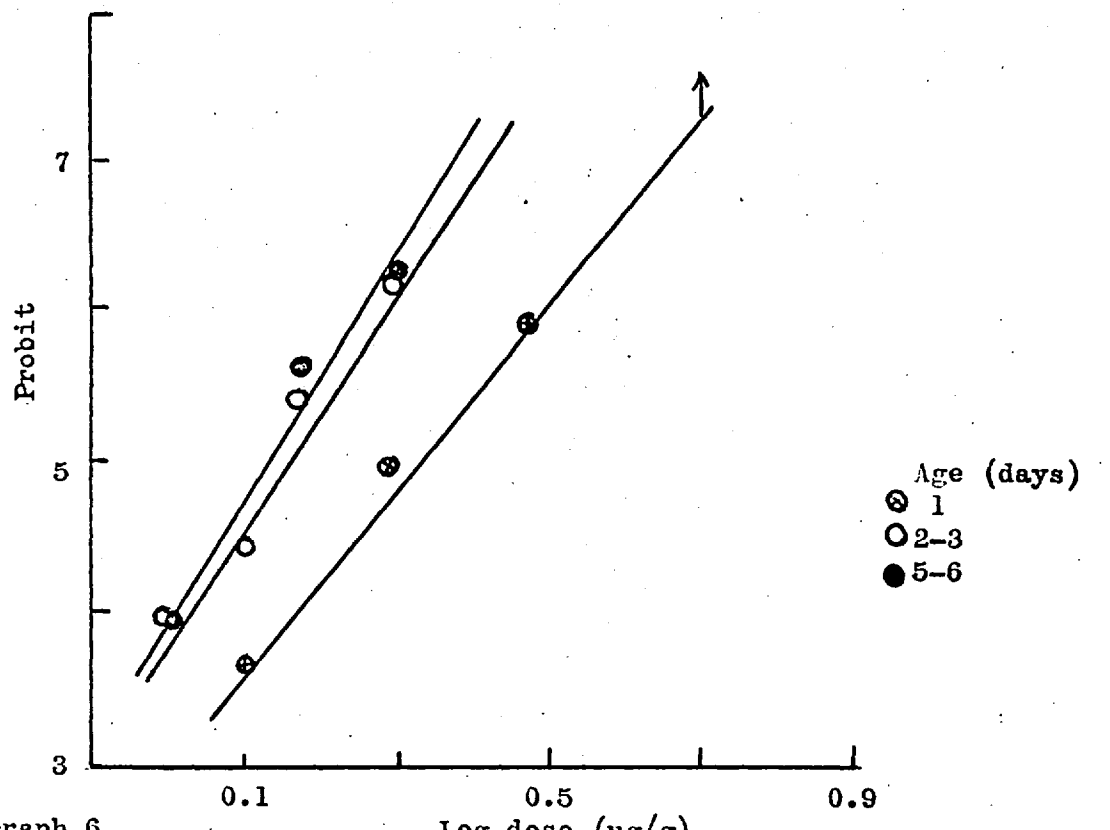
Graph 4

Toxicity of γ-BHC to fifth instar Schistocerca of different ages applied by injection.



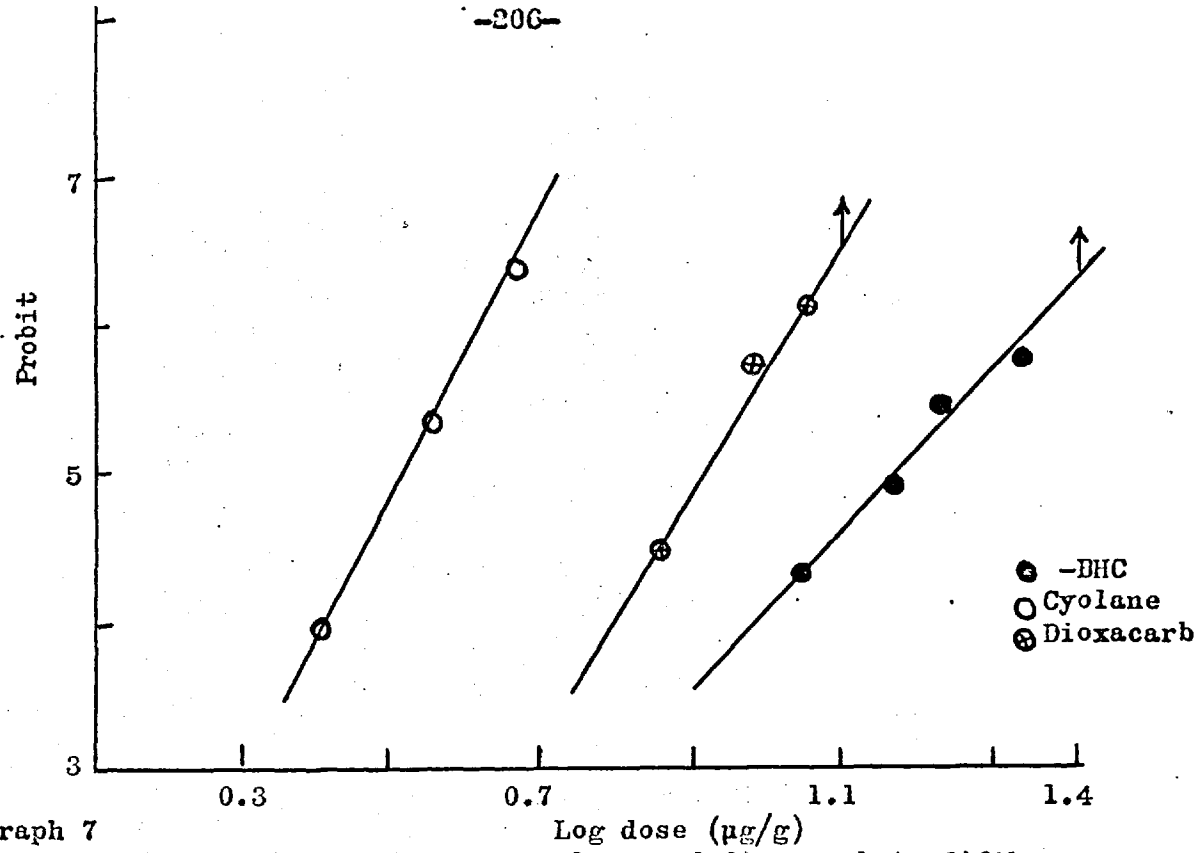
Graph 5

Toxicity of Cyolane to fifth instar Schistocerca of different ages applied by injection.



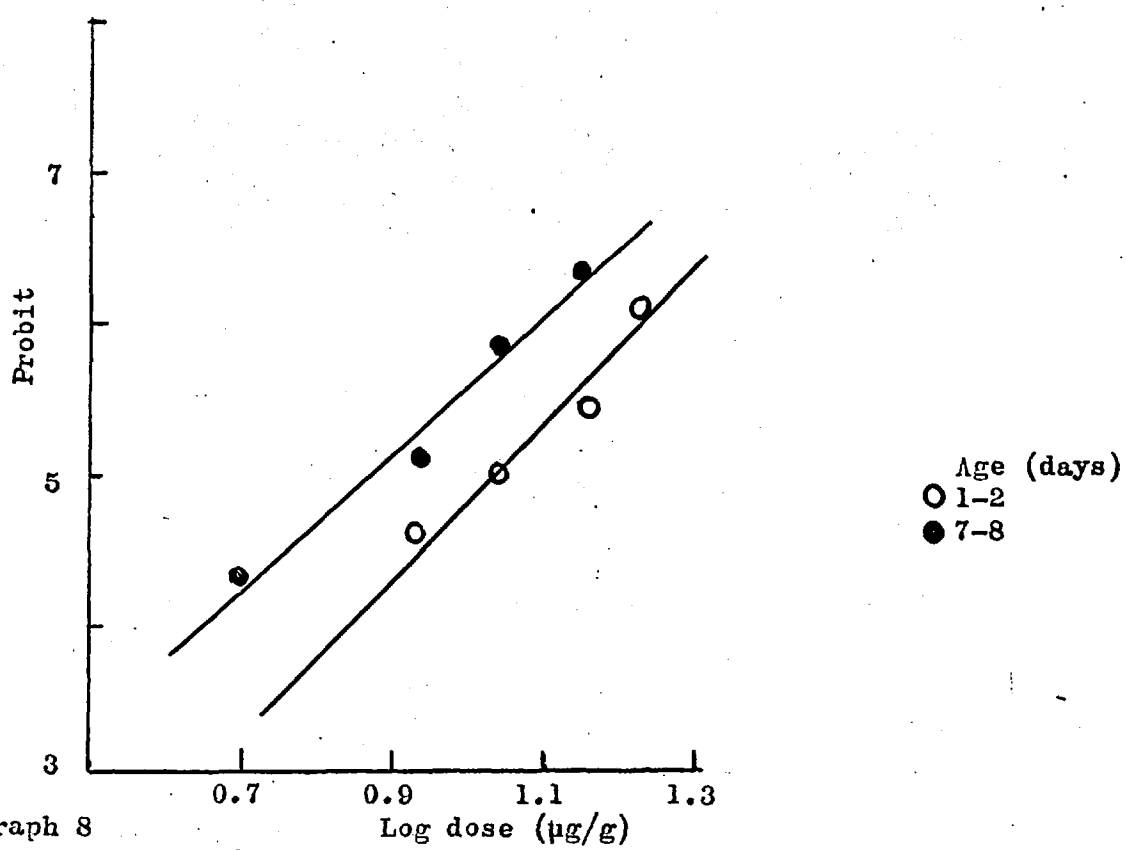
Graph 6

Toxicity of dioxacarb to fifth instar Schistocerca of different ages applied by injection.



