INSECTICIDE RESISTANCE IN THE BLUE TICK, BOOPHILUS DECOLORATUS KOCH, IN SOUTH AFRICA

by

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A THESIS PRESENTED TO RHODES UNIVERSITY
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

African Explosives & Chemical Industries Ltd.,
Research Department,
P.O. Northrand.
SOUTH AFRICA.

December 1959.

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ACKNOWLEDGEMENTS

The author wishes to thank Professor D.W. Ewer and Dr. E. McC. Callan of Rhodes University for assistance and helpful criticism in the preparation of this dissertation.

All the work recorded was undertaken at the Research Department, African Explosives and Chemical Industries Limited, and permission for its use for the purposes of a thesis is gratefully acknowledged. The assistance and encouragement of Dr. F.L. Clark, Dr. H.A.E. Mackenzie and Mr. R. du Toit of the Research Department, A.E. & C.I. Ltd., is also acknowledged.

Thanks are accorded the technical staff of Cooper and Nephews S.Af. (Pty) Ltd. and the staff of Frankenwald Research Station, Witwatersrand University, for assistance in obtaining strains of the blue tick.

The analytical determination of arsenic was undertaken by Dr. A. Mendelowitz of the Analytical Section and determination of thiol compounds by Dr. J.S. Harington of the Entomological Section and Mr. M.E. Thompson and Dr. A.M. Johnston of the Organic Chemistry Section of the Research Department, A.E. & C.I. Ltd., to whom thanks are accorded.

Statistical analysis of results and accurate determination of LC50 values were undertaken by Mr. D.C. Goch, Mr. H. Marr and Mr. S.J. Bozalek of the Statistical Section, Research Department, A.E. & C.I. Ltd.

In the laboratory investigations of the insecticidal response and maintenance of cultures of the various strains of blue tick assistance was rendered by Mrs. X. Proimos, Mrs. M.M. Hall, Mrs. P.E. Cary and Mrs. C.R. Jourdan whose help is gratefully acknowledged.

The author wishes to thank Mrs. A.W. Baiocchi who undertook the typing of the manuscript.

SUMMARY

The blue tick, Boophilus decoloratus Koch, is an ectoparasite of several species of indigenous animals and of domestic stock in South Africa. The species is a vector of a number of pathogenic organisms. For this reason the control of the blue tick on domestic animals is vital in many farming areas.

Dipping or spraying of cattle with insecticides for the control of the blue tick has been effectively practised for many years. However, the development of strains resistant to several insecticides has necessitated a continual investigation of new materials for blue tick control.

Blue tick populations from the eastern coastal area of the country were observed in 1938 to have become tolerant to sodium arsenite treatment at a concentration of 0.16% As_2O_3. Initially benzene hexachloride (BHC) was found to be effective against the arsenic resistant strain. After eighteen months of field use increased tolerance to BHC was first observed. This tolerance increased rapidly and a high degree of resistance to BHC was soon established. In areas affected by the BHC-arsenic resistant strain, DDT was introduced with considerable success as a field control measure.

Resistance to DDT developed in populations from localised areas five or six years after DDT was first introduced. The strain resistant to DDT remained resistant to both sodium arsenite and BHC, but could be effectively controlled with several organo-phosphorus insecticides.

Inland, blue tick populations remained sensitive to all insecticides except in isolated areas around Pretoria and Johannesburg where strains resistant to the chlorinated cyclic hydrocarbon group of insecticides only were detected.

With the availability of a number of strains of ticks differing in susceptibility to insecticides, it was possible to determine the respective levels of tolerance and the relationships between the various types of insecticide resistance.

Laboratory test techniques were devised for determining response to a variety of insecticides using both the fully engorged adult female and unengorged larval ticks. The results are presented in the form of histograms depicting the viability of tick eggs after treatment of adults with varying concentrations of insecticides. In respect of larvae, the results are expressed as probit mortality-log concentration regression curves from which the concentration of insecticide required to produce 50% mortality (LC50) was computed. The LC50 values have been used as a means of comparing the insecticide susceptibility of a number of strains.

Although a strain of blue tick, resistant to sodium arsenite only, was not available, evidence is presented to show that resistance to sodium arsenite does not confer a cross resistance to any other insecticide of the range investigated.

Resistance to BHC resulted in a cross resistance to all the chlorinated cyclic hydrocarbon insecticides investigated. Those investigated were toxaphene, chlordane, dieldrin and aldrin.

Resistance to DDT conferred a cross resistance to dilan and pyrethrum.

The organo-phosphorus insecticides and the carbamate, sevin, were equally effective against all strains of ticks.

These results suggest that the series of insecticides used can be grouped in accordance with the cross resistance displayed to them. The grouping is as follows :-

1. Sodium arsenite
2. The chlorinated cyclic hydrocarbon insecticides: BHC, toxaphene, dieldrin, aldrin and chlordane.
3. DDT, dilan and pyrethrum.
4. The organo-phosphorus and carbamate insecticides: malathion, diazinon, delnav, asuntol, korlan and sevin.

As strains resistant to the organo-phosphorus or carbamate insecticides have not yet appeared in blue tick populations, no information on the relationship between the organo-phosphorus and carbamate insecticides could be obtained. However, in respect of the above grouping, resistance to one insecticide in a group resulted in a cross tolerance in some degree to all insecticides in the same group but to no insecticide in any other group. Resistance to several groups was found to occur in a single population of ticks, but it has been shown that resistance to each group arose independently as a result of continual field treatment.

The distribution of resistant strains of the blue tick in South Africa is discussed, and an attempt is made to explain the reasons for the development of resistance consecutively to sodium arsenite, BHC and DDT along the eastern coast and the absence of resistant populations over most of the inland areas.

It is significant that the time taken to develop resistant populations to sodium arsenite, BHC and DDT was different. The time taken to develop resistant populations is correlated with effectiveness of the insecticide.

This correlation suggests that a resistant population will develop most rapidly to the more effective insecticide. These findings are in agreement with results obtained with other species of insects where it has been shown that resistant populations developed most rapidly where selection pressure is greatest.

The mechanisms of resistance to sodium arsenite, DDT and BHC in strains of the blue tick have been investigated.

(a) Sodium arsenite

Attempts were made to investigate the penetration of sodium arsenite in treated adult ticks. The chemical analysis of micro-quantities of As_2O_3 was not entirely satisfactory, but the results indicate that sodium arsenite does penetrate the cuticle. Because of the

limitations of the analytical method no differences in the penetration of sodium arsenite in the arsenic-sensitive and -resistant strains was observed.

Evidence from the literature suggesting an association of the toxic action of arsenic with sulphydryl compounds is discussed.

The inhibition of sulphydryl compounds by iodoacetic acid, several alpha, beta unsaturated ketones and the heavy metal halides alone, and in combination with sodium arsenite, gave some indication that resistance to arsenic in the blue tick might be associated with increased levels of sulphydryl compounds.

It is shown that glutathione, cysteine and free sulphydryl occurred in greater quantities per unit weight of ticks of the resistant strain as compared with the sensitive strain.

(b) DDT

DDT resistant ticks were found to be cross tolerant to dilan, a mixture of nitroparafene analogues of DDT. This suggests that DDT resistance in ticks may not be the result of an enzymatic dehydrochlorination, a mechanism which has been reported in the literature to exist in DDT resistant flies.

The enzyme extraction procedure developed for the extraction of DDT-dehydrochlorinase from DDT resistant houseflies was applied to DDT resistant ticks. The extract from ticks failed to show any breakdown of DDT "in vitro".

A spectrophotometric examination of extracts of DDT treated resistant and sensitive tick larvae failed to show any appreciable breakdown of DDT to DDE.

In tests using larvae it was found that di-(p-chlorophenyl) methyl carbinol (DMC) which synergises DDT in respect of DDT resistant houseflies did not potentiate the effect of DDT on resistant ticks.

These results indicate that the mechanism of resistance in ticks is different from that in houseflies.

(c) BHC

The first step in metabolism of BHC in houseflies is reported to be the monodehydrochlorination of BHC to pentachlorocyclohexene.

Repeated attempts to detect pentachlorocyclohexene in BHC treated resistant and sensitive adult female and larval ticks failed. It would appear that a different mechanism of resistance to BHC in ticks as opposed to houseflies, is operative.

In conclusion, evidence is presented suggesting that in naturally-occurring blue tick populations there exists, in respect of response to a number of insecticides, a mixed population. One population being more resistant than the other. It is inferred that selection of such a population by treatment with a particular insecticide will result in the build-up of the resistant portion of the population at the expense of the sensitive portion.

PREFACE

The work described in this dissertation was undertaken in an attempt to obtain a better understanding of the causes and relationships of insecticide resistance in Blue Tick, Boophilus decoloratus Koch, which has become an acute problem in some localities of the country. The work was undertaken at the Research Department of African Explosives and Chemical Industries Limited over a period of six years from 1953 to 1958. An attempt has been made to keep up to date with the published literature on the various aspects of these investigations up until the end of 1958. Information gained from the literature subsequent to end of 1958 has been made use of but it has not been possible for a number of reasons to follow all recent developments in the various aspects of insecticide resistance published during 1959.

In the execution of this work assistance has been obtained from colleagues better equipped in the field of organic chemistry, biochemistry and statistics, than the author. Where information so gained has been used it has been duly acknowledged. Considerable assistance was rendered by laboratory assistants who were responsible for performing the considerable amount of routine collection, breeding and testing of the biological material,

The blue tick is not a convenient experimental organism for studies on insecticide resistance. Even with the best facilities the tick cannot be bred satisfactorily and in consequence all supplies had to be collected from naturally-occurring populations. Although this had decided advantages in some aspects of the work, a great deal of useful information might have been obtained if certain strains of ticks could have been maintained. The lack of a standard sensitive reference strain has been a considerable disadvantage which could not be overcome and which has influenced the manner in which this work has been carried out.

Because the tick could not be bred artificially the work could only be undertaken with unfed larvae and fully engorged adult females. Larvae are extremely small and in consequence could only be handled in batches while the fully engorged female is sluggish and contains a large quantity of semi-digested mammalian blood which invariably interfered with chemical or biochemical studies.

INTRODUCTION

When the application of an insecticide to an insect pest fails to achieve control in a manner consistent with past experience, the species is generally assumed to have become "resistant" to the particular insecticide. The term "resistance" is loosely used but has become accepted to mean the acquired ability of an insect population to withstand concentrations of an insecticide to which it formerly succumbed. It is with this interpretation, viz. an acquired increase in tolerance, that the term "resistance" will be used throughout this dissertation.

Resistance to insecticides is generally first noticed in the field when the administered treatment fails to give the expected degree of control. Failure to obtain satisfactory control of an insect pest does not necessarily infer a development of insecticide resistance but could be caused by a number of circumstances. For example, the faulty application of the insecticide, inferior materials, degradation of materials and unfavourable climatic conditions. When field observations suggest that the control achieved is not what would be normally expected, all possible causes of the breakdown of control must be thoroughly and systematically investigated. To establish the existence of an insecticide resistant strain requires the determination of the response of the insect population to a particular insecticide. The measure of response can then be compared with the response under the same conditions of a known "sensitive" population of the same species to the same insecticide.

Instances of resistance in insects are not confined to the modern synthetic hydrocarbon insecticides. Resistance to lime sulphur by the San José scale was first observed in 1908 (Melander, 1914). Resistance to lead arsenate by the codling moth (Hough, 1928) to hydrocyanic acid by Californian red scale (Quayle, 1916) and black scale (Woglum, 1925), to potassium antimonyl tartrate by citrus thrips (Boyce, Persing & Bernhardt, 1942) and the artificially induced resistance to phenothiazine by laboratory populations of Cochliomyia sp. (Kipling, 1942) are some of the earliest reports of established

insecticide resistance to materials in use before the development of the synthetic hydrocarbon insecticides. All these examples, with the exception of the artificially induced resistance of Cochliomyia to phenothiazine, were first noticed as a result of the failure of routine field control measures and later confirmed by detailed laboratory experiments.

Initially, attempts were made to explain the observed increase in tolerance by physical means such as "spiracular closure" in cyanide resistant scale-insects or "the discarding of the first bite of the arsenate treated apple" by codling moth larvae. It was not long, however, before laboratory investigations revealed that resistance to insecticides was a result of genetically transmitted biochemical protective mechanisms.

These early examples of insecticide resistance created little but academic interest, and it was only when resistance to DDT in the housefly was demonstrated (Weismann, 1947) that the problem of insecticide resistance was more urgently investigated. The introduction of DDT for the control of vectors of many serious human diseases allowed the development of vast areas of country previously uninhabitable. The sudden dominance of resistant forms of some medically important insects threatened the usefulness of DDT as a weapon against many epidemic diseases which had previously been so effectively controlled.

Since the first reports of DDT resistance in the housefly there have been many reports of resistance to a variety of insecticides by a number of different species of insects, ticks and mites. The magnitude of the problem is indicated by the following list of species with the insecticides to which they are resistant. No attempt has been made to tabulate all instances of resistance throughout the world and only the first occurrence of each particular insecticide resistance in each species is recorded. Doubtful and inconclusively established cases of resistance have been omitted.

List of insect species known to show insecticide resistance up to the end of 1958

Common Name	Specific Name	Insecticide	Date	Country	Ref.
San Jose Scale	Aspidiotus perniciosus Comst.	Lime sulphur	1913	U.S.A.	Melander (1914)
California Red Scale	Aonidiella aurantii Mask.	Hydrocyanic acid	1916	U.S.A.	Quayle (1916)
Black Scale	Saissetia oleae (Bern.)	Hydrocyanic acid	1916	U.S.A.	Woglum (1925)
Citricola Scale	Coccus pseudo-magnoliarum (Kuw.)	Hydrocyanic acid	1925	U.S.A.	Quayle (1938)
Codling Moth	Carpocapsa pomonella (L.)	Lead arsenate	1928	U.S.A.	Hough (1928)
Citrus thrips	Scirtothrips citri (Moult.)	Potassium antimonyl tartrate	1942	U.S.A.	Boyce et al. (1942)
Imported cabbage worm	Pieris rapae (L.)	DDT, DDD, Methoxychlor Perthane	1952	U.S.A.	McEwan et al. (1952)
Diamondback moth	Plutella maculipennis Curt.	DDT	1953	Java	Akersmit (1953)
Grape Leaf hoppers	Erythroneura elegantula Osborn	DDT	1953	U.S.A.	Stafford et al. (1953)
	E. variabilis Beamer	DDT	1954	U.S.A.	Barnes et al. (1954)
Walnut aphid	Chromaphis juglandicola (Ktlb)	Parathion	1954	U.S.A.	Michelbacher (1954)
European mite	Paratetranychus pilosus (C. & F.)	Parathion	1952	U.S.A.	Lienk (1952)
Two-spotted spider mite	Tetranychus bimaculatus (Harvey)	Parathion	1950	U.S.A.	Garman (1950)
	"	Sodium selenite	1950	U.S.A.	Pratt et al. (1953)
German cockroach	Blatella germanica (L.)	Chlordane	1953	U.S.A.	Heal et al. (1953)
Housefly	Musca domestica L.	DDT	1947	Sweden	Weismann (1947)
	"	Chlordane	1951	Italy	Missiroli (1947)
	"	Benzene hexachloride	1949	U.S.A.	March & Metcalf (1949)
	"	Dieldrin	1949	Italy	Busvine (1953)
	"	Parathion	1956	Denmark	Keiding (1956)
	"	Malathion	1957	U.S.A.	Kilpatrick (1957)

Common Name	Specific Name	Insecticide	Date	Country	Ref.
Mosquitoes	Culex pipiens autogenicus Roub.	DDT	1948	Italy	Mosna (1948)
	Aedes nigromaculis (Lud.)	DDT	1949	U.S.A.	Bohart et al. (1950)
	"	Aldrin		U.S.A.	Gjullin (1952)
	"	Dieldrin		U.S.A.	Gjullin (1952)
	"	Heptachlor		U.S.A.	Gjullin (1952)
	Culex tarsalis Coq.	Chlordane		U.S.A.	Gjullin (1952)
	Culex fatigans (quinquefasciatus) Wied.	DDT	1952	India	Pal et al. (1952)
	Aedes aegypti L.	DDT	1950	Trinidad	Gillette (1956)
	A. stephensi	DDT	1957	Iraq	Gramiccia (1958)
	"	"	"	Iran	Mofidi (1958)
	A. subpictus	DDT	1957	India	Shama (1957)
	A. quadrimaculatus Say.	Dieldrin	1956	U.S.A.	Mathis (1956)
	Anopheles quadrimaculatus Say.	DDT	1952	U.S.A.	Kruse et al. (1952)
	Aedes taeniorhynchus Wied.	DDT & DDD	1950	U.S.A.	Deonier (1950)
	A. melas Theo.	DDT	1947	Nigeria	Muirhead-Thompson (1947)
	A. gambiae Giles	DDT	1947	"	" (1950)
	A. gambiae Giles	Dieldrin	1955	"	Elliott (1956)
	A. gambiae Giles	"	1955		Busvine (1956)
	A. minimus Theo.	DDT	1950	Assam	Bertram (1950)
	A. albitarsus Arrib.	DDT	1952	Venezuela	Gabaldon (1952)
	A. pseudopunctipennis	DDT	1952	"	"
	A. darlingi Root	DDT	1951	Argentine	Pinotti (1951)
	"	"	1951	Brazil Guiana Venezuela	Bustamante (1951)
Stableflies	Stomoxys calsitrans (Linne)	"	1955	Sweden	Weismann (1955)
	Farnia canicularis	"	1955	Spain	" "
Midges	Glyptotendipes sp.	BHC & EPN	1956	U.S.A.	Quarterman (1956)

Common Name	Specific Name	Insecticide	Date	Country	Ref.
Sewage fly	Psycoda alternata	DDT	1949		Bruce (1950)
	Chrysomyia putoria	Organo phos.	1957	B. Congo	Bervorts (1957)
Bedbug	Cimex lectularius Linne	DDT	1948	U.S.A.	Johnson (1948)
	Cimex hemipterus	DDT	1952	Formosa	Quarterman (1958)
	Cimex hemipterus	Dieldrin	1957	Tanganyika	Smith (1957)
	Triatoma infestans Klug.	DDT	1948	Chile	Neghme (1948)
Human body louse	Pediculus humanus corporis DeG.	DDT	1952	Korea	Hurlbut (1952)
	" "	DDT	1953	Japan	Burnett (1953)
Fleas	Pulex irritans Linne	DDT	1950	Equador, Brazil	Vera (1953)
	Pulex irritans Linne	DDT	1952	Greece	Hess (1953)
	" "	DDT	1953	Peru	Simmons (1954)
	Ceratophyllus londinensis	DDT	1950	Brazil	Vera (1953)
	Polygenis spp.	DDT	1950	"	Vera (1953)
	Xenopsylla cheopis (Roth.)	DDT	1950	"	Vera (1953)
	Ctenocephalides felis (Bouche)	DDT	1952	U.S.A.	Kilpatrick (1952)
	Ctenocephalides canis (Curtis)	DDT	1952	U.S.A.	Kilpatrick (1952)
Ticks	Boophilus decoloratus Koch	Sodium arsenite	1940	S.A.	du Toit (1941)
	"	Benzene hexachloride	1948	"	Whitnall (1949)
	"	DDT	1956	"	Whitehead (1956)
	"	Toxaphene	1952	"	Fiedler (1952)
	"	Chlordane	1956	"	Whitehead (1959)
	"	Dilan	1956	"	Whitehead (1958)
	"	Aldrin + Dieldrin	1952	"	Fiedler (1952)
	"				

Common Name	Specific Name	Insecticide	Date	Country	Ref.
	Boophilus microplus (Canestrini)	Sodium arsenite	1937	Australia	Seddon (1951)
	"	Benzene hexachloride	1953	"	Hitchcock (1953)
	"	Toxaphene	1956	"	Norris (1956)
	"	DDT	1957		Stone (1957)
	"	Dieldrin	1957	"	Stone (1957)
Brown dog tick	Rhipicephalus sanguinens	Chlordane	1956		Hansens (1956)
Blowflies	Lucilia cuprina Wied.	Dieldrin	1957	"	Shanahan (1958)
Spotted Alfalfa Aphid	Therioaphis maculata (Buckton)	Parathion	1958	U.S.A.	Stern & Reynolds (1958)

It has been stated (Metcalf, 1955) that resistance to insecticides has appeared in less than 0.1% of more than 5000 insect species which are of economic importance. Although resistance is the exception rather than the rule, it may be noted from the above table that resistance has occurred in species of greatest economic importance and constitutes a serious threat to the health of man, particularly in tropical regions.

In South Africa where the widespread and general use of insecticides has not been as necessary as in many tropical countries, the development of strains of resistant insect species up to the present time has been limited to a few species. The isolated occurrence of red scale resistance to hydrocyanic acid was noted at Fort Beaufort in the Cape Province (Smith, personal communication), but it appeared to develop nowhere else nor did it spread from the original locality. The codling moth was controlled for many years with three or four lead arsenate sprays per year but in time the frequency of treatment had to be increased to such an extent that spraying became impractical and the pest was assumed to be resistant to arsenates. Lead arsenate sprays were replaced by DDT preparations and recent unsubstantiated reports of the failure of DDT field treatments have come to hand. The fruit tree red spider, Tetranychus telarius L., has

developed a high degree of resistance to organo-phosphorus insecticides (Whitnall, 1958). In the veterinary field the blowflies Lucilia spp. have in a very short time developed resistance to dieldrin (McHardy, personal communication). The blue tick Boophilus decoloratus Koch had become resistant to a number of inseoticides and it is the investigation of this problem which forms the subject of this dissertation.

PART I. GENERAL:

(a) Life history, habits and economic importance of the blue tick

Boophilus decoloratus Kock, commonly called the blue tick, is widely distributed throughout the Ethiopian region. Although the species has a variety of hosts among the indigenous African fauna it has become adapted to domestic bovines on which it is a serious ectoparasite. Heavy infestations of the blue tick result in a general debility of stock but economically more serious is the fact that it is a vector of the serious stock diseases, anaplasmosis (gall sickness) and bovine piroplasmosis (red water fever), and spirochaetosis of cattle, horses and other animals. It is also a vector of tick bite fever, a rickettsial disease of man.

Unlike many other species of African ticks the blue tick attaches to a host as a larva and remains on the same animal until mature. As a result of this habit it is commonly referred to as a "one-host tick".

After hatching from the egg the small six-legged larva ascends to the top of the surrounding vegetation and there awaits chance contact with a suitable host. Having achieved this the larva attaches to the skin of the host animal by means of the barbed hypostome and commences to suck blood. After six or seven days the larva moults to become the eight-legged nymph. A further six or seven days brings the nymph to the second moult and imaginal stage. The adult feeds on the same host for a further six or seven days and when fully engorged disengages its mouth parts, drops from the host and conceals itself in any available ground cover. From commencement of engorgement of the larva to the time when the fully engorged female tick leaves the host takes an average of twenty-one days. The life history of the male blue tick is less precisely known, but from field observations the male life history differs from that of the adult female only in that it remains on the host for a longer period than seven days.

Under optimal conditions the fully engorged female tick commences oviposition seven days after dropping from the host. Approximately five thousand eggs are laid over a period of fourteen days: the eggs begin to hatch 33-38 days after being laid. After completion of oviposition the parent female dies. Thus in favourable conditions the life cycle is completed in nine to twelve weeks. In its natural habitat the life cycle of the blue tick is probably very different from that under optimal laboratory conditions. It is well-known that under adverse conditions the adult female will delay oviposition for considerable periods. Under dry and cold conditions the length of time required for the hatching of the eggs may be considerably extended.

In areas of South Africa where climatic conditions are conducive to the development of high blue tick populations, regular insecticide dipping or spraying of cattle at seven day intervals has been practised since approximately 1900. Under such conditions the one-host tick comes in contact with the insecticide three times during its 21-day parasitism of the host animal. This method of control originally afforded a satisfactory safeguard against the diseases carried by the blue tick.

In practice, difficulty has been experienced in the control of blue tick populations because they have in some areas shown a propensity to become resistant consecutively to a number of insecticides which have been used as a means of field control. The blue tick population in some areas of the country has developed tolerance in turn to sodium arsenite, benzene hexachloride and DDT preparations. In other areas resistance has developed to benzene hexachloride only and in still other areas the tick remains sensitive to all insecticides.

(b) <u>An historical review of insecticide resistance in the blue tick</u>

During the period 1938-39 cattle farmers in the Eastern Cape Province experienced difficulty in controlling the blue tick with sodium arsenite preparations which had proved effective for the previous 30 to 40 years. It was shown by field experimentation in 1940

(du Toit, Graf & Bekker, 1941) that lack of control by arsenic preparations was a direct result of the development of increased arsenic tolerance in the blue tick population. The first signs of arsenic resistance appeared in the East London district, but over a period of two or three years the arsenic resistant tick became established along the entire coastal area from Port Elizabeth in the south to Natal in the north.

During 1946 benzene hexachloride preparations came into general use in areas affected by the arsenic resistant tick. Initially, gamma BHC at a concentration of 0.005% was spectacularly effective and it showed promise of being a means of eradicating the blue tick. Eighteen months after the commercial introduction of BHC, signs of BHC resistance were observed, again in the East London district (Whitnall, Thorburn, Whitehead, McHardy & Meerholz, 1949). In a very short time the entire area originally covered by the arsenic resistant tick was also affected by the BHC resistant tick. At that time, from many hundred of tests, no instance was found where the BHC resistant tick was not also resistant to arsenic (Whitnall, Thorburn, Whitehead, McHardy & Meerholz, 1952). At the same time no instance of ticks resistant to sodium arsenite alone was ever found.

A single instance of the development of BHC resistance without prior resistance to arsenic was recorded from the Pretoria district (Bekker, 1953).

In areas with the problem of resistance to both arsenic and BHC, commercial control of these strains was achieved by the introduction of DDT preparations. DDT at 0.2% never gave effective control of adult female blue ticks in the laboratory and to achieve 100% control at least 0.5% pp'DDT was necessary. These observations were verified in the field, but in spite of this DDT at 0.2% kept cattle satisfactorily free of nymphal and adult ticks after three dippings at weekly intervals. The conclusions drawn were that DDT was effective in the field at the concentration used by virtue of its toxic effect on

larvae only. This was, in addition, supported by the fact that DDT had no effect at 0.2% in the field on two- and three-host ticks where control measures were directed mainly against the adult stage.

During 1954 specimens of the blue tick were received from a farm in the East London district (Allandale ticks), where DDT was not giving satisfactory field control and DDT resistance was suspected. Laboratory tests on larvae and adults showed conclusively that the blue tick from this locality was twelve-fold more tolerant to DDT than sensitive ticks from Frankenwald Research Station, Transvaal (Whitehead, 1956). The strain resistant to DDT was also resistant to BHC and to sodium arsenite in spite of the fact that the two latter materials had not been used in that area for six and ten years respectively. Specimens of blue ticks from East London resistant to arsenic and BHC but not to DDT are still common, which suggests that resistance to DDT and resistance to BHC and arsenic is unrelated.

Most of the investigation of insecticide resistance in the blue tick was undertaken in the field. The limited laboratory investigations which were carried out at that time are inadequate for determining relationships in the different types of insecticide resistance.

In the work described in the following chapters the different resistant strains of ticks available have been examined, using laboratory techniques specially designed to detect differences in insecticide response in both larval and adult ticks. From the results obtained from these tests it has been possible to show not only relationships in types of resistance but also to throw some light on heterogeneity to insecticide response of tick populations. The results have also explained the rather curious distribution of resistant strains of the blue tick in South Africa.

PART II. INSECTICIDE RESPONSE OF THE BLUE TICK IN SOUTH AFRICA

(a) Source of tick material

In this work large numbers of blue ticks from different areas of the country were required for the various tests conducted. Response of different strains to insecticide treatment could only be assessed by comparison with ticks susceptible to all insecticides. For an absolute basis of comparison it would have been most satisfactory to obtain a strain of the blue tick from a population which had not previously come in contact with any insecticide. Such a population could not be obtained in South Africa. An attempt was made to obtain blue tick specimens from the Gwembe Valley, Northern Rhodesia, where there was no record of the field use of insecticides for tick control. Gwembe Valley ticks were regarded as naturally susceptible to all insecticides and termed "primitive" by Matthysse (1954). An application for a permit to import blue ticks from Northern Rhodesia was refused by the South African Veterinary authorities and alternative arrangements had to be made.

In the initial experiments undertaken during 1954-1956, the reference sensitive strain, termed "Frankenwald ticks", were obtained from Frankenwald Research Station, Transvaal. At that time there had been no field indication of increased tolerance to any insecticide in Frankenwald ticks. The ticks used were collected from naturally occurring populations on range cattle. On occasions, the supply from this source was not sufficient and it was necessary to augment the supply by infesting cattle with laboratory hatched larvae originally obtained from the Frankenwald range cattle. During 1958 further work along the same lines was undertaken with a selected number of recently developed insecticides. An investigation of the Frankenwald strain of ticks at this stage showed a development of resistance to a number of insecticides and precluded their use as a basis of comparison. This difficulty was overcome by obtaining adult blue ticks from the Johannesburg abattoir which have been referred to as "Transvaal 1958" ticks. Before these ticks were used they were examined on each occasion for resistance to

arsenic, BHC and DDT and compared with the original data obtained with ticks from Frankenwald during 1954-1956.

Most of the resistant strains were obtained from farms in the East London district and were usually available in large numbers. According to the type of resistance, East London ticks have been referred to as :-

(i) "Ferndale ticks", resistant to sodium arsenite and the BHC group of insecticides.

(ii) "Allandale ticks", resistant to sodium arsenite, the BHC group and DDT.

(iii) "Nagaza Park 1958", resistant to sodium arsenite, the BHC group and DDT. Nagaza Park 1958 ticks were identical to Allandale ticks and were only used because the source of supply of ticks from Allandale was no longer available for the tests conducted during 1958.

Ticks from the East London area were collected in the field, enclosed in compressed cardboard containers and despatched by air to the laboratory where they were received not later than thirty-six hours after removal from the host.

It has already been indicated that ticks from Frankenwald underwent a change in response to insecticides between 1956 and 1958. This necessitated a re-evaluation of the susceptibility of Frankenwald ticks to a number of insecticides and these ticks have been referred to as "Frankenwald 1958".

In a number of tests larval ticks were used. Larvae were obtained by placing adult female ticks in small glass tubes loosely stoppered with cotton wool. To minimise the possibility of selecting larvae from a single parent, ten or more adult female ticks were placed in each tube. Tubes were stored in a constant conditions room at 25°C and 85% RH. Under these conditions complete hatch of egg batches was usual.

In the tests using larvae the various strains were referred to in the same terms already described for adult ticks.

It is emphasised that in all tests ticks were obtained from natural populations. No artificial breeding was undertaken. This eliminated the possibility of making comparisons with inbred laboratory strains which have not been subjected to the selective agencies existing in their natural habitat. Attention has been called to the fact that the use of laboratory bred colonies of insects can give rise to erroneous results because of a lower vigour resulting from continuous laboratory inbreeding. (Brown, 1950).

Note: Throughout this work the blue tick has been compared with insects. Chemical compounds toxic to ticks have been referred to as "insecticides" and not as "acaricides". This has arisen from the fact that the ticks, although belonging to the class Acarina, behave more like insects in respect of response to toxic chemicals than do the mites. The term "acaricide" has become accepted to indicate a chemical which is toxic to mites. Generally, the accepted acaricides are not effective against ticks whereas most of the insecticides are.

(b) Tests to determine insecticide response

In establishing the existence of resistant strains of insects it is necessary to compare the response to a toxicant of a suspected resistant strain with the response to the same toxicant of a known sensitive strain. The difference in response between strains is usually expressed as a ratio with the response of the sensitive strain equated to 1. To obtain such a comparison a suitable method of determining response is necessary.

Individual insects in a population differ one from another in their response to a toxicant and as a result an insect population will show a variation in response over a range of insecticidal dosages.

There are three ways of measuring the response to a toxicant (Finney, 1952) (Busvine, 1957) .

(i) Direct assay which necessitates determining the exact dose required to kill the individual or batches of individuals. Some

information on the distribution of susceptibility among individuals of a population has been obtained in different ways (Yeager & Munson, 1945; Sheppard, 1939) but generally this procedure is impracticable with insects.

(ii) Determination of the quantitative response. This procedure is sometimes used in determining response of insect populations to toxicants and takes into account, for example, the survival times after treatment with various concentrations of toxicants. The practical difficulties in determining survival times accurately seriously limits this procedure but it has been used effectively in some instances (Gregoire, Cotteleer & Pouplard, 1958).

(iii) Determination of the quantal response. In this procedure the response is measured as "occurring" or "not occurring" or "all or nothing" response and the criterion is usually death. Measurement is made of the proportion of each batch responding by being killed. The method based on quantal response is most convenient and practical when dealing with the response of insect populations for assay purposes or for determining increases in tolerance in populations.

The response of batches of insects exposed to doses of a toxicant increasing by an arithmetic progression will not be normally distributed. Thus in the region of near 100% mortality large increases in dose will produce only slight increases in response. This is illustrated in diagram 1.

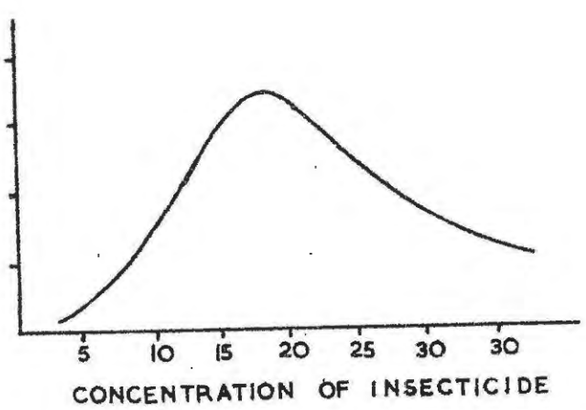


Diagram 1. Illustrating the distribution of response in a population exposed to doses of a toxicant increasing by an arithmetical progression.

In many biological processes equal increments in response are produced only when the stimulus is increased by a constant proportion (Bliss, 1935). Thus if batches of an insect population are exposed to doses of a toxicant which are increased logarithmically, the distribution of response approaches normality and when response is plotted against log dose a normal sigmoid curve is obtained (Diagram 2).

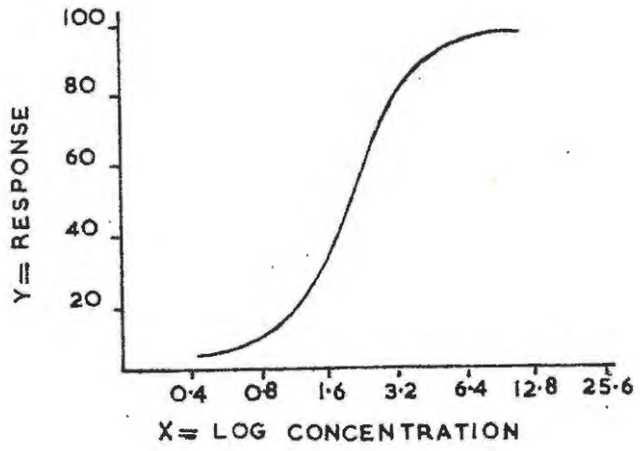


Diagram 2. The sigmoid curve obtained when response is plotted against the log concentration.

At the point of inflection of the sigmoid curve which corresponds to 50% mortality the change of x for an increase in y is least and hence the value of x at this point will be subject to the least error and errors in measurment will be minimal. For this reason the dose required to produce 50% kill of a population is most commonly used in biological assay of insecticides and in determining differences in response of populations to various toxicants. The dose required to produce 50% mortality is usually expressed as the 50 per cent lethal dose (LD.50) or median lethal dose (MLD) although the latter is not recommended as it can be confused with "minimum lethal dose" (Busvine, 1957). Where tests are conducted with concentrations of insecticides such as in exposure to treated plates or where batches of insects are immersed in concentrations of insecticides, the concentration required to produce 50% mortality is usually expressed as the LC50 (Busvine, 1957).

It is possible to obtain a reasonable measure of the LC50 simply by plotting the percentage mortality against the log dose. However, in specialised cases the values for as high as LC99 are sometimes required and the accuracy of the dosage-mortality values in this section of the sigmoid curve are considerably reduced. Furthermore, when dealing with resistant populations it is often not possible for practical reasons to obtain figures of the percentage mortality above, say, 25% kill at extreme dose. From mortalities of lower than 25% it is not possible to construct a sigmoid curve from which LC50 values can be obtained. This difficulty has been overcome by plotting the percentage mortality on a probability scale using logarithm - probability graph paper (O'Kane, 1930) or by transforming the percent mortality values into probability units (probits) and plotting these arithmetically against log dose (Bliss, 1935). Having obtained the LC50 which is usually expressed as the dosage or concentration of the insecticide, an accurate comparison of the difference in response of populations can be obtained.

In all tests with blue tick larvae, batches were exposed to insecticide concentrations increasing logarithmically. The percent mortalities obtained at the various concentrations was converted to probits and plotted against the insecticide dose expressed as a percent concentration. The best fitting log dose-probit regression line was then drawn through the points. From the regression lines the LC50 could be obtained by reading off the dose corresponding to the point at which the regression line intersects the line representing probit 5.

Blue tick larvae are easily obtained in large numbers, require no feeding, and in consequence there are no complications due to variations in the state of engorgement. In these respects they are eminently suitable for insecticide response tests. Unfortunately larvae are extremely small and active, and are difficult to handle without specialised techniques. Many different methods of handling batches of larvae in tests were investigated before a satisfactory one was evolved.

In attempts to devise a larval test procedure, the effect of relative humidity (R.H.), temperature, and age of larvae on the reproducibility of results was first investigated.

Humidity was found to have a considerable influence on larvae in insecticide response tests. By conducting a wide variety of experiments it was found that reproducible results could only be obtained when larvae were bred, treated, and stored between treatment and mortality counts at 85% R.H. It was noted from these experiments that the R.H. affected various strains of ticks differently. This suggests a difference in vigour which might be correlated with locality from which the ticks were obtained.

The age of larvae also had considerable bearing on the insecticide response. It was found that larvae younger than 10 days and older than 35 days were more susceptible to insecticides, and that water-treated control larvae in the same age range often could not survive the 24 hour storage period between treatment and mortality counts. Larvae between 10 and 30 days old gave a consistent response and all subsequent tests were conducted with larvae in this age range.

Because an atmospheric temperature of 25°C was found to be satisfactory and convenient to maintain, the effect of temperature on larval response to insecticides was not investigated as thoroughly as the effect of relative humidity and age.

(1) Immersion technique using larvae

For all tests larvae bred under constant conditions of temperature and humidity (25°C and 85% R.H.) were available.

The immersion of larvae was carried out in an adaptation of an instrument originally described by McIntosh (1947). Basically the apparatus consisted of a constant temperature bath containing a rotating end-over-end shaker to which a glass tube could be clamped. For the purpose of the larval test the bath was maintained at

25°C and the shaker rotated at 32 r.p.m. The modified apparatus is shown in Plate 1.

Larvae were removed from the breeding tubes in batches of between 100 and 200 with a small camel-hair brush and brushed into glass dipping tubes measuring 2 cm in diameter and 5 cm in length. Approximately 10 ml of the insecticide dispersion in water was then added, the tube stoppered, secured in the shaker of the immersion apparatus, and rotated for 1 minute. Larvae exposed to dispersions of insecticides were then separated from the insecticide suspension by pouring the contents of the dipping tube through a separating apparatus (Plate 2). This consisted of two flanged glass cylinders of approximately 4 cm diameter placed one above the other. A circular disc of 40 mesh copper gauze with a thin rubber washer was placed between the faces of the two flanges and the whole clamped together with spring clips. The apparatus was conveniently used on a filter flask as recovery vessel although the application of suction had no advantage. The insecticide suspension, together with the larvae, was poured through the separating apparatus. The dipping tube was then rinsed with 10 ml of distilled water containing 0.05% Triton X100 and the washings also poured through the funnel. The larvae which remained adhering to the copper gauze were further washed with 10 ml of water. After washing the under-surface of the copper gauze disc it was removed and held against a pad of absorbent paper to remove the surplus water. The gauze was then placed in the centre of a sheet of 12.5 cm Whatman No. 1 filter paper, the paper loosely folded in half (Plate 3) and the edges crimped between a set of serrated rollers (Plate 4). It was found essential for the filter paper from which envelopes were constructed to be in equilibrium with an atmosphere of 85% R.H. As a precaution, all filter paper used in the tests was stored for at least three days prior to use in these conditions. Filter papers unequilibrated for humidity resulted in considerable mortality even with water-treated control batches of larvae. The arched gauze disc in the centre of the folded filter

Plate 1. Immersion apparatus used for treating both larvae and adult ticks.