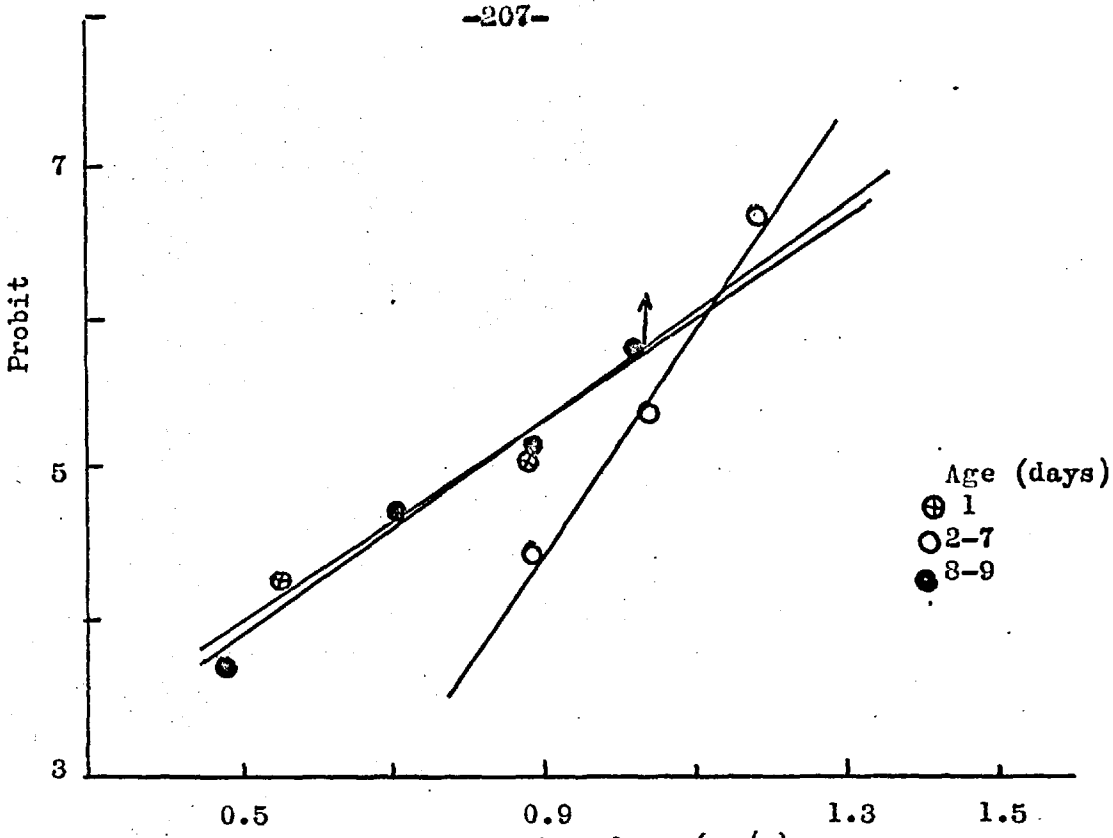
Graph 7

Toxicity of γ-BHC, Cyolane and dioxacarb to fifth instar Schistocerca applied orally.

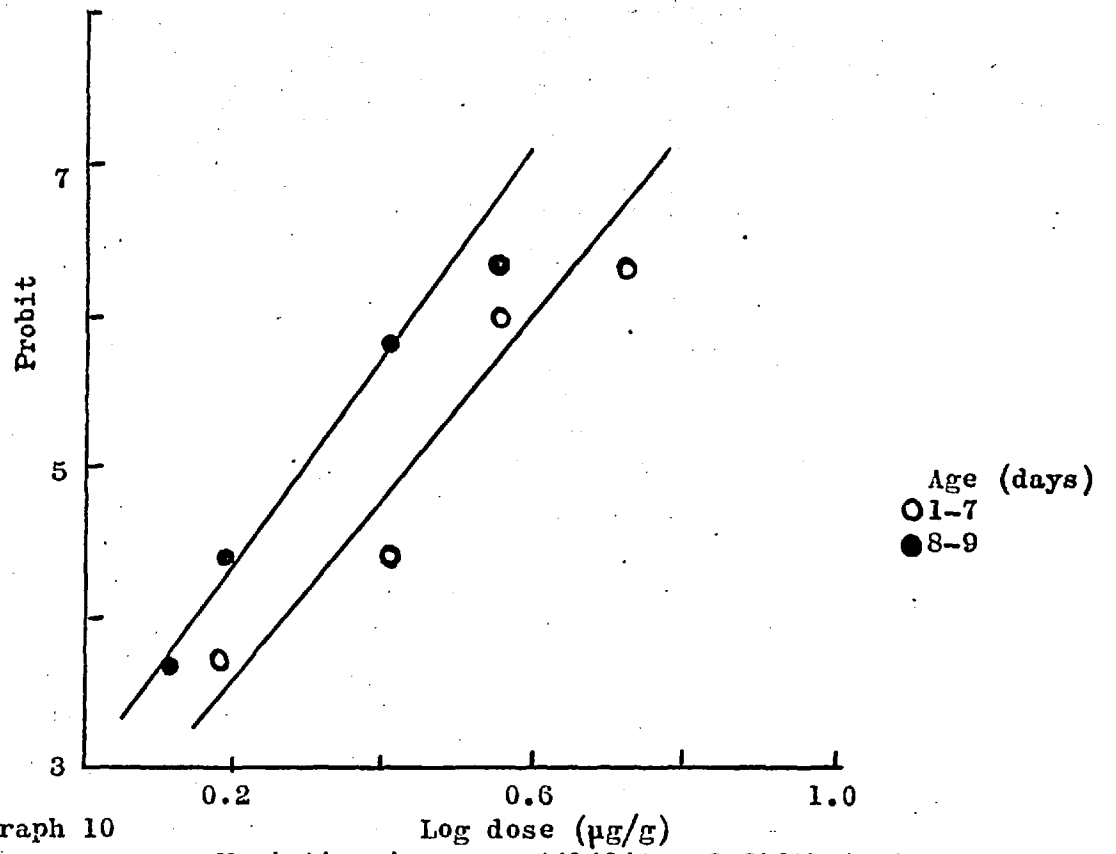


Graph 8

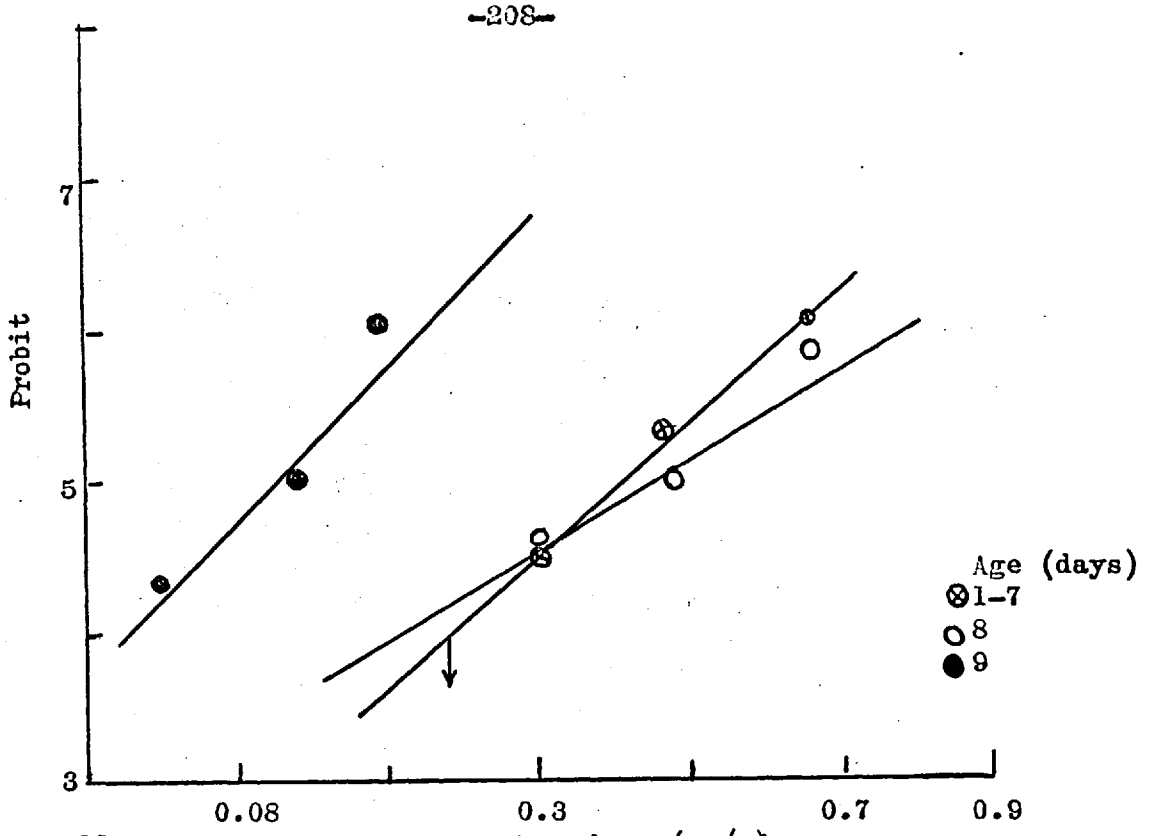
Effect of the stomach toxicity of γ-BHC in relation to age.



Graph 9 Variation in susceptibility of fifth instar Schistocerca to γ-BHC applied topically.

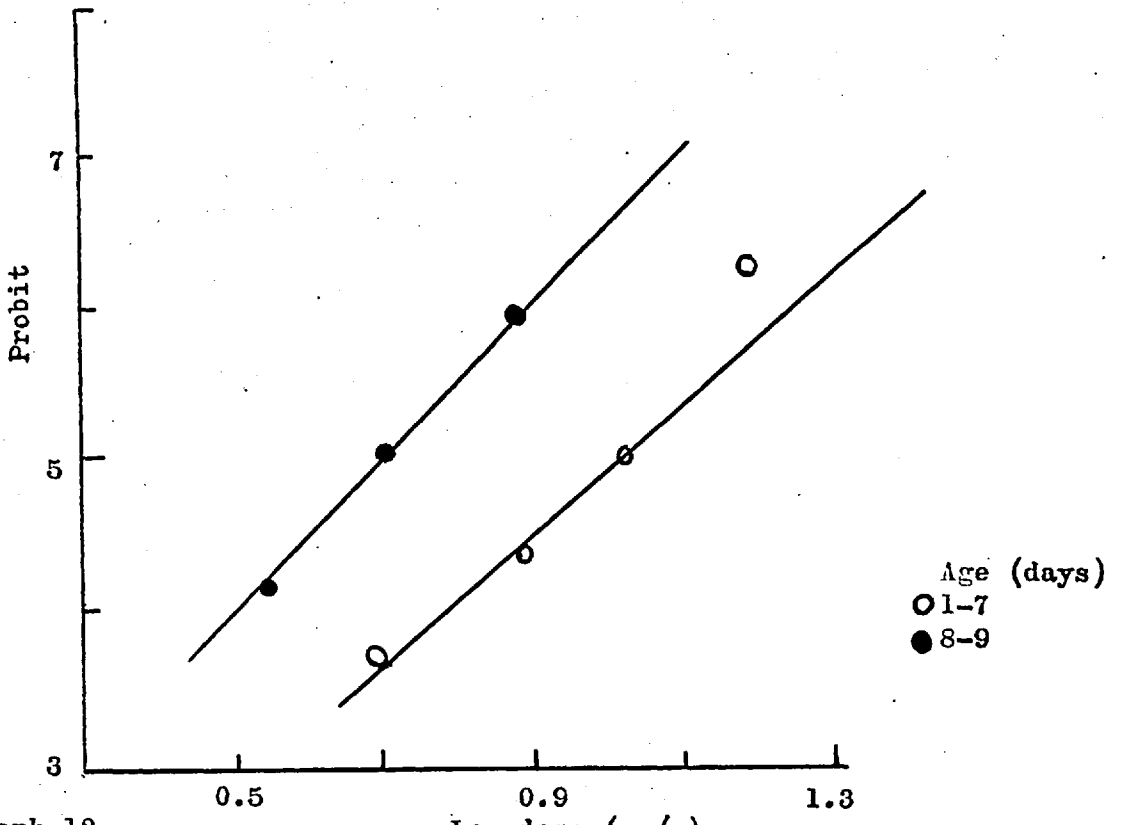


Graph 10 Variation in susceptibility of fifth instar Schistocerca to Cyolane applied topically.