Plate 2. Apparatus used for separating tick larvae from water dispersed insecticides.

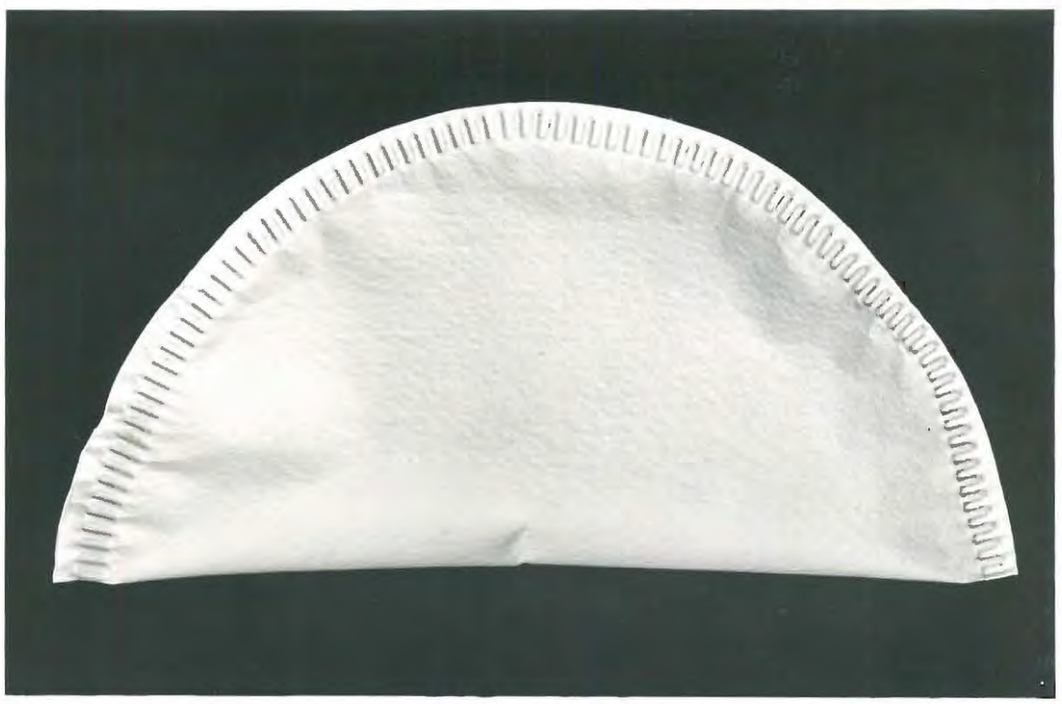


Plate 3. Filter paper envelope in which insecticide treated tick larvae were stored between treatment and mortality count.

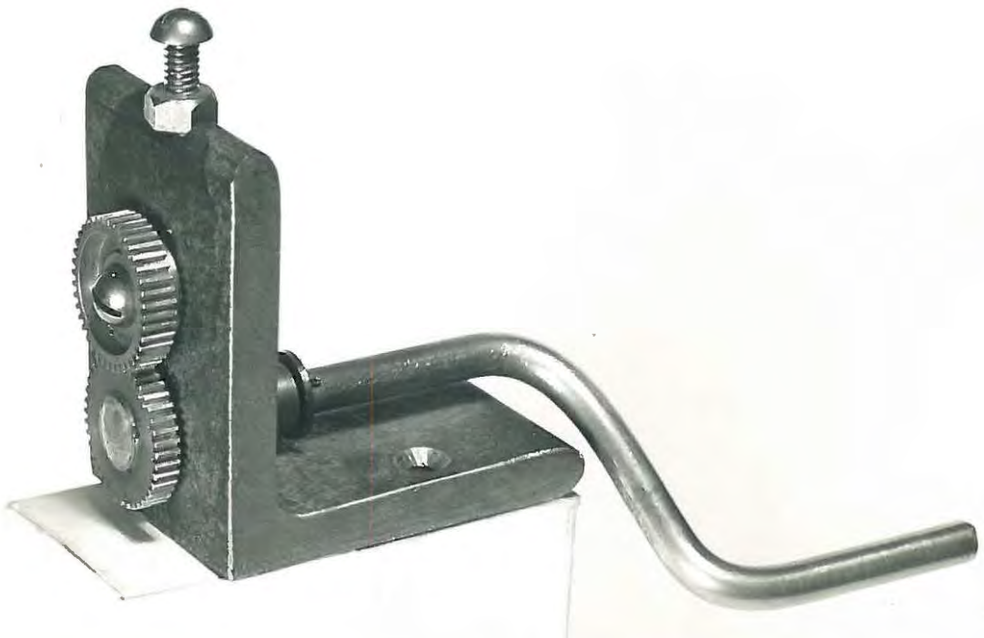


Plate 4. Serrated rollers for crimping the edges of filter paper envelopes in which tick larvae were stored.

paper was sufficient to keep the walls of the envelope well apart. This method was found to be the most convenient as the larvae were effectively confined and a large number of envelopes could be stored in a minimum of space.

Tests with each insecticide concentration were replicated five times giving a total of between 700 and 1000 larvae per insecticide concentration. A water-treated control was included with all tests and in any tests where mortality in the control was more than 5%, the entire series was discarded.

Use was not made of Abbott's correction factor in cases where mortality in the controls was higher than 5% as it was considered that whatever influence was responsible for mortality in the untreated batches it might not be constant for the different concentrations of insecticide.

Results of tests were analysed by the method of probits from which the concentration of insecticide required to give 50% kill was determined. In cases where the dose required to give 50% mortality was so high that it could not be obtained by experiment, the LC50 was obtained by extrapolation.

(2) Immersion technique using fully engorged adult female ticks

The fully engorged adult tick has been used frequently as a means of testing for insecticide tolerance both in South Africa (Whitnall & Bradford, 1947) and in Australia (Hitchcock, 1953). The test procedure, although basically similar to that used for larvae, is considerably simpler as the adult tick is much more amenable to handling. Adult ticks, however, could seldom be obtained in such large quantities as larvae. This immediately decreases the accuracy of the tests in which adult ticks were used. Furthermore, the adult female tick is extremely sluggish and it is not possible to determine accurately whether it is dead or alive. For this reason results of the tests with adult ticks did not give a direct measure of mortality. The index of toxicity

was assessed by observing the effect on oviposition and the viability of the eggs produced. From the point of view of the field effectiveness of a particular insecticide such a procedure was most satisfactory but a precise determination of LC50 necessary in comparing strains for determining resistance, could not be obtained. However, by comparing the results of test on adults and larvae from the same strain it was found that the tests using adults were fairly reliable for detecting resistance in gross terms although they could not give an expressible degree of comparison between strains. The method using adults, although easy to manipulate required almost six weeks before a complete record of the viability of eggs was obtained. Where possible both larval and adult tests were performed on all strains of ticks but on a few occasions only results from adults could be obtained.

The dipping test technique using fully engorged female blue ticks was adapted from that described by Whitnall and Bradford (1947). Fully engorged female ticks were dipped in a range of concentrations of insecticide wettable powder or miscible oil preparations in the same apparatus used for the larval test. Adult ticks were dipped in a similar manner to larvae, except that a larger dipping tube was necessary to accommodate the greater bulk of adult ticks. Batches of 20 to 50 adult ticks, selected for uniformity of engorgement, were placed in glass tubes measuring 2.5 cm in diameter and 7.5 cm in length. The appropriate concentration of insecticide suspension, preconditioned to 25°C was poured over the ticks, the tube well stoppered and rotated in the constant temperature bath at 32 r.p.m. for two minutes. After immersion the contents of the tube were poured into a petri dish. The ticks were immediately removed with forceps and placed on pads of cotton wool moistened with the same insecticide concentration used for dipping. Treated ticks were allowed to drain in this manner for half an hour and were then placed in convenient sized glass tubes loosely stoppered with cotton wool. Control batches treated with water alone were included in all tests. If more than 5% of the female ticks in the control batches failed to lay viable eggs the entire test was discarded. Tubes containing

treated ticks were stored in trays at 25°C and 85% RH. A record was kept of the number of ticks that laid eggs, size of egg batches and the percentage hatch.

Treatment with sodium arsenite (Figs. 2 & 3) appeared to have little direct effect on adult ticks and even after treatment in the high arsenic concentrations some eggs were usually laid. The difficulty in observing mortality in adult ticks has already been discussed. If a tick was considered dead or alive by the fact that it laid or did not lay eggs it might have been very misleading from a point of view of practical control. In view of this, results of all tests using adult ticks were based on the viability of eggs produced. In the histograms is recorded the percentage of ticks which laid eggs; the percentage "kill" which was indicated by the percentage of ticks which failed to lay eggs; the percentage hatch and, finally, the percent control which is the percentage of ticks failing to produce viable eggs. The percent control formed a convenient basis of comparison between strains of ticks differing in their response to insecticides. The percentage control is not a measure of actual mortality although, in some circumstances, it could be. It was considered that the percent control was a more realistic measure of the effect of an insecticide on adult ticks than the percent 'kill", derived from counts made of treated ticks which failed to lay eggs.

(c) Results of tests conducted with fully engorged adult female blue ticks

To enable convenient reference and a better understanding of the histograms and probit regression curves, the following is a list of strains of ticks used in the tests.

1. Frankenwald ticks - Sensitive to all insecticides.
2. Ferndale ticks - Resistant to sodium arsenite and the BHC group of insecticides.
3. Nagaza Park 1958 ticks - Resistant to the BHC group of insecticides, DDT and related compounds and to pyrethrum. Originally resistant to sodium arsenite but a suggestion of some reversion to sensitivity in respect of this insecticide.

4. Allandale ticks - Resistant to sodium arsenite, the BHC group of compounds, DDT and related materials.

5. Frankenwald 1958 ticks - Resistant to the BHC group with an indication of "vigour tolerance" to several other compounds.

6. Transvaal 1958 ticks - Sensitive to all insecticides.

(1) Sodium arsenite

The blue tick from the East London district was shown to be resistant to sodium arsenite preparations by du Toit et al. (1941) and in other areas of the eastern coastal belt by Whitnall and Bradford (1947)

From the histograms (Figs. 2 and 3) it will be noted that complete control of Frankenwald ticks was obtained with sodium arsenite at 0.16% As_2O_3 whereas the same concentration on Frankenwald 1958 ticks resulted in only 43% control. A concentration of 0.32% As_2O_3 resulted in approximately equal effect on both strains. This indicates a slight increase in tolerance to arsenic at low concentrations in Frankenwald 1958 ticks and suggests that some form of selection has been taking place which has had the effect of eliminating the more sensitive proportion of the tick population at Frankenwald Research Station. If log dose-probit mortality lines were drawn using precent control as mortality, the result would show a slight change in slope and a consequent slight change in LC50 but no change in the concentration of arsenic required to produce near 100% control. The slight increase in LC50 is too small to mean a definite increase in tolerance to arsenic, but is suggestive of "vigour tolerance" (Hoskins and Gordon, 1956).

Both Ferndale and Allandale ticks were less affected than Frankenwald ticks by the same concentrations of As_2O_3. This represents a small increase in tolerance to sodium arsenite; it is, however, sufficient to preclude arsenic from field use because of the limits of tolerance to sodium arsenite by cattle. The normal concentration of

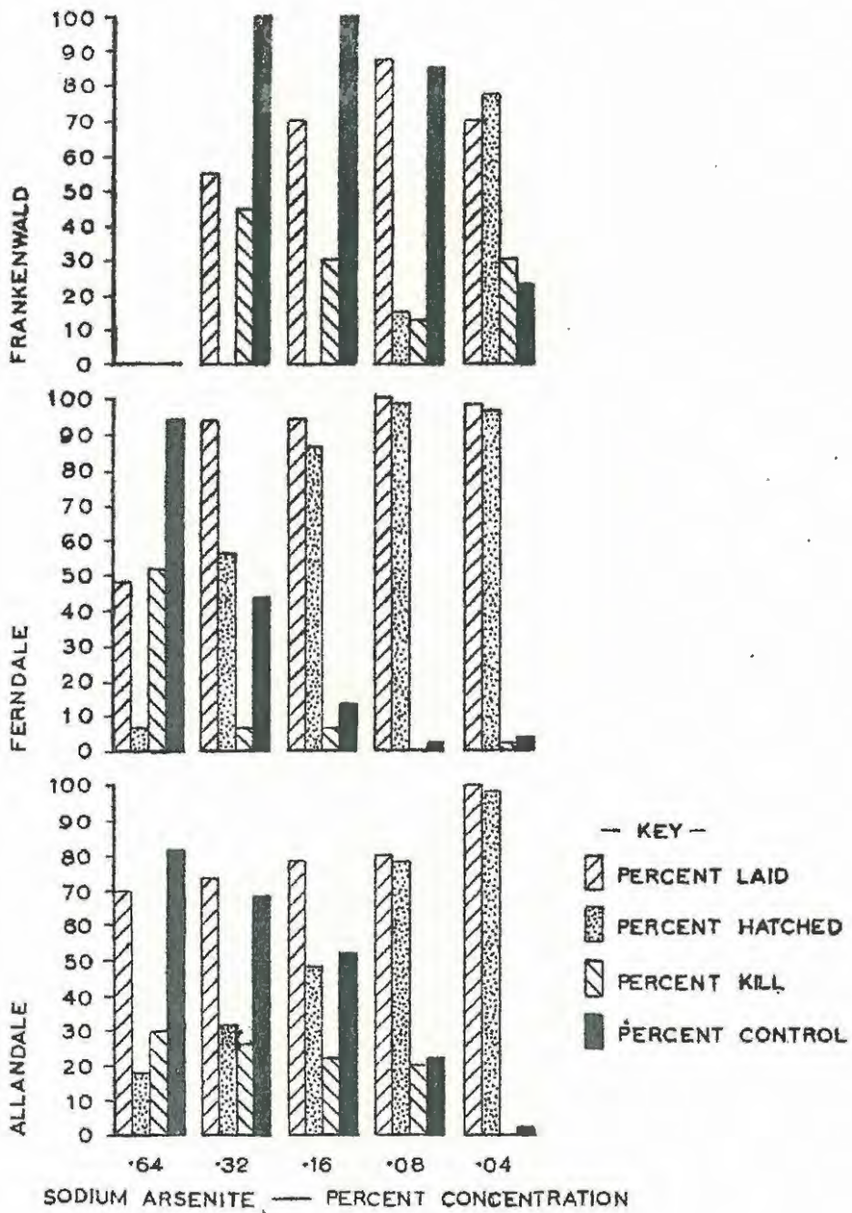


Fig. 2 The effect of sodium arsenite concentrations on fully engorged adult female blue ticks obtained from different localities.

sodium arsenite for field dipping is 0.16% As_2O_3 when treatment is at seven day intervals. The histograms (Fig. 2) suggest that a three- to four-fold increase in sodium arsenite concentration would effectively control the resistant strains from Ferndale and Allandale.

The histogram obtained for Transvaal 1958 ticks (Fig. 3) shows a slightly increased tolerance when compared with Frankenwald ticks but again only at the lower concentrations of As_2O_3.

Nagaza Park 1958 ticks examined three years later than Ferndale and Allandale ticks, which are all from the same area, show a slight decrease in tolerance to sodium arsenite although by comparison were still more resistant than the original Frankenwald tick (Figs. 2 & 3). This result suggests that ticks from some areas of the East London district are showing signs of losing some of their acquired resistance to sodium arsenite. Although the exact history of the dipping practice on the farm, Nagaza Park, is not known, it is on record that arsenic preparations have not been used for more than twelve years.

A particular feature of the action of sodium arsenite is the fact that treated batches of adult female ticks are not all killed at concentrations even as high as 0.64% As_2O_3. At this and at lower concentrations many treated ticks will lay apparently normal eggs. However, many of these do not hatch.

In Table 1 are presented the percentage ticks from batches of various strains which laid eggs after treatment in three concentrations of sodium arsenite together with the percentage of batches of eggs which hatched.

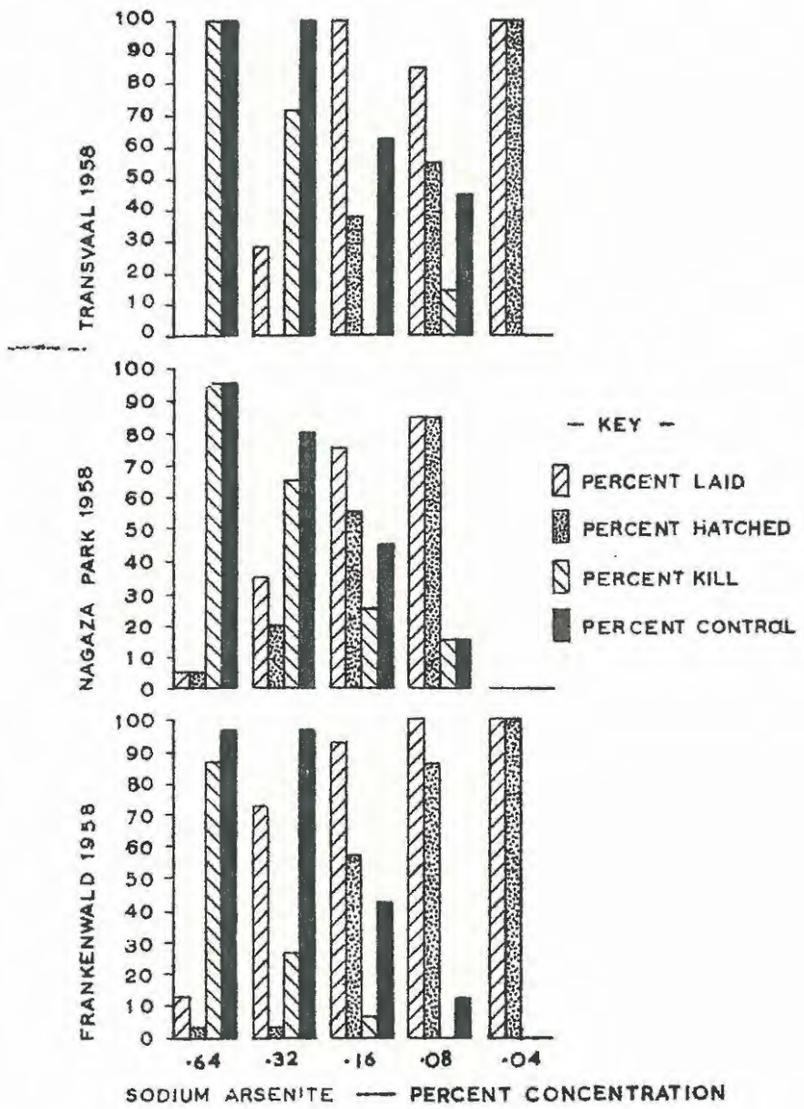


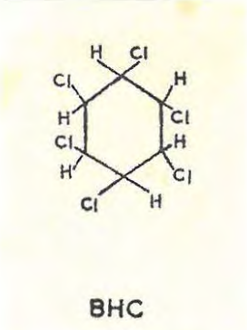
Fig. 3 The effect of sodium arsenite concentrations on fully engorged adult female blue ticks obtained from different localities.

Table 1. The percentage lay and percentage hatch of eggs from batches of adult female ticks of various strains treated in three concentrations of sodium arsenite.

Strain of tick	0.64% As_2O_3		0.32% As_2O_3		0.16% As_2O_3	
	% Lay	% Hatch	% Lay	% Hatch	% Lay	% Hatch
Frankenwald	-	-	55	0	70	0
Ferndale	86.5	48	94	56	94	86.5
Allandale	48	70	74	32	78	48
Transvaal 1958	38	0	28	0	100	38
Nagaza Park 1958	55	5	35	20	76	55
Frankenwald 1958	58	12	72	4	93	58

It will be observed that a considerable proportion of the treated female ticks laid eggs but in all cases many of the batches of eggs failed to hatch. These results indicate that sodium arsenite at the concentration used did not kill the adult female tick but it nevertheless exerted a considerable controlling influence by virtue of the fact that many of the eggs were non-viable. This phenomenon is also observed in the field where sodium arsenite treatment of tick infested cattle will not result in an immediate control of the adult female tick but prolonged treatment does result in a definite decrease in the incidence of ticks (Whitnall, McHardy, Whitehead and Meerholz, 1951).

(2) Gamma BHC : the gamma isomer of 1, 2, 3, 4, 5, 6 - hexachlorocyclohexane



BHC

Treatment of Frankenwald ticks with 0.01% gamma BHC resulted in 92% control but this degree of control could not be attained with as much as 2% gamma BHC on Ferndale and Allandale ticks (Fig. 4).

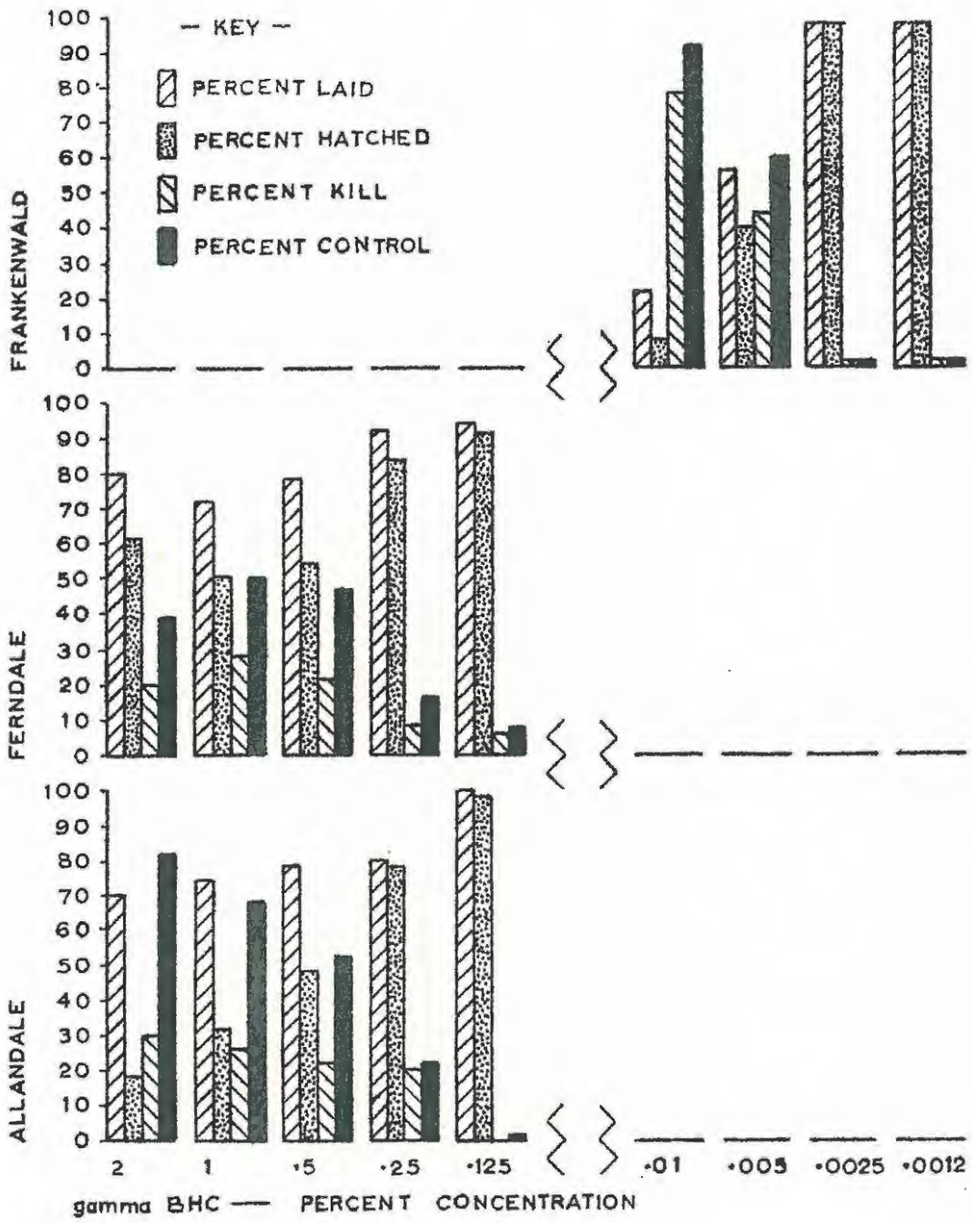


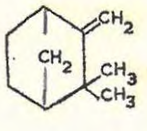
Fig. 4 The effect of gamma BHC concentrations on fully engorged adult female blue ticks from different localities.

The results indicate a clear-cut resistance to gamma BHC by the latter two strains of ticks. There was a slight difference in response between Allandale and Ferndale ticks which, because of the erratic nature of the results with the latter strain, might be accounted for by experimental error.

Slightly higher concentrations of gamma BHC were necessary on Transvaal 1958 ticks (Fig. 5) to achieve a result similar to that obtained with Frankenwald ticks, but the increase was not sufficiently high to suggest the development of a degree of resistance in Transvaal 1958 ticks. The histogram obtained for gamma BHC on Nagaza Park 1958 ticks (Fig. 5) was similar to that obtained with Ferndale ticks and again indicates a high degree of resistance.

Frankenwald 1958 ticks (Fig. 5) also responded in the same manner as Ferndale ticks to gamma BHC displaying a similar degree of resistance. This observation establishes that Frankenwald 1958 ticks had developed resistance to gamma BHC between 1956 and 1958. It is significant that over this period no gamma BHC was used on Frankenwald Research Station and the dipping and spraying undertaken on the property was with toxaphene.

(3) Toxaphene Octachlorocamphene. (The exact chemical structure of toxaphene is not known. It is prepared by chlorinating the bicyclic terpene, camphene (Metcalf, 1955)



CAMPHENE

A hundred percent control was obtained with 0.5% toxaphene on Frankenwald ticks and considerably less control on Ferndale and Allandale ticks (Fig. 6). The effect of toxaphene on ticks from Frankenwald Research Station was re-examined three years later

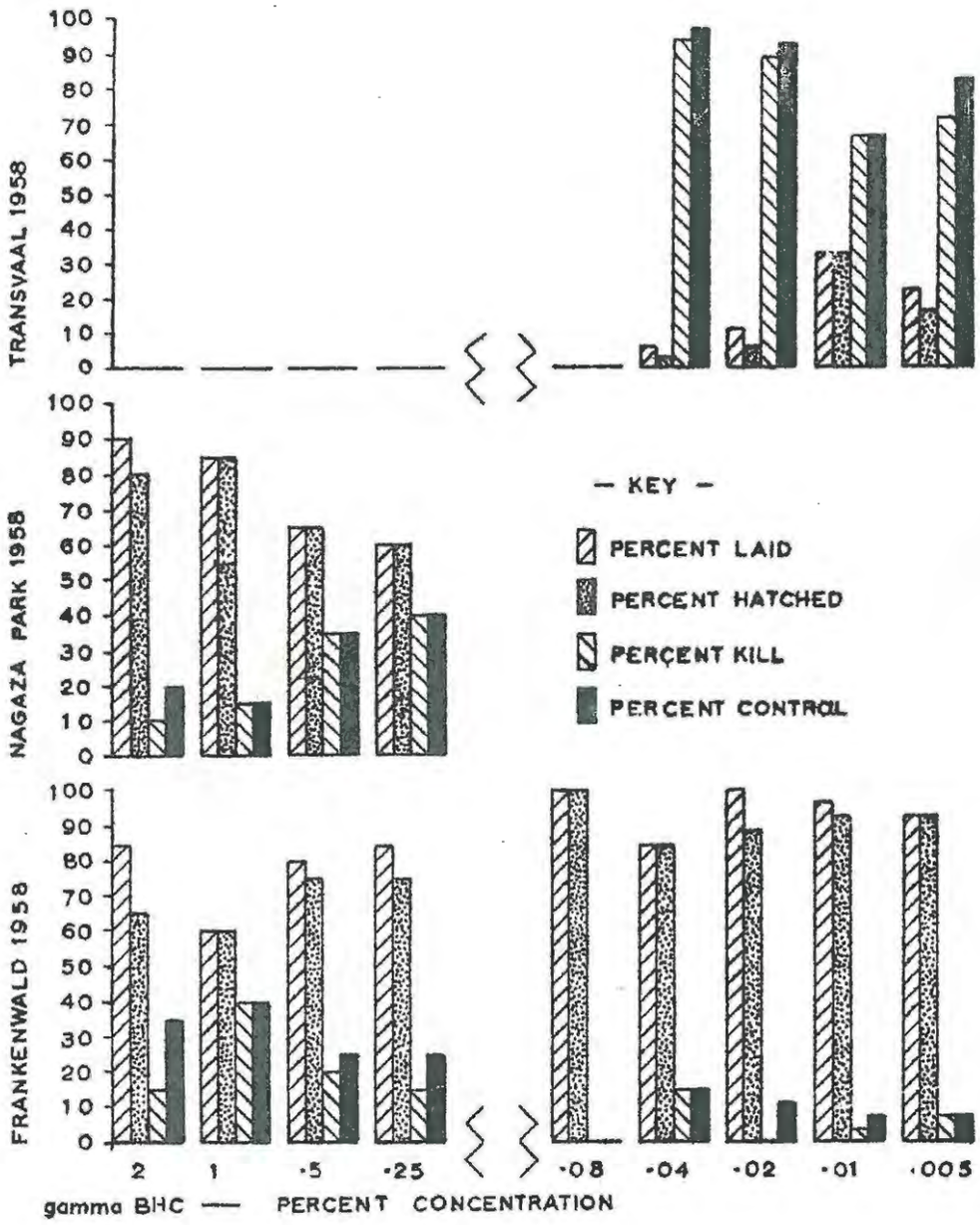


Fig. 5 The effect of gamma BHC concentrations on fully engorged adult female blue ticks from different localities.

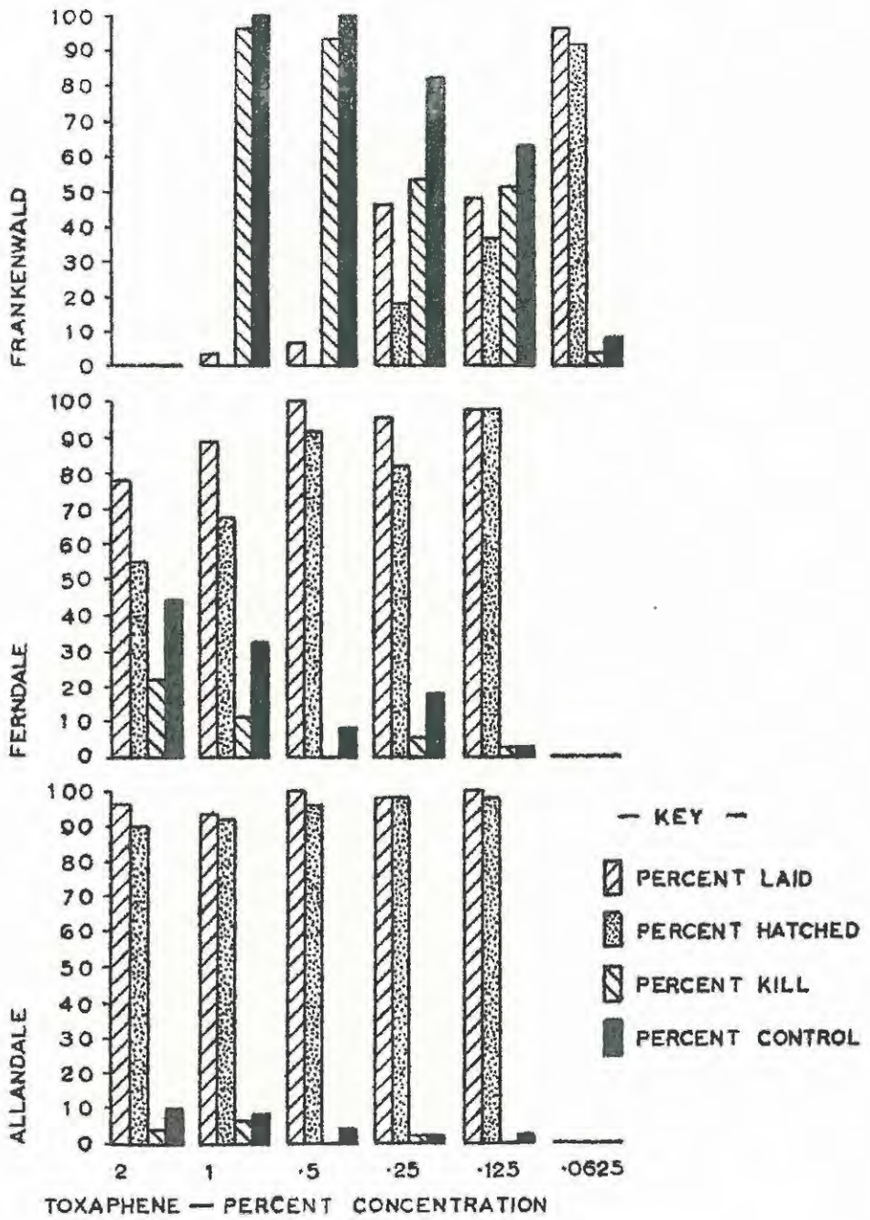
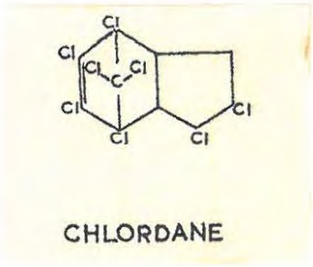


Fig. 6 The effect of toxaphene concentrations on fully engorged adult female blue ticks obtained from different localities.

(Frankenwald 1958 ticks) (Fig. 7) and the results showed a degree of resistance similar to that occurring in Ferndale and Allandale ticks.

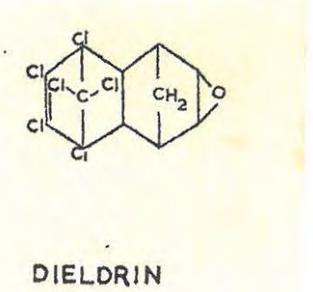
(4) Chlordane — 4, 5, 6, 7, 8, 8 - hexachloro-3a, 4, 7, 7a tetrahydro-4, 7-methanoindene.
(Syn. Chlordene, chlordan)



CHLORDANE

The control with 0.25% chlordane on Frankenwald ticks was 96% but 0% and 4% on Ferndale and Allandale ticks respectively (Fig. 8) indicating a high degree of resistance to chlordane in the latter two strains. Frankenwald 1958 ticks (Fig. 7) also showed a high degree of resistance to chlordane which was slightly lower than the chlordane resistance in Ferndale and Allandale strains.

(5) Dieldrin — 1, 2, 3, 4, 10, 10 - hexachloro-6, 7-epoxy-1-4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-endo, exo-5, 8-dimethanonaphthalene.



DIELDRIN

A control of 94% was obtained with 0.0625% dieldrin on Frankenwald ticks (Fig. 9), whereas treatment of Ferndale ticks with 2% dieldrin resulted in 4% control and 28% in Allandale ticks. This indicates a high degree of resistance in the latter two strains. A high degree of resistance was also obvious in Frankenwald 1958 ticks (Fig. 10) similar in degree to the dieldrin resistance in Allandale and Ferndale ticks.

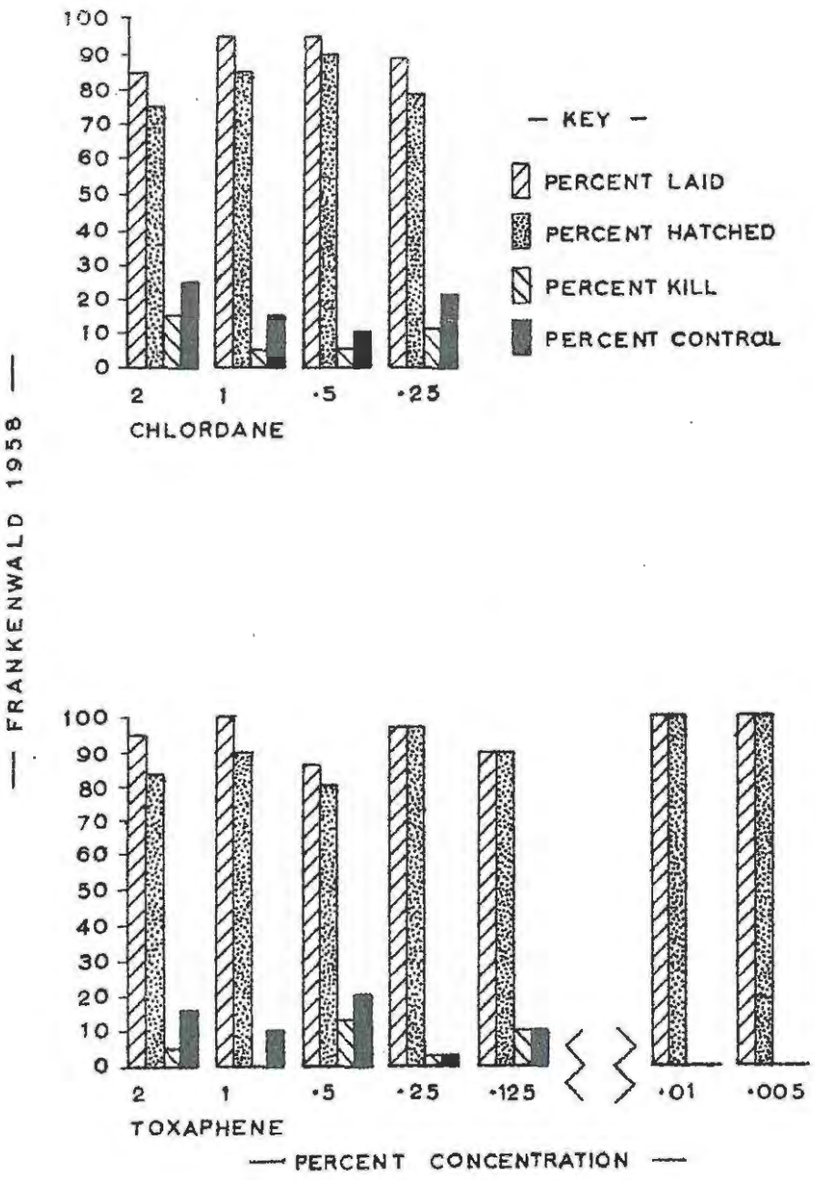


Fig. 7 The effect of chlordane and toxaphene concentrations on fully engorged adult female blue ticks from Frankenwald Research Station.