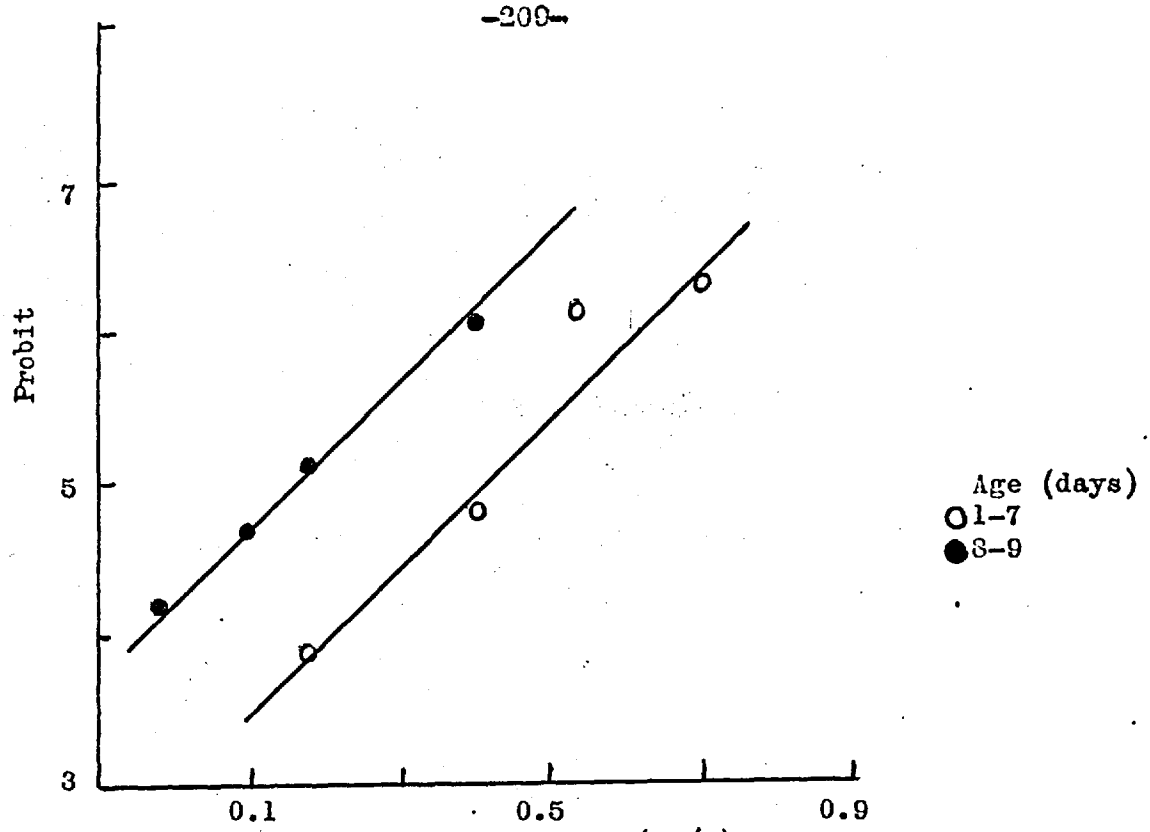
Graph 11

Variation in susceptibility of fifth instar Schistocerca to dioxacarb applied topically.



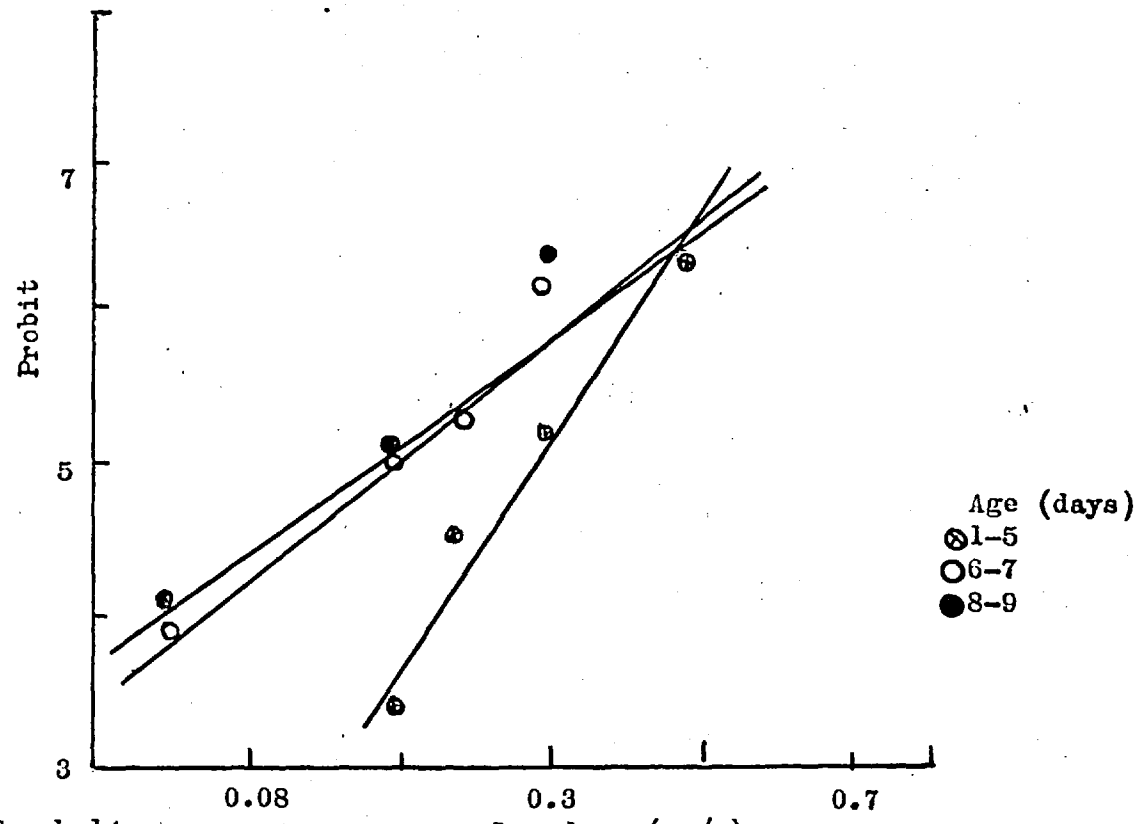
Graph 12

Variation in susceptibility of fifth instar Schistocerca to γ-BHC applied by injection.



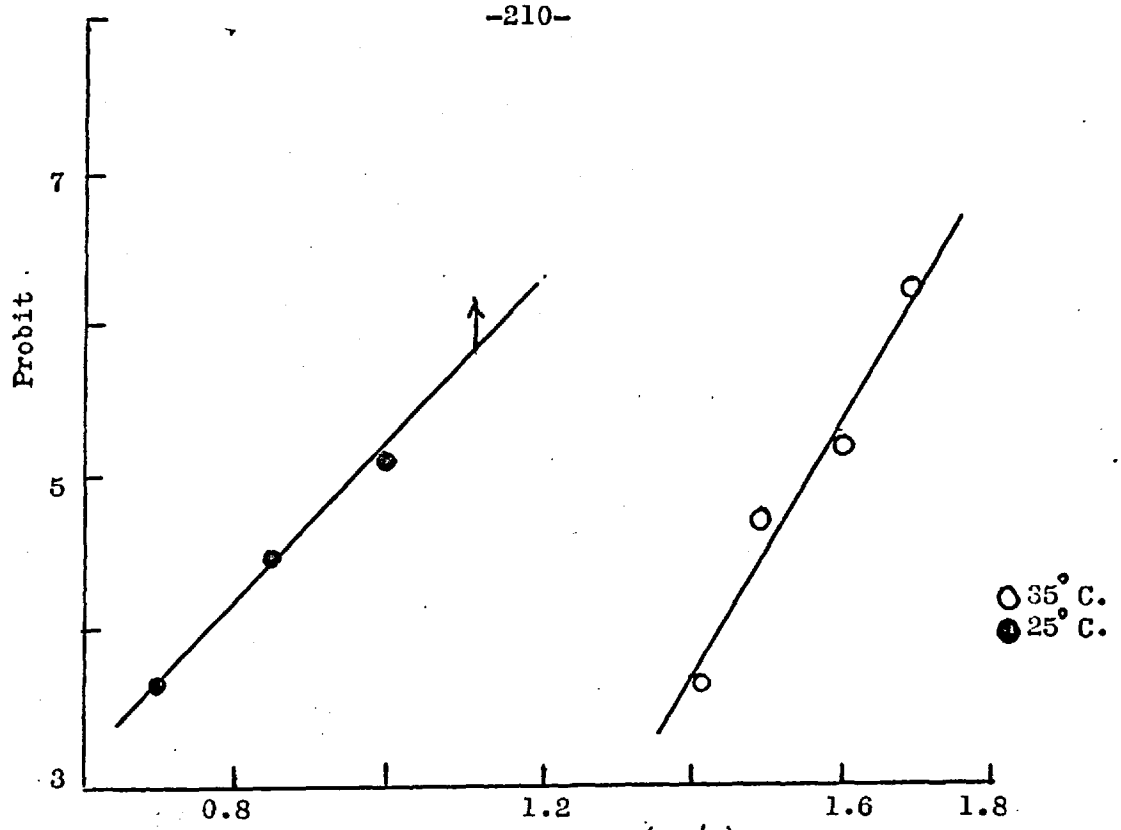
Graph 13

Variation in susceptibility of fifth instar Schistocerca to Cyolane applied by injection.