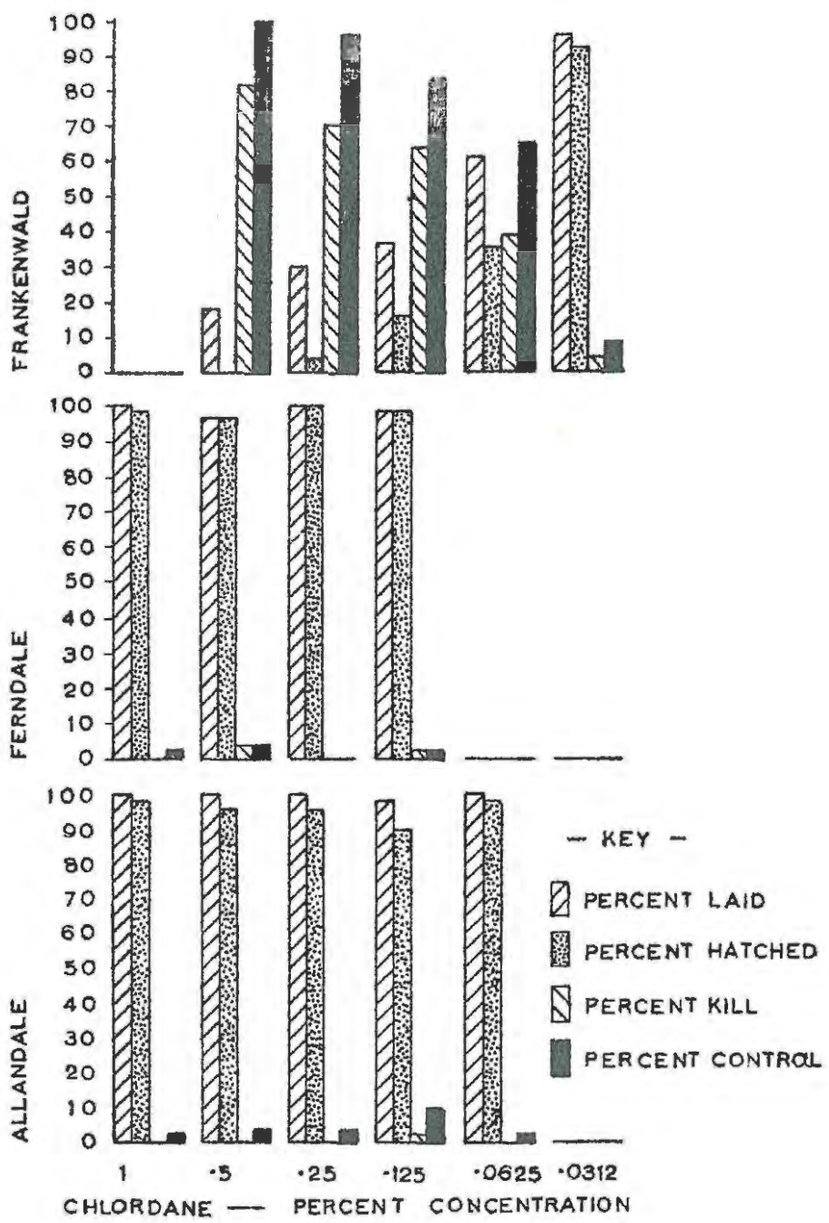


Fig. 8 The effect of chlordane concentrations on fully engorged adult female blue ticks obtained from different localities.

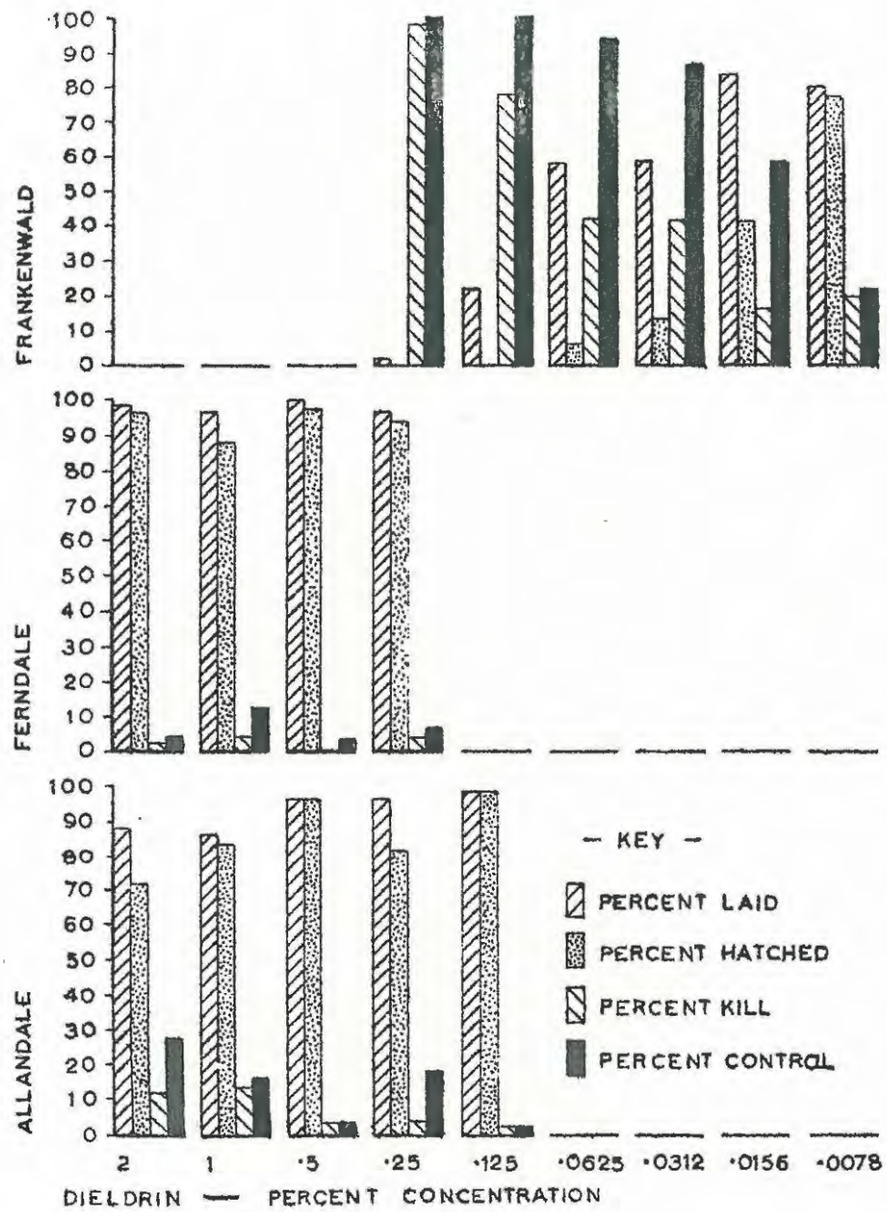
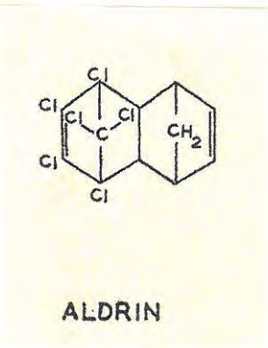


Fig. 9 The effect of dieldrin concentrations on fully engorged adult female blue ticks obtained from different localities.

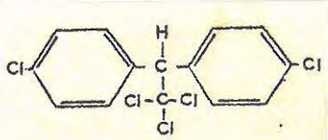
(6) Aldrin 1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 8, 8a-hexahydro-1, 4-endo, exo-5, 8-dimethanonaphthalene



ALDRIN

Treatment of Frankenwald ticks with 0.125% aldrin resulted in a control of 92%. The same treatment applied to Ferndale and Allandale ticks resulted in only 2% control (Fig. 11). Aldrin treatment of Frankenwald 1958 ticks (Fig. 10) also showed a high degree of resistance in this strain although it was not quite of the same order as with aldrin resistance in Ferndale and Allandale ticks.

(7) pp'DDT 2,2-bis-(p-chlorophenyl)-1, 1, 1-trichloroethane



DDT

DDT treatment at 0.5% resulted in a hundred percent control of Frankenwald ticks, 94% control of Ferndale ticks and 2% control of Allandale ticks (Fig. 12). By comparison, Allandale ticks showed a well-developed resistance to DDT. The histogram obtained by treating Transvaal 1958 ticks (Fig. 13) with DDT indicates a tick population slightly more tolerant to DDT than either Frankenwald or Ferndale ticks but considerably less resistant than Allandale ticks. Nagaza Park 1958 ticks (Fig. 13) show the same marked DDT resistance as Allandale ticks. Frankenwald 1958 ticks show a surprising degree of increased tolerance to DDT when compared with Frankenwald ticks; were slightly more tolerant

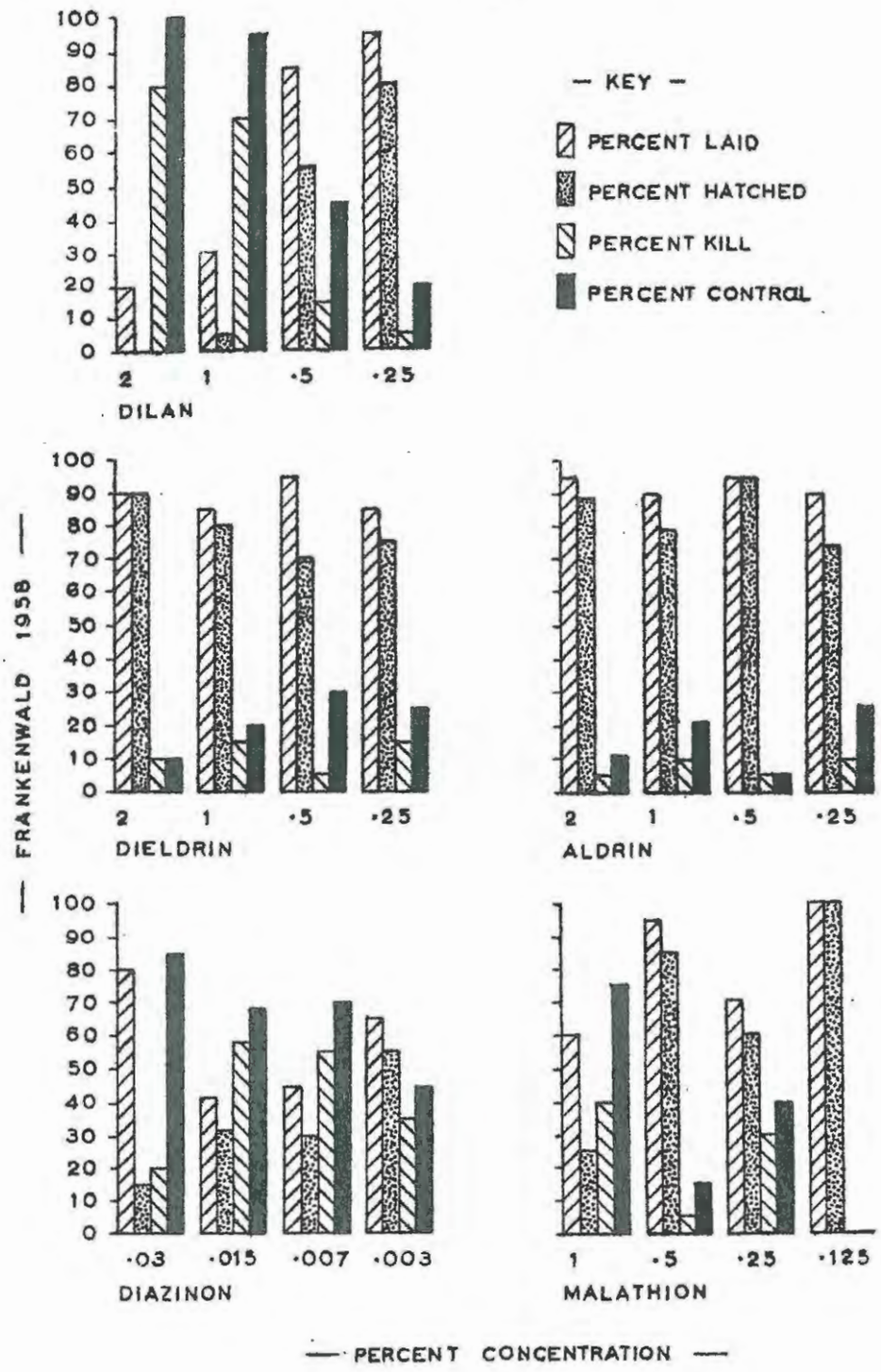


Fig. 10 The effect of dilan, dieldrin, aldrin, diazinon and malathion on fully engorged adult female blue ticks obtained from Frankenwald Research Station.

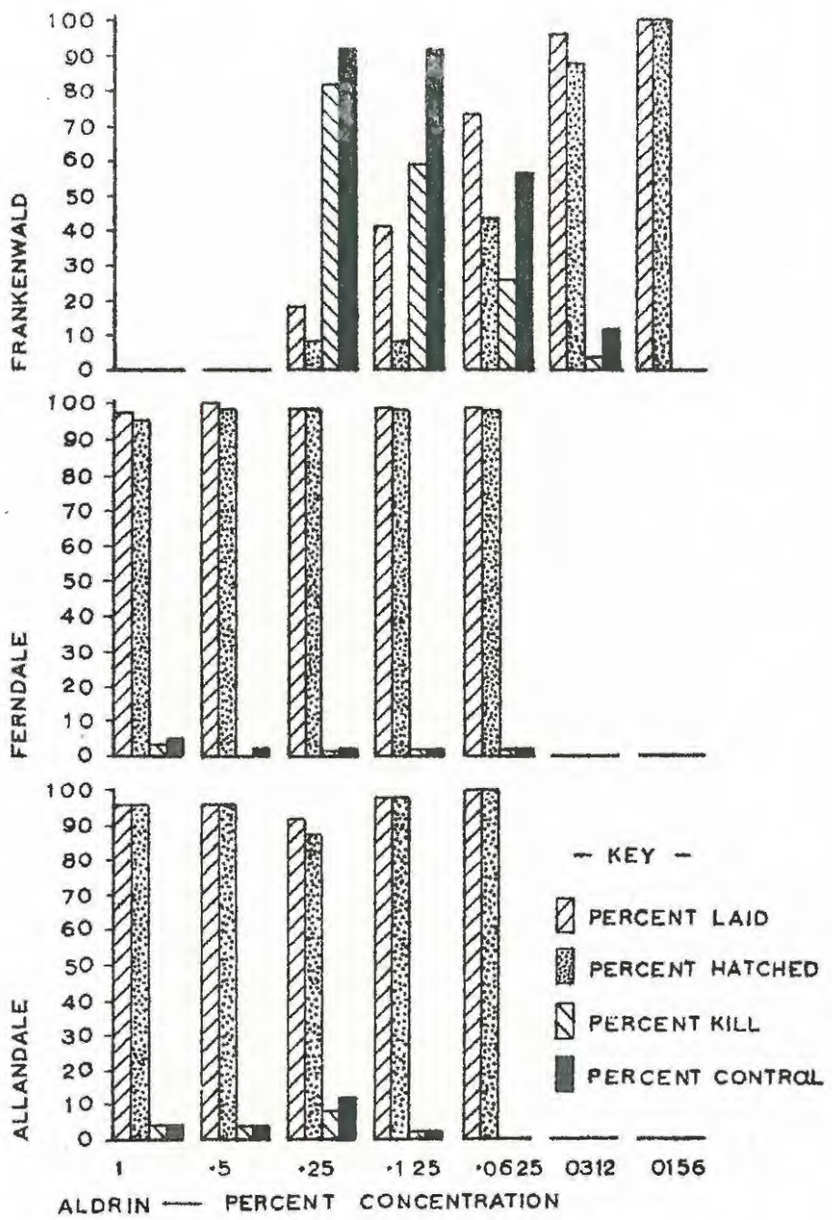


Fig. 11 The effect of aldrin concentrations on fully engorged adult female blue ticks obtained from different localities.

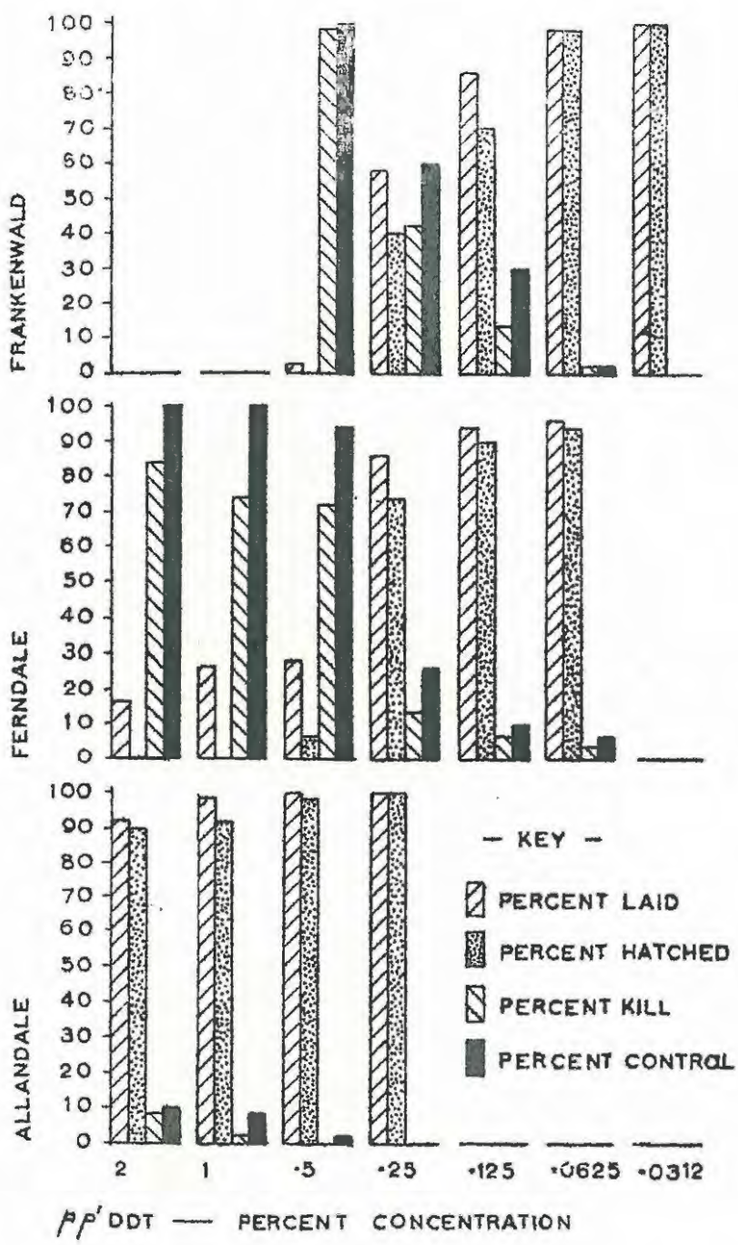


Fig. 12 The effect of pp'DDT concentrations on fully engorged adult female blue ticks obtained from different localities.

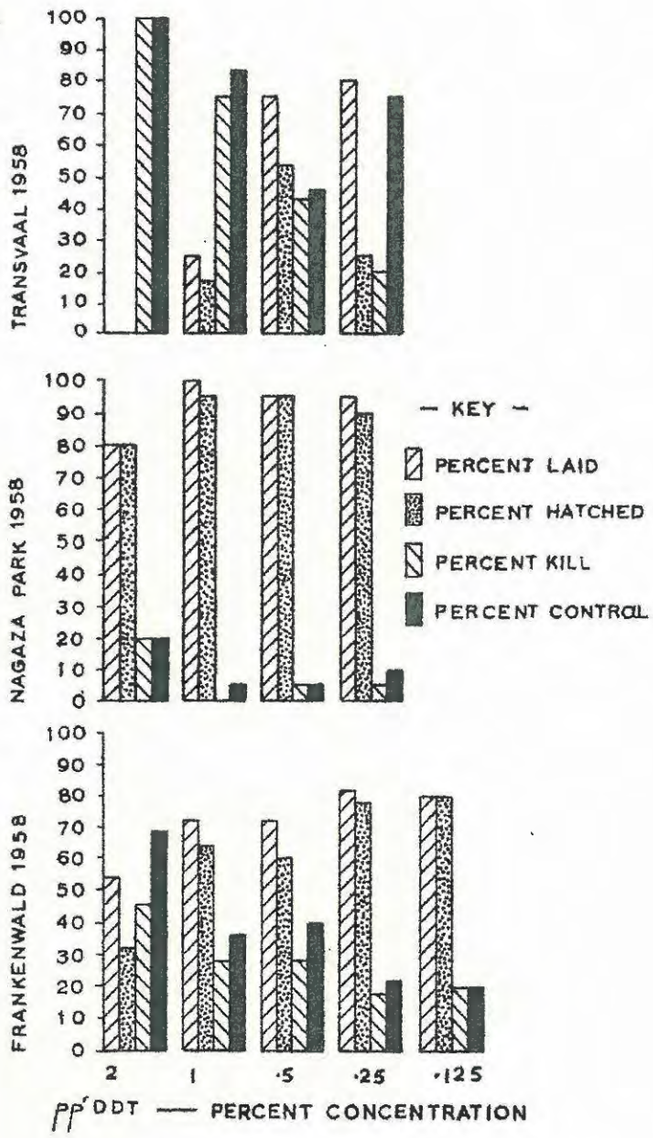
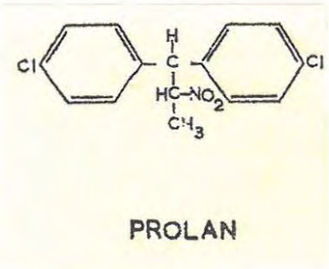


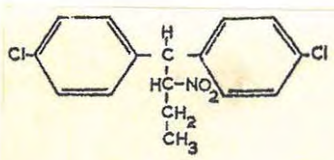
Fig. 13 The effect of pp'DDT concentrations on fully engorged adult female blue ticks obtained from different localities.

than Transvaal 1958 ticks but considerably less resistant than Allandale and Nagaza Park 1958 ticks.

(8) Dilan a mixture of Prolan, 1, 1 bis-(p-chlorophenyl)-2-nitropropane and Bulan, 1, 1-bis-(p-chloropheryl)-2-nitrobutane



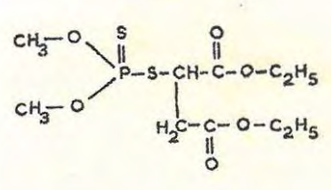
PROLAN



BULAN

It was necessary to use dilan at fairly high concentrations to achieve control of fully engorged female ticks. On Frankenwald ticks 2% dilan treatment resulted in 99% control and 100% control with Ferndale ticks (Fig. 14). The same concentration of dilan on DDT-resistant Allandale ticks resulted in only 20% control indicating a fairly high degree of resistance by comparison with the effect of dilan on Frankenwald and Ferndale ticks. Treatment of Frankenwald 1958 ticks (Fig. 10) with dilan gave an effect very similar to that on Frankenwald ticks. Because of the shortage of supply of Transvaal 1958 and Nagaza Park 1958 ticks, the effect of dilan on these strains could not be determined.

(9) Malathion 0,0-dimethyl s-(1, 2-dicarboethoxyethyl) dithiophosphate



MALATHION

Malathion was equally effective against Frankenwald, Ferndale and Allandale ticks (Fig. 15). Frankenwald 1958 ticks (Fig. 10) were slightly less affected than the above strains but the difference in

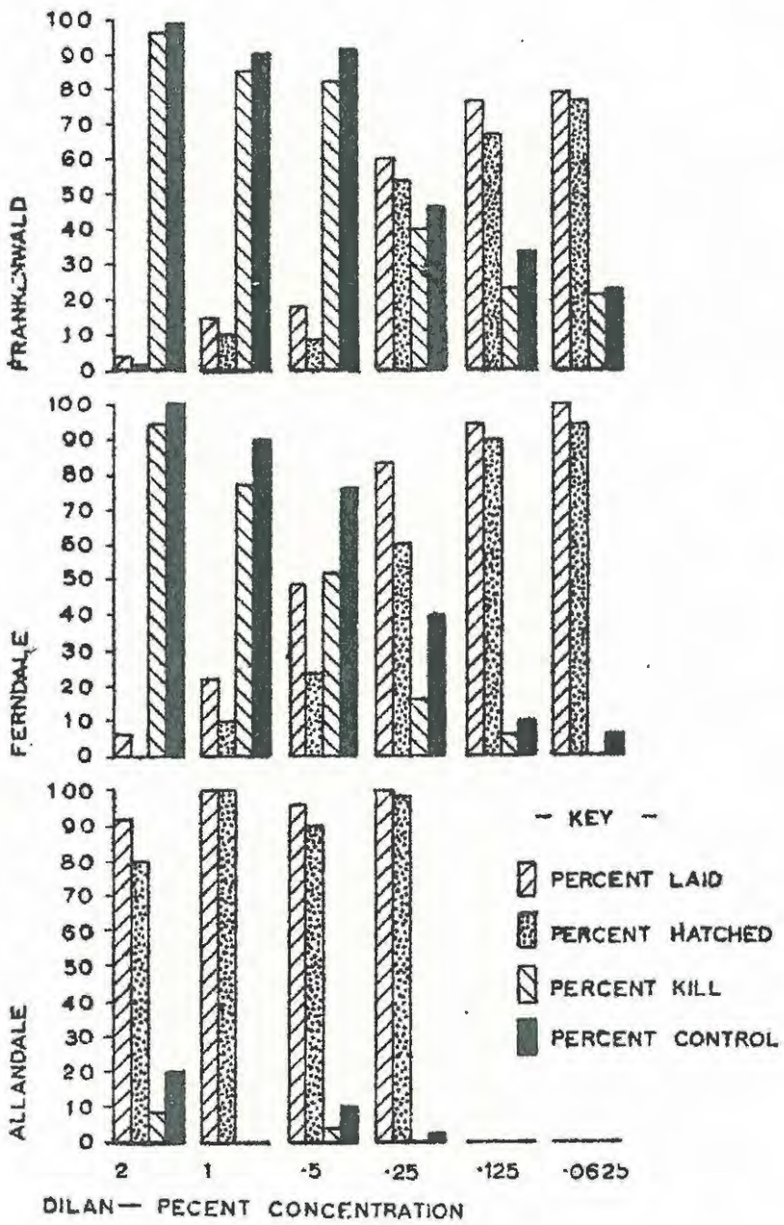


Fig. 14 The effect of dilan concentrations on fully engorged adult female blue ticks from different localities.

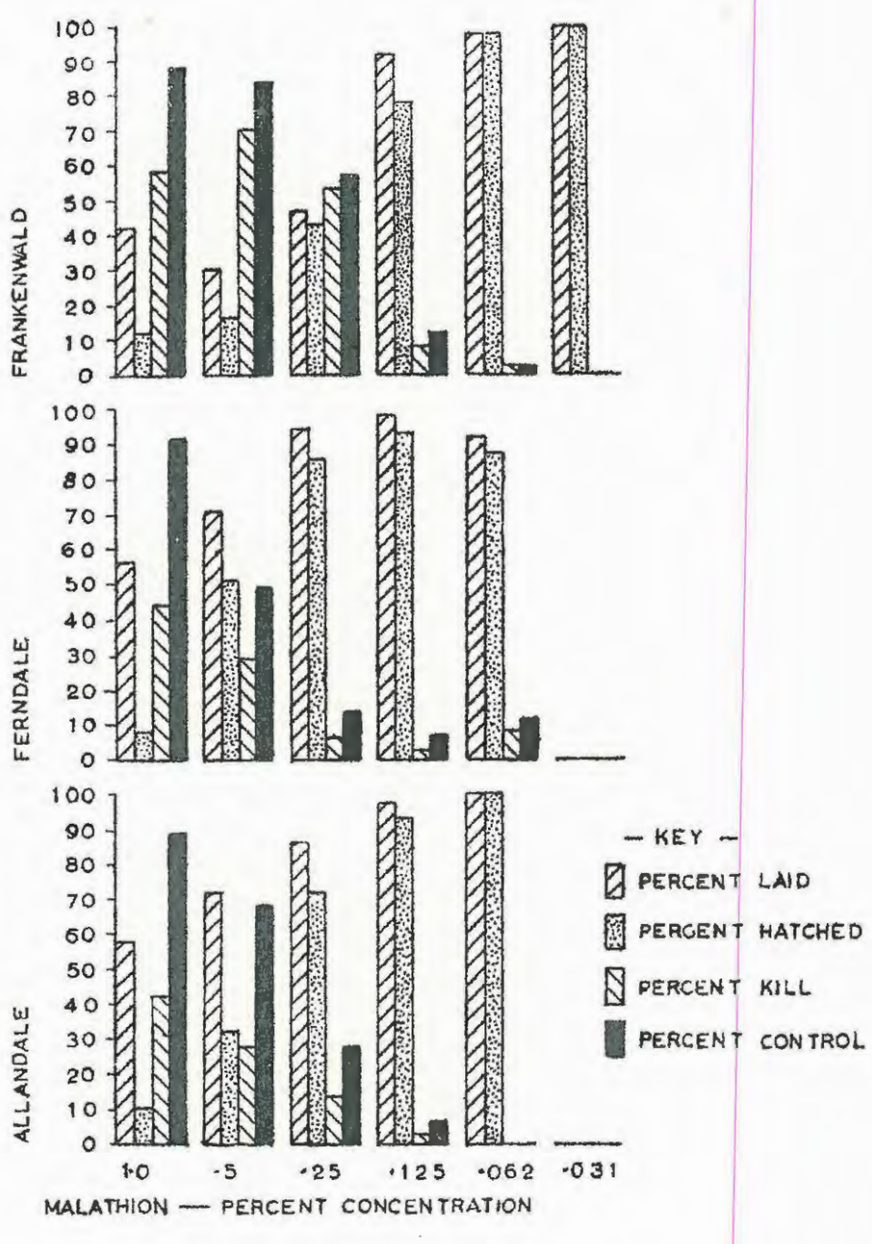
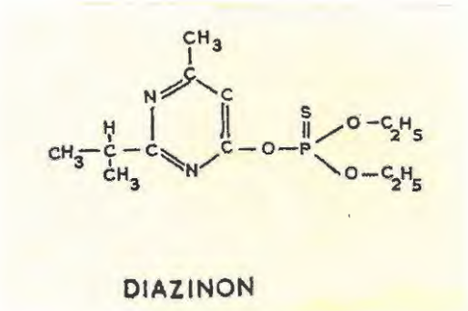


Fig. 15 The effect of malathion concentrations on fully engorged adult female blue ticks from different localities.

response was too small to suggest the development of resistance. As a result of a shortage of supply of Transvaal 1958 and Nagaza Park 1958 ticks the effect of malathion on these strains was not determined.

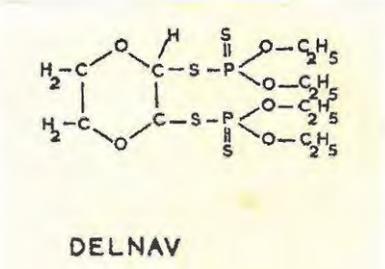
(10) Diazinon 0, 0-diethyl 0(-2-isopropyl-4-methylpyrimidyl) -(6) thionophosphate



DIAZINON

Frankenwald, Ferndale and Allandale ticks were all equally affected by diazinon (Fig. 16). On Frankenwald 1958 ticks (Fig. 10) diazinon was only slightly less effective than on the above strains of ticks. The effect of diazinon on Transvaal 1958 and Nagaza Park 1958 ticks was not investigated.

(11) Delnav 2, 3-p-dioxanedithiol-bis-(0, 0-diethylphosphoro-dithioate)



DELNAV

Delnav has only recently become available and for this reason could not be used on Frankenwald, Ferndale and Allandale ticks. On Frankenwald 1958, Nagaza Park 1958 and Transvaal 1958 ticks, delnav produced a similar response (Fig. 17).

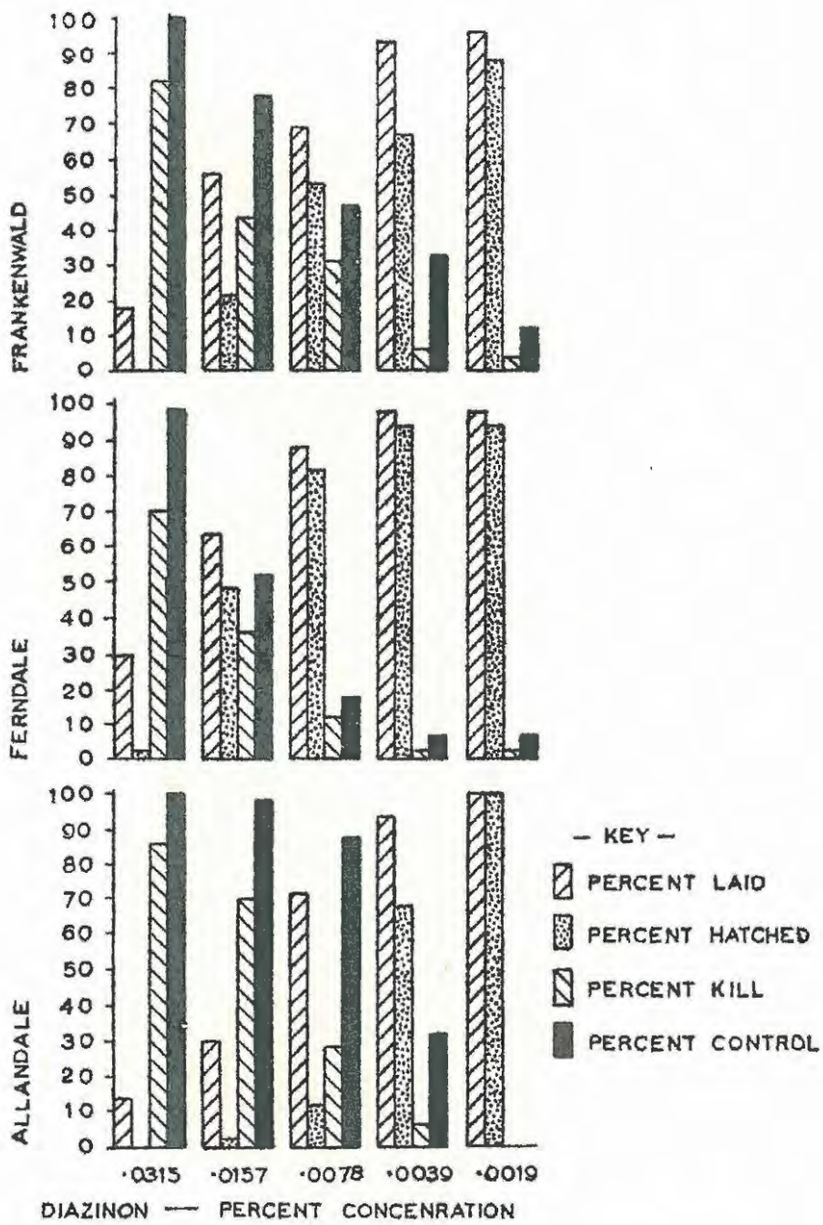


Fig. 16 The effect of diazinon concentrations on fully engorged adult female blue ticks obtained from different localities.

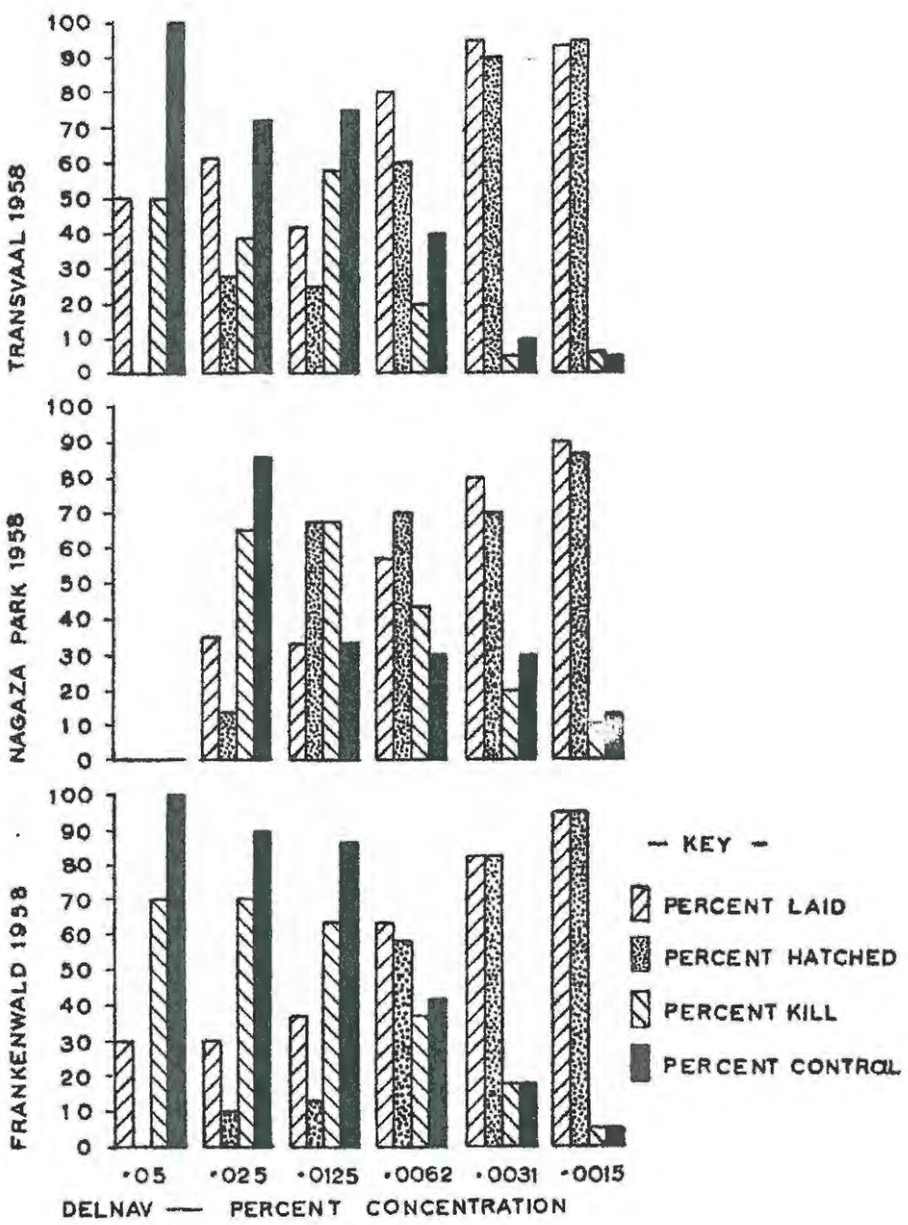
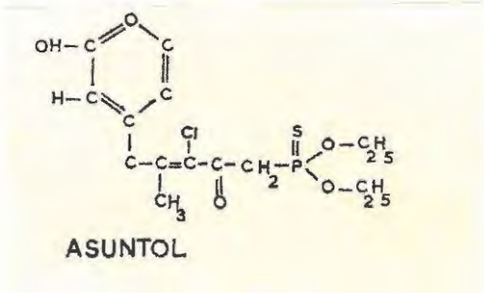


Fig. 17 The effect of delnav concentrations on fully engorged adult female blue ticks obtained from different localities.

(12) Asuntol — O, O-diethyl O, 3-chloro-4-methyl-7-coumarinyl thiophosphate



Frankenwald 1958, Nagaza Park 1958 and Transvaal 1958 ticks were all equally affected by asuntol (Fig. 18). Asuntol was not available for examination against Frankenwald, Ferndale and Allandale ticks.

(13) Korlan — O,O-dimethyl-O-(2, 4, 5-trichlorophenyl) phosphorothioate

Korlan which is being used with some success as a systemic against the ox warble fly in North America has a very low toxicity to ticks in comparison with a number of other organo-phosphorus compounds. Against the three strains of blue tick, namely, Frankenwald 1958, Nagaza Park 1958 and Transvaal 1958, it was equally effective (Fig.19). Results of the effect of korlan on these three strains of ticks were rather erratic, which might be attributed to the high concentrations used.

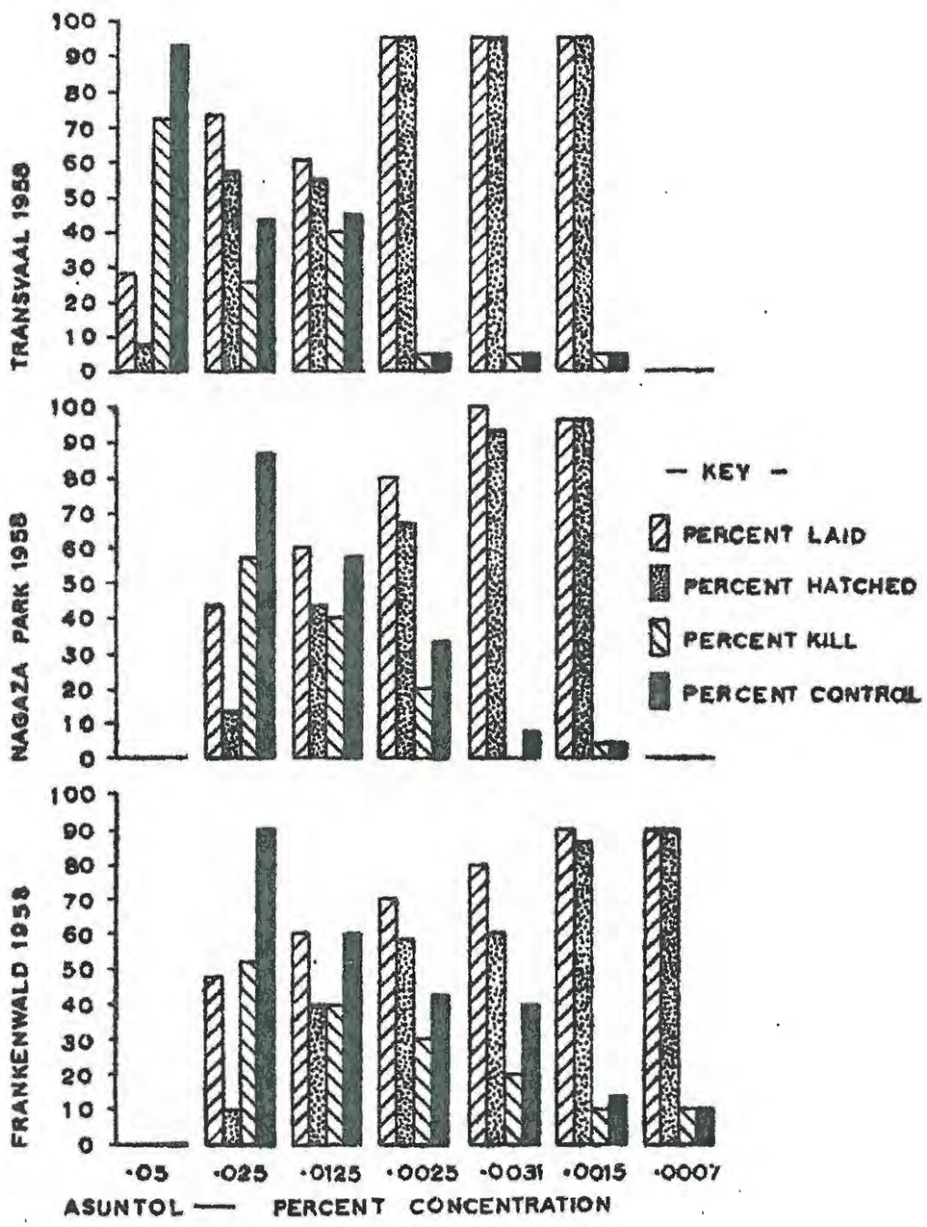


Fig. 18 The effect of asuntol concentrations on fully engorged adult female blue ticks obtained from different localities.

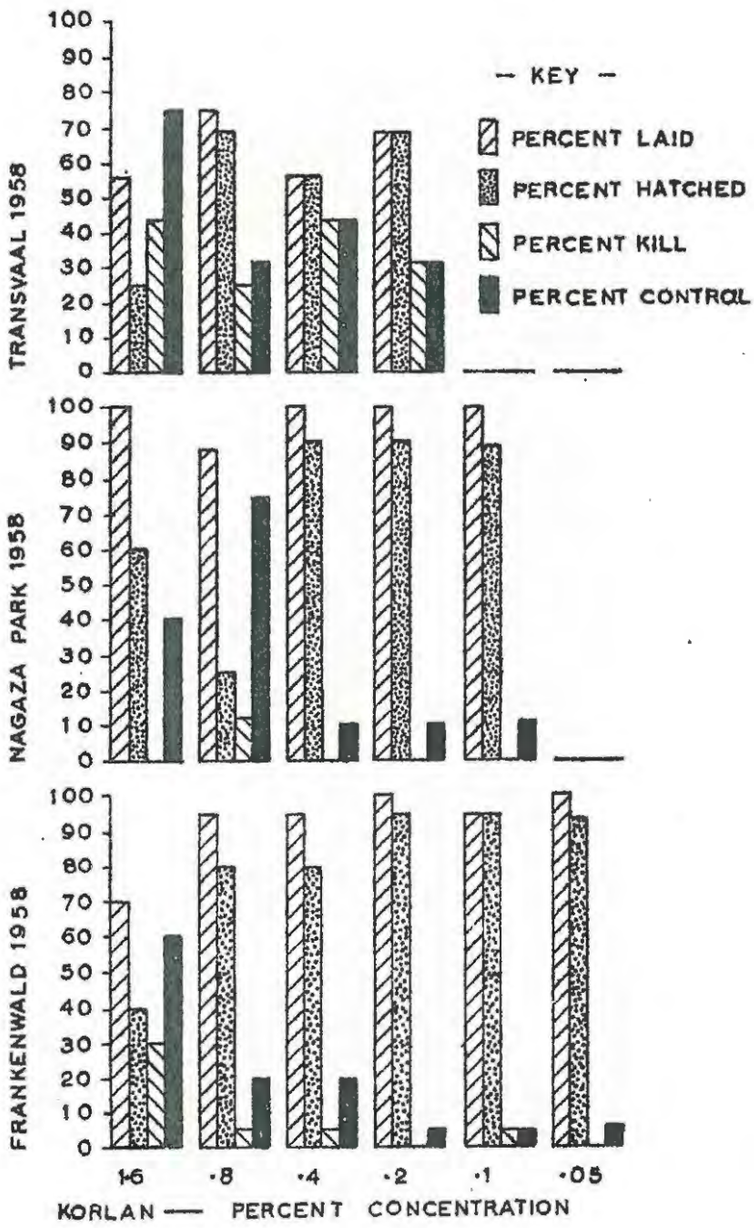
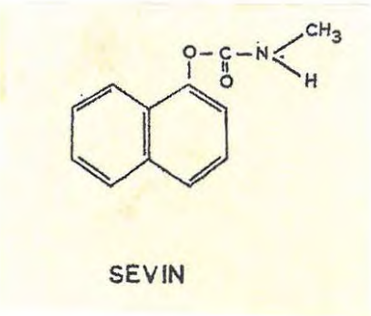


Fig. 19 The effect of korlan concentrations on fully engorged adult female blue ticks obtained from different localities.

(14) Sevin N-methyl-1-naphthylcarbamate



SEVIN

The recently introduced carbamate insecticide, sevin, was examined for effectiveness against Frankenwald 1958, Nagaza Park 1958 and Transvaal 1958 ticks and found to be equally effective against all three strains (Fig. 20).

(15) Pyrethrum A mixture of insecticidal compounds occurring in several species of plants belonging to the genus Chrysanthemum (= Pyrethrum)

Pyrethrum was equally effective against Frankenwald 1958 and Transvaal 1958 ticks but slightly less effective against Nagaza Park 1958 ticks (Fig. 21). The fact that Frankenwald 1958 adult ticks which were resistant only to the BHC group were more affected by pyrethrum than were arsenic-BHC-DDT resistant Nagaza Park 1958 adult ticks suggests that the slight increased tolerance to pyrethrum displayed by Nagaza Park 1958 adult ticks is associated with resistance to either sodium arsenite or DDT.

Note: In all the above tests the insecticides used were prepared in wettable powder form with the exception of dilan, korlan and pyrethrum, which were only available as miscible oils.

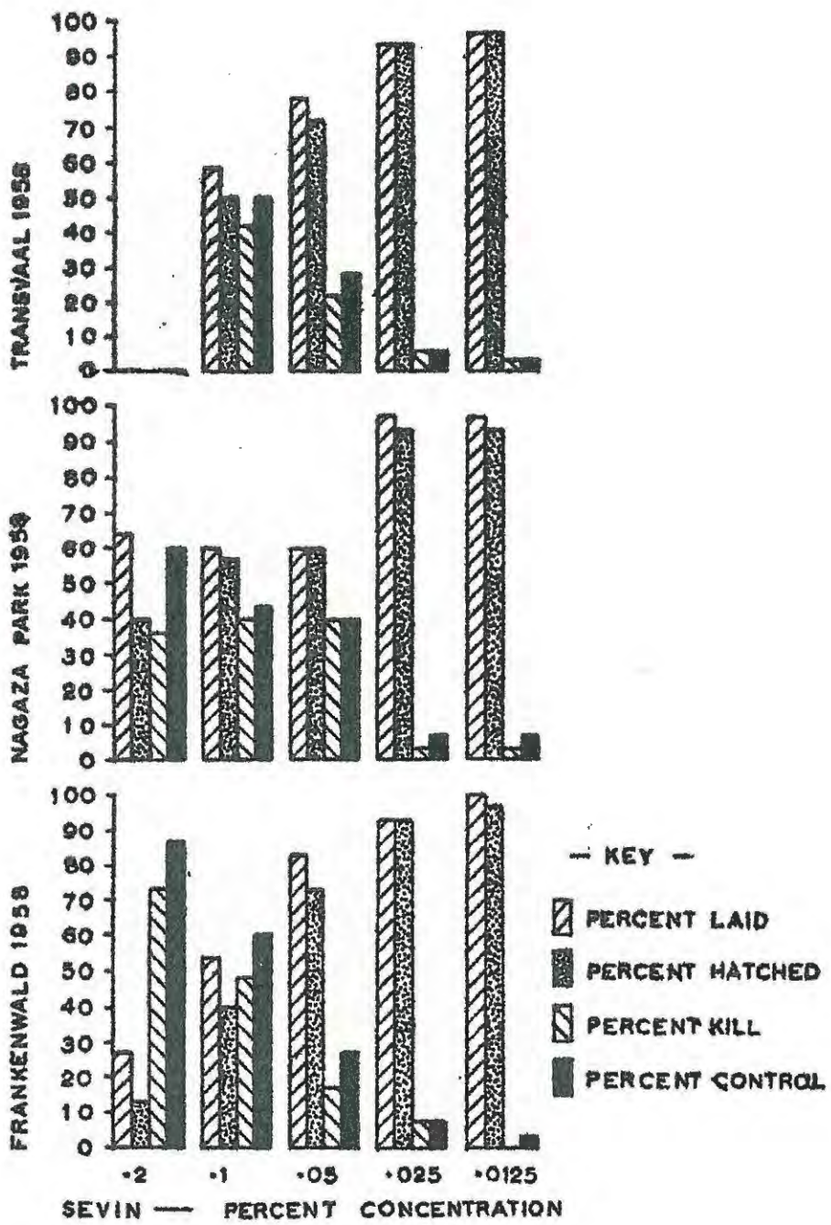


Fig. 20 The effect of sevin concentrations on fully engorged adult female blue ticks obtained from different localities.

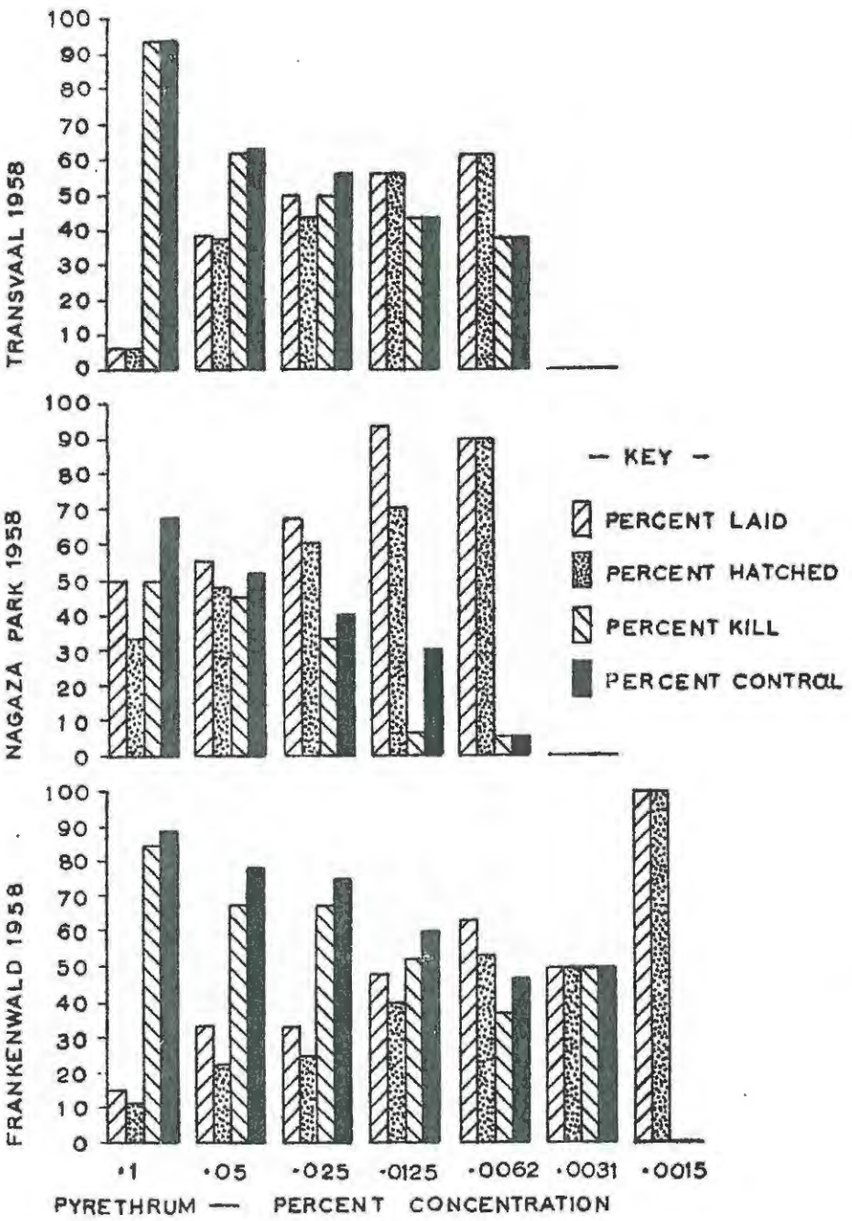


Fig. 21 The effect of pyrethrum concentrations on fully engorged adult female blue ticks obtained from different localities.

(d) Results of tests conducted with unengorged blue tick larvae

The test using blue tick larvae devised to determine response to a range of insecticide concentrations under controlled conditions enabled the treatment of large numbers of larvae. This enabled the precise determination of LC 50 values to which statistical methods for the determination of accuracy could be applied. With this information it was possible to detect significant differences in response to insecticides in a variety of strains of larval ticks.

In all tests percentage mortalities were transformed to probits and plotted against the log concentration of the insecticide dosage. The probit mortality-dosage regression lines were calculated and the slope of the lines determined.

Results were obtained in two series of experiments. The first series was conducted during the period 1954 to 1956 and use was made of larvae from Frankenwald and Ferndale. The designation given to strains of larvae is the same as that for the series of adult tick tests. The second series was conducted during 1958. The strains of tick larvae used in the second series of tests have been termed Frankenwald 1958, Transvaal 1958 Nagaza Park 1958 and Ferndale 1958: these were the same strains as those used in tests conducted with adult ticks. The number of larvae available for the second series was limited and their response to all the insecticides available could not be investigated.

Many attempts to obtain reproducible results with water soluble sodium arsenite using larvae in the test already described were made without success.

Tests were carried out during 1954-1956 using the following insecticides on Frankenwald and Ferndale larvae :-

gamma BHC	(Fig. 22)
Toxaphene	(Fig. 23)
Chlordane	(Fig. 24)
Dieldrin	(Fig. 25)

Aldrin	(Fig. 26)
pp'DDT	(Fig. 27)
Malathion	(Fig. 28)
Diazinon	(Fig. 29)

The following insecticides were used on Frankenwald 1958, Nagaza Park 1958, Ferndale 1958 and Transvaal 1958 strains of larvae :-

Delnav	(Fig. 30)
Asuntol	(Fig. 31)
Korlan	(Fig. 32)
Sevin	(Fig. 33)
Pyrethrum	(Fig. 34)

Finally, the effect of DDT on Transvaal 1958 and Frankenwald 1958 larvae was investigated (Fig. 35) in order to clarify the results obtained with DDT on these strains of adult ticks.

An examination of the results presented in Table 2 indicates a high degree of increased tolerance to several insecticides by Ferndale larvae when the LC50 values are compared with those obtained with Frankenwald larvae.

Table 2. LC50 values, factors of increased tolerance, slope of regression lines and standard deviation of LC 50 for various strains of blue tick larvae treated with a number of insecticides

Insecticide	Strain of tick larvae	LC50 % concentration	Factor of increased tolerance	Slope of regression line	Standard deviation
gamma BHC	Ferndale	0.2395	8×10^2	.4103	.08059
	Frankenwald	0.000298	1	.4188	.05437
Toxaphene	Ferndale	0.8292	9×10^2	.3155	.1023
	Frankenwald	0.000870	1	.3947	.05483
Chlordane	Ferndale	30156*	7×10^6	.0947	.0435
	Frankenwald	0.00426	1	.3495	.06222
Dieldrin	Ferndale	26971*	2×10^7	.0985	3.4201
	Frankenwald	0.00123	1	.4190	.04364
Aldrin	Ferndale	71031*	3×10^7	.0953	3.2482
	Frankenwald	0.00179	1	.4068	.04891
pp'DDT	Ferndale	0.00345	1	.4542	.08272
	Frankenwald	0.00491	1.43	.3778	.04953
	Transvaal 1958	0.02154	6.25	.4725	.07927
	Frankenwald 1958	0.01045	3.03	.520	.0682

Table 2 (continued)

Insecticide	Strain of tick larvae	LC50 % concentration	Factor of increased tolerance	Slope of regression line	Standard deviation
Pyrethrum	Frankenwald 1958	0.0000094609	2.41	.6443	.0429
	Ferndale 1958	0.0000096261	2.45	.6695	.03993
	Nagaza Park 1958	0.000072401	18.3	.6857	.03586
	Transvaal 1958	0.000003955	1	.6500	.01752
Malathion	Ferndale	0.08199	1	1.3073	.02533
	Frankenwald	0.1034	1.26	.8311	.02752
Diazinon	Ferndale	0.000756	1.02	.5045	.04764
	Frankenwald	0.000742	1	.4203	.05819
Delnav	Frankenwald 1958	0.00029881	1.28	.5771	.04013
	Ferndale 1958	0.00023298	1	.4216	.08154
	Nagaza Park 1958	0.00031505	1.35	.5220	.05490
	Transvaal 1958	0.0002705	1.16	.2900	.01723
Asuntol	Frankenwald 1958	0.00034651	1.38	.7244	.03614
	Ferndale 1958	0.00053108	2.12	.8766	.03574
	Nagaza Park 1958	0.00071353	2.85	.9680	.02906
	Transvaal 1958	0.0002504	1	.9050	.046139
Korlan	Frankenwald 1958	0.0018804	2.87	.9104	.02775
	Ferndale 1958	0.00065464	1	.7772	.03234
	Nagaza Park 1958	0.0029262	4.5	.8108	.03501
	Transvaal 1958	0.003421	5.25	.4350	.0639
Sevin	Frankenwald 1958	0.0040430	1	.2938	.1037
	Ferndale 1958	0.0056642	1.4	.6350	.03683
	Nagaza Park 1958	0.0048756	1.2	.3758	.06356
	Transvaal 1958	0.005868	1.45	.3000	.04273

* By extrapolation

Insecticides to which increased tolerance was shown by Ferndale larvae are gamma BHC, toxaphene, chlordane, dieldrin and aldrin. The effect of pp'DDT on Ferndale and Frankenwald larvae was similar; a concentration of DDT 1.4 times greater being required to produce the same result on Frankenwald larvae as was obtained with Ferndale larvae. This difference was not significant ($P > 0.05$). It will be observed in respect of DDT that the LC50 value for Frankenwald 1958 larvae was 2.1 fold higher than it was for Frankenwald larvae which were examined two years previously during 1956. However, this apparent difference in response is not significant ($P > 0.05$). Based on LC50 values Transvaal 1958 larvae were 4.3 times more tolerant to pp'DDT than Frankenwald larvae and two-fold more tolerant than Frankenwald 1958 larvae. These differences were not significant ($P > 0.05$) and it must be accepted that Ferndale, Frankenwald,

Frankenwald 1958 and Transvaal 1958 larval ticks all respond similarly to DDT and that the differences in LC50 values are a result of unavoidable errors in technique.

Ferndale and Frankenwald ticks were similarly affected by both malathion and diazinon. Delnav was equally effective against Ferndale 1958, Frankenwald 1958 and Transvaal 1958 larvae but a slightly higher concentration was required to produce the same response in Nagaza Park 1958 larvae. However, the difference between the response of Nagaza Park 1958 larvae and the other three strains was not significant ($P > 0.05$). Similar results were obtained with Frankenwald 1958, Transvaal 1958 and Ferndale 1958 larvae treated with korlan. Likewise these three strains responded similarly to asuntol. With both korlan and asuntol treated larvae, the LC50 values were slightly higher than those obtained with Frankenwald 1958, Transvaal 1958 and Ferndale 1958 strains but the difference was not significant ($P > 0.05$). When using pyrethrum Frankenwald 1958 and Ferndale 1958 were 2.41 and 2.45 fold respectively more tolerant than Transvaal 1958 larvae. However, this difference was not significant ($P > 0.05$). Nagaza Park 1958 larvae showed an 18.3 fold increase in tolerance as compared with the Transvaal 1958 strain. The difference in tolerance displayed by Nagaza Park 1958 larvae when compared with Transvaal 1958, Frankenwald 1958 and Ferndale 1958 strains of larvae was statistically significant ($P < 0.05$). Pyrethrum has never been used in South Africa for tick control and the resistance to pyrethrum in Nagaza Park ticks is clearly a cross tolerance conferred by resistance previously developed to some other insecticide. As the DDT-BHC-arsenic resistant Nagaza Park 1958 tick was the only strain cross tolerant to pyrethrum, and since the BHC-arsenic resistant Ferndale 1958 ticks, BHC resistant Frankenwald 1958 ticks, and the insecticide sensitive Transvaal 1958 ticks were all equally susceptible to pyrethrum, the only conclusion that can be arrived at is that the blue tick resistant to DDT is automatically cross resistant to pyrethrum.

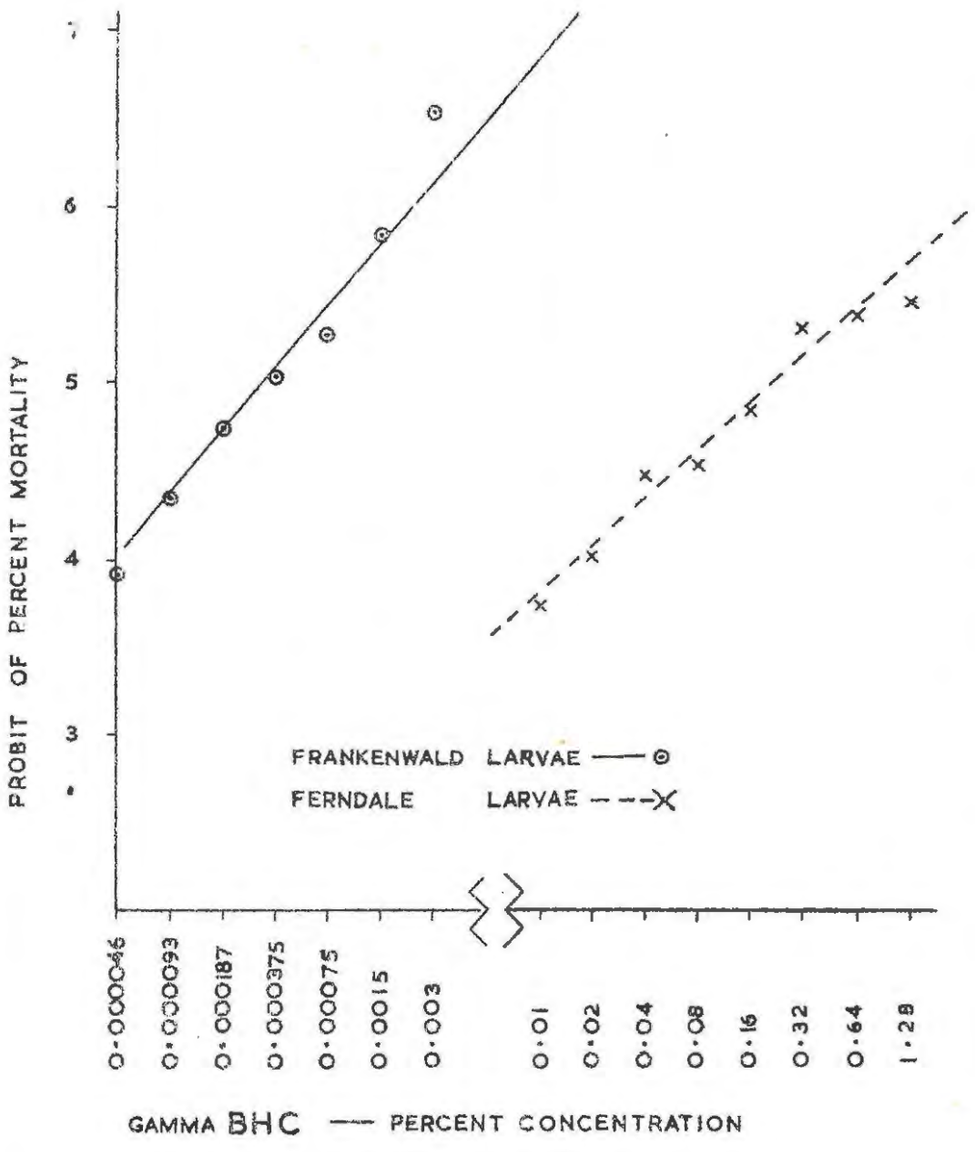


Fig. 22 Log. concentration-probit mortality lines obtained with Frankenwald and Ferndale tick larvae treated with gamma BHC.

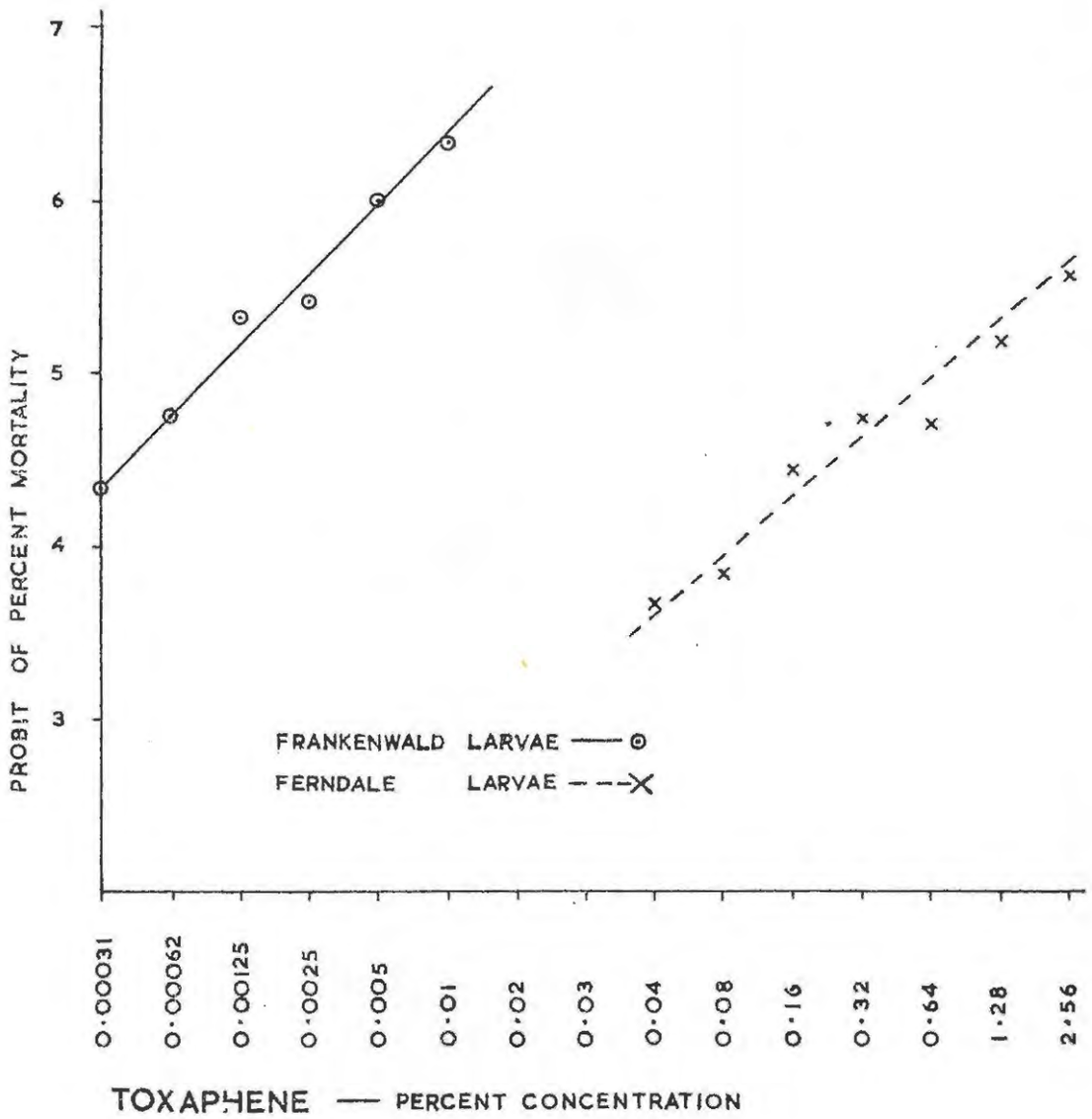


Fig. 23 Log. concentration-probit mortality lines obtained with Frankenwald and Ferndale tick larvae treated with toxaphene.

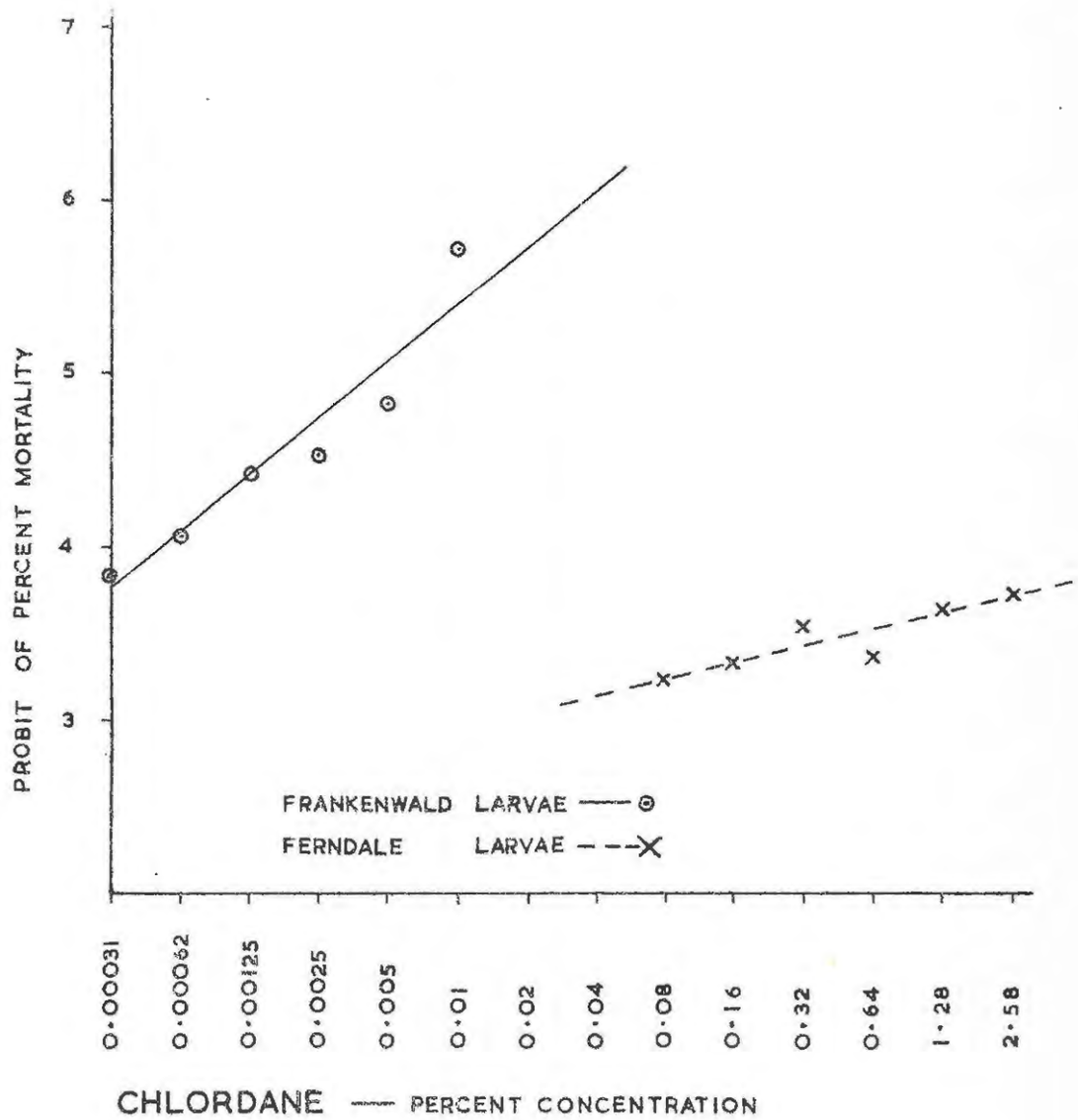


Fig. 24 Log. concentration-probit mortality lines obtained with Frankenwald and Ferndale tick larvae treated with chlordane.

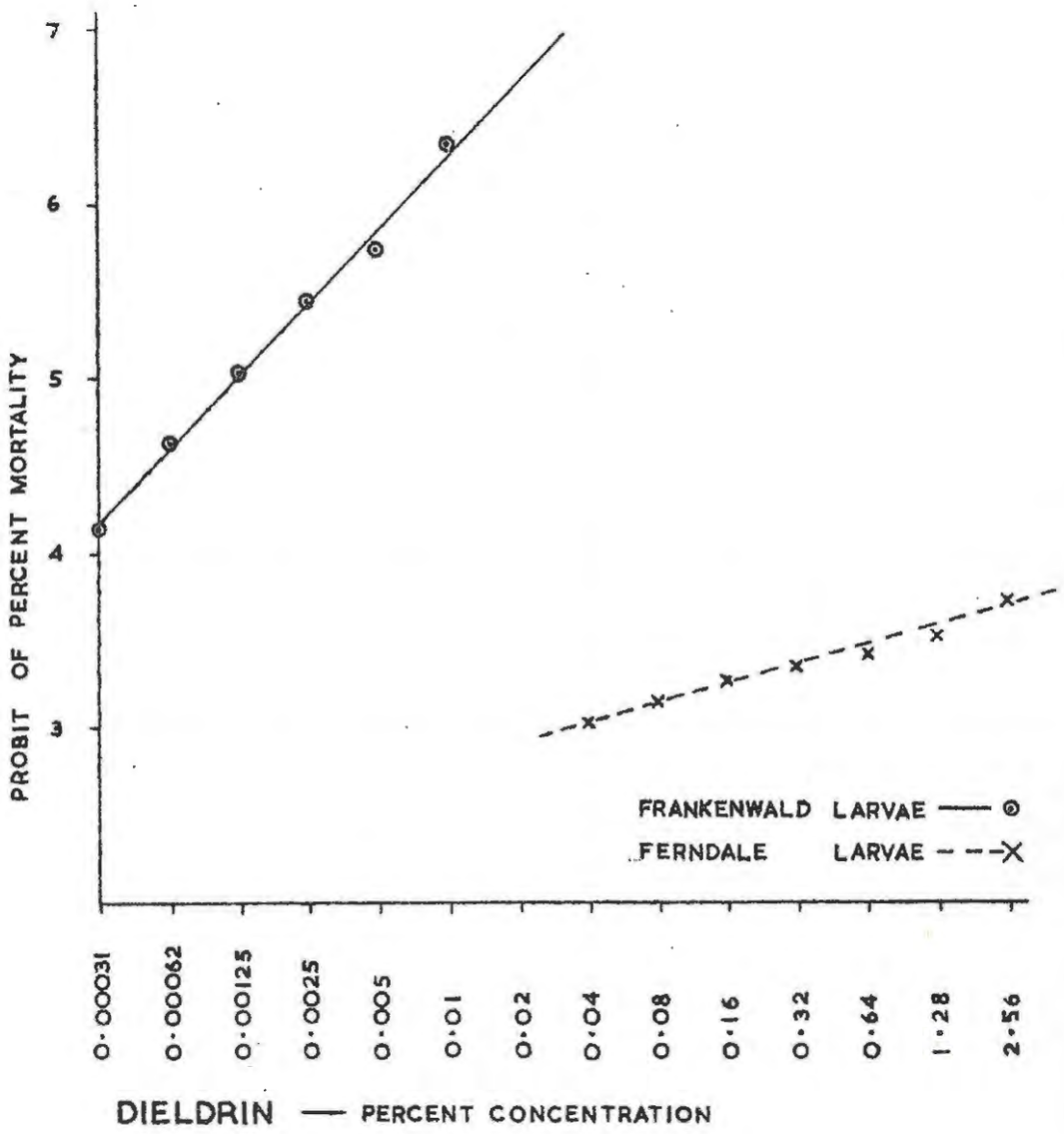


Fig. 25 Log. concentration-probit mortality lines obtained with Frankenwald and Ferndale tick larvae treated with dieldrin.

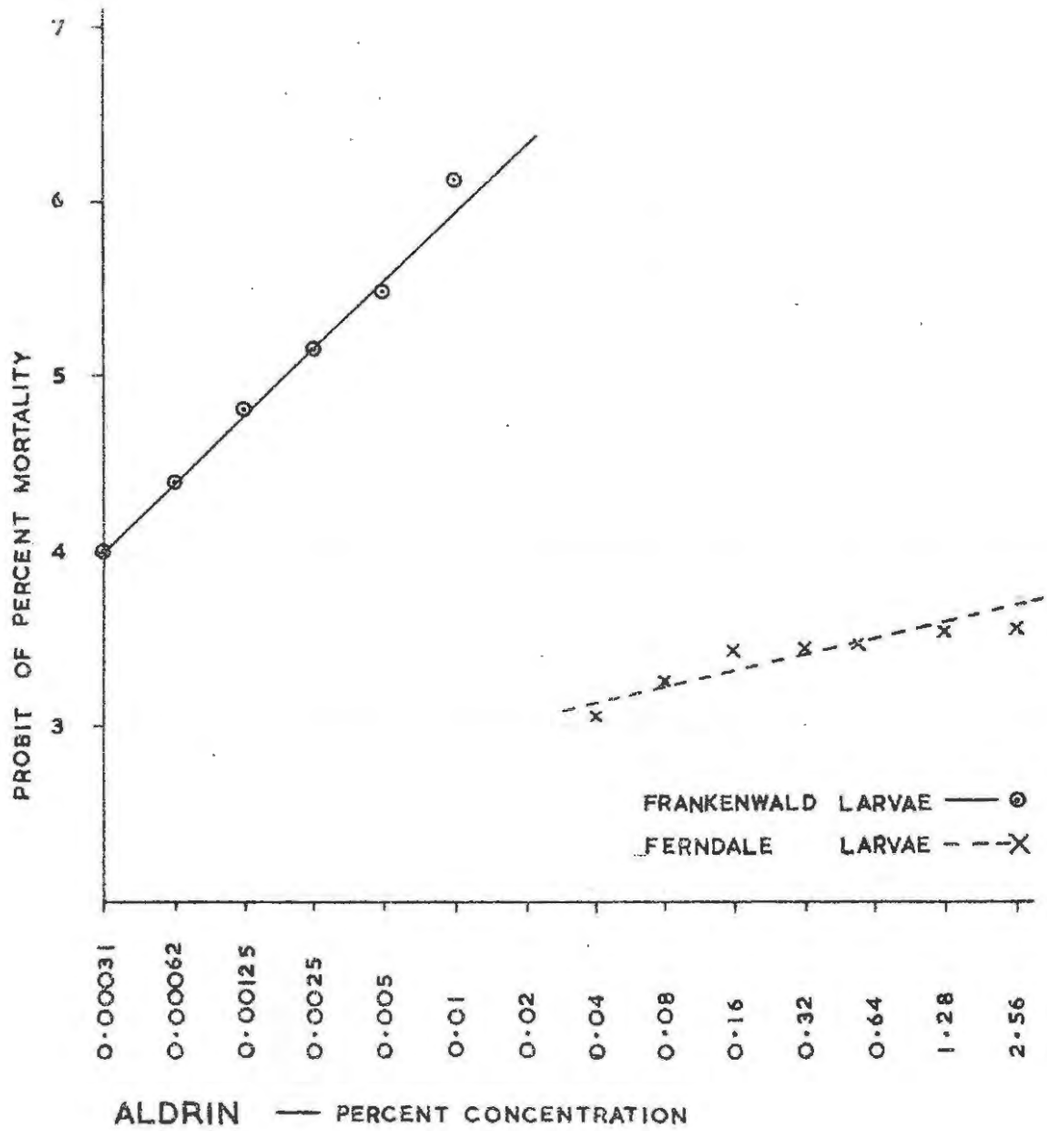


Fig. 26 Log. concentration-probit mortality lines obtained with Frankenwald and Ferndale tick larvae treated with aldrin.