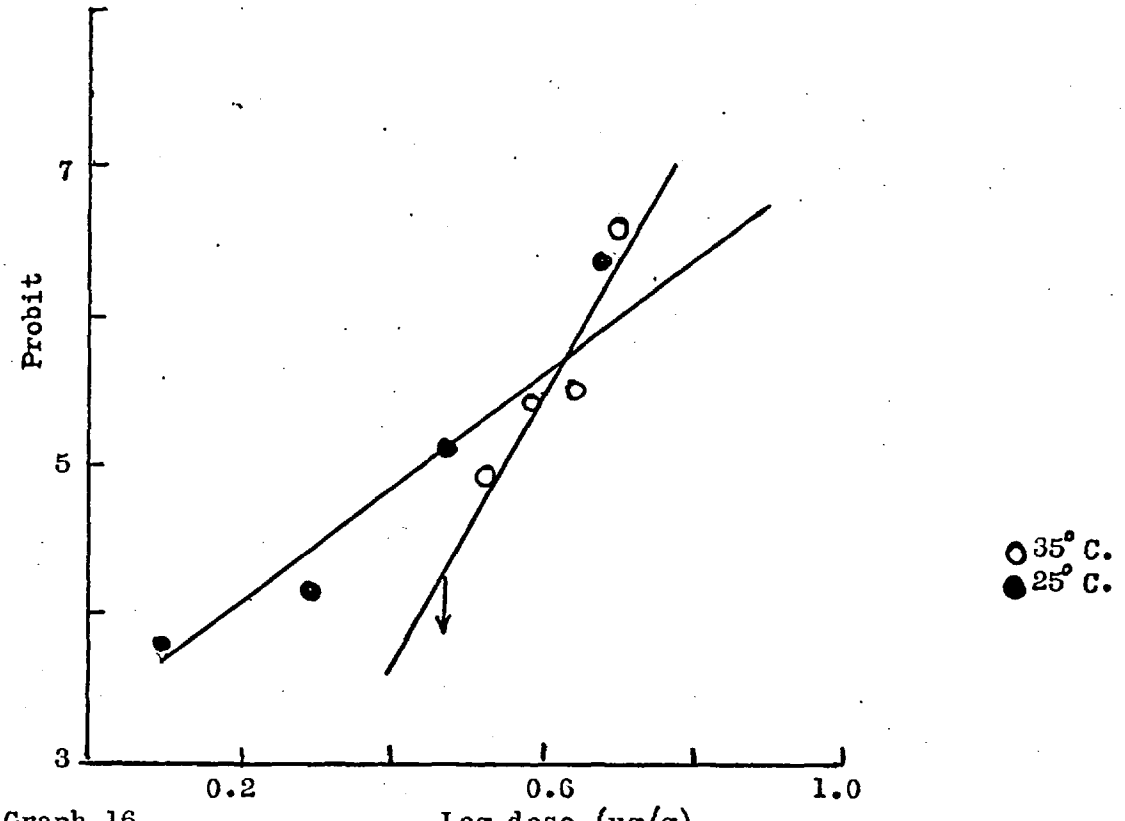


Graph 14

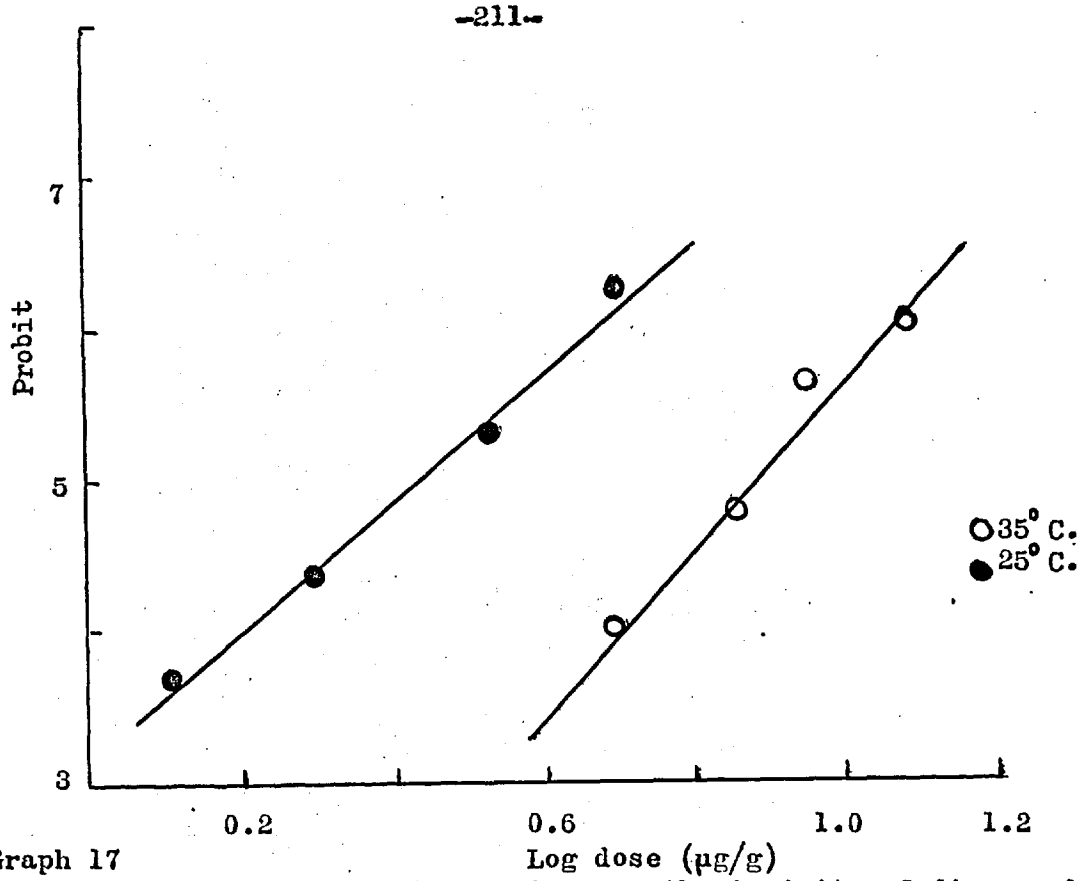
Variation in susceptibility of fifth instar Schistocerca to dioxacarb applied by injection.



Graph 15
Effect of temperature on the toxicity of γ-BHC.

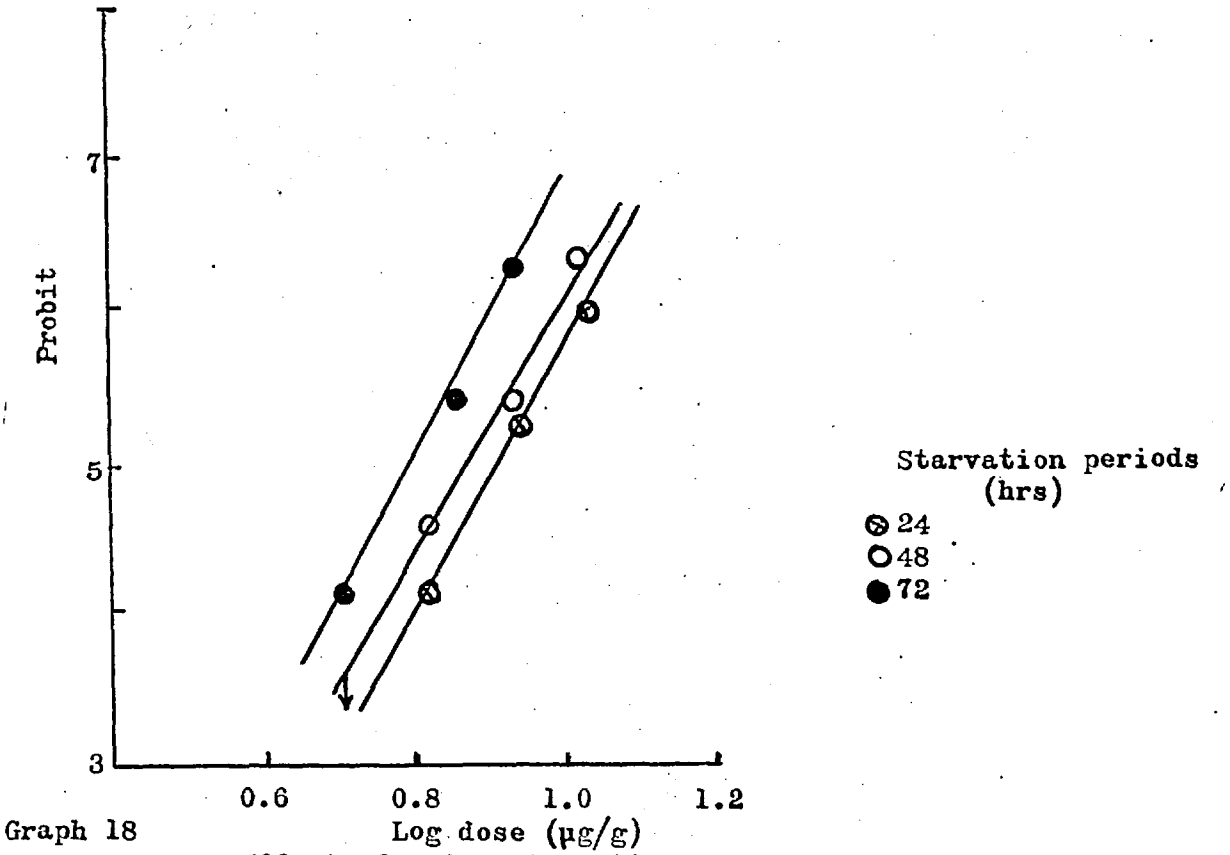


Graph 16
Effect of temperature on the toxicity of Cyolane.



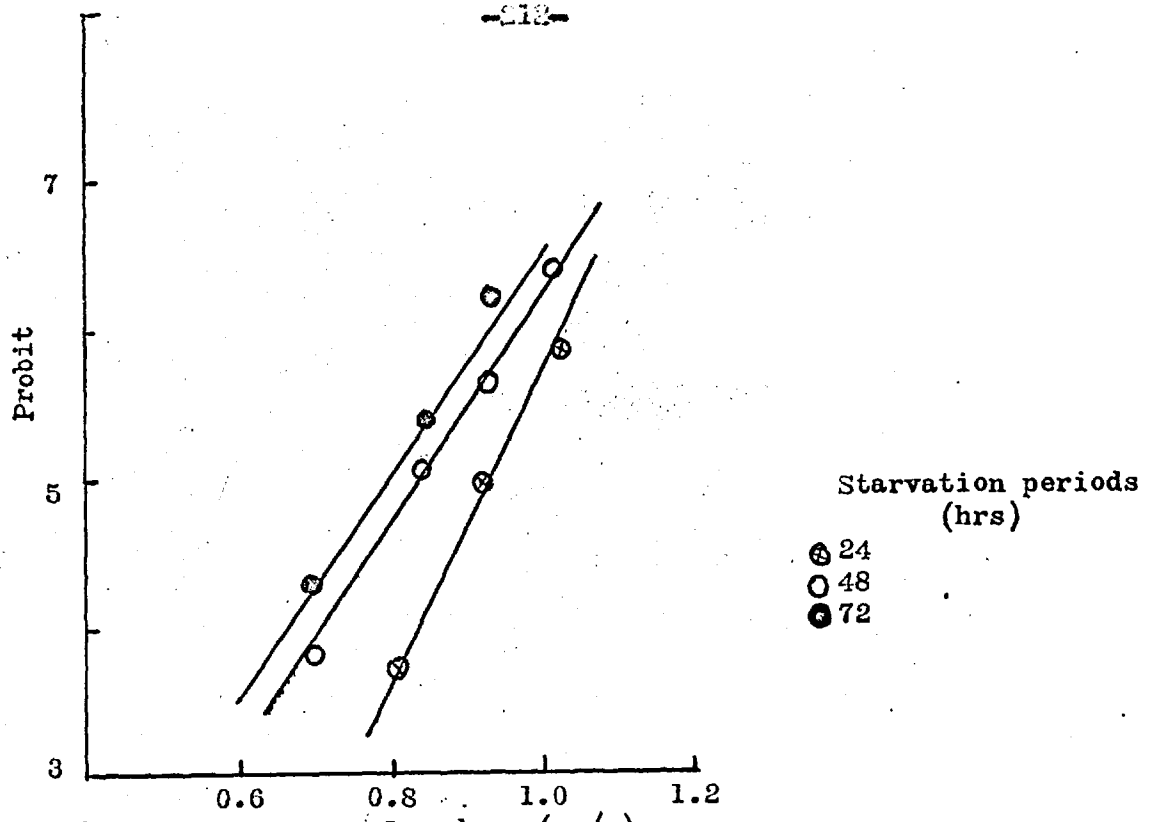
Graph 17

Effect of temperature on the toxicity of dioxacarb.