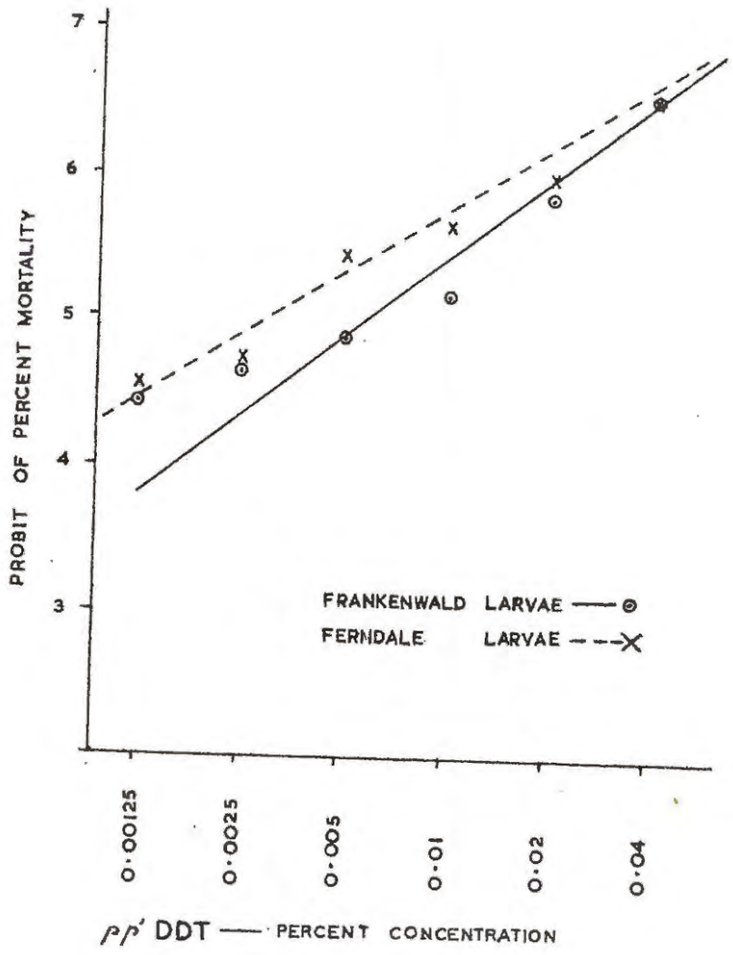


Fig. 27 Log. concentration-probit mortality lines obtained with Frankenwald and Ferndale tick larvae treated with pp'DDT.

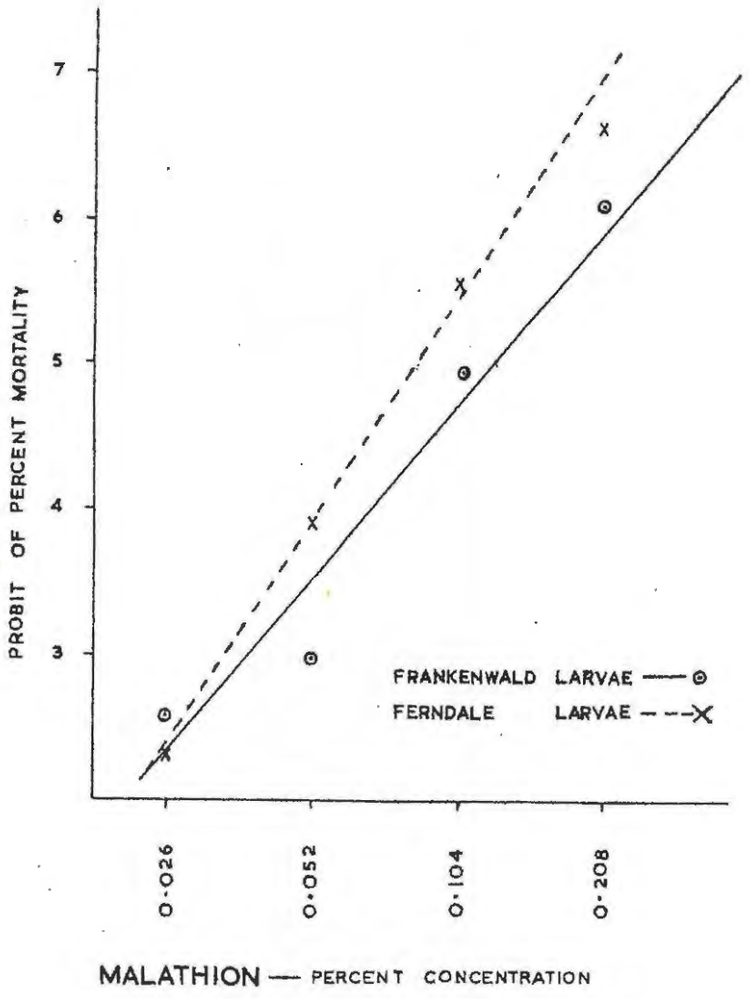


Fig. 28 Log. concentration-probit mortality lines obtained with Frankenwald and Ferndale tick larvae treated with malathion.

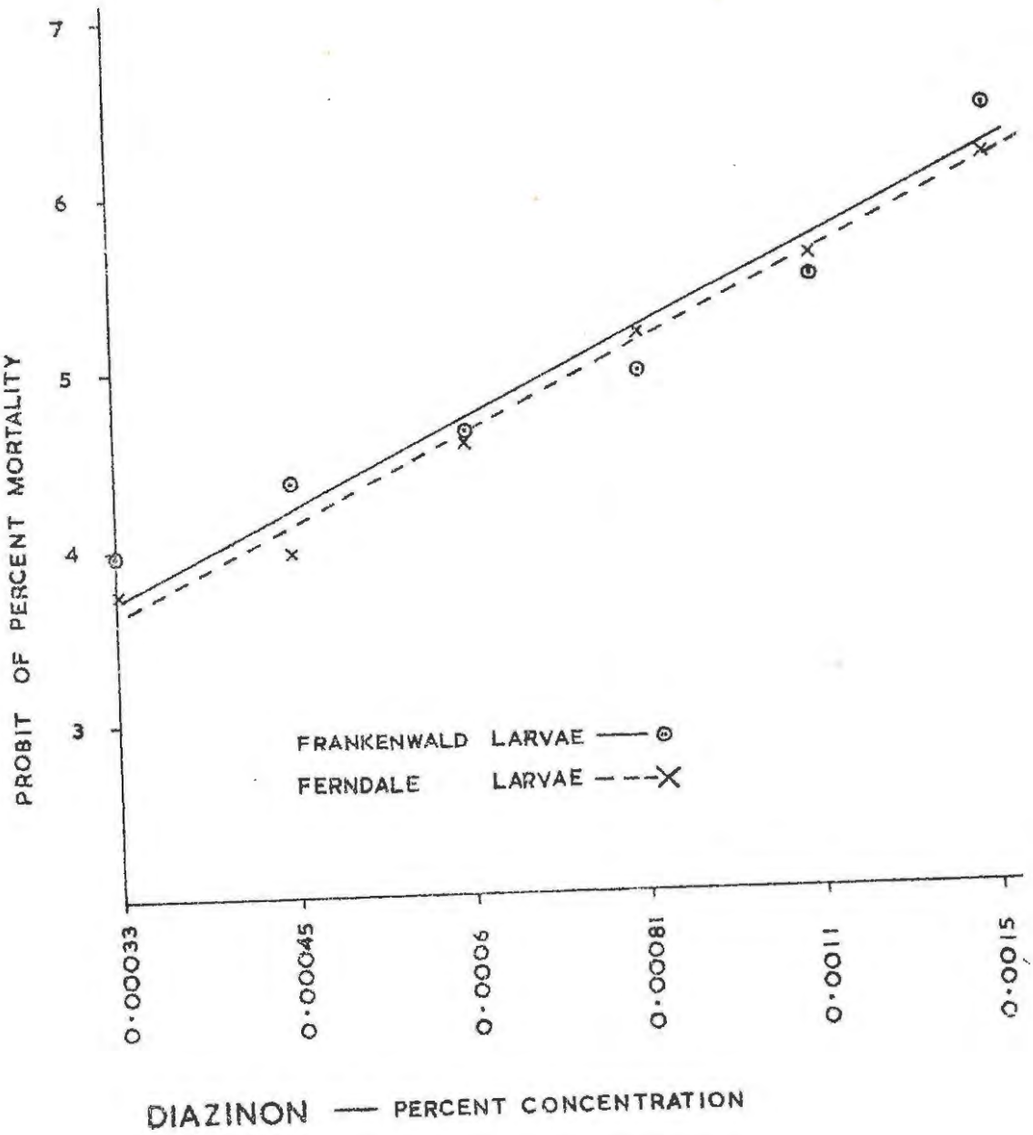


Fig. 29 Log. concentration-probit mortality lines obtained with Frankenwald and Ferndale tick larvae treated with diazinon.

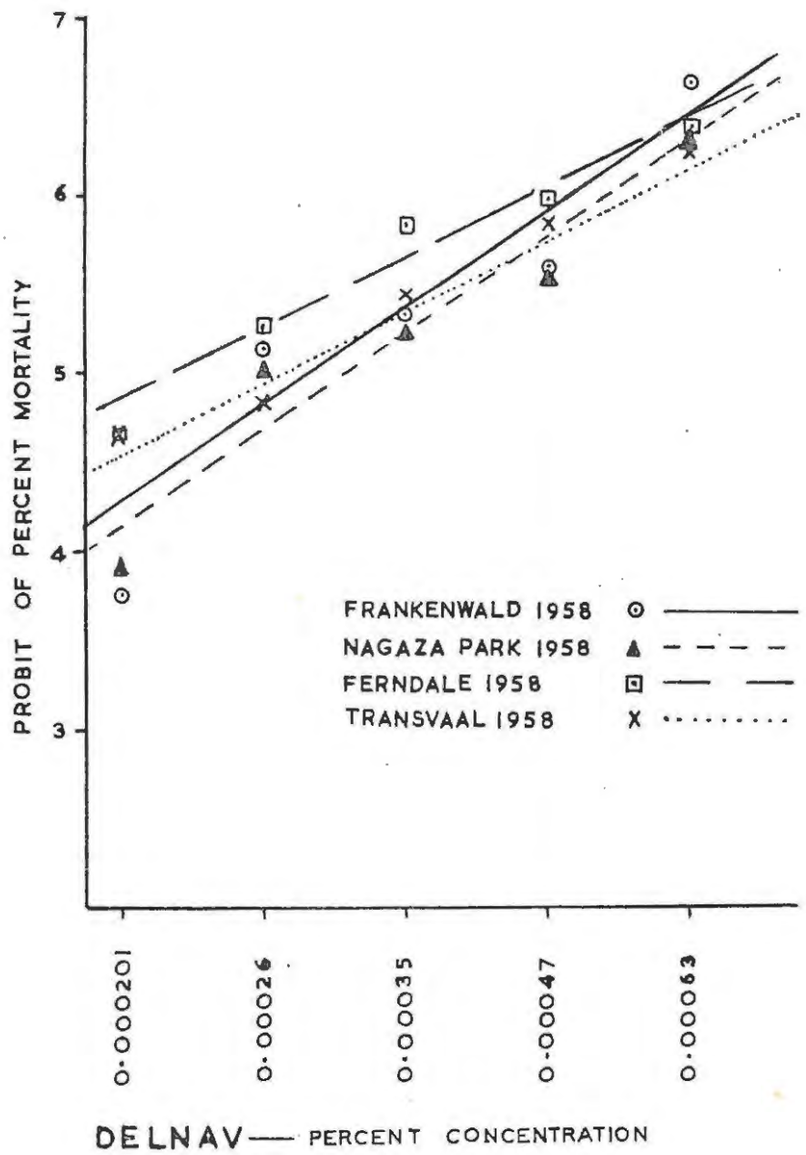


Fig. 30 Log. concentration-probit mortality lines obtained with Frankenwald 1958, Nagaza Park 1958, Ferndale 1958 and Transvaal 1958 tick larvae treated with delnav.

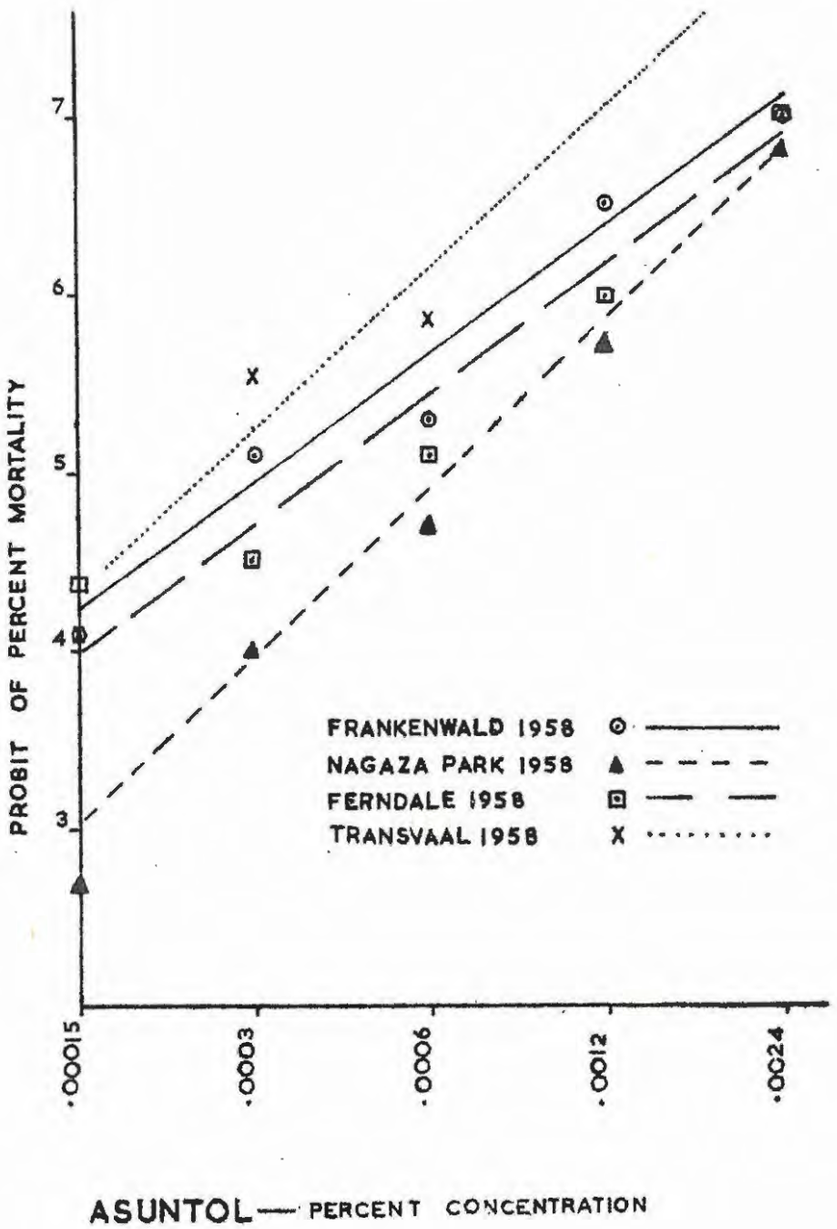


Fig. 31 Log. concentration-probit mortality lines obtained with Frankenwald 1958, Nagaza Park 1958, Ferndale 1958 and Transvaal 1958 tick larvae treated with asuntol.

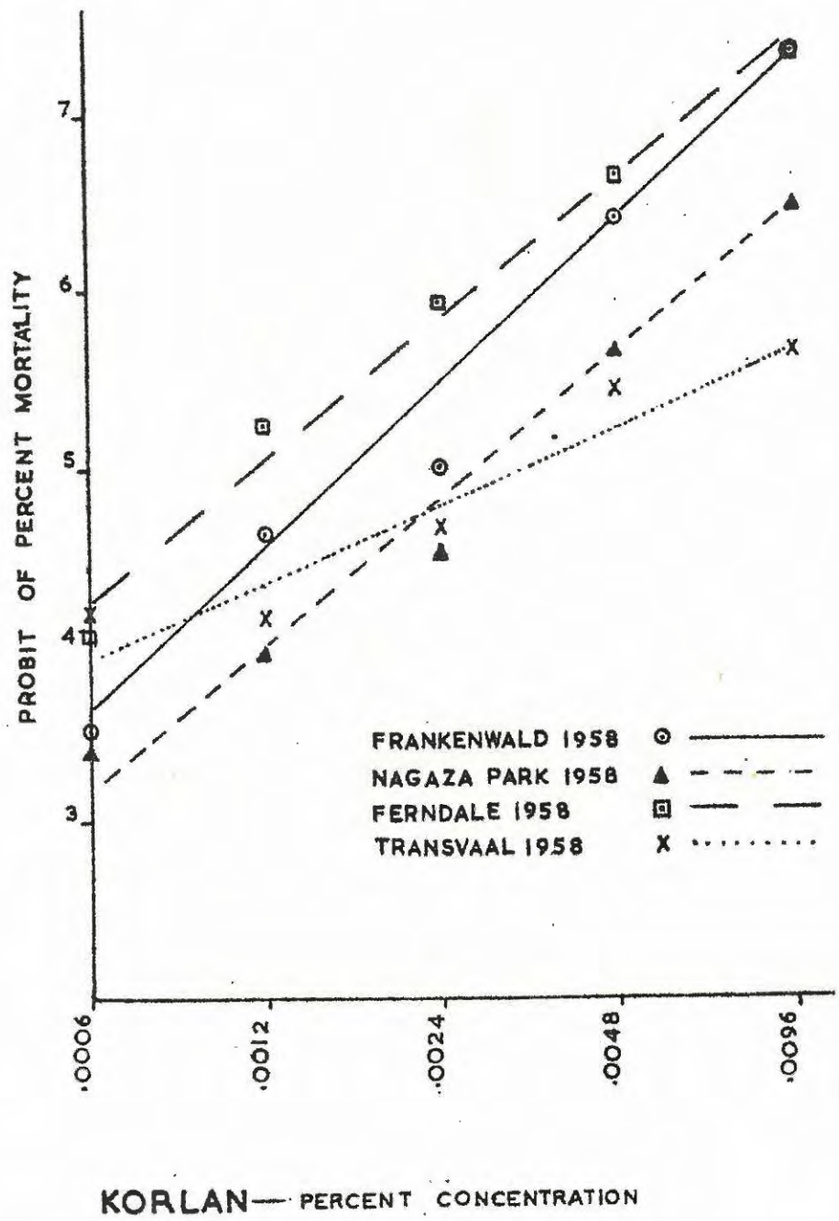


Fig. 32 Log. concentration-probit mortality lines obtained with Frankenwald 1958, Nagaza Park 1958, Ferndale 1958 and Transvaal 1958 tick larvae treated with korlan.

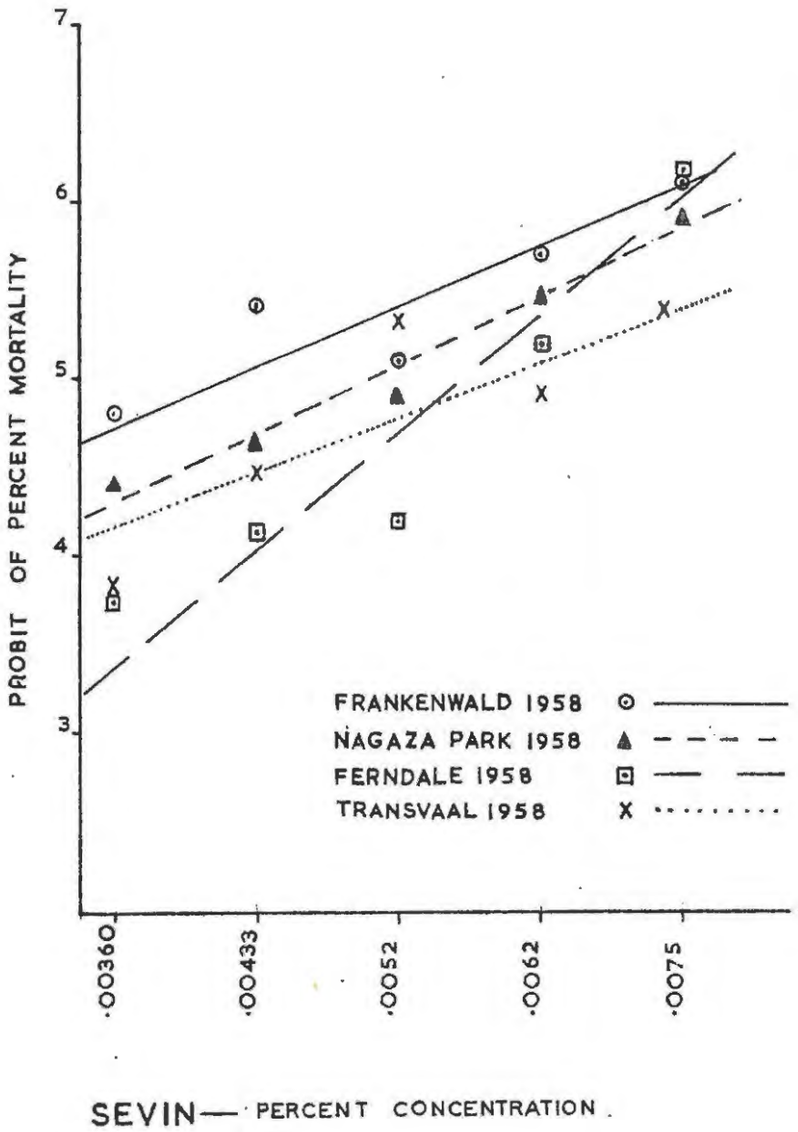


Fig. 33 Log. concentration-probit mortality lines obtained with Frankenwald 1958, Nagaza Park 1958, Ferndale 1958 and Transvaal 1958 tick larvae treated with sevin.

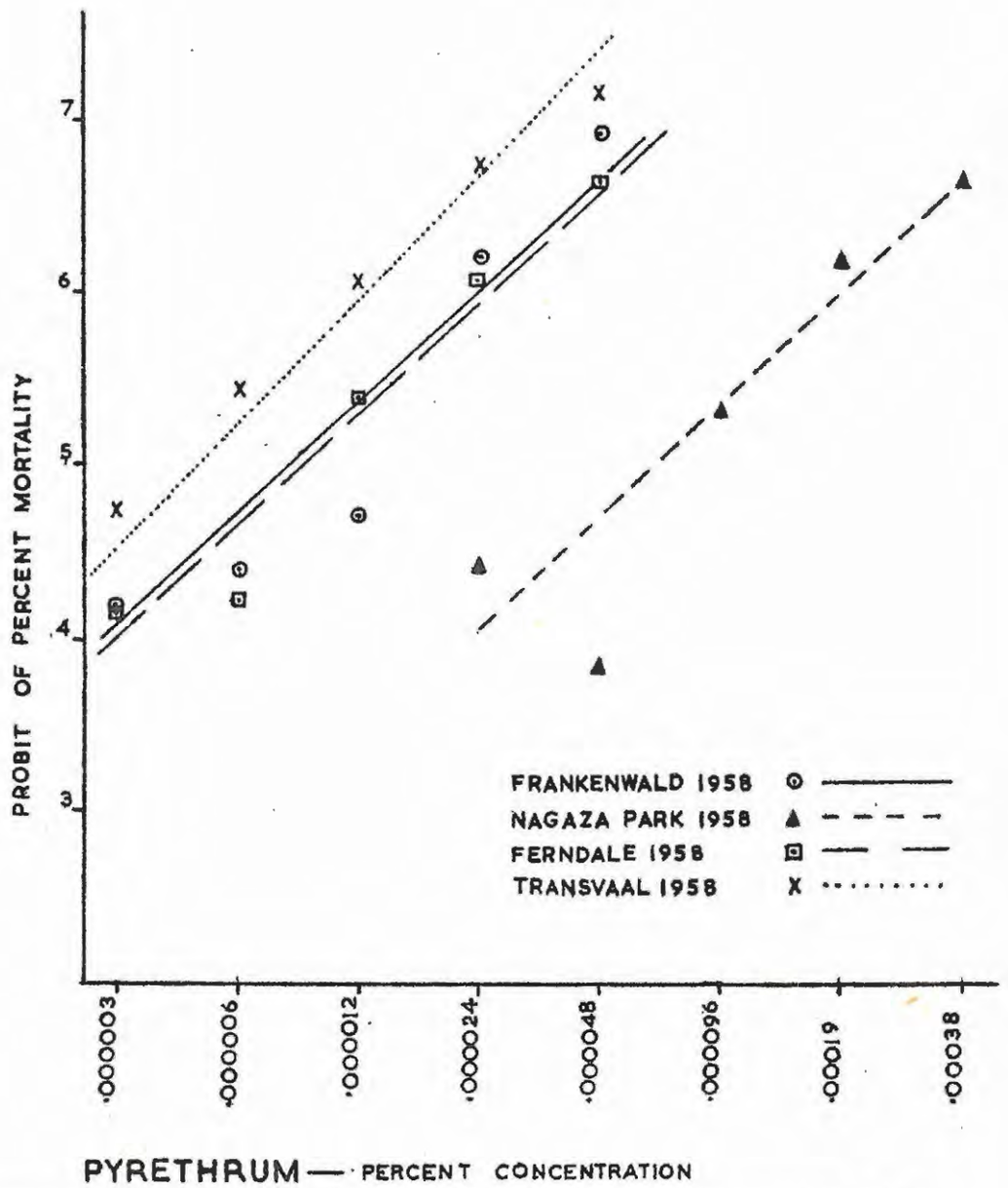


Fig. 34 Log. concentration-probit mortality lines obtained with Frankenwald 1958, Nagaza Park 1958, Ferndale 1958 and Transvaal 1958 tick larvae treated with pyrethrum.

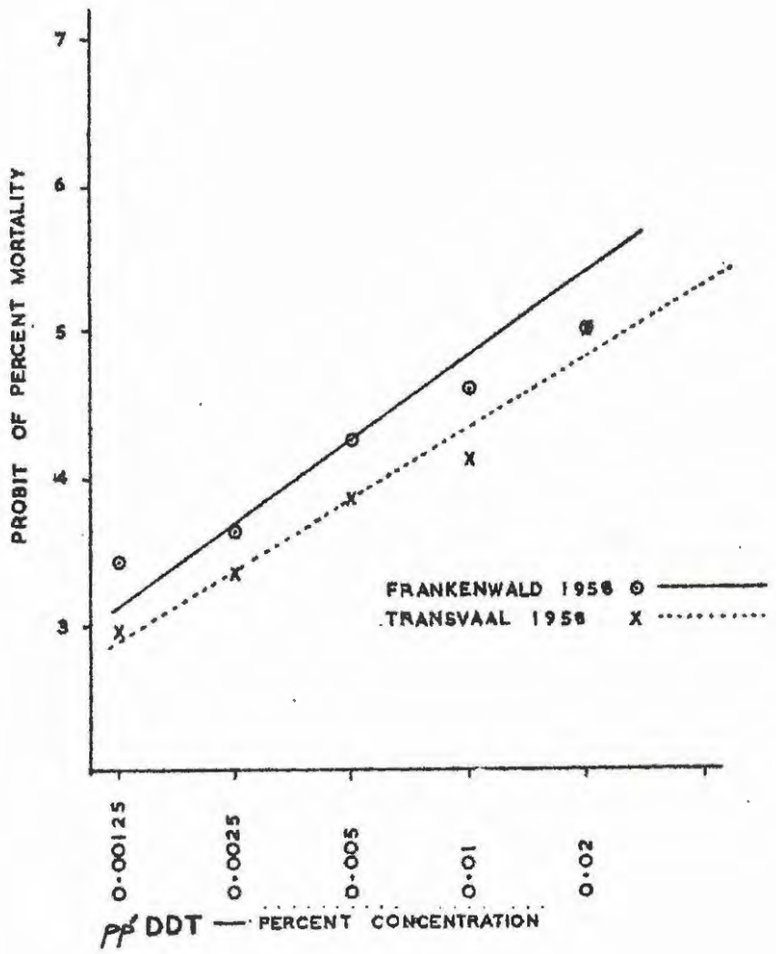


Fig. 35 Log. concentration-probit mortality lines obtained with Frankenwald 1958 and Transvaal 1958 tick larvae treated with pp'DDT.

Sevin was equally effective against all four strains of larvae including the arsenic-BHC-DDT resistant Nagaza Park 1958 strain.

The comparative effectiveness of insecticides on ticks is of considerable practical importance. With the accurate determination of the LC50 for a number of insecticides presented in Table 2 it is possible to place the series of insecticides used on sensitive Frankenwald and Transvaal 1958 larvae in order of effectiveness. In order of decreasing effectiveness the series of insecticides is as follows :-

1. Pyrethrum
2. gamma BHC
3. Asuntol
4. Delnav
5. Diazinon
6. Toxaphene
7. Dieldrin
8. Aldrin
9. Korlan
10. Chlordane
11. pp'DDT
12. Sevin
13. Malathion

(e) Summary of conclusions drawn from results of tests conducted with both larval and adult female ticks

A summary of results of tests with larvae and adult female ticks is presented in Tables 3 and 4.

Frankenwald ticks

Originally Frankenwald ticks were assumed to be sensitive to all insecticides and were used as a standard strain with which other strains were compared. Frankenwald 1958 ticks were found to display increased tolerance to gamma BHC, toxaphene, chlordane, dieldrin and aldrin, but were sensitive to sodium arsenite, DDT, dilan, malathion, diazinon, delnav, asuntol, korlan, sevin and pyrethrum (Tables 3 and 4).

Ferndale ticks

The Ferndale strain of ticks showed an increased tolerance to sodium arsenite, gamma BHC, toxaphene, chlordane, dieldrin, and aldrin but were sensitive to pp'DDT, dilan, malathion, diazinon, delnav, korlan, sevin and pyrethrum (Tables 3 and 4).

Allandale ticks

Allandale ticks showed increased tolerance to sodium arsenite, gamma BHC, toxaphene, chlordane, dieldrin, aldrin and pp'DDT but were sensitive to malathion and diazinon (Tables 3 and 4).

Nagaza Park 1958 ticks

This strain of ticks showed an increased tolerance to gamma BHC, toxaphene, chlordane, dieldrin, aldrin, DDT, dilan and pyrethrum. Although the tolerance of Nagaza Park 1958 ticks to sodium arsenite was greater than that of the arsenic sensitive Frankenwald strain there was an indication of a drop of tolerance to sodium arsenite as compared with other strains of arsenic resistant ticks (Tables 3 & 4 and Figs. 2 & 3).

Nagaza Park 1958 ticks were sensitive to malathion, diazinon, delnav, asuntol, korlan and sevin (Tables 3 and 4).

Transvaal 1958 ticks

The Transvaal 1958 strain of ticks showed no statistically significant increase in tolerance to sodium arsenite, pp'DDT, gamma BHC, delnav, asuntol, korlan, sevin and pyrethrum (Tables 3 & 4).

Table 3. A summary of the status of resistance to a number of insecticides by several strains of blue tick larvae by comparison of LC 50 values with that of a sensitive strain

Insecticide	Frankenwald	Ferndale	Ferndale 1958	Transvaal 1958	Nagaza Park 1958	Frankenwald 1958
gamma BHC	S	R				
Toxaphene	S	R				
Chlordane	S	R				
Dieldrin	S	R				
Aldrin	S	R				
DDT	S	S		S	R(Whitehead 1956)	S
Pyrethrum			S	S	R	S
Malathion	S	S				
Diazinon	S	S				
Delnav			S	S	S	S
Asuntol			S	S	S	S
Korlan			S	S	S	S
Sevin			S	S	S	S

Key: S = sensitive
R = resistant

Table 4. Summary of the effect of a number of insecticides on various strains of the adult female blue tick

Insecticide	Percent Concentration	Percent control of various strains of ticks					
		Frankenwald	Ferndale	Allandale	Transvaal 1958	Nagaza Park 1958	Frankenwald 1958
Sodium arsenite	0.32 As_2O_3	xxxx	xx	xxx	xxxx	xxxx	xxxx
	0.16	xxxx	x	xxx	xxx	xx	xx
gamma BHC	0.01	xxxx	x	x	xxx	x	x
Toxaphene	0.25	xxxx	x	x			x
Chlordane	0.25	xxxx	x	x			x
Dieldrin	0.0625	xxxx	x	x			x
Aldrin	0.125	xxxx	x	x			x
DDT	0.5	xxxx	xxxx	x	xx	x	xx
"	0.25	xxx	xx	x	xxx	x	x
Dilan	0.5	xxxx	xxxx	x			xx
"	0.25	xx	xx	x			x
Pyrethrum	0.1				xxxx	xxx	xxxx
Malathion	1.0	xxxx	xxxx	xxxx			xxxx
Diazinon	0.03125	xxxx	xxxx	xxxx			xxxx
Delnav	0.025				xxx	xxxx	xxxx
Asuntol	0.025				xx	xxxx	xxxx
Korlan	1.6				xxxx	xx	xxx
Sevin	0.1				xxx	xx	xxx

Key : xxxx = 75% to 100% control
xxx = 50% to 75% control
xx = 25% to 50% control
x = 0% to 25% control

(f) The development of insecticide resistance in blue tick populations

All individuals of an insect population treated with a toxicant do not respond alike. If the response, usually measured as a percentage mortality of batches of insects exposed to a toxicant is plotted graphically against the log dose the distribution of response of individuals in the population approaches normality as shown in the accompanying diagram.

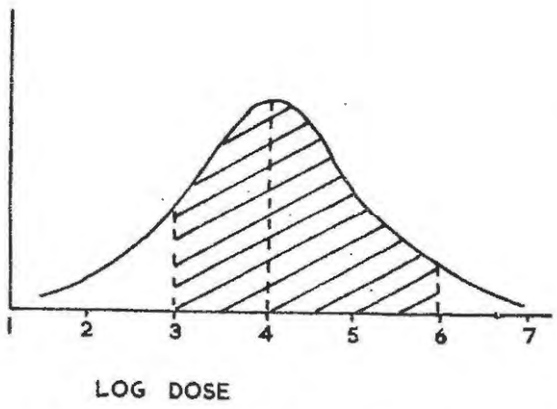


Diagram 3. The distribution of response to a toxicant in an insect population.

The area between the two ordinates 3 and 6 represents the proportion of the population having tolerances lying between these dosage limits. The area between ordinates 1 and 4 represents 50% of the population and at a dosage represented by 4, 50% of the population would be killed. Such a distribution of response to toxicants is usually very similar in populations of the same species occurring naturally in different localities although the distribution of response might be very different in different species even from the same locality. Except where insecticide resistance has arisen, the response of the blue tick from several localities appears very similar. By comparing LC50 values for several strains of tick larvae (Table 5) obtained by treatment with malathion, diazinon, delnav, asuntol and sevin to which the blue tick is not resistant, very little difference between the strains was observed with any one insecticide.

Table 5. LC50 values for several strains of tick larvae from different localities

Locality of tick larvae	LC50 Percent insecticide concentration				
	Malathion	Diazinon	Delnav	Asuntol	Sevin
Ferndale	.08199	0.00075	.000232	.000531	.00566
Frankenwald 1958			.000298	.000346	.00404
Frankenwald	0.1034	0.000742			
Nagaza Park 1958			.000315	.000713	.00487
Transvaal 1958			.000270	.000250	.00586

In none of these examples was the difference in LC50 greater than three-fold and in all cases the differences were not statistically significant ($P > 0.05$). From the histograms (Figs. 15, 16, 17, 18 and 20) similarity in response in the adult tick populations is also noticeable. The similarity of response to sodium arsenite and gamma BHC between adult ticks used in tests by Whitnall and Bradford (1947) in 1945 may be compared in Table 6 with an entirely different source of ticks used in similar tests carried out during the period 1954-1956 and 1958. Similar response in an identical test was also obtained with the Australian cattle tick, Boophilus microplus (Canestrini) (Hitchcock, 1953) (Table 6).

Table 6. The effect of sodium arsenite and gamma BHC on adult female Boophilids from Australia and different localities in South Africa

	Year of examination	Locality	Concentration	Percent control	
				Resistant strain	Sensitive strain
Sodium arsenite					
B. microplus	1953	Queensland, Australia	0.04% As_2O_3	4	25
B. decoloratus	1945	Eastern Cape, S.A.	0.04% As_2O_3	5	24
"	1958	Transvaal, S.A.	0.04% As_2O_3	4	23
BHC					
B. microplus	1953	Queensland, Australia	0.005%	0	62
B. decoloratus	1945	Eastern Cape, S.A.	0.005%	0	60
B. decoloratus	1958	Transvaal, S.A.	0.005%	7	83
B. microplus	1953	Queensland, Australia	0.2%	10	100
B. decoloratus	1945	Eastern Cape, S.A.	0.25%	9	100
B. decoloratus	1958	Transvaal, S.A.	0.25%	25	93

Differences in response to insecticides is conveniently shown by differences in LC50. However, a comparison of LC50 values of different strains shows only a measure of the degree of resistance already existing and is of no use in predicting what might be taking place within the entire population. Hoskins and Gordon (1956) making use of the results of a number of workers showed that with continued selection of a population the LC50 value gradually increased with a lessening of the slope of the log dose-probit mortality line which indicates an increase in the standard deviation of the response of the population. With continued selection the degree of resistance reached a plateau with a greatly increased LC50 value but with the log dose-probit mortality line parallel with that of the original population i.e. the same standard deviation.

In the initial stages of selection the weaker individuals of the population will be eliminated and a log dose-probit mortality line will tend to become steeper with only a slight increase in LC50 value. After continued selection the proportion of the more resistant individuals would increase and in time the slope of the log dose-probit mortality line would become progressively less until maximum resistance is reached. This sequence of changes in a population under selection pressure is illustrated in the following diagram.

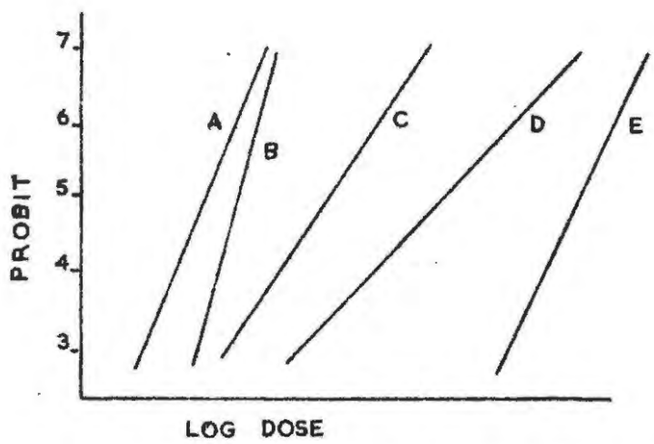


Diagram 4. **Diagram illustrating the change in slope of the log dose-probit mortality curve characteristic of the development of resistant populations.**

The log dose-probit regression line (A) represents the distribution of response in an unselected sensitive population. The line (B) represents the distribution in response in the initial stages of selection when the more sensitive individuals are being eliminated. The lines (C) and (D) are a result of a later stage in the selection process where the more resistant individuals are increasing in proportion. The line (E) represents the maximum resistance where although the response is at a much higher level the standard deviation of the distribution of response once again becomes the same as that of the original population (A).