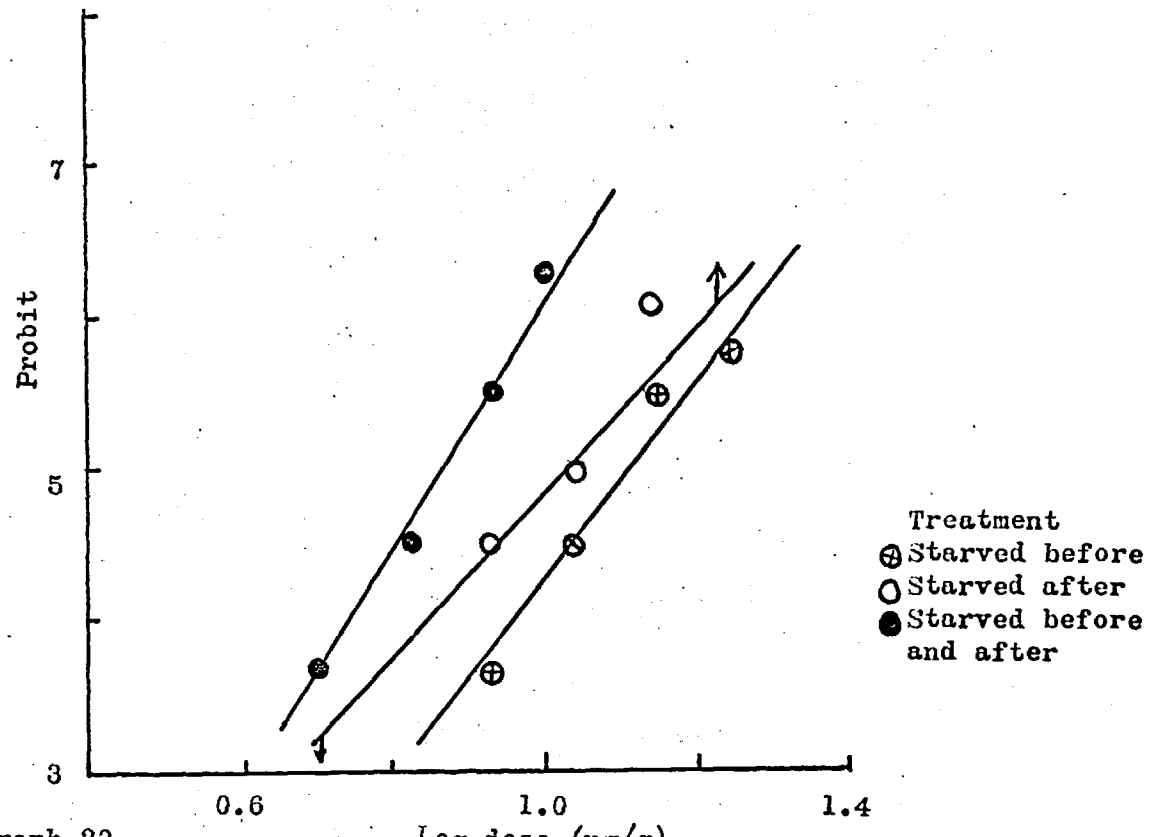


Graph 18

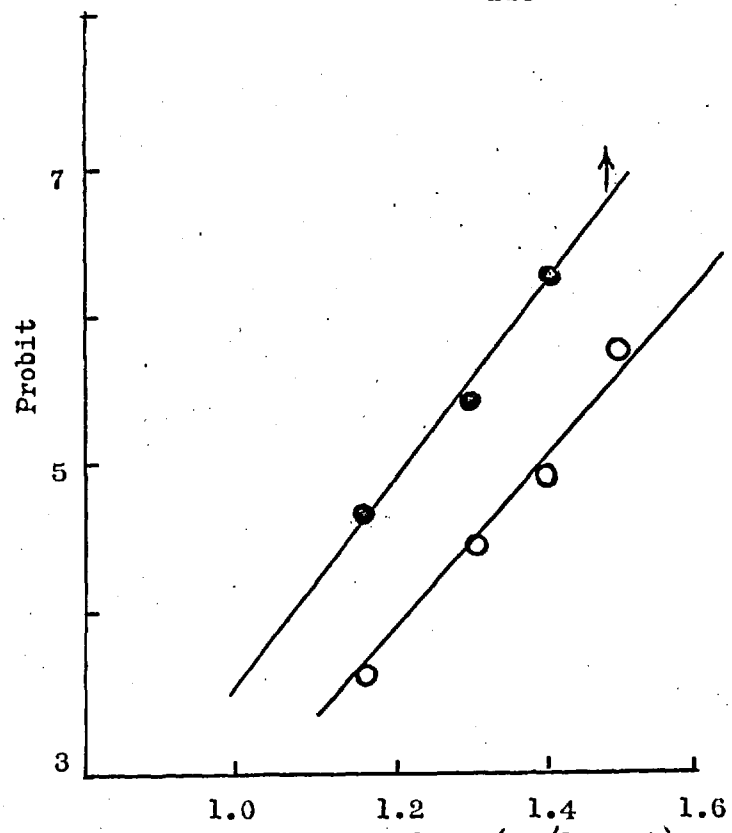
Effect of prior starvation on the toxicity of γ-BHC applied topically.



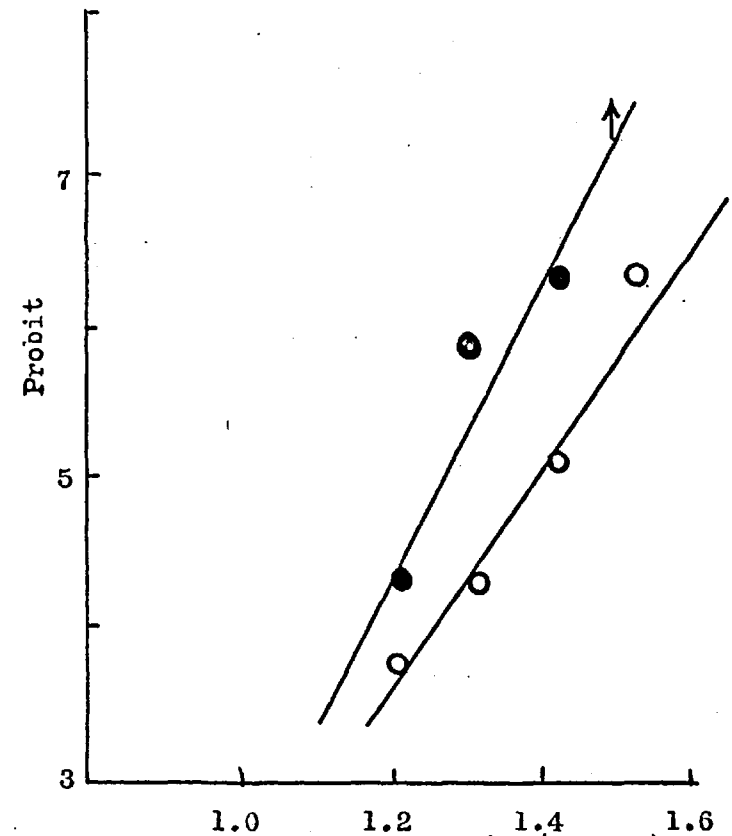
Graph 19
Effect of prior starvation on the toxicity of γ-BHC applied by injection.



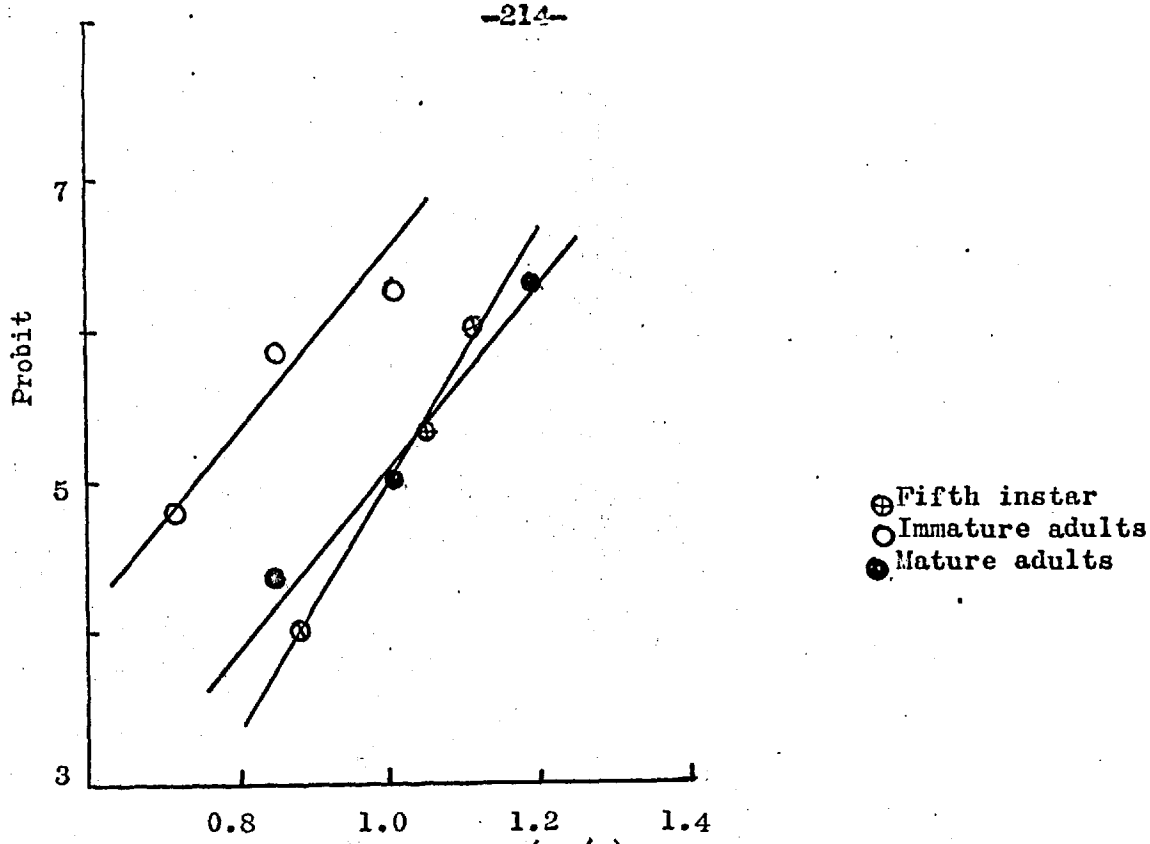
Graph 20
Effect of starvation on the stomach toxicity of γ-BHC.