The practical importance of these observations is that by determining the slope of the log dose-probit mortality line some indication can be obtained on the behaviour of a population as a whole.

The results of insecticide response tests on tick larvae (Part II (d)) indicate that with strains that have developed resistance to chlordane (Fig. 24), dieldrin (Fig. 25), and aldrin (Fig. 26) the slope of the log concentration-probit mortality line has lessened considerably with the slopes significantly different. This suggests, according to Hoskins and Gordon's hypothesis that considerable selection had taken place in respect of these three insecticides but that the process was not yet complete suggesting that a population of maximum resistance had not yet been attained.

With gamma BHC (Fig. 22) and toxaphene (Fig. 23) to which some strains of larvae show a high tolerance, the slope of the log concentration-probit mortality lines of resistant strains were not significantly different from that of the sensitive strains suggesting that the resistant strains had reached a plateau. This indicates that resistance to gamma BHC and toxaphene are at the highest level which can arise under the existing conditions of selection.

Although it has already been shown that resistance to gamma BHC in ticks resulted in a cross-resistance to toxaphene, chlordane, dieldrin and aldrin (Ferndale ticks) and resistance to toxaphene resulted in a cross tolerance to gamma BHC, chlordane, dieldrin and aldrin (Frankenwald 1958 ticks), these results and the figures presented in Table 7 indicate a clear difference between resistance to gamma BHC and toxaphene on the one hand and chlordane, dieldrin and aldrin on the other.

Table 7. LC50 values and the slope of the log concentration-probit mortality lines obtained with a number of chlorinated cyclic hydrocarbon insecticides on resistant and sensitive strains of tick larvae

Insecticide	LC50 values % concentration		Factor of increased tolerance in the resistant strain	Slope of log concentration -probit mortality line	
	Sensitive strain (Frankenwald)	Resistant strain (Ferndale)		Frankenwald	Ferndale
gamma BHC	0.000298	0.2345	8×10^2	0.4188	0.4103
Toxaphene	0.00087	0.829	9×10^2	0.3947	0.3155
Chlordane	0.00426	30156 *	7×10^6	0.3495	0.0947
Dieldrin	0.00123	26971 *	2×10^7	0.4190	0.0985
Aldrin	0.00179	71031 *	3×10^7	0.4068	0.0953

* Obtained by extrapolation

If it is assumed that, because of cross resistance in the chlorinated cyclic hydrocarbon group of insecticides, the mechanism of resistance is similar, then it is suggested by the results presented in Table 7 that such a mechanism is much more efficient in detoxifying chlordane, dieldrin and aldrin than it is for BHC and toxaphene. The possibility of a mechanism of detoxification in respect of chlordane, dieldrin and aldrin in addition to a mechanism for the detoxification of BHC and toxaphene cannot, however, be excluded.

It may be significant that resistance to the chlorinated cyclic hydrocarbon group has developed as a result of field

selection with gamma BHC (Ferndale ticks) or toxaphene (Frankenwald 1958 ticks) and that the selection resulting from the field treatment with these two insecticides has now reached a maximum. Chlordane, dieldrin and aldrin have never been used commercially for the control of the blue tick and resistance to these compounds has been as a result of selection with either BHC or toxaphene. As the blue tick cannot be bred satisfactorily under artificial conditions the effect of selection with chlordane, dieldrin and aldrin on strains without a prior resistance to BHC or toxaphene could not be determined.

The test procedure for determining log concentration-probit mortality lines was not satisfactory with sodium arsenite solutions and in consequence probit regression lines could not be obtained. However, it is significant that tests conducted with the adult tick using sodium arsenite showed a similar response as compared with the response obtained twelve years later (Table 6). This indicates that the response to sodium arsenite by the adult female arsenic-resistant blue tick has not changed after a period of twelve years, suggesting that resistance to sodium arsenite has reached its maximum in the arsenic-resistant strain under the prevailing conditions of selection.

In respect of DDT to which the blue tick from some localities is resistant no larval response data is available.

These results suggest that the development of insecticide resistant populations of the blue tick is a result of a selection of a characteristic already existing in the populations.

The mechanism of the development of resistant populations has been extensively studied in the housefly. From these investigations two explanations are possible .

(a) Post-adaptation, where a physiological change is induced by the toxicant.

(b) Pre-adaptation, where genetic differences already exist in a population and the toxicant acts as a selective agent favouring the resistant genotypes (Crow, 1957).

Most of the evidence available favours the theory that insecticide resistance is a pre-adaptive phenomenon. Susceptible houseflies treated repeatedly with levels of BHC and DDT which caused no mortality were found subsequently to be more sensitive to these materials (Hadaway and Barlow, 1956). Larvae of the wax moth which survived treatment with small doses of DDT, nicotine and pyrethrum were found to be more susceptible when treated with the same insecticides seven days later (Beard, 1952 (a) and (b)). Resistance in houseflies could not be induced by frequent treatment with levels of DDT which gave no mortality (Harrison, 1952). DDT resistance could be induced in lice by treatment at levels giving low mortality but could not be induced when DDT was applied at sub-lethal levels (Cole, 1956). DDT was found to have no effect on the mutation rate in Drosophila when applied to larvae or adults from which it was concluded that DDT was in no way responsible for producing a mutation which could give rise to a resistance factor.

It may be concluded from this evidence that resistance to insecticides arises from pre-adaptive factors occurring initially in the population. The insecticide is simply a selecting agent, frequent application of which will eventually give rise to a population in which the selected strain is predominant.

No evidence is available on whether insecticide resistance in the blue tick is a pre- or post-adaptive phenomenon but as resistant blue tick populations have arisen and display characteristics of insecticide resistance similar to many insect populations it is reasonable to assume that the phenomenon is pre-adaptive.

Busvine and Harrison (1953) suggested that the speed with which resistance would become evident in a population was dependent on the following three factors :-

(a) The frequency of occurrence and the effectiveness of resistant genes in the natural populations of the insect.

(b) The intensity of selection, i.e. the magnitude of the population exposed to the insecticide and the proportion killed.

(c) The number of generations per year.

The frequency of occurrence of the resistance factor or resistant gene would be extremely difficult, if not impossible, to determine in tick populations in South Africa although a detailed study of tick populations from areas with a history of no insecticidal treatment might be very informative. As tick populations with a history of no previous treatment with insecticides no longer exist in South Africa and the importation of such strains from Central Africa was not permitted the only course open was to examine the history of the development of resistance to several insecticides in tick populations. As the intensity of selection and number of generations per year have been reasonably constant in respect of tick populations a knowledge of the speed with which resistant populations have developed might give some indication of the frequency of occurrence of the resistance gene and its mode of inheritance.

Resistance to sodium arsenite in B. decoloratus populations developed relatively slowly (Whitnall and Bradford, 1947 and 1949). Arsenic resistance only became noticed in the blue tick population from some localities after a period of thirty to forty years of field treatment in sodium arsenite preparations. Since the development of resistance, treatment with sodium arsenite in these areas has been discontinued yet resistance to arsenic has remained at a fairly constant level and only recently has there been an indication of a slight drop in arsenic tolerance (Nagaza Park 1958 ticks). Resistance to BHC was first observed in the short time of eighteen months after the original use of BHC preparations in the field (Whitnall and others, 1952). Resistance to toxaphene in the Frankenwald 1958 strain of ticks also arose after less than two years of field treatment. In the Australian species of tick, B. microplus (Canestrini) resistance to toxaphene was found to have developed fairly rapidly (Norris and Stone, 1956).

The same species also developed resistance to BHC in less than two years (Hitchcock, 1953). A high degree of resistance to dieldrin was built up after only four sprayings of cattle infested with B. microplus although this strain of ticks may have had a previously developed low level of BHC resistance (Stones and Meyers, 1957).

With the development of resistance to BHC particularly along the eastern coastal belt of South Africa, DDT was introduced as a general field dipping or spray material. DDT although effective mainly against larvae at the concentration used, continued to give good control of B. decoloratus for some time and it was five years before signs of the development of resistance were observed (Whitehead, 1956).

In Australia the position was similar and DDT resistance became manifest approximately five years after its first introduction as a field spray (Stone, 1957). From the point of view of effectiveness in tick control, sodium arsenite is the least effective, followed in order of effectiveness by DDT and BHC. Resistance to the least effective material, i.e. sodium arsenite, took the greatest length of time to develop, whereas resistance to the highly effective BHC appeared in a comparatively short time while DDT took an intermediate position in respect of both effectiveness and the length of time taken to develop a resistant population in the field.

Whitnall (1958) expressed the opinion that resistance was likely to develop more rapidly with the more effective insecticides. A number of species of mosquito have developed resistance to a variety of insecticides in recent years. In some species resistance to DDT only became apparent after five or more years of field treatment. In other species dieldrin resistance developed in a considerably shorter time. Davidson (1956 and 1958) using the discriminating dose technique was able to show that in Anopheles gambiae Giles, the inheritance of dieldrin-BHC resistance was monofactorial and that the gene for resistance was semi-dominant. By cross breeding the strains obtained by the

discriminating dose technique a population was obtained comprised of sensitive, hybrid and resistant individuals in a 1:2:1 ratio. The response of the hybrids to dieldrin was intermediate between that of resistant and sensitive but they were sufficiently resistant to survive the concentrations of dieldrin used in the field. The inheritance of DDT resistance in A. sundaicus was found by the same technique to be monofactorial and the gene for resistance was recessive. Cross breeding of individuals selected with discriminating dosages resulted in a final population containing sensitive and resistant individuals in a 3:1 ratio. Once having established the inherent nature of insecticide resistance in mosquitoes, Davidson concluded that the higher the selection pressure the more quickly a resistant population would develop, i.e. the more effective the insecticide, the higher the dose or the more effective the attack on all stages of the life cycle the more rapidly would a resistant population develop. Selection of a population where the resistant characteristic was semi-dominant would result in the build-up of a resistant population more rapidly than where the characteristic was recessive.

Using a similar technique Shanahan (1959) found in Lucilia cuprina Wied, which had developed populations resistant to dieldrin comparatively rapidly, that the characteristic of inheritance of dieldrin resistance was also semi-dominant.

In the field control of the blue tick, treatments are applied at weekly intervals in areas of high tick incidence and because of its one host characteristics all stages of the tick come in contact with the insecticide. It is thus not surprising that of all the species of ticks in South Africa the rapidly breeding blue tick has been the first to build up insecticide resistant populations. The speed with which a blue tick resistant population will develop resistance to a particular insecticide is related directly to the selection pressure of the insecticide and the nature of the inheritance of the resistance factor. A highly effective insecticide resistance which is inherited by a semi-dominant gene

will result in the selection of a resistant population in a comparatively short time.

Because of the time taken to develop resistance to BHC and DDT in tick populations is similar to that in mosquitoes the conclusion might be drawn that the inheritance of BHC resistance in ticks is semi-dominant while the inheritance of DDT resistance is recessive. However, the situation is probably more complex than this as it has already been shown that DDT acts mainly against larval stages whereas BHC is active against all stages which immediately suggests that the selection pressure is greater in respect of BHC and this factor alone might be responsible for the more rapid build up of BHC resistant populations. On the same assumption it is possible that the inheritance of arsenic resistance in the blue tick may be recessive but the reason for the greater length of time taken to develop arsenic resistant blue tick populations as compared with DDT may also be because sodium arsenite is less effective than DDT.

The organo-phosphorus insecticides, delnav and asuntol, were introduced at the end of 1958 for the control of the arsenic-BHC-DDT resistant blue tick strains. Judged by LC50 values these two materials are very slightly less efficient than BHC on sensitive larvae and it will be interesting to observe what time period will be required to develop an organo-phosphorus resistant strain.

(g) The distribution of resistant strains of the blue tick

The appearance of strains of the blue tick resistant in turn to sodium arsenite, gamma BHC and DDT was always first observed in the East London district. Strains resistant to arsenic and BHC now occur in a narrow coastal belt extending from Mossel Bay in the south to Zululand in the north. DDT resistant strains occur in localised areas in the East London district but reports have come to hand that DDT resistant strains are now appearing north and south of East London along the coast.

In a distribution map of B. decoloratus in South Africa, Theiler (1949) showed that this species of tick was limited in its distribution by humidity and did not survive in areas with an average annual rainfall of below 15 inches. The moist and equable climatic conditions of the Eastern coastal belt are most conducive to blue tick development. The greater the reproduction potential the more necessary does conscientious field control become. In short, the climatic conditions of the coastal belt gives rise to a cycle of events which increases the selection pressure which is a condition favouring the selection of the more resistant individuals of the population. There is no reason to believe that resistant populations would not arise in inland areas but the process would be expected to be slower because of the reduced reproductive potential resulting from less favourable climatic conditions. This, in fact, has been the case as strains resistant to the BHC group of insecticides have developed in isolated areas of the Transvaal, but it has taken five to six years or more of field treatment for the resistant populations to become evident.

A point of interest is the fact that insecticide resistance has only developed in populations of the "one host" blue tick and in none of a variety of other species of ticks against which insecticidal treatment is extensively practised. It is probable that the "two" and "three" host characteristic of these species is responsible for a reduced selection pressure and consequent lack of the development of resistance. With the "one host" blue tick which is primarily a parasite of domestic stock a seven-day dipping programme will result in three treatments of a tick during its twenty-one day parasitism of the host animal. Dipping or spraying in these circumstances will result in treatment of all parasitic stages, namely larval, nymphal and adult. It has been shown that insecticide treatment of all stages of an insect results in the development of resistance more rapidly than if the insect is treated at one stage of its life cycle (Bruce and Decker, 1950).

"Two" and "three" host ticks, as the name implies, leave the host once or twice as the case may be before reaching maturity. Many of the "two" and "three" host ticks require to alternate host variety and it is likely in these circumstances that they will come in contact with the insecticide treatment less frequently than in the case of the blue tick. In short, selection pressure in respect of the "two" and "three" host ticks, because of their habits is lower than in the blue tick and in consequence resistant populations would take a far longer time to develop.

Note: Investigations still being undertaken have shown that the "two" host red tick Rhipicephalus evertsi from the East London district has developed a marked tolerance to toxaphene, a preparation of which was being used on the farm concerned. As far as has been established toxaphene resistant R. evertsi are cross tolerant to gamma BHC and dieldrin but not to sodium arsenite or the organo-phosphorus and carbamate insecticides.

(h) Cross tolerance to insecticides in the blue tick

In a review, Metcalf (1955) making use of the results of a number of workers (Barber & Schmitt, 1948; Barber & Schmitt, 1949; Busvine, 1951, Busvine, 1953b; Decker & Bruce, 1952; March & Metcalf, 1949; Tonelli, 1950), showed that houseflies resistant to DDT were also resistant in varying degrees to DDT analogues in spite of not having ever previously come in contact with these materials. Cross tolerance was highest in the analogues which most closely resembled DDT. Flies resistant to DDT were not resistant or only slightly resistant to gamma BHC and related chlorinated cyclic hydrocarbon insecticides, to the nitroparafenes and organo-phosphorus compounds. The thiocyanate insecticides (thanite and lethane), the carbamate insecticide (pyrolan) and pyrethrum were fully effective against DDT-resistant flies. BHC-resistance in houseflies resulted in a cross resistance to chlordane, dieldrin and other chlorinated cyclic hydrocarbons. Busvine (1953a) artificially selected resistant flies with chlordane and produced a high degree of resistance to dieldrin and gamma BHC but only a 2.2 fold increase in resistance to DDT.

Although Metcalf (1955) mentions a number of unexplained anomalies in the general pattern of cross tolerance to insecticides, he was able to group a number of insecticides according to cross tolerance and suggested that this indicated a common mechanism of resistance within groups but that the mechanism differed between groups. Metcalf's grouping was as follows :-

(1) DDT and analogues
(2) gamma BHC and chlorinated cyclic hydrocarbons
(3) Nitroparafenes
(4) Organo-phosphorus compounds
(5) Pyrethroids
(6) Thiocyanates
(7) Carbamates.

Metcalf concluded from the findings in Drosophila (Weirner & Crow, 1951), Aedes taeniorhynchus and A. solicitans (Keller & Chapman, 1953; MacCreary Darsie & Cannon, 1953), Culex tarsalis and Aedes nigromaculis (Gjullin, Isaak & Smith, 1953), Blatella germanica (Fisk & Isert, 1953; Grayson, 1954; Heal, Nash & Williams, 1953), Pediculus humanus corporis (Busvine, 1953a, and Eddy, 1952) that the classification of insecticides according to cross tolerance could be applied generally to a number of species of insects in which insecticide resistance had developed.

In the blue tick it was shown by field and laboratory experiments that the arsenic-resistant strain was susceptible to gamma BHC. Although resistance to gamma BHC developed very rapidly in arsenic-resistant ticks initially, this development was independent of resistance to arsenic and there was no suggestion of any cross tolerance (Whitnall & Bradford, 1947). Bekker (1953) reported a BHC-resistant strain of tick susceptible to sodium arsenite in the Pretoria district which is further evidence suggesting that resistance to arsenic and BHC are not related. Arsenic-BHC-resistant ticks showed some increased tolerance to toxaphene (Whitnall et al., 1952) which can be related to BHC resistance rather than arsenic resistance, as toxaphene was originally effective against the strain of tick resistant to arsenic only (Thorburn, personal communication).

The arsenic-BHC resistant strain of tick was found to be also resistant to dieldrin, aldrin, toxaphene, chlordane and to parathion but not to DDT and some of its analogues (Fiedler, 1952).

An examination of the results obtained in a series of tests conducted on both adult and larval ticks (Part II) allows a number of conclusions to be drawn. The arsenic-BHC-resistant strain of tick from Ferndale (Figs. 2, 3, 4, 22) was also highly resistant to toxaphene (Figs. 6, 23) chlordane (Figs. 8, 24) dieldrin (Figs. 9, 10, 25) and aldrin (Figs. 10, 26) which are all chlorinated cyclic hydrocarbon insecticides. With the exception of toxaphene none of these insecticides has been used on a commercial scale in the district from which Ferndale ticks were obtained and resistance to all these insecticides is obviously a result of cross tolerance conferred by resistance to BHC. Using the more precise larval test it was possible to show that response to BHC in the Ferndale strain was 8×10^2 times greater than in the sensitive Frankenwald strain (Table 1). The level of resistance to toxaphene was slightly higher than that to BHC and greater still to chlordane and dieldrin and with the greatest degree of resistance to aldrin.

No degree of DDT resistance in Ferndale ticks could be detected (Figs. 12, 27). The Allandale strain of ticks was highly resistant to DDT (Fig. 12) as well as being resistant to BHC and the other chlorinated cyclic hydrocarbons (Figs. 4, 6, 8, 10, 11). This indicated that DDT resistance had developed independently in Allandale ticks and was unrelated to resistance to BHC or sodium arsenite. It will be noted from the structural formulae shown in Part II that DDT is a straight-chain chlorinated aliphatic compound attached to which are two monochlorinated aromatic rings whereas BHC is a chlorinated cyclic aliphatic compound with no aromatic moiety. As there is no cross tolerance between resistance to DDT and BHC in ticks it is likely that the mechanism of resistance to these two compounds is different. From this it may be inferred that chemical structure has some direct bearing on the mechanism of resistance to

insecticides in ticks.

The arsenic-BHC-DDT-resistant Allandale tick was as resistant to dilan as it was to DDT (Figs. 12, 14). This result was surprising as it has been shown that DDT-resistant flies are susceptible to the nitroparafene analogues of DDT (Metcalf, 1955). This divergence in the cross tolerance pattern in ticks and houseflies suggests a difference in the mechanism of resistance in the two species. As dilan cannot be dehydrochlorinated and resistance to dilan and DDT appears to be the result of a similar mechanism, it may be concluded that dehydrochlorination of DDT is not the basic cause of resistance in ticks as it is reported to be in houseflies. This interpretation is supported by the finding, reported in Part III, that DDT-dehydrochlorinase could not be detected in DDT-resistant ticks.

The organo-phosphorus insecticides malathion (Figs. 10, 15, 28), diazinon (Figs. 10, 16, 28), korlan (Figs. 19, 32), delnav (Figs. 17, 30) and asuntol (Figs. 18, 30) although individually different in their toxic potency were each reacted to similarly by the arsenic-BHC-resistant Ferndale and arsenic-BHC-DDT resistant Allandale and Nagaza Park 1958 strains of larvae. From these observations it is concluded that resistance to sodium arsenite, BHC or DDT in the blue tick does not confer a cross tolerance to the organo-phosphorus insecticides. This observation is not in agreement with that of Fiedler (1952) who found BHC-resistant larvae 2.5 times more tolerant to Ticodol (parathion) than a sensitive strain. Fiedler, although claiming that this difference is a result of cross tolerance, gives no indication of the statistical treatment of his results. In experiments with organo-phosphorus insecticides already described differences in LC50 values as high as 5.25 fold (Table 2) were not significant at the 5% probability level.

A re-examination of ticks from Frankenwald Research Station during 1958 (Frankenwald 1958 ticks) showed a marked degree of resistance to BHC, toxaphene, chlordane, dieldrin and aldrin (Figs. 7, 9). The level of resistance to these materials was roughly of the same order as that obtained three years previously with both Ferndale and Allandale ticks. The Frankenwald 1958 ticks now BHC resistant showed in tests with both adult and larval ticks, a slight but not statistically significant increase in tolerance to DDT (Figs. 13, 35), which was not as high as the significant difference detected in Allandale ticks and probably represents a "vigour tolerance" rather than a true developed resistance. An examination of the larvae (Fig. 35) gave an LC50 for Frankenwald ticks of 0.005% as opposed to 0.0125% with Frankenwald 1958 tick larvae showing a 2.5 fold increase in tolerance which was similar to the effect obtained by Busvine (1953a) when he examined the effect of DDT on flies selected with chlordane and which he interpreted as indicative of a "vigour tolerance". Further evidence that the increase in tolerance to DDT in Frankenwald 1958 ticks was not associated with the resistance developed to BHC was obtained by treatment with dilan (Figs. 10, 14). Whereas DDT-resistant Allandale ticks were also resistant to dilan (Fig. 14) no resistance to dilan could be detected in Frankenwald 1958 ticks (Fig. 10).

Treatment of Frankenwald 1958 ticks with malathion and diazinon (Fig. 10) resulted in the same response as obtained with the original Frankenwald sensitive tick (Figs. 15, 16) which is further evidence confirming that resistance to BHC confers no cross tolerance to insecticides in the organo-phosphorus group.

The BHC-resistant Frankenwald strain of adult tick showed a slight increase in tolerance to sodium arsenite when compared with Frankenwald sensitive ticks but only at the lower concentrations (Figs. 2, 3); this is indicative of a decrease in heterogeneity rather than a development of resistance.

The effect of pyrethrum (Figs. 21, 35) and the carbamate insecticide, sevin, (Figs. 14, 23) was determined on both larvae and adult ticks known to be arsenic-BHC-DDT resistant (Nagaza Park 1958), BHC resistant (Frankenwald 1958) and sensitive to all insecticides (Transvaal 1958).

Sevin was equally effective against all strains as was pyrethrum to Frankenwald 1958, Ferndale 1958 and Transvaal 1958 larvae, Frankenwald 1958 and Transvaal 1958 adult ticks, but there was a surprising degree of tolerance to pyrethrum shown by Nagaza Park 1958 larvae and adults.

The response to pyrethrum was almost identical in the BHC-resistant Frankenwald 1958 larvae and arsenic-BHC-resistant Ferndale 1958 larvae but insecticide sensitive Transvaal 1958 larvae were three-fold more susceptible. This difference was not significant ($P > 0.05$). The arsenic-BHC-DDT resistant Nagaza Park 1958 larvae were 18.5 times more resistant to pyrethrum than Transvaal 1958 larvae. As Ferndale 1958 larvae which were arsenic-BHC-resistant and BHC-resistant Frankenwald 1958 larvae were only three-fold more tolerant than sensitive Transvaal 1958 larvae it must be concluded that the high degree of resistance to pyrethrum displayed by Nagaza Park 1958 larvae is associated with resistance to DDT which is the same conclusion arrived at in respect of tests conducted with the same strains of adult ticks. This observation is surprising as in most other studies of cross tolerance to insecticides DDT resistance is not related to resistance to pyrethrum (Metcalf, 1955). However, one exception is the example described by Busvine (1953b) where it was found that an Italian strain of houseflies resistant to DDT was also markedly resistant to pyrethrum. The previous history of insecticidal treatment of the Italian strain is not given but as pyrethrum is frequently used against houseflies the independent and prior development of pyrethrum resistance in the Italian strain cannot be excluded. A Sardinian strain of flies resistant to chlordane, dieldrin, aldrin, BHC, toxaphene and DDT was susceptible to pyrethrum.

No strains of tick resistant to organo-phosphorus compounds or the carbamate insecticides have as yet arisen and it was thus not possible to prove that no cross tolerance between these insecticides exist. However, as the cross tolerance pattern in ticks closely resembles that of houseflies, with the exception of the relationship between DDT-resistance and resistance to dilan and pyrethrum, it is possible that any mechanism of resistance in ticks to either organo-phosphorus insecticides or sevin, may not be related. As the pyrethrum resistant Nagaza Park 1958 strain is as susceptible to both organo-phosphorus compounds and sevin it is clear that pyrethrum resistance could not be related to any resistance which might develop to these two compounds.

With these results of an analysis of resistance displayed by various strains of the blue tick, it is possible to arrange the insecticides in accordance with cross tolerance in groups as follows :-

1. Sodium arsenite
2. DDT, dilan and pyrethrum
3. Chlorinated cyclic hydrocarbons: gamma BHC, toxaphene chlordane, dieldrin, aldrin
4. Organo-phosphorus compounds: malathion, diazinon, delnav, asuntol, korlan and the carbamate, sevin.

As no resistance to organo-phosphorus or carbamate compounds has been observed in blue tick populations, there are no grounds, at the present time, for placing these two classes of insecticides in different groups in respect of cross tolerance.

The development of resistance to any one particular insecticide within a group automatically resulted in resistance to all insecticides in the same group, but not to insecticides in another group. Resistance to several groups of insecticides is possible as in the case with ticks resistant to sodium arsenite, gamma BHC and DDT, but resistance to each group arose independently and is unrelated.

PART III. THE MODE OF ACTION AND MECHANISM OF RESISTANCE TO SODIUM ARSENITE, DDT AND BHC IN THE BLUE TICK

(a) Sodium arsenite

The use of sodium arsenite as a dipping material for the control of ticks on livestock developed slowly and much of the earlier development work was simply a process of trial and error, in many cases carried out by farmers and stockmen themselves. Arising from such work and the researches of government officials and the technical staff of interested companies, arsenic preparations were used for tick control at a concentration of 0.16% As_2O_3 with a dipping interval of 7 days. Where the interval between dippings was increased to 14 days, concentrations of 0.32% As_2O_3 were permissible. In declared East Coast Fever areas, dipping in 0.12% As_2O_3 at 5-5-4 day intervals was enforced by legislation.

Sodium arsenite preparations at recommended concentrations were adequate for the control of most South African tick species with the exception of those with a predelection for protected body sites e.g. Otobius megnini Duges, which is found in the ears and Rhipicephalus evertsi Neumann, which attaches under the tail.

From time to time claims were made for various additives which were reputed to increase the effect of arsenical dips. The effect of many of these ingredients which included such materials as various grades of mineral oil and plant extracts were investigated scientifically but without exception none of these materials could be shown to improve the effect of sodium arsenite.

The development of resistance to arsenic preparations increased the enthusiasm with which arsenic potentiators were sought, but the results were nevertheless as fruitless.

A phenomenon substantiated by the observations of a number of farmers in many districts was the effect of weather conditions on sodium arsenite efficiency. Overcast weather was apparently conducive to good tick control. Re-wetting of a dipped animal by rain before it had dried thoroughly often resulted in a severe scalding and a consequent loss of hair of dipped livestock.

These observations led to an investigation of the effect of humidity on the tick-killing properties of sodium arsenite. Ticks were dipped in a range of sodium arsenite dilutions using the laboratory technique already described. Batches were kept for 24 hours after dipping in conditions approaching 100% humidity, while other batches were placed in desiccators for the same period with concentrated sulphuric acid as a desiccant. No difference in the effect of sodium arsenite under the different conditions of humidity could be detected. The addition of "humectants" such as 2% ethylene glycol and 0.1% glycerine likewise in no way increased the effect of sodium arsenite solutions.

It has been shown (Part II (c)) that the three- to four-fold increase in the effectiveness of sodium arsenite would be required to reinstate arsenic as a control measure for the resistant strain of blue tick. It is theoretically possible that this could be achieved by increasing the penetration of arsenic.

It was not clear at this stage how much of a topically applied amount of sodium arsenite actually penetrated the tick cuticle. Whitnall and Bradford (1947) reported that in their investigations, applied sodium arsenite was taken up by the integument and that no arsenic could be detected inside the tick. In the light of innumerable more recent studies on the penetration of insecticides, this result is surprising and it is more than likely that the failure to detect arsenic internally in arsenic treated ticks was as a result of the limitations of the analytical method used. With this in mind, the penetration of sodium arsenite in the blue tick was investigated.

A microdrop applicator, a modification of that described by (Hewlett, 1954) was available (Plate 5) and after calibration of the instrument with P^{32} labelled sodium dihydrogen phosphate the actual amount of sodium arsenite per tick required to produce 100% kill in the Frankenwald strain was determined. This lay between the equivalent of 30

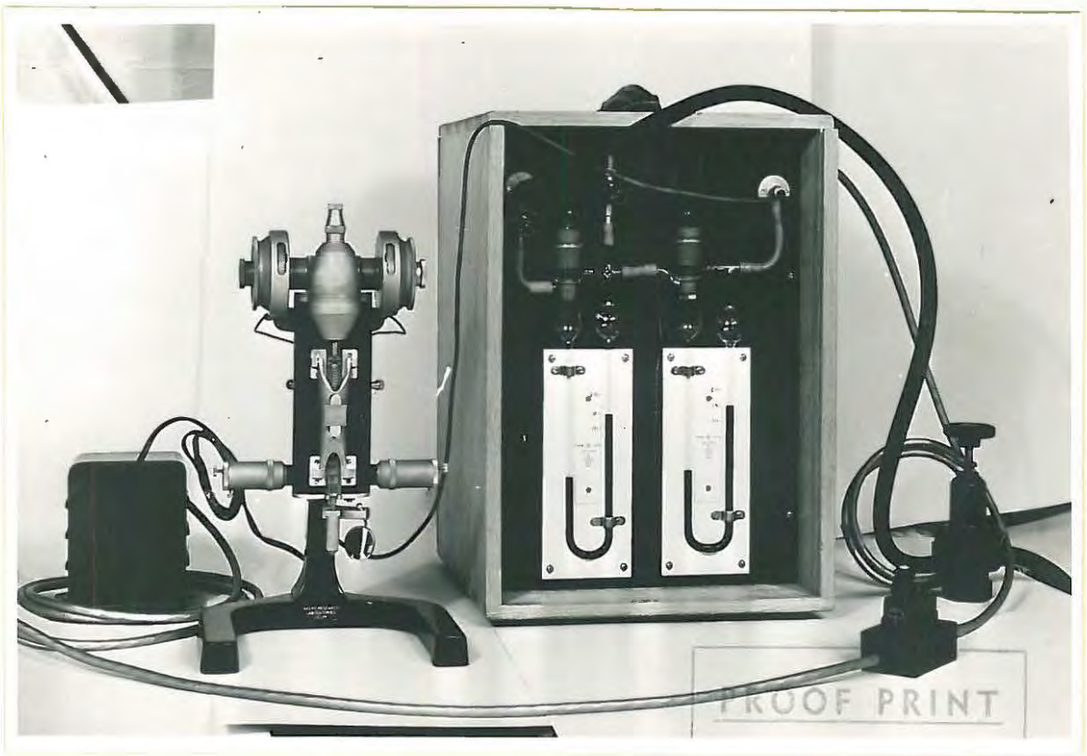


Plate 5. The microdrop applicator.

and 35 micrograms of As_2O_3 per fully engorged female tick. Ticks were presumed dead if they showed no movement after heat stimulation 48 hours after treatment.

In initial experiments, varying amounts of sodium arsenite were deposited from the microdrop applicator in small "cellophane" cups. These were then transferred immediately to kjeldahl flasks, in which the first steps of the analytical procedure were carried out.

The analytical procedure used was a modification of a method evolved by Sandell (1942) which involved the separation of arsenic from interfering elements by the evolution of arsine (Mendelowitz, personal communication). (All analytical determinations were carried out by the Analytical Services Section of the Research Department, A.E. & C.I. Ltd.)

It was established in the original calibration of the microdrop applicator that the instrument was subject to a 5% error at the concentrations of sodium arsenite used. Taking this fact into account the analytical recovery of As_2O_3 from the cellophane cups was good. When the same procedure was repeated by depositing measured droplets of sodium arsenite on adult female fully engorged ticks placed in the cellophane cups, the accuracy of As_2O_3 determinations declined. With a knowledge of the limitations of both the microdrop applicator and the analytical method in mind several experiments were undertaken in an attempt to find out the fate of measured amounts of topically applied sodium arsenite in fully engorged female blue ticks.

To prevent movement after treatment, all legs were removed from the experimental batches of fully engorged female ticks. Each tick was placed in a cellophane cup and the appropriate number of drops of sodium arsenite solution applied mid-dorsally with the microdrop applicator. The treated ticks were then stored in constant conditions at 25°C and 85% RH. After 24 hours, ticks were removed and examined individually under a microscope without removing them from the individual cellophane cups. The original sodium arsenite droplet, which was easily

discernible, was covered with a layer of quick-drying synthetic lacquer. Each tick was then washed three times in a separating funnel with 5 ml of a 0.05% lissapol solution. The ticks were then individually frozen with solid carbon dioxide and the lacquer-covered drop of sodium arsenite removed by cutting away a small section of cuticle with a scalpel. While the tick was still frozen, it was comparatively simple to remove the entire cuticle which, in these conditions, peeled off from the frozen internal contents of the tick. All the portions were placed separately in kjeldahl flasks and subjected to As_2O_3 analysis. The most satisfactory results of an analysis of a batch of 8 ticks subjected to a topical application of 20 micrograms of As_2O_3 are presented in Table 8.

Table 8. The amount of As_2O_3 recovered from adult ticks topically treated with 20 micrograms of As_2O_3 applied as sodium arsenite

No.	Weight of tick in grams	Cellophane cup	Washings	Lacquer-covered drop	Washings plus lacquer-covered drop	Cuticle	Inside	Total Recovered
1	.3722	4.7	1.9	7.2	9.1	1.1	6.1	21.3
2	.3604	1.3	1.5	14.2	15.8	7.2	1.1	25.7
3	.2712	0.79	4.4	0.7	5.1	1.3	10.7	18.2
4	.2944	0	2.5	12.0	14.5	2.1	0	16.7
5	.2301	3.7	2.1	2.8	4.9	1.1	3.3	13.2
6	.2239	0.79	9.5	6.4	15.9	.79	3.3	20.7
7	.2815	.33	9.7	0.3	10.0	2.6	4.8	17.8
8	.2644	9.3	16.1	0.7	16.8	3.6	2.3	32.2
(Results expressed in micrograms of As_2O_3)								

The total As_2O_3 recovered from each tick varied considerably from the amount applied and the reproducibility among replicates was poor. Until a more accurate method for determining micro-quantities of As_2O_3 in biological material is available it would be unwise to attempt to build up a picture of the distribution of topically applied sodium arsenite in the blue tick.

These results do, however, clearly indicate that topically applied sodium arsenite does penetrate the cuticle and can be found within the cuticle and inside the tick. There is also some evidence of the excretion of arsenic as, with one exception, arsenic was found in all the cellophane cups.

Attempts to investigate differences in penetration of arsenic in resistant and sensitive ticks were undertaken but the results obtained were of little significance due to lack of reproducibility within the replicates. These results are presented in Table 9.

Table 9. The amount of As_2O_3 recovered from sodium arsenite topically treated arsenic-resistant and sensitive adult female ticks 24 hours after treatment. (Results expressed in micrograms As_2O_3)

Arsenic-resistant strain (32 micrograms As_2O_3 applied per tick)

No.	Weight of tick in grams	Cellophane cup	Washings	Lacquer covered drop	Cuticle and inside	Total recovered
1	.2614	0	0	25.344	.1980	25.542
2	.2555	.132	11.233	15.312	0	26.677
3	.2277	0	21.846	0	4.026	25.872
4	.2804	1.386	28.512	1.65	1.056	32.604
5	.2394	.33	4.95	0	1.584	5.557
6	.2391	0	8.91	18.084	4.620	31.614
7	.2325	0	2.244	1.056	3.036	6.336
8	.2272	0	31.548	2.838	3.234	37.62
9	.2320	0	4.29	20.79	6.204	31.284
10	.2294	0	0	20.79	4.158	24.948

Table 9 (continued)

Arsenic-sensitive strain (20 micrograms As_2O_3 applied per tick)

No.	Weight of tick in grams	Cellophane cup	Washings	Lacquer covered drop	Cuticle	Inside	Total recovered
1	.2730	4.35	3.6	12.35	0	2.5	22.80
2	.3369	5.15	8.35	19.5	9.4	12.0	54.40
3	.2559	2.25	4.65	5.5	2.75	0	15.15
4	.2781	0	5.5	1.8	5.0	7.95	20.25
5	.2862	0	0	13.5	20	.7	34.20
6	.2516	0	2.25	13.6	8.75	2.0	26.60
7	.2911	0	0	12.5	6.25	1.45	20.20
8	.3173	4.65	0	0	8.35	12.0	25.00
9	.2539	.85	4.65	15.5	0	3.75	24.75
10	.2672	1.0	3.95	0	0	.35	5.30

There is a wealth of evidence to show that resistance to DDT in houseflies is not related to penetration (March & Metcalf, 1949) but no such evidence could be found in the literature in respect of the arsenicals. In view of the difficulty experienced in determining micro-quantities of sodium arsenite the possibility of a biochemical protective mechanism such as that shown to exist in respect of DDT in DDT-resistant houseflies was investigated.

Before protective mechanisms against arsenic toxicity could be investigated, a more thorough knowledge of the mode of toxic action of arsenic preparations generally was necessary. An examination of the limited literature on this subject suggested lines which have been followed and are here discussed. In the past, several workers suggested that the toxic action of arsenic might be associated with the affinity of arsenic for sulphydryl groups. Voegtlin (1923) was one of the first to demonstrate this when he found that the trypanocidal activity of arsenicals could be reversed and even prevented by the addition of amorphous glutathione, cysteine and other related -SH containing compounds.

In mammals the lethal action of arsenic could be offset by the administration of glutathione and several other -SH containing materials (Voegtlin, 1925). An association between arsenic toxicity in insects and sulphydryl compounds was suggested by Fink (1927) who found that the glutathione content of ten different species of insects decreased following the injection of both arsenic trioxide and arsenic pentoxide.

Lyman and Barron (1937) indicated that the role of glutathione was that of a regulator of the rate of reversible oxidation-reduction reactions.

Barron and Singer (1943) pursued the idea of the regulatory function of glutathione further, when they found that 17 out of 32 enzymes examined were inactivated when treated with -SH deactivating agents and furthermore, were reactivated by the addition of glutathione. This suggested that glutathione was essential to a number of enzyme systems where it played the role of keeping the enzymes possessing the -SH grouping in the reduced state. It thus becomes apparent that the immobilisation of glutathione by arsenicals could disrupt oxidation-reduction and many other enzyme systems dependent on the -SH grouping and thereby bring about a serious state of intoxication.