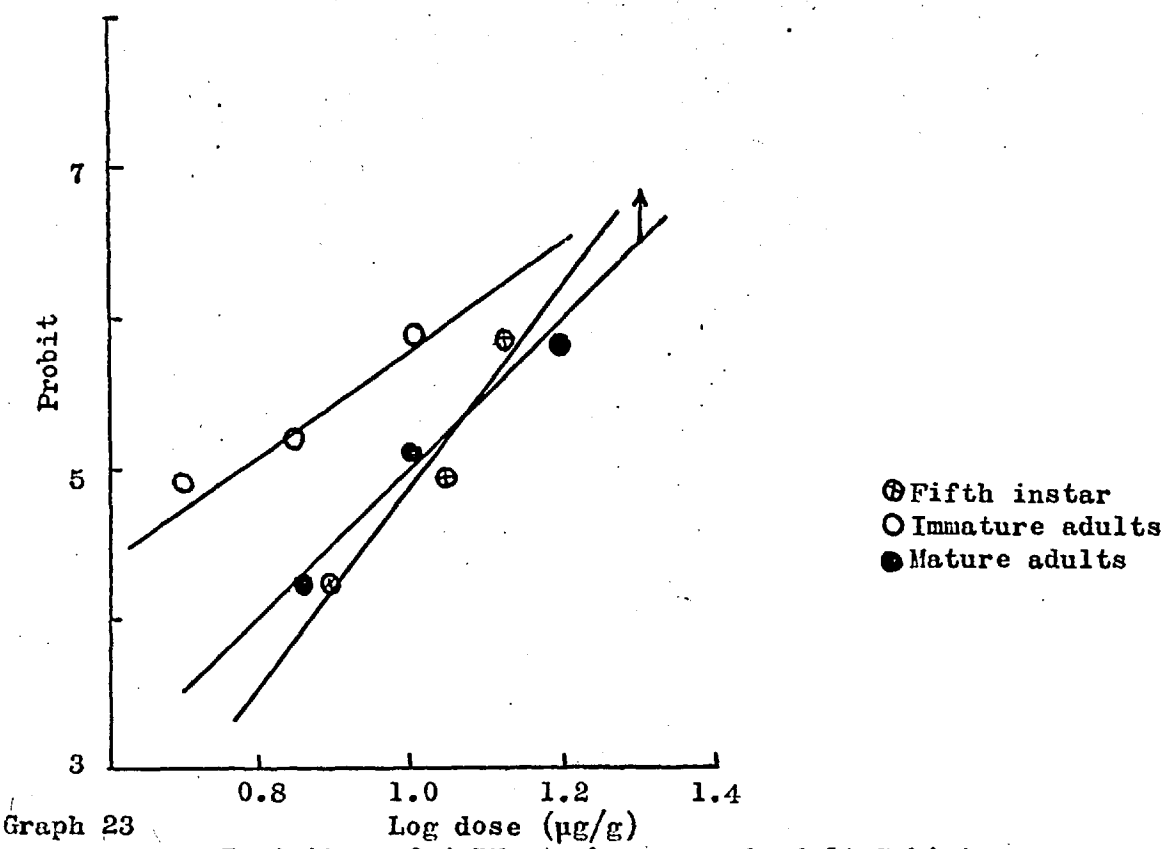
Graph 21
Effect of weight on the toxicity of γ-BHC.



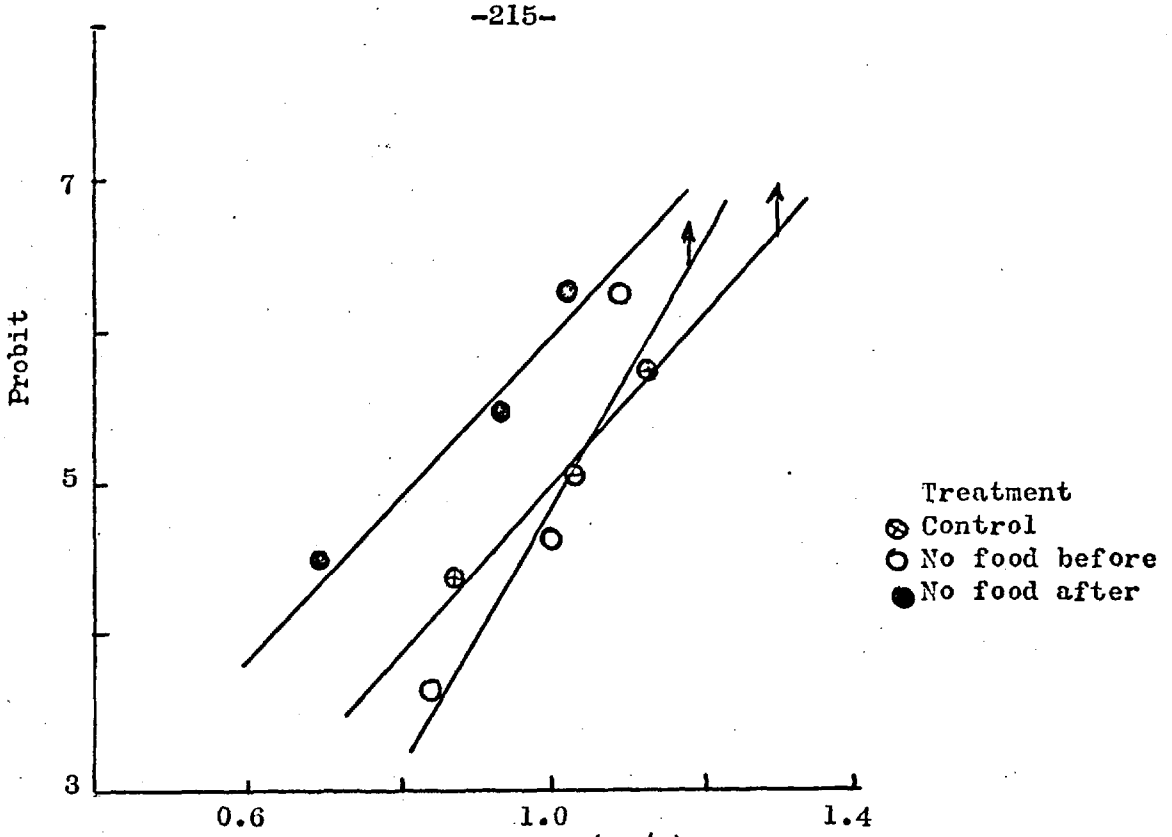
Graph 21
Effect of weight on the toxicity of γ-BHC.



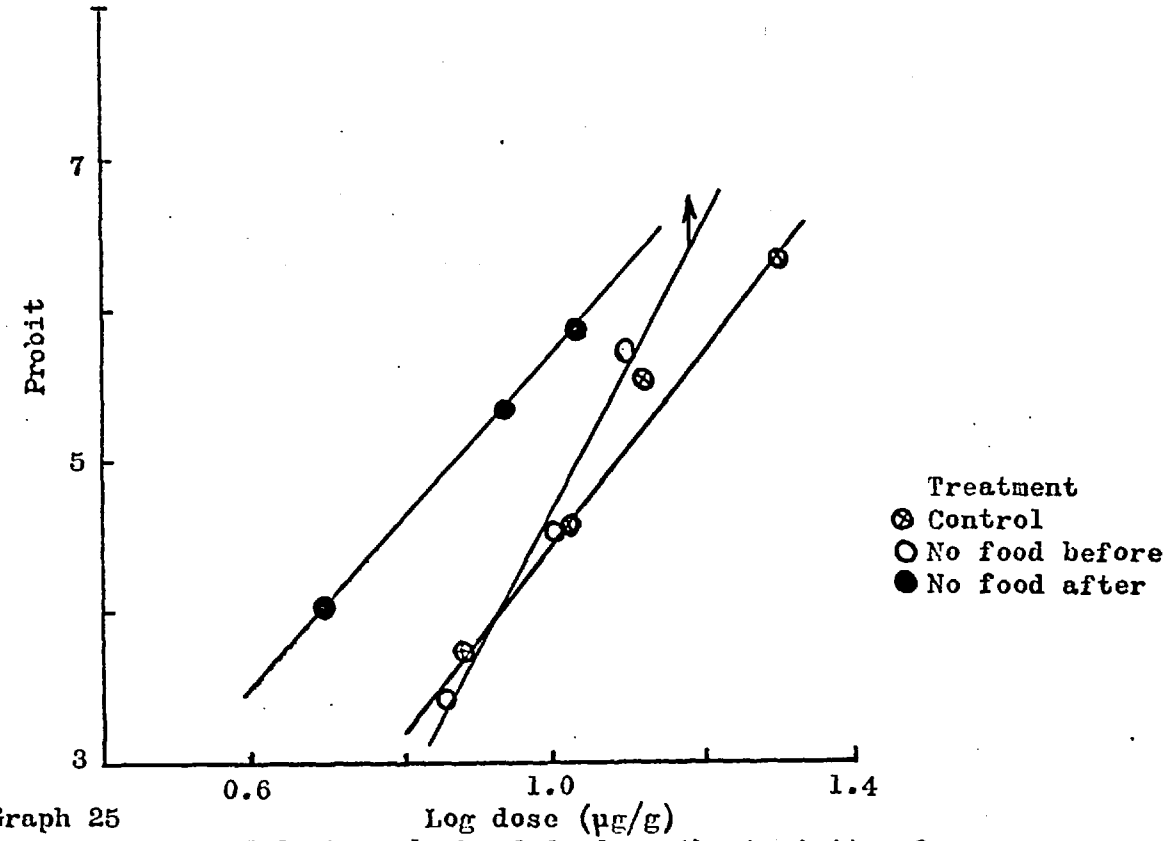
Graph 22 Toxicity of γ-BHC to hoppers and adult Schistocerca applied topically.



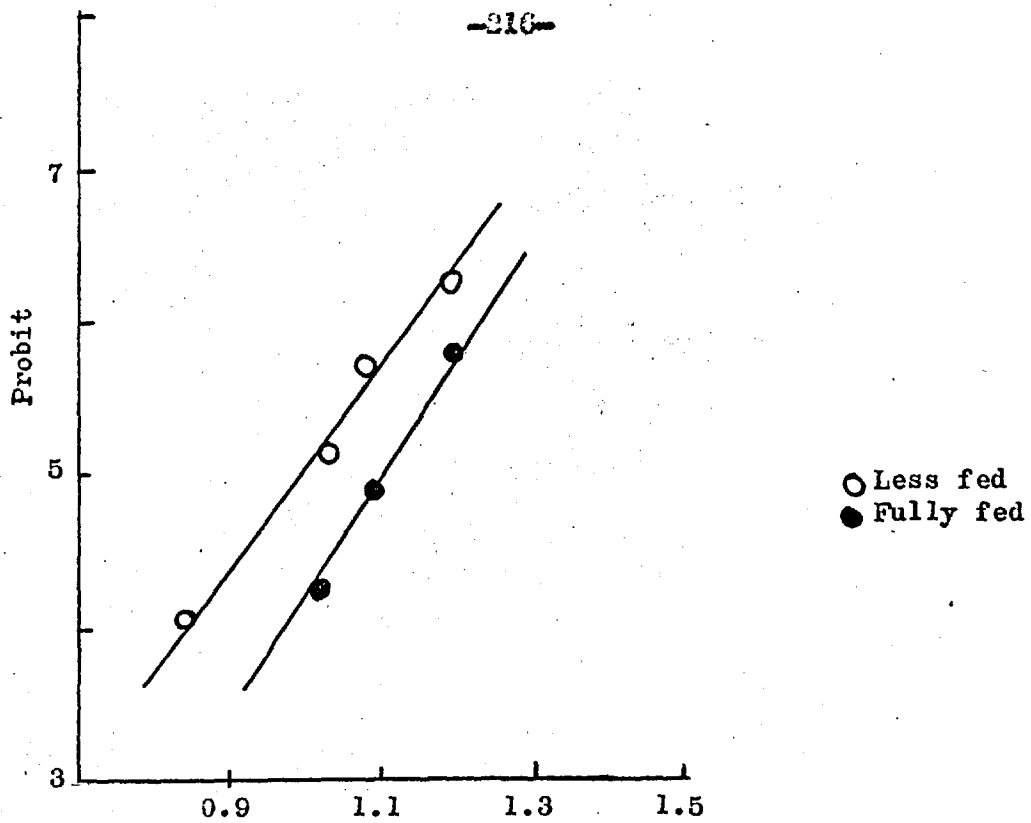
Graph 23 Toxicity of γ-BHC to hopper and adult Schistocerca applied by injection.



Graph 24 Effect of lack of food on the toxicity of γ-BHC applied topically.

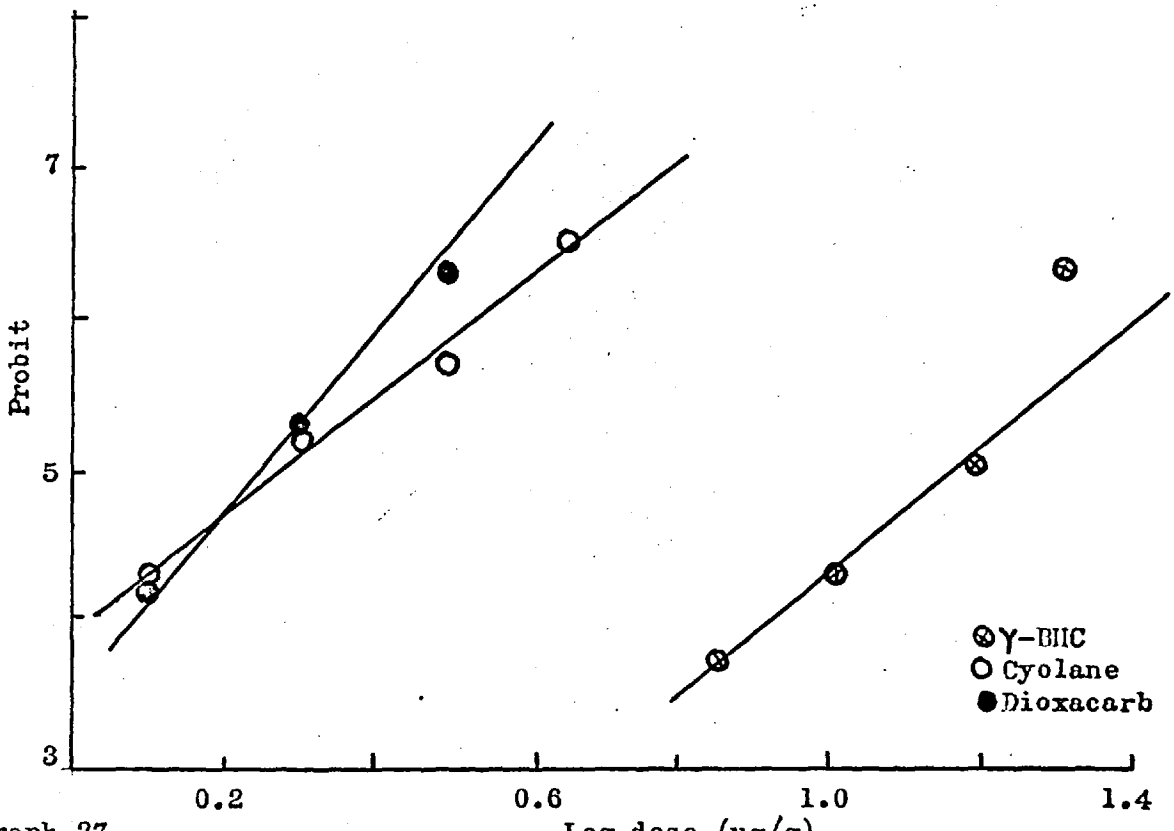


Graph 25 Effect of lack of food on the toxicity of γ-BHC applied by injection.



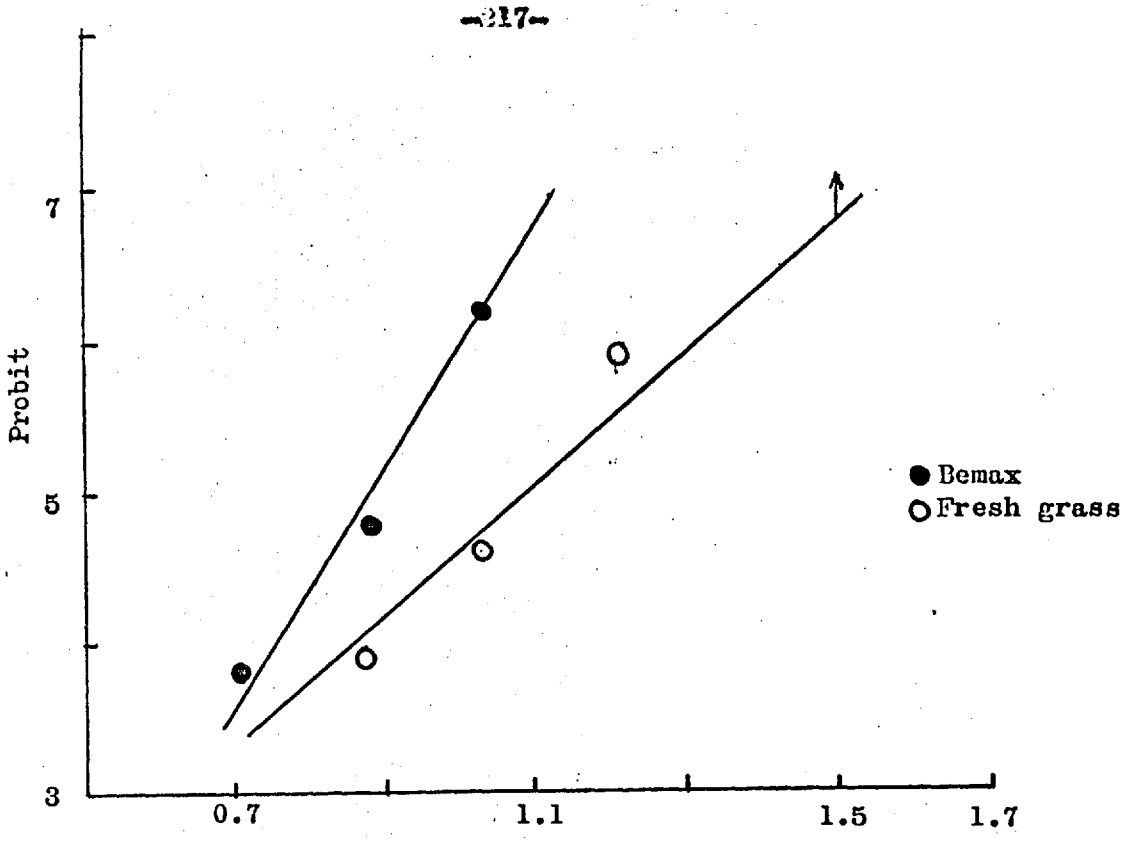
Graph 26

Effect of reduced feeding on the toxicity of γ-BHC applied topically.



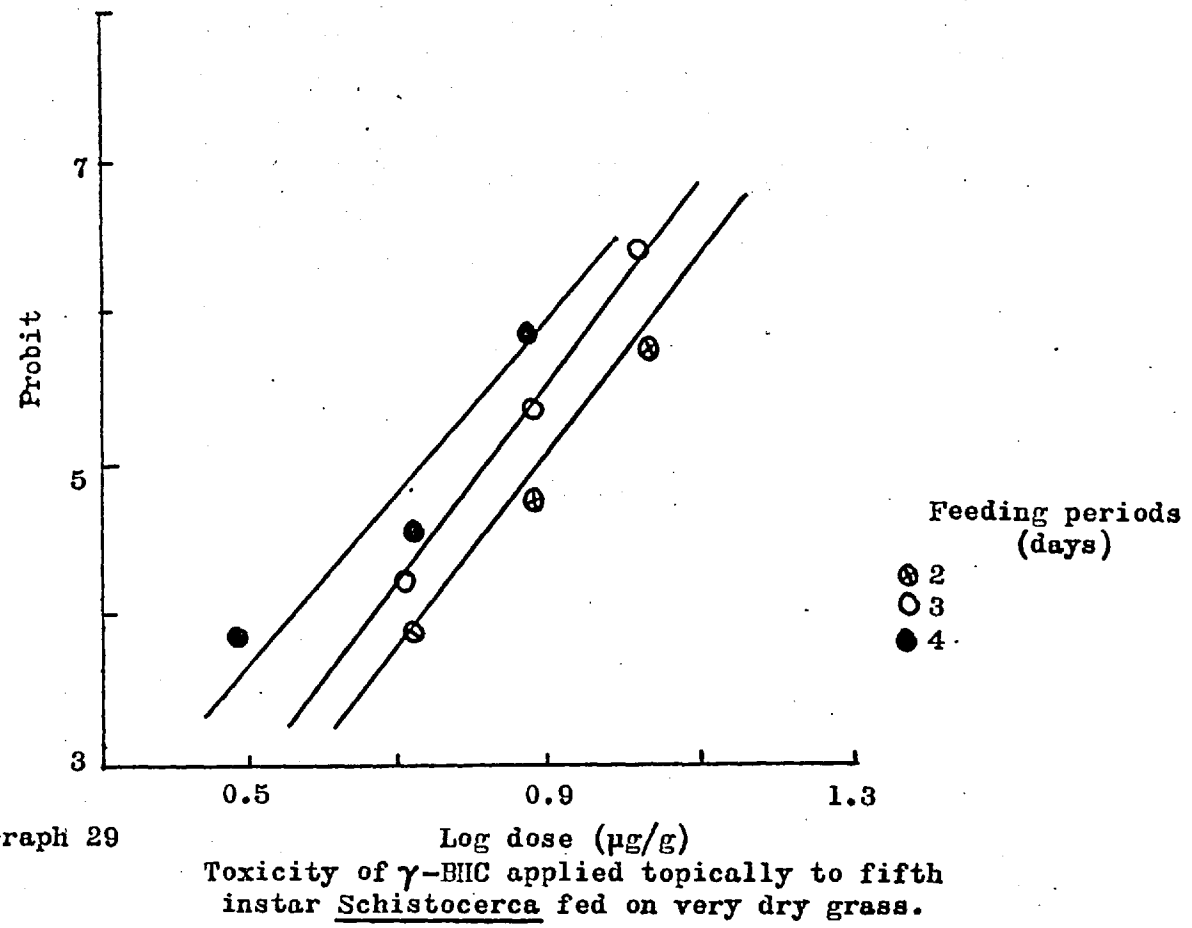
Graph 27

Effect of diet on the toxicity of γ-BHC, Cyolane and dioxacarb applied topically.