Forgash (1951) investigated the effect of a number of insecticides on glutathione in Periplaneta americana (L.) and concluded that only sodium trioxide, dibasic sodium arsenate and cupric chloride reduced the glutathione content. Injections of sublethal concentrations of As_2O_3 into roaches resulted in a rapid fall in glutathione content. The glutathione level had returned to normal 24 to 48 hours after injection, suggesting the existence of a mechanism capable of regenerating glutathione very rapidly. It was also found that considerable inhibition of glutathione was necessary before intoxication occurred as 50% reduction in glutathione occurred at a dose level of As_2O_3 corresponding to the amount required to produce 50% mortality. It was also found that injection of glutathione into P. americana prior to treatment with As_2O_3 protected

against arsenic toxicity and treatment with glutathione after arsenic had a similar but less marked effect. (Forgash, 1951).

Susceptibility to As_2O_3 varies in male and female P. americana, the male being more susceptible. Determination of glutathione in the two sexes indicated a greater amount in the male which is the reverse of what might have been expected assuming a direct relationship between As_2O_3 toxicity and glutathione content. Furthermore, the final instar nymphs have less glutathione than male or female adults, yet were equally affected by As_2O_3 (Forgash, 1956). These findings at first sight appeared to invalidate the earlier assumptions that arsenic action was directly associated with glutathione. In a latter publication, however, Forgash (1957) investigated glutathione levels in various tissues of P. americana and found the greatest amounts in the alimentary canal followed in order by muscle tissue and fat body. The greatest reduction of glutathione after As injection was in the fat body and this is put forward as an explanation for the difference in response to As_2O_3 in the sexes. The female roach contains approximately eight times more fat than the male.

These findings have not entirely elucidated the mechanism of toxic action of arsenicals but suggest very clearly that -SH containing compounds are vitally concerned and that the levels of -SH compounds, glutathione in particular, are directly related to the amount of arsenic required to produce toxicity.

It has been postulated that the basis of the action of arsenical vesicants is an interference with the thiol-dependent pyruvate oxidase system (Sexton, 1949). It was on this basis that British Anti-Lewisite (2, 3-dimercaptopropanol) was developed as an antidote to the war gas vesicants. The dimercaptopropanol functioned by providing thiol groups which reacted with the arsenical preferentially thus protecting the thiol enzymes (Peters and Wakelin, 1946).

No references could be found in the literature on the comparative levels of thiol containing enzymes in arsenic resistant and sensitive insects. It is logical in view of the findings already mentioned to consider the possibility that arsenic resistance in insects is a result of a greater abundance of particular thiol containing compounds which might act in a manner similar to British Anti-Lewisite. This possibility was investigated in the arsenic resistant ticks in two ways :-

(a) by attempting to inhibit reduced glutathione and thiol enzymes generally with known inhibitors;

(b) by comparing the levels of glutathione, cystine, cysteine and total free sulphydryl in arsenic sensitive and resistant ticks.

(1) Treatment of arsenic-sensitive and resistant adult blue ticks with glutathione inhibitors

A number of known -SH inhibiting materials commonly used by enzymologists were obtained or synthesized and were applied to ticks using the dipping technique already described. The effect of these materials alone or followed by treatments in dilutions of sodium arsenite was assessed by the percent control achieved. Where materials were not water-soluble, water miscible preparations were prepared by dissolving the material in a suitable solvent and adding 0.01% of an appropriate emulsifying agent. The three classes of compound used were monoidoacetic acid, several alpha beta unsaturated ketones and the heavy metal halides. The results of these tests are presented in the following tables.

Table 10. The effect of treatment of adult blue ticks with several alpha beta unsaturated ketones, ferric chloride and ferrous chloride alone and followed in some cases with treatment in varying concentrations of sodium arsenite

(a) Benzalacetophenone, sodium arsenite alone and in combination on arsenic sensitive adult ticks

Concentration % As_2O_3	% Concentration Benzalacetophenone	Percent Control		
		Arsenic alone	Benzalacetophenone alone	Arsenic plus Benzalacetophenone
0.32	0.5	100	0	100
0.16	0.25	100	0	100
0.08	0.125	95	0	70
0.04	0.0625	30	0	40
0.32	0.5			100
0.16	0.5			100
0.08	0.5			95
0.04	0.5			64

Water treated control resulted in 0% control

(b) Mesityl oxide on arsenic-resistant and -sensitive adult ticks

Mesityl oxide percent concentration	Percent control	
	As-resistant strain	As-sensitive strain
1.0	2	45
0.5	6	35
0.25	4	20
0.0625	4	35
Water control	0	5

Table 10 (continued)

(c) Furfuralacetophenone, sodium arsenite alone and in combination on arsenic sensitive adult ticks

Concentration % As_2O_3	Concentration Furfuralacetophenone	Percent Control		
		Arsenic alone	Furfuralacetophenone alone	Arsenic and Furfuralacetophenone combined
0.32	0.5	100	25	
0.16	0.25	100	20	
0.08	0.125	75	65	
0.04	0.0625	15	0	
0.32	0.0625			100
0.16	0.0625			100
0.08	0.0625			50
0.04	0.0625			80
0.32	0.5	100	25	95
0.16	0.5			100
0.08	0.5			90
0.04	0.5			75

Water treated control resulted in 0% control

(d) Cyclopropylfuryl propenone on arsenic resistant adult ticks

Cyclopropylfurylpropenone percent concentration	Percent Control
1.0	0
0.5	20
0.25	0

Table 10 (continued)

(e) Ferric and ferrous chloride on arsenic sensitive adult ticks

%Concentration	Percent Control	
	Ferric chloride in 2% HCl	Ferrous chloride in 2% HCl
2	70	100
1	60	40
0.5	50	60
0.25	40	0

Control treated in 2% HCl resulted in 10% control

Benzalacetophenone alone had no effect on adult ticks at the concentrations used, neither did it enhance the effect of subsequent treatment with sodium arsenite. Mesityl oxide alone had little effect on the arsenic resistant strain of adult ticks but had some effect on the arsenic sensitive strain indicating a cross tolerance between resistance to arsenic and the -SH inhibiting mesityl oxide. Furfuralacctophenone showed a slight toxic effect when used alone on arsenic sensitive ticks and treatment with concentrations of 0.0625% and 0.5% followed with concentrations of sodium arsenite gave a slightly increased percent control when compared with arsenic alone at the same concentrations. Cyclopropylfurylpropenone alone at the concentrations used had little effect on adult arsenic resistant ticks, and could not be examined preceding arsenic treatment or on arsenic sensitive ticks because of a shortage of tick material at that time.

The heavy metal halides, ferric and ferrous chloride both show considerable effect on arsenic sensitive ticks. Both these materials are unstable in water solution and were stabilised with 2% hydrochloric acid. For this reason ferric and ferrous chloride treatments were not followed with sodium arsenite treatments as it was considered that the acidity of the solution might affect the alkaline sodium arsenite resulting in effects which would be difficult to interpret.

Note: At the time when these tests were conducted the supply of arsenic sensitive and resistant ticks was very limited and considerably curtailed the extent of the experiments conducted. This limited the treatment of ticks to batches of between ten and twenty which undoubtedly accounts for some of the erratic results obtained.

Monoiodoacetic acid at 1% and 0.5% was highly toxic to both arsenic resistant and arsenic sensitive adult female ticks.

Table 11. The effect of treatment with iodoacetic acid and iodoacetic acid followed by sodium arsenite on adult blue ticks.

Percent concentration		Percent control	
Sodium arsenite % As_2O_3	Iodoacetic acid	Arsenic resistant strain	Arsenic sensitive strain
0.32	1	100	100
0.16	1	100	100
0.08	1	100	100
0.04	1	100	100
-	1	100	100
0.32	0.5	100	98
0.16	0.5	100	100
0.08	0.5	100	98
0.04	0.5	87	84
-	0.5	93	100
0.32	0.25	100	90
0.16	0.25	100	68
0.08	0.25	67	95
0.04	0.25	45	15
-	0.25	16	0
0.32	-	44	100
0.16	-	14	100
0.08	-	2	85
0.04	-	4	23
Water treated control		0	0

(Batches of 50 adult ticks were used for each treatment)

At a concentration of 0.25%, iodoacetic acid had little effect on either strain although the percent control of the resistant tick appeared slightly higher. Treatment of ticks with 0.25% iodoacetic acid followed half an hour later by treatment with various concentrations of sodium arsenite greatly increased the percent control of the resistant strain but inexplicably reduced the percent control obtained on the sensitive strain.

The results obtained by treatment of the arsenic resistant strain of adult tick with 0.25% iodoacetic acid followed by treatment with sodium arsenite, the greater effect of mesityl oxide on the arsenic sensitive strain than on the arsenic resistant strain, and the increase of effectiveness of sodium arsenite by pretreatment of the arsenic sensitive strain with furfuralacetophenone suggests the existence of an arsenic detoxifying mechanism in ticks which is associated with thiol containing compounds. The results obtained with 0.25% iodoacetic acid followed by sodium arsenite and the indication of resistance to mesityl oxide by arsenic resistant ticks suggest that thiol-dependent detoxifying compound or compounds may occur in higher concentration in the arsenic resistant strain.

These results prompted the investigation of the comparative levels of glutathione, cysteine and its precursor cystine and total free sulphydryl in various stages of arsenic-resistant and -sensitive ticks. At this stage the investigation was handed over to the Organic Chemistry section, Research Department, African Explosives & Chemical Industries Limited, who were better equipped for the detailed analytical methods required. A brief resume of these results follows.

(2) Levels of thiol containing compounds in arsenic-resistant and -sensitive ticks

An examination of eggs midway between oviposition and eclosion by a method adapted from that of Mason (1929, 1930) showed a sulphydryl level in the eggs from an arsenic resistant strain of tick twice as great as that in the arsenic sensitive strain (Thompson and Johnston, 1958).

Further work on arsenic resistant and sensitive blue tick larvae was carried out by Harington (personal communication) and is as follows :-

Cystine and cysteine were determined in resistant and sensitive larvae by the method of Park and Speakman (1952), glutathione by the glyoxalase manometric method of Woodward (1935), and total free sulphydryl by the method of Snell and Snell (1953).

The levels of cystine and cysteine in larvae were determined in both resistant and sensitive larvae in two series of experiments. In the first series seven determinations of cystine and cysteine were carried out on each strain and in the second series sixteen determinations of cystine on resistant larvae and twenty-four on sensitive larvae. Twenty-four determinations of cysteine in both sensitive and resistant larvae were undertaken. The means of these determinations are presented in Table 12.

Table 12. Cystine and cysteine content of arsenic-sensitive and arsenic-resistant blue tick larvae. Results are expressed in mg/g of tick material.

	Cystine		Cysteine	
	Arsenic-resistant larvae	Arsenic-sensitive larvae	Arsenic-resistant larvae	Arsenic-sensitive larvae
1st series	1.459	0.228	0.666	0.191
2nd series	2.808	0.992	None detected	None detected

The results of these determinations are difficult to interpret. There was considerable variation in the individual determinations which may have been caused by sampling errors but in spite of this, the greater amount of cystine and cysteine found in resistant larvae compared with sensitive larvae was statistically significant. In the second series of determinations cystine was found in greater amounts in resistant larvae, but no cysteine could be detected in twenty-four separate determinations. These differences could not be explained but suggest that the levels of cystine-cysteine vary with time.

From twenty-four individual determinations of glutathione in arsenic-resistant larvae and ten in arsenic-sensitive larvae the mean levels were 0.4271 and 0.1684 mg/gram of tick larval material. Again there was considerable variation between individual replicates but at the 5% probability level the difference between the means was significant. The glutathione content of arsenic-resistant larvae was from 1.99 to 3.25 fold greater than in the arsenic-sensitive strain.

The determination of free sulphydryl (that -SH which is not bound and not already accounted for in reduced glutathione and cysteine) was also undertaken. Eighteen individual determinations for free sulphydryl on arsenic-resistant larvae and twelve on arsenic-sensitive larvae were carried out. The means for these determinations were 70.8 micrograms/gram for arsenic-resistant larvae and 33.7 micrograms/gram for arsenic-sensitive larvae. These differences were significant at the 1% probability level and showed a free sulphydryl level in arsenic-resistant larvae 1.59 to 2.88 times higher than in arsenic-sensitive larvae.

Determination of glutathione, cystine, cysteine and free sulphydryl in adult female ticks was also attempted (Harington, personal communication) but the variation between determinations was too great to allow any conclusions to be drawn.

In conclusion, the evidence supplied by these experiments suggests that the action of sodium arsenite in ticks is associated with thiol-containing materials and that resistance to sodium arsenite in ticks is associated with an increase level of thiol-containing materials. Further, it appears, as might be expected, that no particular, but several or many, thiol compounds occur in higher concentration in arsenic-resistant ticks thereby imparting an increased tolerance to sodium arsenite.

The means of determinations of glutathione, cystine, cysteine and free sulphydryl in larvae have shown an approximate increase

of between 2 and 6.6 fold in the arsenic resistant strain as compared with the arsenic sensitive strain. The order of increase of -SH compounds in the arsenic resistant strain of larvae is approximately of the same magnitude as the increase in tolerance to sodium arsenite in the arsenic resistant adult tick.

(b) pp'DDT

The mode of toxic action of DDT in insects remains unelucidated and in consequence has been subject to a great deal of speculation. The inhibition of enzyme systems by DDT has been investigated by several workers; the cytochrome oxidase system (Johnston, 1950; Morrison and Brown, 1954) was unaffected by DDT. DDT had no effect on cholinesterase (Tobias, Kollros and Savit, 1946). DDT in high concentrations was found to inhibit succinoxidase in houseflies but this system was also inhibited by the non-toxic DDT analogues (Anderson, March and Metcalf, 1954).

Mullins (1954) proposed a physical basis for the action of DDT. It was postulated that a molecule, provided with attractive forces properly distributed, could orientate itself in the interspaces surrounding lipoprotein molecules, resulting in distortion which could give rise to a leak of ions and consequent nervous excitation. However, proof of this theory is apparently impossible to obtain (Kearns, 1956).

The development of resistance to DDT in a number of insects, the housefly in particular, has resulted in a concentration of effort on the resistance problem rather than on a study of the mode of toxic action.

Weismann (1947) noted in DDT resistant flies from Sweden that the membranes of the tarsal pulvilli were thicker than in a strain of susceptible flies, but it was later discovered that the membranes of the pulvilli of Swedish flies were normally thicker than in many other strains of resistant flies from other areas. Tarsal membrane thickness was characteristic of flies from Sweden rather than a characteristic of DDT

resistant flies generally (Hoskins and Gordon, 1956). The behaviour characteristics of many insects have been put forward as a possible cause of insecticide resistance. Resistant flies tended to settle on untreated feed troughs and dairy floors rather than on treated walls and ceilings (King and Gahan, 1949). Busvine (1951) suggested that DDT resistance might be due to resistant flies being irritated sooner by DDT and in consequence they would move away from the treated area or be knocked down sooner so preventing the accumulation of a lethal dose. Behaviour characteristics do appear to exist and undoubtedly can play some part in insecticide resistance but injection experiments (March and Metcalf, 1949) show that restricted uptake and penetration play little part in resistance to DDT.

A by-pass mechanism is a further possible explanation of insecticide resistance in insects. In such a mechanism it is assumed that the insecticide acts by blocking a pathway of some vital physiological process and resistance results when the blocked pathway is by-passed by an additional physiological mechanism. This mechanism of resistance was put forward as an explanation for HCN resistance in scale insects (Yust and Sheldon, 1952) and DDT resistance in houseflies (Sacktor, 1950).

A study of the metabolic breakdown of DDT in insect tissues has been most rewarding in the elucidation of the mechanisms of resistance to DDT. This subject has been well reviewed by several authorities (Chadwick, 1955; Metcalf, 1955; Dahm, 1957).

Using C^{14} labelled DDT on American cockroaches it was found that topically applied DDT was quickly absorbed and widely distributed internally. Most of the absorbed DDT was excreted in the faeces in the form of metabolites (Robbins and Dahm, 1955). DDT topically applied to DDT-resistant strains of the housefly were shown to be converted to the non-toxic 1,1-dichloro-2, 2-bis (p-chlorophenyl) ethylene (DDE). The conversion of DDT to DDE was far greater in the resistant strain of flies than in DDT-sensitive flies (Perry and Hoskins, 1950; Sternburg, Kearns and Bruce, 1950). It was demonstrated that the conversion of DDT to DDE

in DDT-resistant flies was catalysed by an enzyme which was called DDT-dehydrochlorinase and which could not be detected in DDT-sensitive flies (Sternburg, Vinson and Kearns, 1953). From this work it was concluded that DDT-resistance in flies was partly explained by the enzymatic conversion of DDT to DDE, but that other factors such as penetration and absorption in the fat body might also contribute to resistance and these factors might vary from insect to insect or even in different strains of the same insect.

The mechanism of DDT resistance in the blue tick was investigated along similar lines to that carried out in houseflies. DDT-resistant ticks were found to be also resistant to dilan, a mixture of the nitropropane and nitrobutane analogues of DDT. These insecticides have no chlorine in the alkyl chain thus cannot be dehydrochlorinated. Prolan was found to be effective against DDT-resistant flies (Busvine, 1953; Fulmer and Hoskins, 1951; March and Metcalf, 1950) and this observation may be considered to support the dehydrochlorination theory of DDT resistance. Resistance to dilan by DDT-resistant blue ticks thus suggests that the mechanism of DDT resistance is different from that in houseflies. Winteringham (1952) came to a similar conclusion when the DDT-resistant strain of housefly with which he was working was found to be resistant to prolan. In view of this, an investigation of the possible existence of DDT-dehydrochlorinase in DDT-resistant ticks was undertaken. The procedure used was identical with that of Sternburg, Kearns and Moorefield (1954). Both "acetone powders" and partially purified extracts of DDT-resistant blue tick larvae were prepared. Using these preparations as a source of DDT-dehydrochlorinase no "in vitro" degradation of DDT could be detected. As DDT-resistant houseflies were not available it could not be established that the method was satisfactory with the equipment available and no definite conclusion could be drawn from these results although other results have since come to hand which support a theory that DDT-resistance in the blue tick is not as a result of DDT breakdown by DDT-dehydrochlorinase. Roulston (1957) using DDT-resistant Boophilus

microplus (Canestrini) could not show that DDT resistance was as a result of the conversion of topically applied DDT to DDE. Neither could he show the existence of DDT-dehydrochlorinase in DDT-resistant Boophilus microplus which he was able to detect in the Canberra-strain of DDT-resistant houseflies.

As the lack of completely suitable equipment and a known DDT-resistant strain of houseflies threw some doubt on the observations that DDT-dehydrochlorinase did not exist in DDT-resistant ticks it was decided to investigate the breakdown of DDT by determining both DDT and DDE after DDT treatment of DDT-resistant tick larvae.

Herriott (1946) showed that pp'DDT in ethanol absorbed very slightly at a wave-length of 250 mμ. Absorption increased with a decrease in wave-length to a peak at 235 mμ. DDE (2,2-bis(p chlorophenyl) 1,1-dichloroethylene) absorbed strongly at a wave-length of 250 mμ with the absorption decreasing slowly to almost zero at 300 mμ. The ultra-violet absorption curves of DDT and DDE are shown in diagram 5.

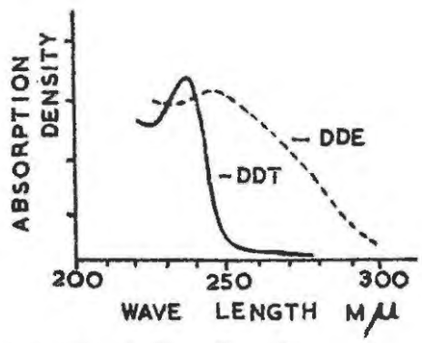


Diagram 5. Ultraviolet absorption curves for DDT and DDE.

Thus by determining the absorption density at a wave-length of 250 mμ the presence of DDE in a solution containing DDT could be detected.

Sternburg and Kearns (1952) described a method for chromatographically separating several breakdown products of DDT in insect tissues. By this method DDT could be separated from DDE. By combining some aspects of both these methods the presence or absence of DDT and DDE in DDT-treated resistant tick larvae was investigated.

Initially pure pp'DDT and pure pp'DDE were obtained by recrystallisation. Chromatographic columns were prepared according to the

method of Sternburg and Kearns (1952). In the initial experiments difficulty was experienced with the activated alumina as the proprietary brand mentioned in the method was not available in this country. Eventually the most satisfactory separation of DDT and DDE was obtained by using alumina prepared as follows :-

> Dry HCl washed alumina was heated at 360°C in a Wild Barfield furnace with intermittent stirring for 2 hours. It was then cooled in a desiccator and 1% water added and agitated on a set of rollers for three hours.

DDT (0.5 mg) and DDE (0.5 mg) were dissolved in 5 ml spectrographically pure petroleum ether (30-60° boiling range) and poured through the alumina column. This was followed by two 5 ml quantities of petroleum ether applied as soon as the preceding quantity had sunk away. This was followed by 60 ml of petroleum ether. The entire petroleum ether eluate was collected. (The petroleum ether available in this country was not sufficiently pure for spectrographic work and a pure product had to be prepared as follows :-

> Petroleum ether (30-60° boiling range) was shaken with successive quantities (10% v/v) of oleum until the oleum fraction remained colourless. The petroleum ether was then shaken up with successive quantities (10% v/v) saturated $KMnO_4$ in 10% H_2SO_4 until the colour of the $KMnO_4$ ceased to fade. The petroleum ether was then shaken with successive quantities of distilled water until both fractions remained colourless. The ether was separated from the water and placed in a flask with a quantity of $CaCl_2$ for half an hour with occasional shaking. The ether was then distilled and left to stand over sodium wire for 24 hours to facilitate complete drying.

When the last of the petroleum ether had sunk into the alumina a clean flask was placed under the column and 25 ml of CCl_4 was poured through. The flasks containing the petroleum ether fraction and the CCl_4 fraction were evaporated down to 5 ml on a steam bath with a stream of compressed air. The last 5 ml was taken down to dryness with a stream of compressed air only as at this stage care has to be taken to avoid volatilization. The flasks were then successively rinsed with spectrographically pure methanol and made up to 25 ml in a volumetric flask. The absorption density was determined over a range of wave-lengths

on a Zeiss spectrophotometer using pure methanol as a blank (Herriott, 1946). Subsequently, it was found that the petroleum ether prepared as above was satisfactorily pure to allow a direct reading on the spectrograph thus eliminating the necessity for using methanol. In these circumstances the CCl_4 fraction still had to be reduced to dryness but could then be taken up in pure petroleum ether.

The results obtained with a mixture of pure DDT and DDE are shown graphically in Fig. 36.

Having established the method for detecting DDT and DDE spectrographically, batches of DDT-resistant and -sensitive tick larvae weighing approximately 1 g were exposed for 24 hours to 0.5 mg pure pp'DDT deposited from petroleum ether in glass stoppered flasks. After exposure a convenient quantity of petroleum ether was added to the flasks, rinsed and poured into a pestle and mortar. A convenient quantity of ground glass and anhydrous Na_2SO_4 was added and the larvae ground to a fine slurry. The slurry was filtered through Whatman No. 1 filter paper and successively rinsed with a total amount of 150 ml of purified petroleum ether. The filtrate was concentrated to a volume of 5 ml by evaporation on a steam bath with a stream of air. The concentrate was then poured through the activated alumina column as previously described. Determinations were carried out on three replicates of DDT-resistant and -sensitive tick larvae and in all cases the absorption spectrum obtained from the CCl_4 fraction was almost identical with that obtained with pure pp'DDT. The petroleum ether fraction showed no absorbance over a wavelength range of 250 to 300 m

These results indicated that DDT applied to both DDT-resistant and -sensitive larvae was not being converted to DDE. Further determinations were carried on both strains of tick larvae but as no DDE could be detected in the initial experiments it was decided to omit the chromatographic separation and determine the absorption density directly on petroleum ether extracts of treated larvae 24 hours after exposure. The mean results of three separate determinations on DDT-resistant and -sensitive larvae are shown in Fig. 37.

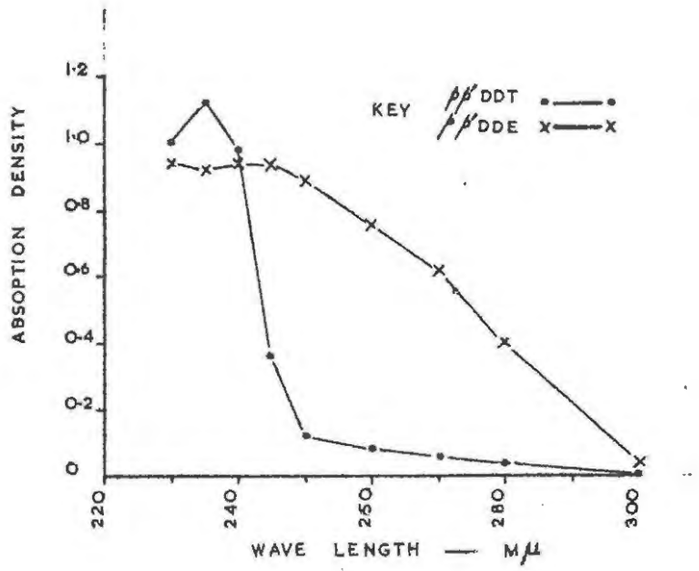


Fig. 36 Ultraviolet absorption curves for pure pp'DDT and pp'DDE separated chromatographically from a mixture.

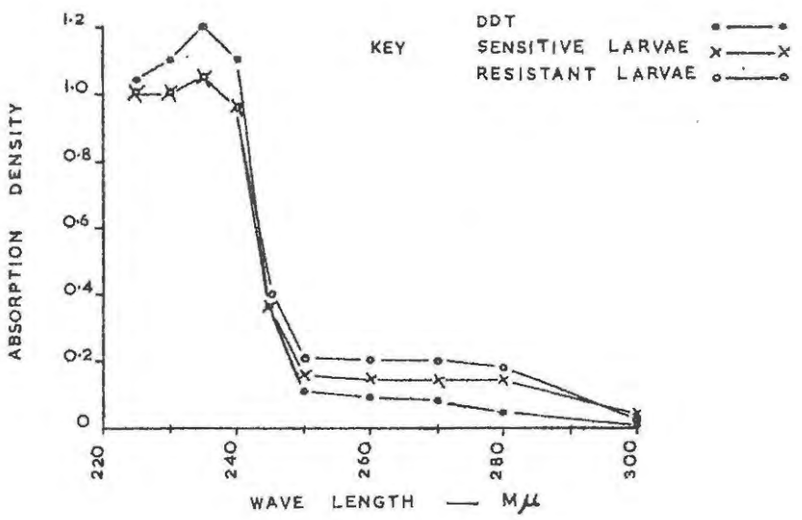


Fig. 37 Ultraviolet absorption curves for DDT and petroleum ether extracts from DDT treated resistant and sensitive tick larvae.

It will be observed that the graph of the absorption density for pure pp'DDT (Fig. 36) was basically similar to that of the extracts from DDT treated resistant and sensitive larvae (Fig. 37). At a wave-length of 250 mμ the absorption density appears to be slightly higher for extracts from DDT resistant and sensitive larvae than for pure DDT. The resistant larval extracts gave consistently higher absorption densities at wave-lengths greater than 250 mμ. This may indicate that some DDE is being formed in both strains of larvae and that the breakdown of DDT to DDE was highest in the resistant strain. However, these differences are so small it appears hardly likely that such a process could give rise to an effective protective mechanism. In all cases DDT-sensitive larvae exposed to 0.5 mg pp'DDT were dead after 24 hours while the resistant strain exposed in a similar manner were all alive. It would have been expected that had the mono-dichlorination of DDT been the protective mechanism in resistant ticks, far larger amounts of DDE would have been detected.

Perry, Mattson and Buckner (1953) showed that di-(p-chlorophenyl) methyl carbinol (DMC or Kelthane) synergised DDT in respect of DDT resistant flies. They postulated that DDT-dehydrochlorinase reacted preferentially with DMC, thus blocking the normal DDT resistance mechanism. If the DDT resistance mechanism was similar in DDT-resistant ticks DMC should also be synergistic to DDT on DDT -resistant ticks. Tests using a range of concentrations of DDT, DMC and combinations of both compounds were applied to DDT-resistant Nagaza Park 1958 and DDT sensitive Transvaal 1958 larvae in a manner similar to the technique described in Part II (b) (2). The results of these tests are presented in Table 13.

Table 13. The effect of DDT, DMC and combinations thereof on DDT-resistant and -sensitive tick larvae

% Concentration DDT	% Concentration DMC	% Mortality of larvae					
		DDT alone		DMC alone		DDT & DMC combined	
		R	S	R	S	R	S
.02	.2	3	100	5	6.6	5	98
.01	.1	2	100	2.5	6	5	82
.005	.05	3	98	1.6	10	1	33
.0025	.025	4.5	95	1.5	8.1	4	21
.00125	.0125	0	69	1.5	3.7	4	9
R = Resistant strain				S = Sensitive strain			

These results show that DMC has not potentiated the action of DDT in respect of DDT-resistant larvae. On DDT sensitive larvae the addition of DMC to DDT appears to have reduced the effectiveness of DDT.

In conclusion, the results obtained with dilan and pyrethrum; the failure of DMC to synergise DDT; the apparent absence of DDT-dehydrochlorinase and the absence of DDE after DDT treatment of DDT-resistant tick larvae indicate that the mechanism of DDT resistance in the blue tick is entirely different from that which has been shown to exist in houseflies.

(c) gamma BHC

Considerably less work on the mode of action of gamma BHC in comparison with that on DDT has been carried out. Slade (1945) suggested that gamma BHC may be isosteric with the B-vitamin, meso-inositol and may thus block some vital metabolic process. However, considerable doubt has been cast on Slade's theory by the work of Metcalf (1947) who could find no antidotal effects from meso-inositol treatment of gamma BHC intoxicated insects and of Dresden and Keijgsman (1948) who failed to antagonise gamma BHC poisoning by simultaneous injections of meso-inositol and gamma BHC into P. americana.

Mullins (1955) proposed a physical basis for the action of gamma BHC similar to that proposed for the action of DDT. He showed theoretically that only the gamma isomer of BHC could disrupt a lipoprotein membrane and on this basis explained the lack of insecticidal activity of the other BHC isomers.

Resistance to gamma BHC has developed in a number of insect species but, as with the mode of action, has been studied to a lesser extent than in DDT.

Alpha and delta isomers of BHC were found to be broken down to a greater extent by BHC-resistant houseflies than by sensitive flies and this was suggested as a possible mechanism of resistance (Oppenoorth, 1955). Bradbury and Standen (1953) investigated the fate of labelled gamma BHC in resistant and sensitive houseflies. It was found that both strains of flies metabolised BHC to a number of unidentified water-soluble products which were excreted. The rate of conversion was considerably greater in the resistant strain. It was also found that the rate of pick-up of gamma BHC was slower in the resistant housefly and that the more rapid conversion and reduced pick-up lowered the BHC found inside resistant flies to a quarter of that in a susceptible strain.

Sternburg and Kearns (1956) showed that the mono-dehydrochlorination product of gamma BHC, pentachlorocyclohexene was formed in BHC-treated houseflies and that the rate of formation was accelerated in the BHC-resistant strain. These results were confirmed by Bradbury and Standen (1958) who showed that the BHC-resistant strain of housefly enjoyed a considerable advantage over susceptible flies in the rate of detoxication of BHC. Although the dehydrochlorination of BHC is basically similar to that in DDT, DDT-dehydrochlorinase was not found to be responsible for BHC-dehydrochlorination (Sternburg and Kearns, 1956).

With gamma BHC-resistant blue ticks an attempt was made to find the intermediate BHC metabolic product, pentachlorocyclo-hexene in BHC-treated adult ticks. BHC was applied with a microdrop

applicator to adult ticks which were immobilised by removing the legs. Twenty-four hours after treatment the ticks were washed three times in a separating funnel with n-hexane, then macerated in a Waring blender and extracted with carbontetrachloride. The concentrated extract was treated by the method of Schechter and Hornstein (1952) whereby gamma BHC is dechlorinated with zinc and acetic acid to give benzene and subsequently nitrated with a mixture of fuming nitric acid and concentrated sulphuric acid to form dinitrobenzene. Any pentachlorocyclohexene in the extract would yield chlorobenzene on reduction with zinc and acetic acid and subsequent nitration would yield dinitrochlorobenzene. Dinitrobenzene and dinitrochlorobenzene could then be separated by the reverse phase chromatographic method developed by Sternburg and Kearns (1956). This entailed spotting of the material on strips of Whatman No. 1 chromatographic paper which were then sprayed with a 5% solution of maize oil (cottonseed oil used by Sternburg was not obtainable) in ethyl ether and allowed to dry. The paper strips were run in 3:1 water:methyl alcohol as the mobile phase until the solvent front was a few centimeters from the top of the paper. The paper strips were removed from the solvent and allowed to dry at room temperature. When free of solvent, the papers were sprayed with a 1% solution of sodium polysulphide in water. According to the method, a purple colour is developed by the dinitrobenzene spot and an orange colour with dinitrochlorobenzene. From numerous experiments only the purple spot of the dinitrobenzene could be detected in BHC-treated tick extracts. Pentachlorocyclohexene was also looked for in BHC-resistant and -sensitive larvae. The experimental procedure was essentially the same as that with adult ticks except that larvae were exposed to BHC treated surfaces for two hours in glass stoppered conical flasks. The treated larvae were ground in a pestle and mortar and subjected to the reduction and nitration procedure as already described for adult ticks. The chromatographic examination of the extracts from five replicates of resistant and sensitive larvae again showed only a single purple spot corresponding to dinitrobenzene.

Attempts were made to import BHC-resistant flies so that extracts from gamma BHC treated BHC-resistant flies could be compared with extracts from ticks but the necessary permit was refused by the Quarantine Authorities and the project was discontinued.

C O N C L U S I O N

The blue tick, Boophilus decoloratus Koch, from various areas of South Africa has been shown to be resistant to sodium arsenite, gamma BHC and DDT. The resistance to these materials is not related and suggests that the mechanisms of resistance to these three insecticides is entirely different.

Resistance to sodium arsenite is of a low order and imparts resistance to no other commercially available insecticide. Although the existence of resistance to arsenic alone could not be detected in any of the strains of ticks examined, should this arise in any blue tick population any other insecticide of the range examined would be satisfactory in controlling purely arsenic resistant ticks.

Resistance to gammaBHC confers a resistance to a number of insecticides which are chemically similar. The development of resistance to BHC which now exists in some areas of the Transvaal, automatically precludes the use of all chlorinated cyclic hydrocarbon insecticides such as toxaphene, chlordane, dieldrin and aldrin.

Resistance to DDT will preclude the use of dilan and pyrethrum but will not result in an automatic resistance to gamma BHC and other chlorinated cyclic hydrocarbons.

In instances where resistance to sodium arsenite, the BHC group of insecticides and the DDT group co-exist in a tick population, field control can be achieved with several organo-phosphorus insecticides and the carbamate insecticides. Although no resistance in any strain of tick could be detected in respect of the latter two groups of insecticides, it is unlikely in the event of resistance to one group that resistance to the other group would be automatic.

With the exception of a slight loss of tolerance to sodium arsenite in Nagaza Park 1958 ticks, no instances of a reversion to susceptibility to any insecticide to which resistance had been acquired, could be detected. In view of this there appears little possibility of

being able to reinstate, after a number of years, any insecticide to which resistance has developed.

From the evidence obtained in these investigations it is not possible to predict with any certainty how long any particular insecticide would remain effective in the field. It is clear, however, that resistant populations will develop most rapidly in areas where selection pressure is greatest. From the evidence available at the present time in respect of a number of species of insects, the development of resistant populations appears to be a result of continued selection of a characteristic already existing in the population.

The higher the proportion of resistant individuals in the initial unselected population and the greater the selection pressure the sooner a resistant population will develop.

The information gained from past experience suggests that the tick populations have some individuals which are initially tolerant to the concentrations of all insecticides used in the field and there is no reason to believe that the same might not hold for new insecticides developed in the future.

These conclusions suggest that in order to maintain control of the blue tick, new insecticides will have to be developed which can be brought into use as soon as the existing insecticides become ineffective.

Alternatively, an attempt will have to be made to block the mechanism of resistance, thereby reinstating the compound to which a population has become tolerant. Progress in the latter field, presumably because of the limitations of the present knowledge of insect physiology and biochemistry has been slow and a "break through" in this field cannot be anticipated in the immediate future.

In respect of the blue tick the increase in tolerance to arsenic in resistant strains is only 3 to 4 fold. With BHC the increase in resistance is greater than 600 fold while with DDT the increase in tolerance is approximately 12 fold.

In the case of resistance to BHC little information has been obtained in respect of the mechanism of resistance. As the increase in tolerance by the resistant strains is so high the possibility of reinstating BHC as a material for tick control appears remote.

In respect of DDT it has been shown that the mechanism of resistance is not one of an enzymatic breakdown of DDT to DDE. An investigation of alternative mechanisms for the detoxication of DDT in resistant strains of ticks might prove profitable.

It has been shown that strains of ticks resistant to sodium arsenite contain greater quantities of several sulphydryl compounds. As the increase in tolerance to sodium arsenite in resistant strains is comparatively low, further investigation of this mechanism might result in the re-establishment of arsenic for the control of the arsenic-resistant blue tick.

APPENDIX

Statistical analysis of insecticide response data

In the first series of insecticide tests conducted with Frankenwald and Ferndale larvae it was noted that the points (probit of mortality plotted against log concentration) were not always on a straight line. Initially it was thought that the deviations from linearity were the result of experimental error but considerable replication of the tests failed to make any appreciable difference. Examples of the type of regression lines obtained can be seen in Figs. 22, 27, 29. It appeared from the results of these tests that the distribution of insecticide response from the population taken as a whole was not normally distributed. It was obvious that a single regression line did not fit the points satisfactorily and that two separate lines drawn through the points would give a more satisfactory indication of the distribution of insecticide response.