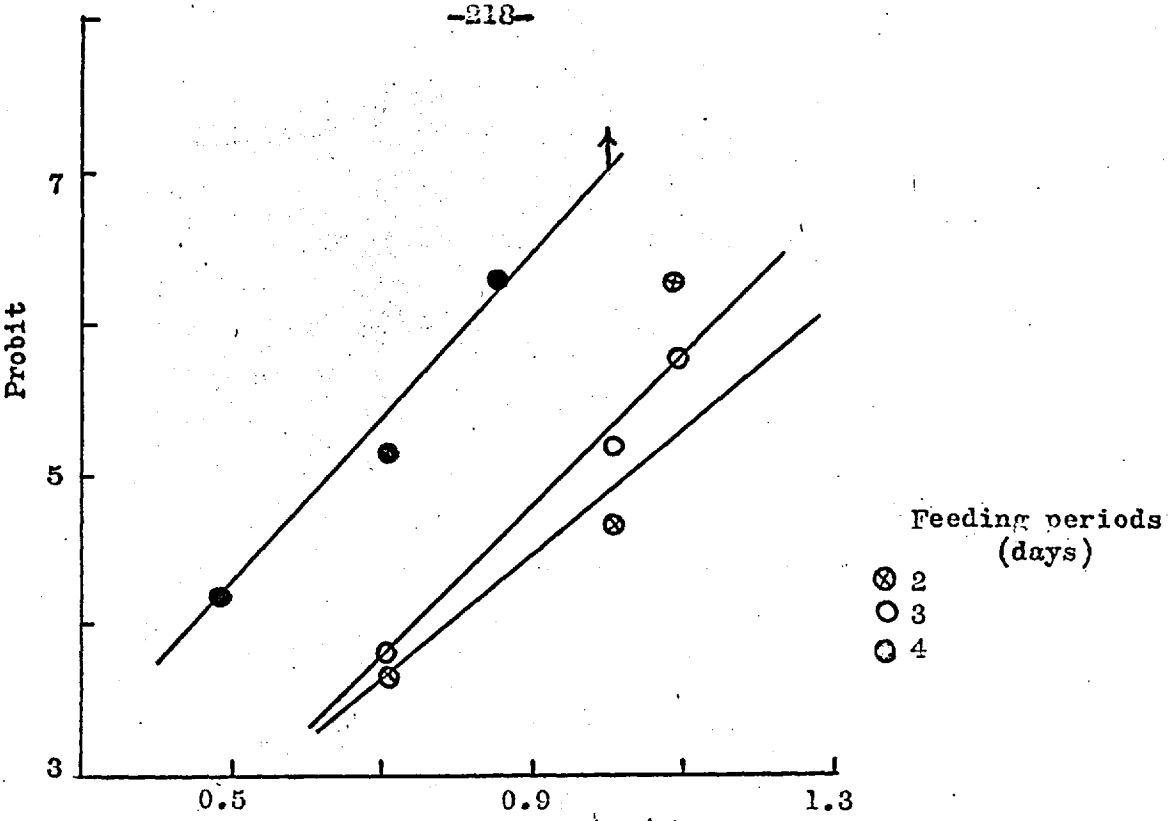
Graph 28

Effect of diet on the resistance to γ-BHC applied topically.



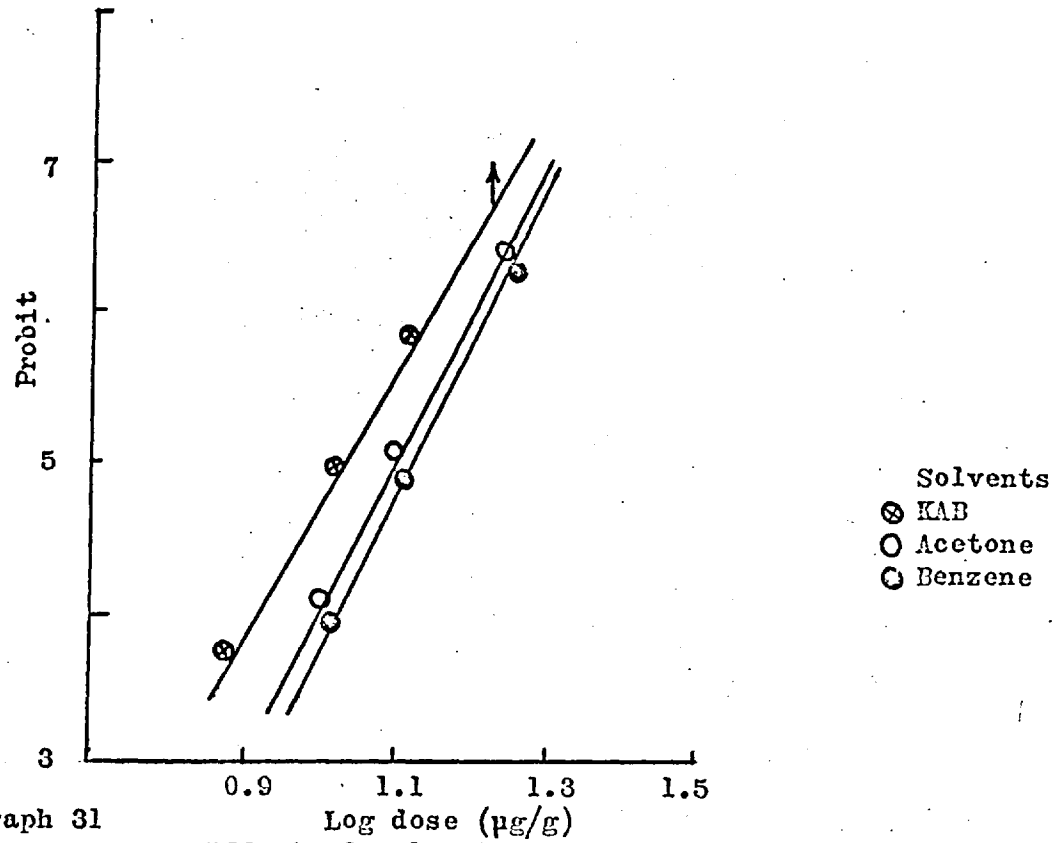
Graph 29

Toxicity of γ-BHC applied topically to fifth instar Schistocerca fed on very dry grass.



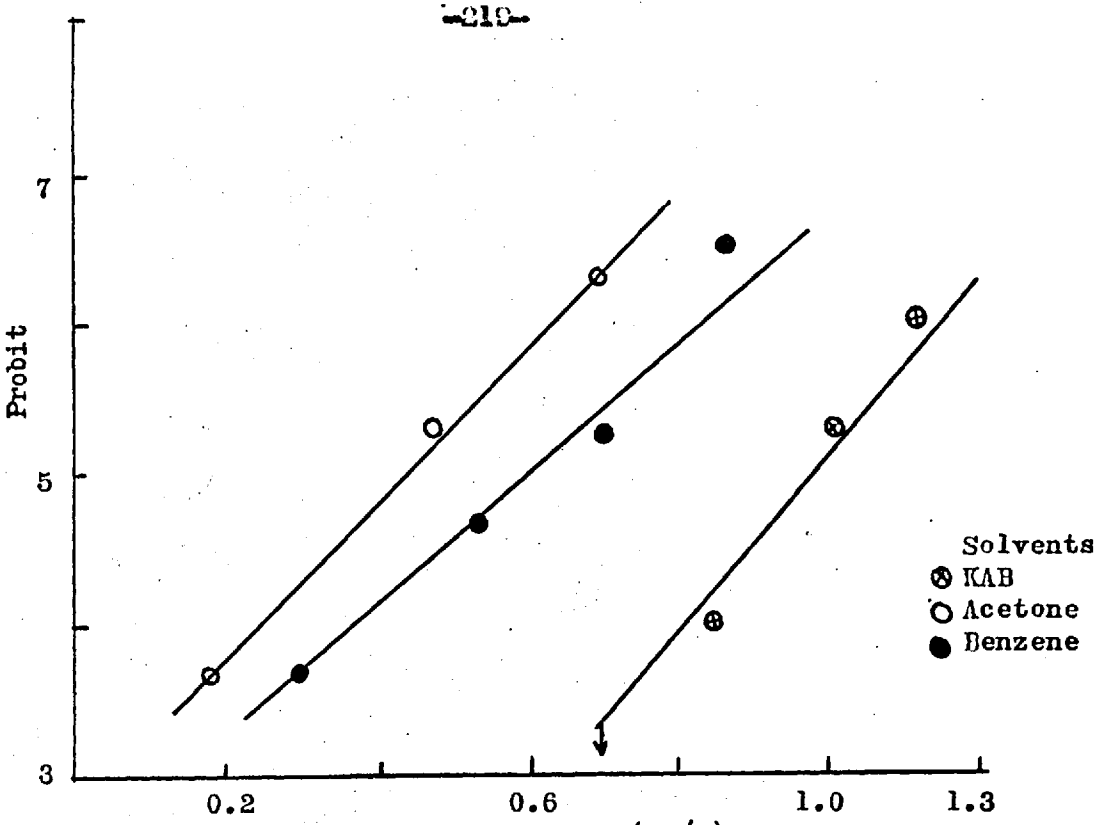
Graph 30

Toxicity of γ-BHC applied by injection to fifth instar Schistocerca fed on very dry grass.



Graph 31

Effect of solvents on the toxicity of γ-BHC applied topically.



Graph 32

Effect of solvents on the toxicity of γ-BHC applied by injection.