This observation prompted the idea that in naturally-occurring ticks there might be two or more distinct populations each normally distributed in respect of response to insecticides. All the experimental results obtained with larvae in the first series of tests were submitted to the Statistical Section of the Research Department, African Explosives and Chemical Industries Limited, with the request that this aspect, i.e. the existence of double populations in naturally-occurring populations of ticks be investigated. Mr. H. Marr's analysis of the results was as follows :-

" In determining insecticide response each test using between 100 and 200 larvae for each concentration of insecticide was replicated five times. When the response from the replicated experimental determinations were plotted individually, (log concentration against probit mortality) the distribution of the points appeared to

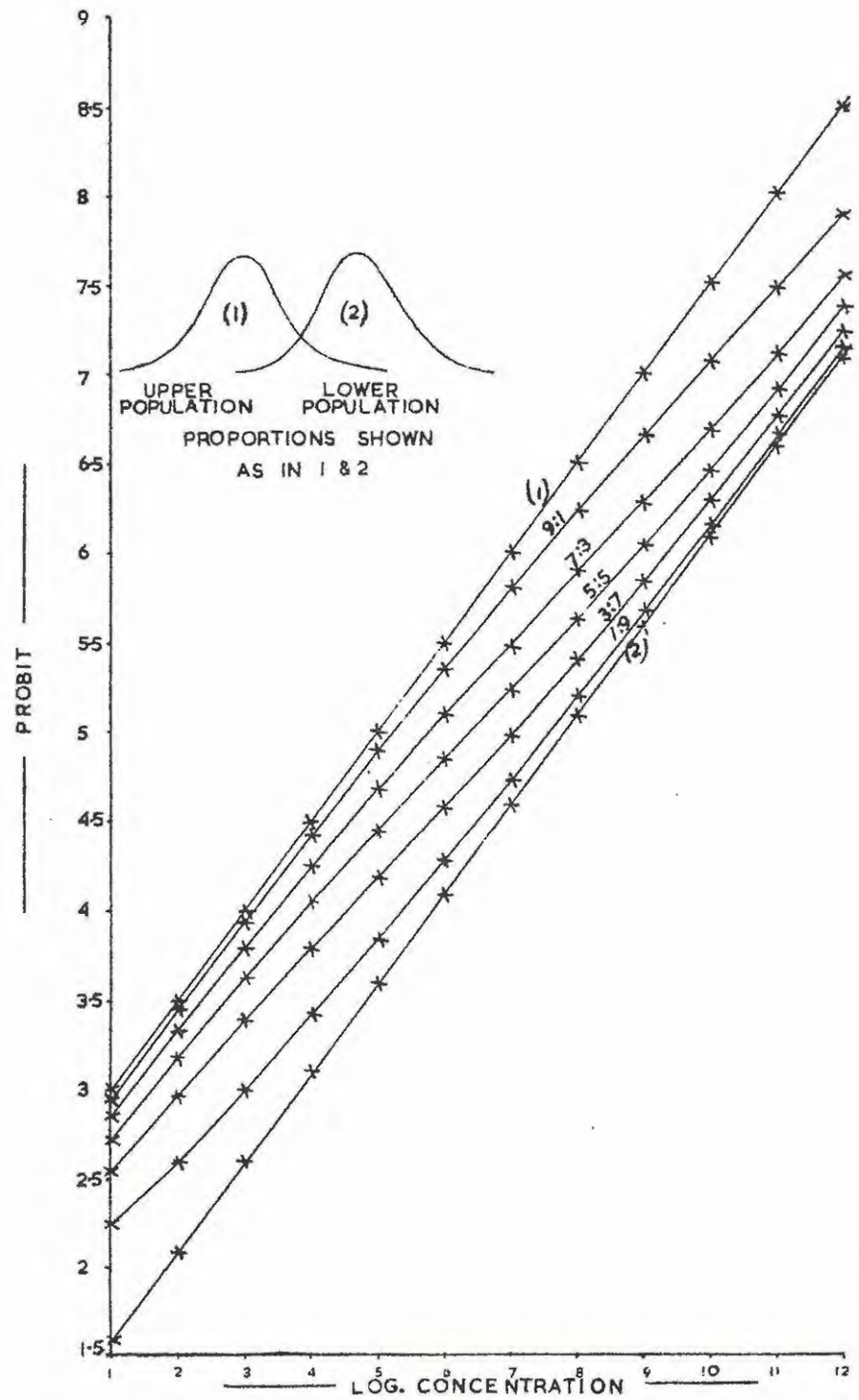


Fig. 38 Graph used as a guide in the selection of groups of points.

Standard deviation of both populations were equal.

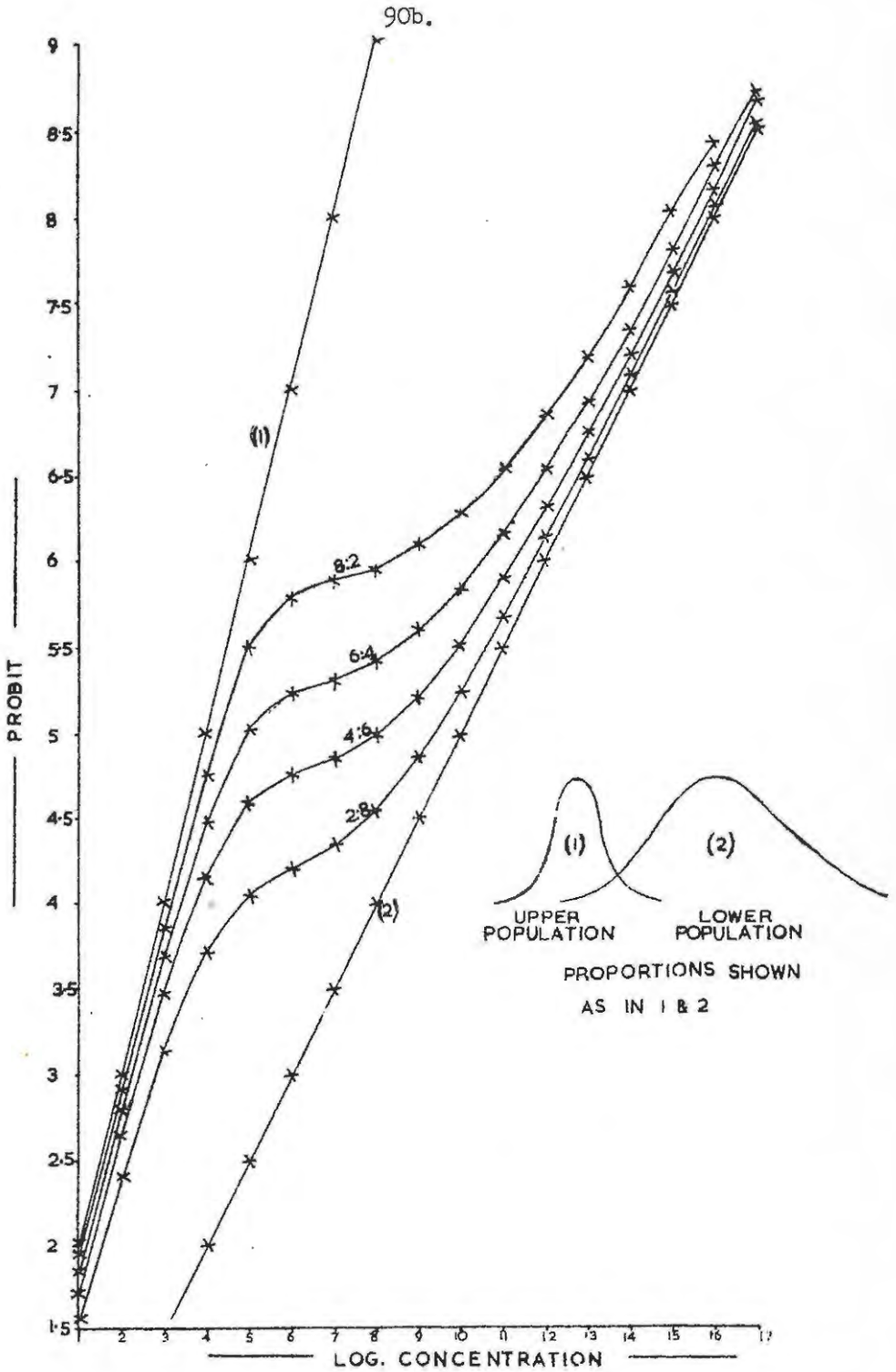


Fig. 39 Graph used as a guide in the selection of groups of points. Standard deviation of the upper population was half that of the lower population.

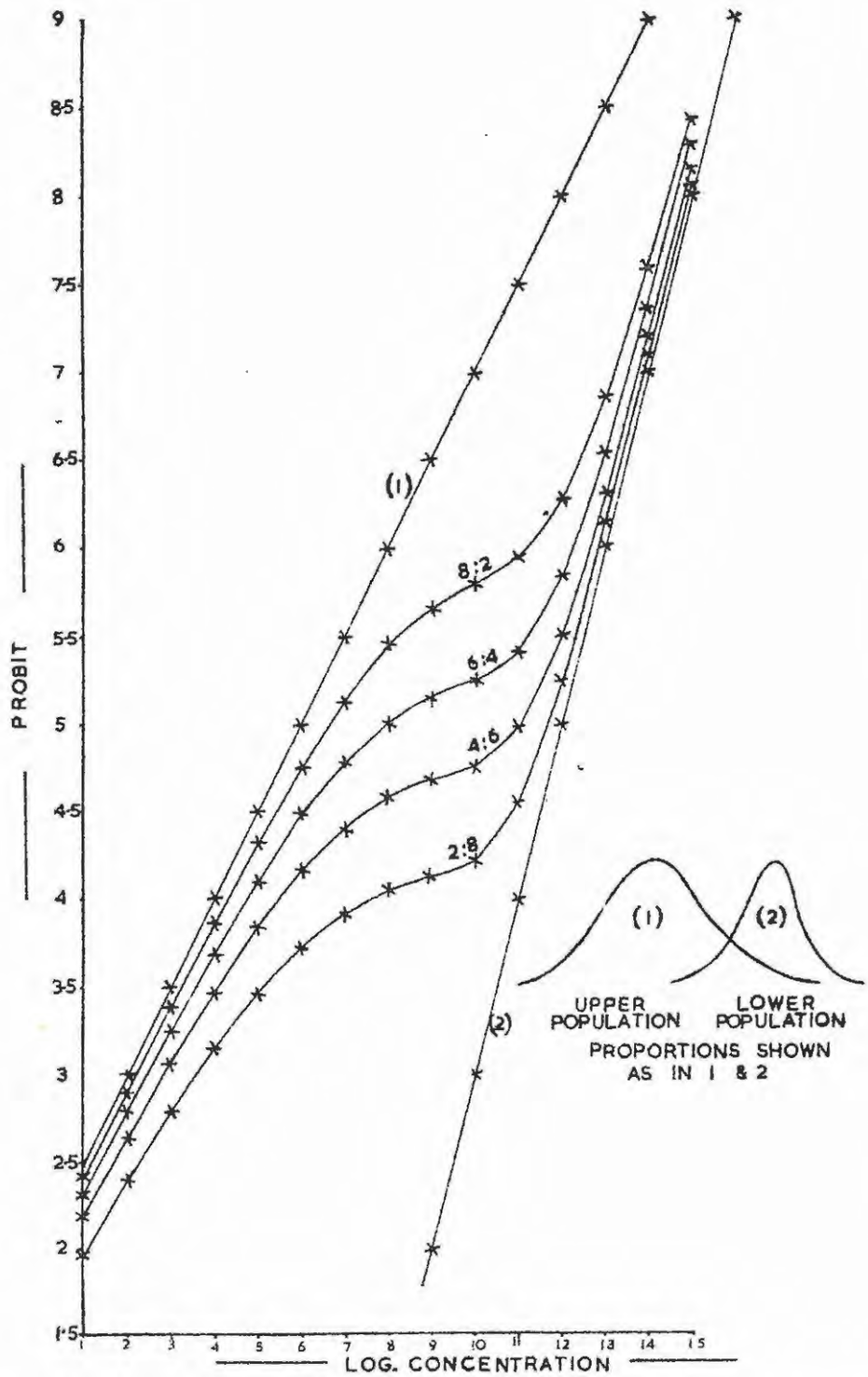


Fig. 40 Graph used as a guide in the selection of groups of points. Standard deviation of the upper population was twice that of the lower population.

fall into two families as indicated in Diagram 6.

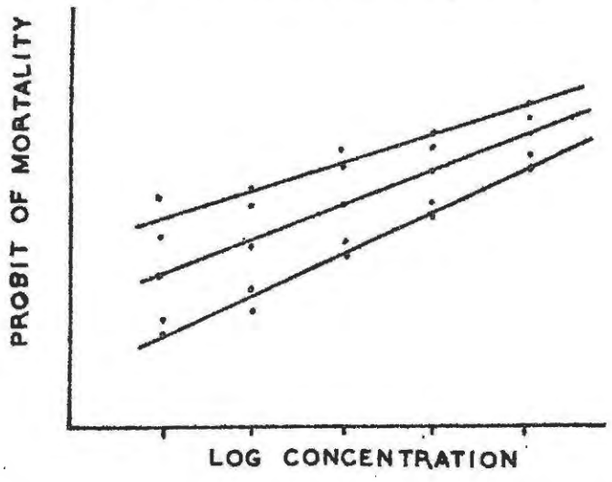


Diagram 6. Diagrammatic representation of the probit mortality plotted against log concentration of the individual replicates.

The pattern suggested that two lines could be drawn separately through upper and lower sets of points, indicating two separate populations, the upper more sensitive to insecticides than the lower with intermediate points arising from a mixture of both populations.

As a guide in the empirical separation of the points into two families, a series of probits obtained from normality tables was drawn for different proportions of two theoretical normal populations (Figs. 38, 39, 40). In Fig. 38 the two normal distributions have equal standard deviations while in Figs. 39 and 40 the standard deviation of one distribution is twice that of the other.

By using the graphs (Figs. 38, 39, 40) as a guide, groups of points were selected so that the means feel roughly on a straight line. Intermediate points corresponding to a mixed population were omitted from the groupings. In this way the distribution of response in the separated populations were obtained (Figs. 41 to 48). Log concentration-porbit mortality regression lines were calculated for the separated populations and for the population taken as a whole (Figs. 41 to 48).

As a measure of closeness of fit of the regression lines to the experimental points in upper and lower populations X^2 values of deviations from the lines were calculated. These values were not recorded because of varying degrees of freedom but comparisons are

facilitated by the X^2 probabilities from upper and lower populations which are recorded in Table 14. Probabilities of less than 0.05 indicate a significant discrepancy. It will be noted from Table 14 that only in the case of Ferndale ticks treated with toxaphene was there a significant departure from linearity. In respect of Ferndale larvae treated with dieldrin there appeared to be only one population whereas with Ferndale ticks treated with malathion there appeared to be no upper population.

Table 14. LC50 values and X^2 probability of goodness of fit for separated populations of tick larvae treated with a number of insecticides

Insecticide	Strain of ticks	LC50 values % concentration			X^2 probability of goodness of fit			
		Upper Population	Lower Population	Whole Population	Upper Population	Lower Population	Double line	Single line
Diazinon	Ferndale	0.000599	0.00104	0.000756	0.40	0.20	0.29	1.3
"	Frankenwald	0.000832	0.000899	0.000742	0.85	0.81	0.95	0.0005
Chlordane	Ferndale	131.25	70060.0	30156.0	0.98	0.87	0.99	0.38
"	Frankenwald	0.00284	0.00608	0.00426	0.38	0.31	0.39	0.0005
DDT	Ferndale	0.00268	0.00533	0.00345	0.84	0.93	0.96	0.0005
"	Frankenwald	0.00558	0.00938	0.00491	0.89	0.97	0.97	0.0005
BHC	Ferndale	0.0825	0.3792	0.2395	0.81	0.55	0.72	0.0005
"	Frankenwald	0.000284	0.000463	0.000298	0.98	0.82	0.99	0.035
Dieldrin	Ferndale	26971.0	-	-	0.992	-	-	-
"	Frankenwald	0.00109	0.00136	0.00123	0.91	0.89	0.98	0.07
Toxaphene	Ferndale	0.3948	1.4242	0.8292	0.0005	0.71	0.0007	0.0005
"	Frankenwald	0.000694	0.00110	0.000870	0.70	0.99	0.97	0.005
Aldrin	Ferndale	54431.0	2761.3	71031.0	0.999	0.999	0.999	0.65
"	Frankenwald	0.00166	0.00238	0.00179	0.90	0.08	0.35	0.04
Malathion	Ferndale	-	0.0914	0.08199	-	0.25	-	0.0005
"	Frankenwald	0.08199	0.1394	0.1034	0.015	0.25	0.025	0.0005

As a check on the validity of separating the points into two groups a single probit regression line was fitted to all points assuming they belonged to a single population. The LC50 values calculated for these lines, together with their X^2 probabilities, are presented in Table 14. If the X^2 probability of no discrepancy of the two fitted lines is greater than the X^2 probability of no discrepancy for the single line, then an improved fit has resulted from grouping the points into two populations. With the exception of the effect of malathion and dieldrin on Ferndale larvae all the results indicate that it is statistically more accurate to fit two separate regression lines to the experimental results than a single line."

These results suggest that in the strains of tick larvae examined there is some heterogeneity in response to most of the insecticides used. If this interpretation is correct it would appear that in strains of ticks regarded as sensitive to a number of insecticides there is already a nucleus of a resistant population. Selection of a population in which the distribution of response to insecticides is heterogenous would result ultimately in the build-up of the resistant strain and a decrease in the proportion of the sensitive strain.

Attempts were made to obtain strains of ticks from areas of Africa where no insecticides had ever been used in order to examine the distribution of response to insecticides in a completely unselected population. Although such strains of ticks do exist in Central Africa their importation was prohibited. It was thus not possible to compare results obtained with tick larvae from South Africa where insecticides for tick control have been applied for more than fifty years with larvae from areas where there was no history of any insecticidal treatment.

Wilson-Jones and Davidson (1958) showed by using a large number of points on the dosage-mortality curve in respect of dieldrin on resistant A. gambiae the gently sloping but not linear curve could be

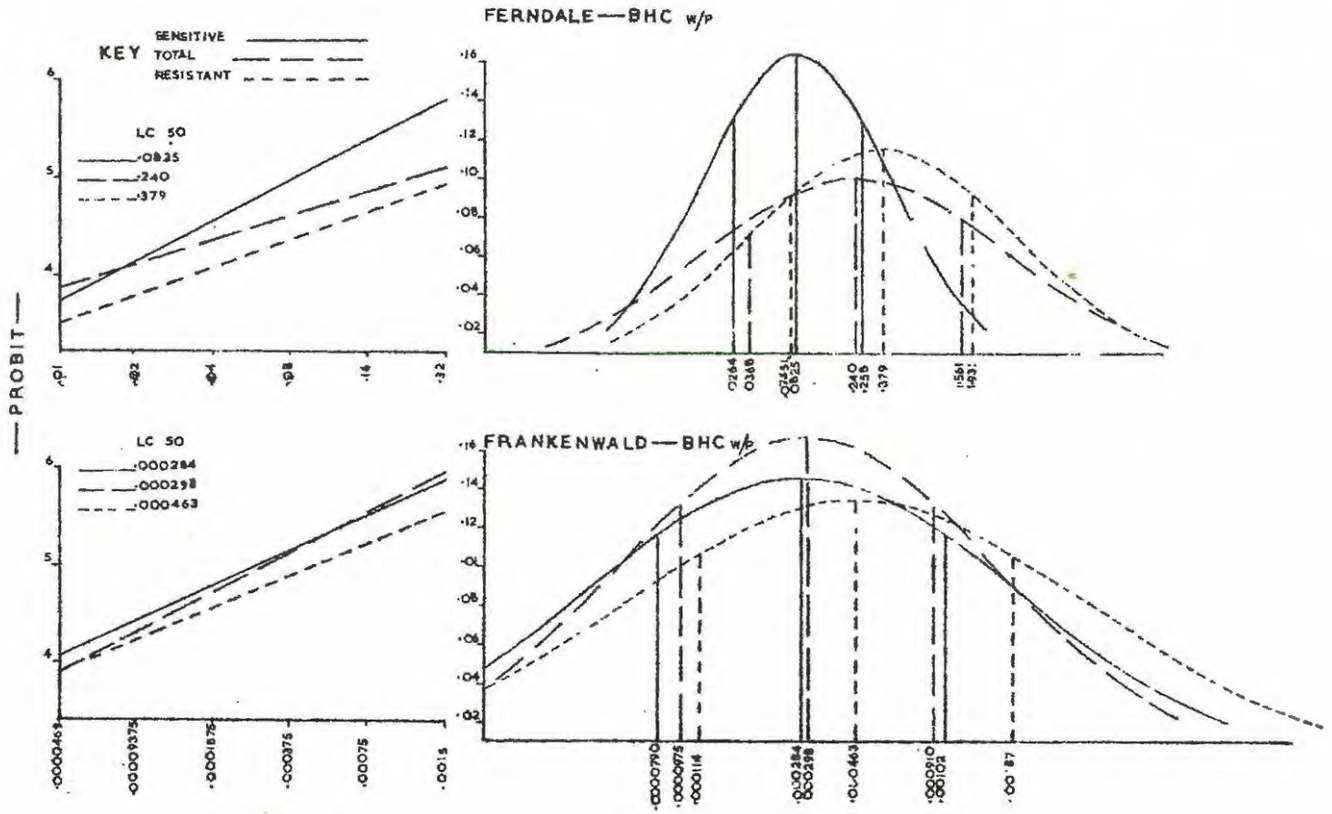


Fig. 41 The separation of tick larvae from Ferndale and Frankenwald into different populations in respect of their response to gamma BHC.

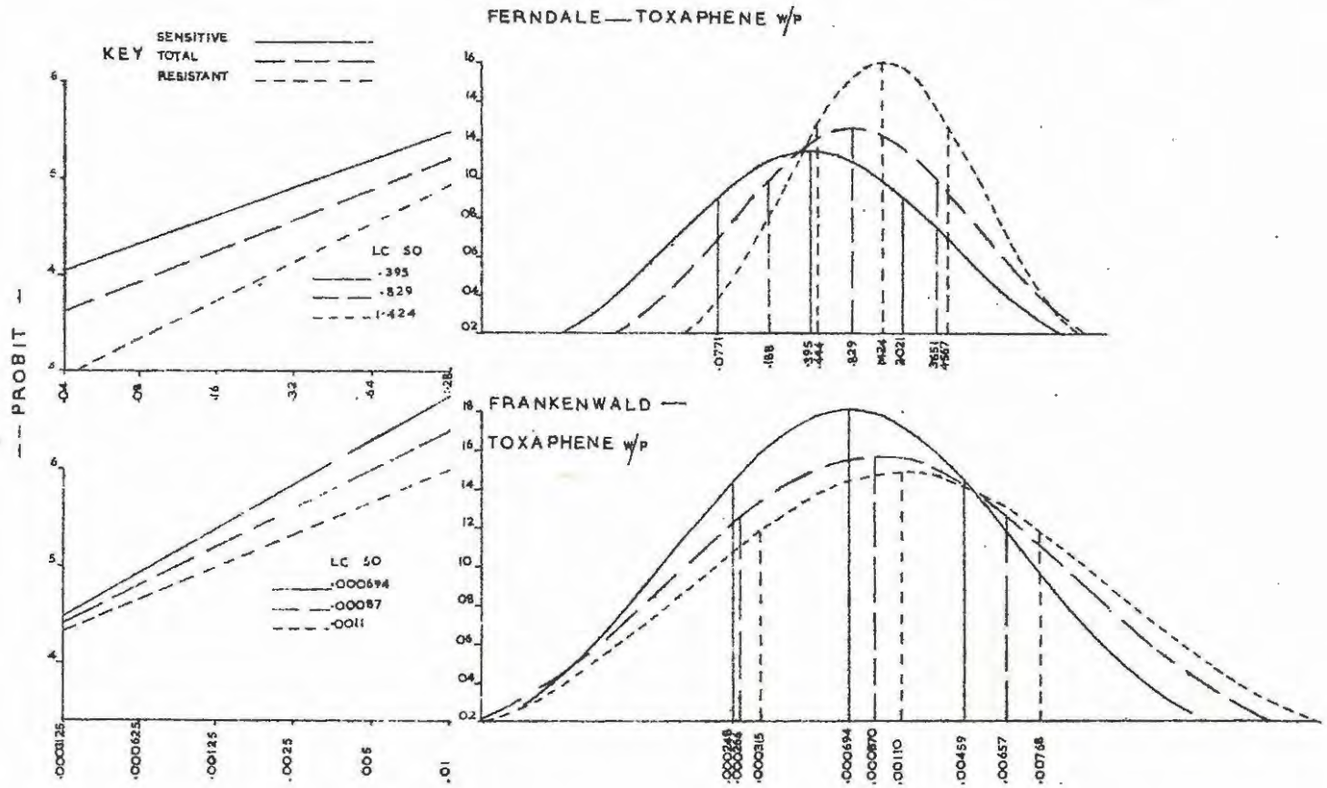


Fig. 42 The separation of tick larvae from Ferndale and Frankenwald into different populations in respect of their response to toxaphene.

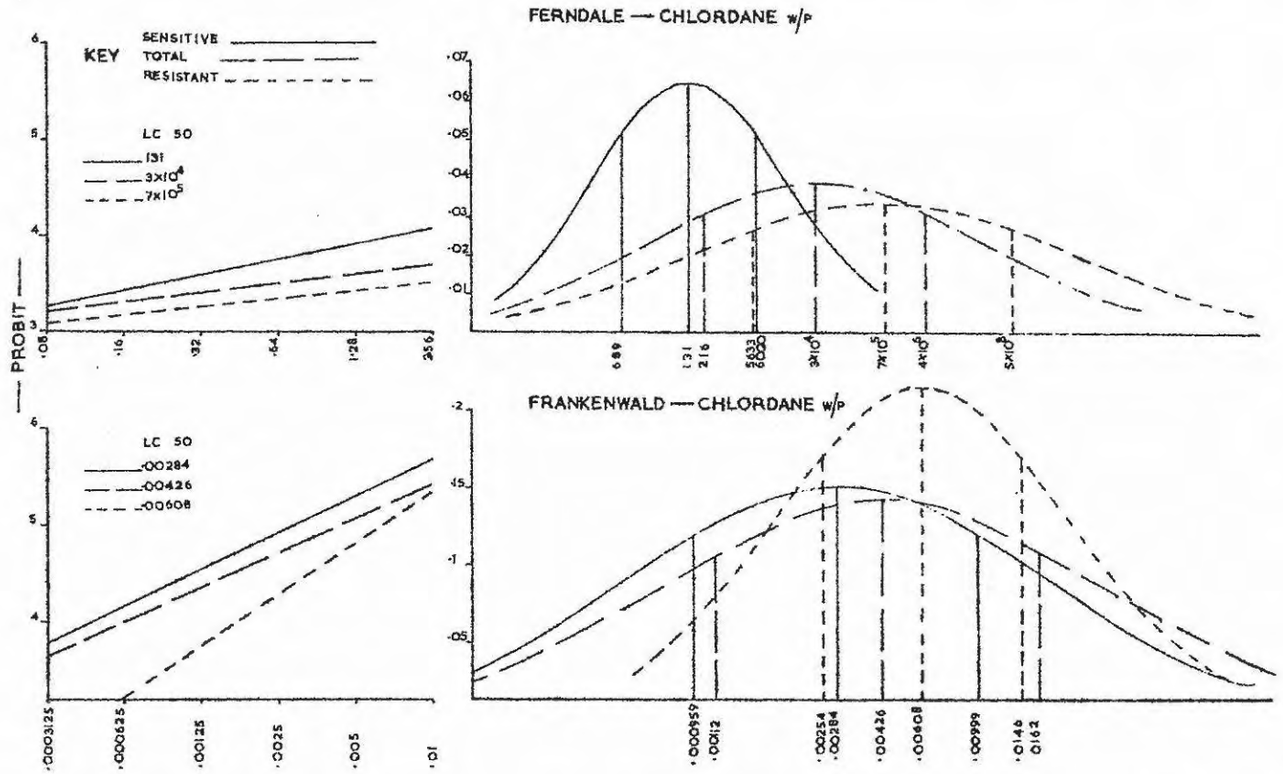


Fig. 43 The separation of tick larvae from Ferndale and Frankenwald into different populations in respect of their response to chlordane.

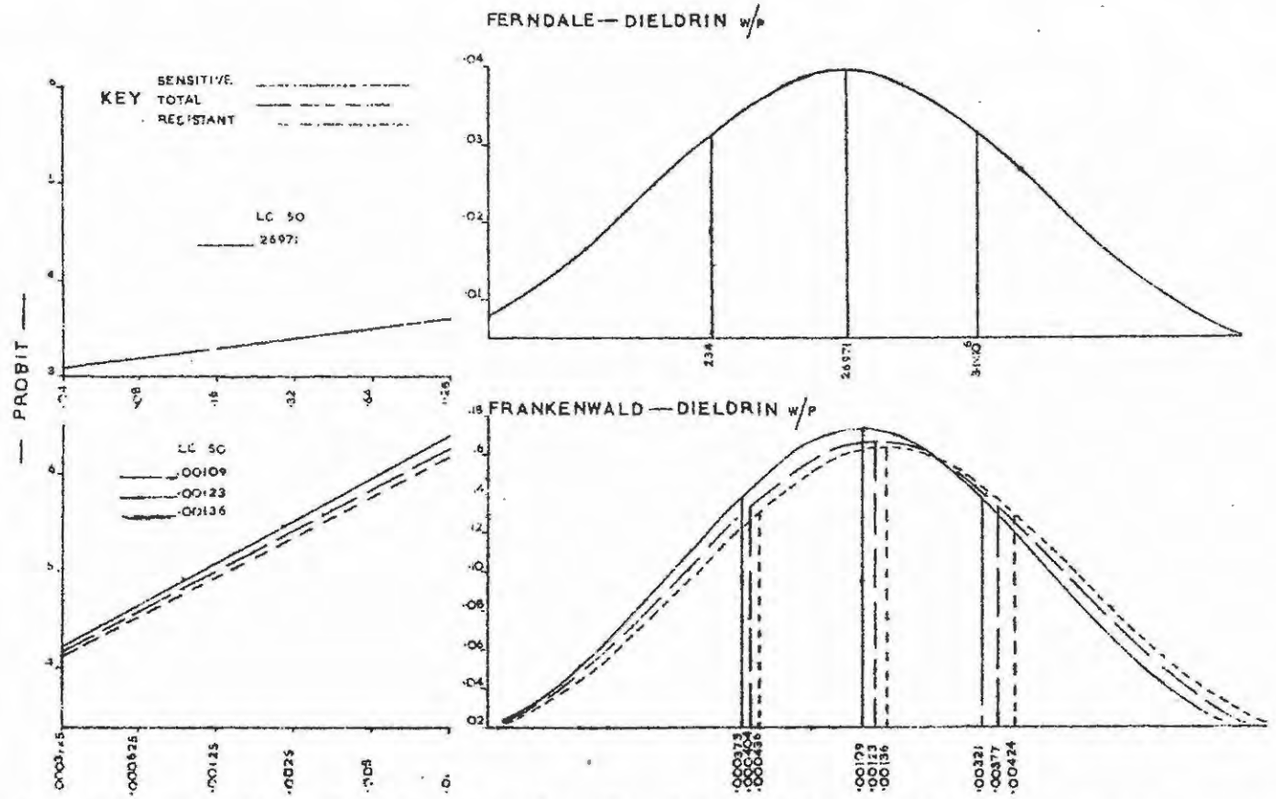


Fig. 44 The separation of tick larvae from Ferndale and Frankenwald into different populations in respect of their response to dieldrin.

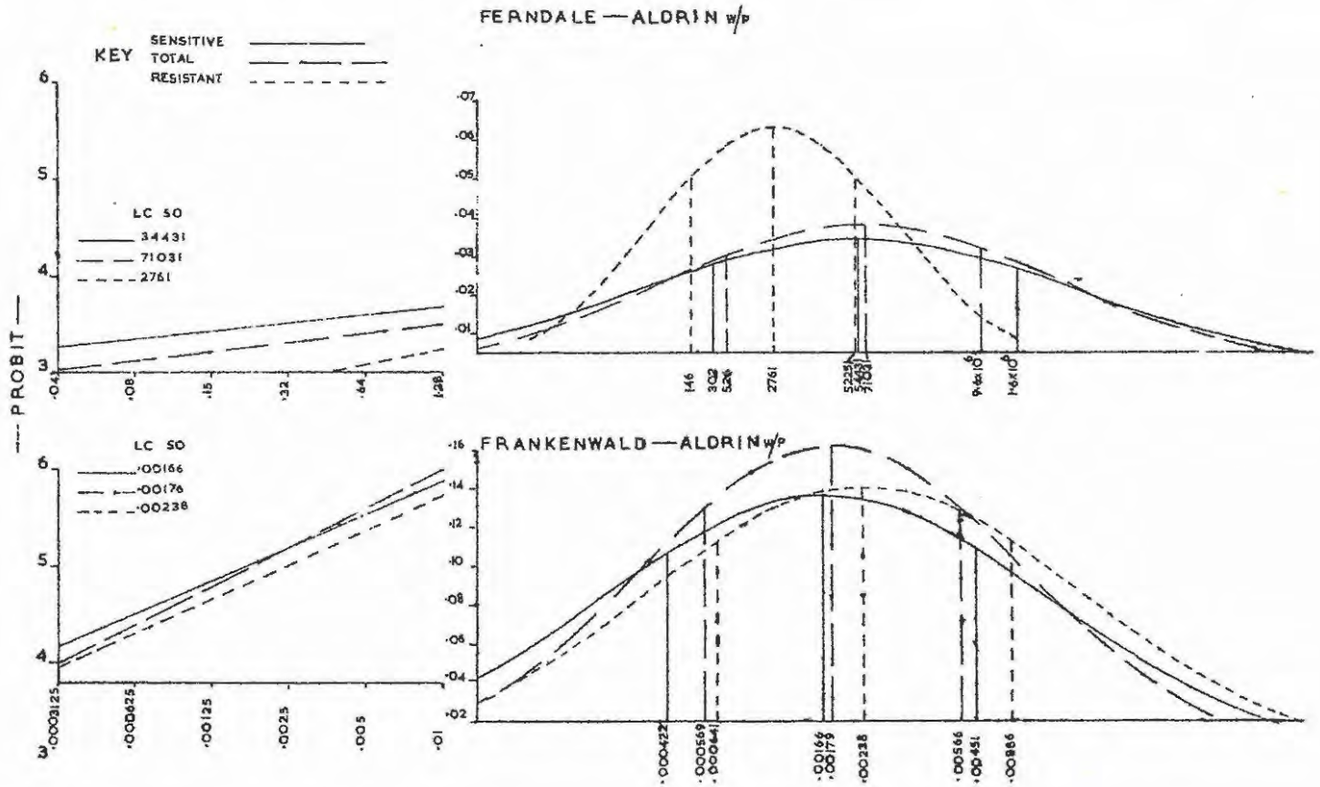


Fig. 45 The separation of tick larvae from Ferndale and Frankenwald into different populations in respect of their response to aldrin.

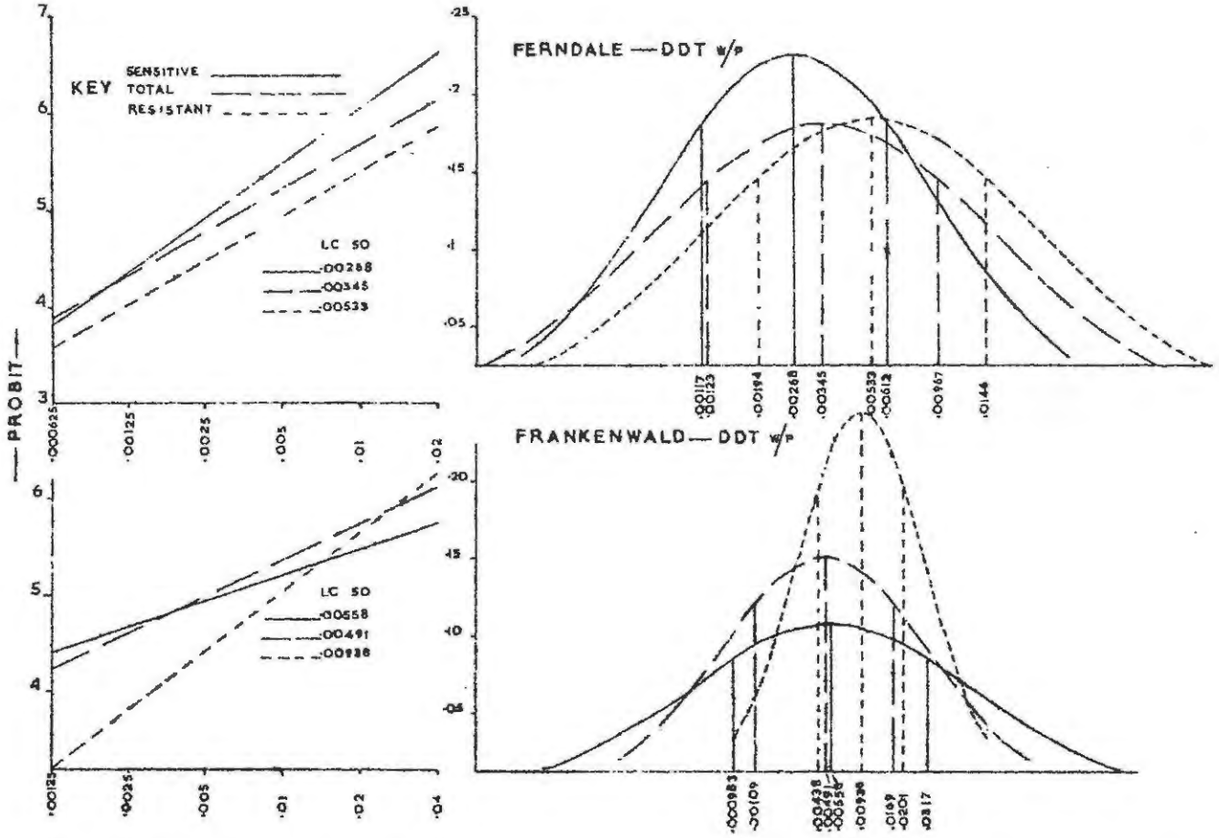


Fig. 46 The separation of tick larvae from Ferndale and Frankenwald into different populations in respect of their response to DDT.

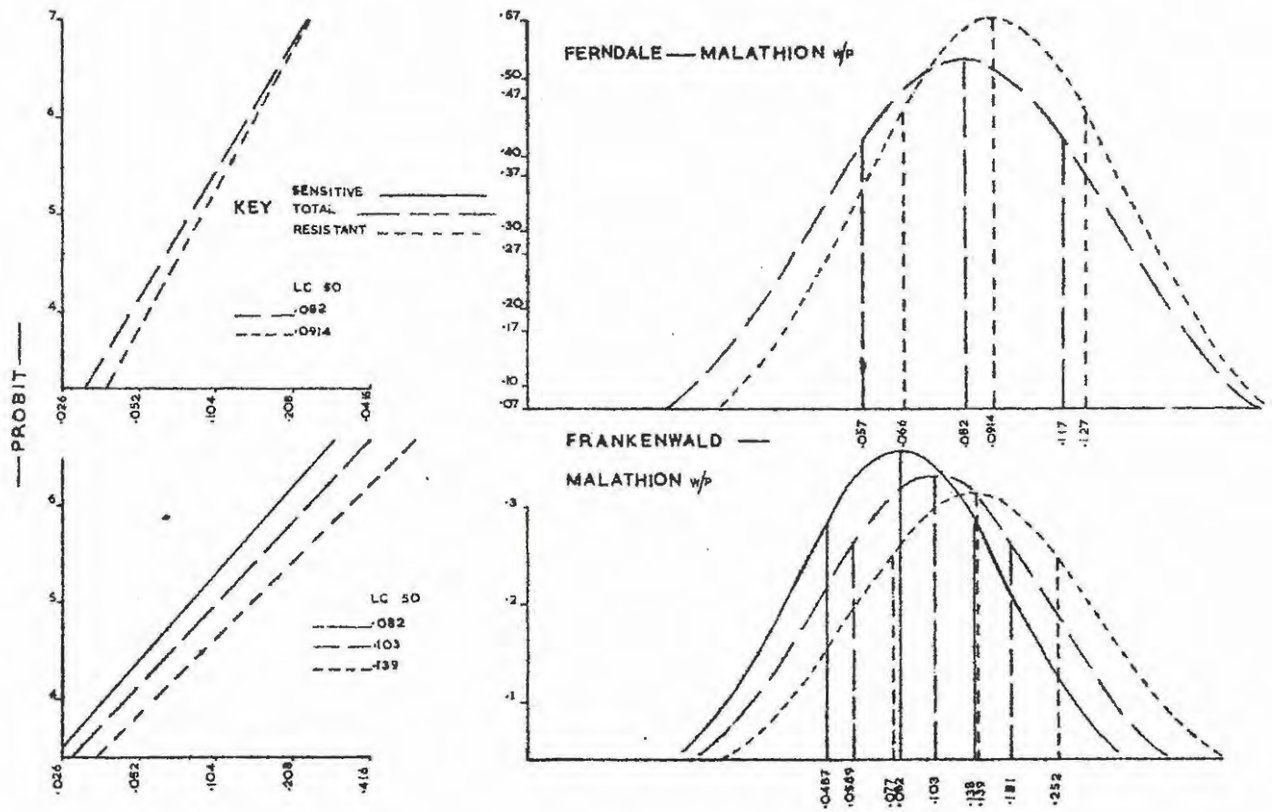


Fig. 47 The separation of tick larvae from Ferndale and Frankenwald into different populations in respect of their response to malathion.

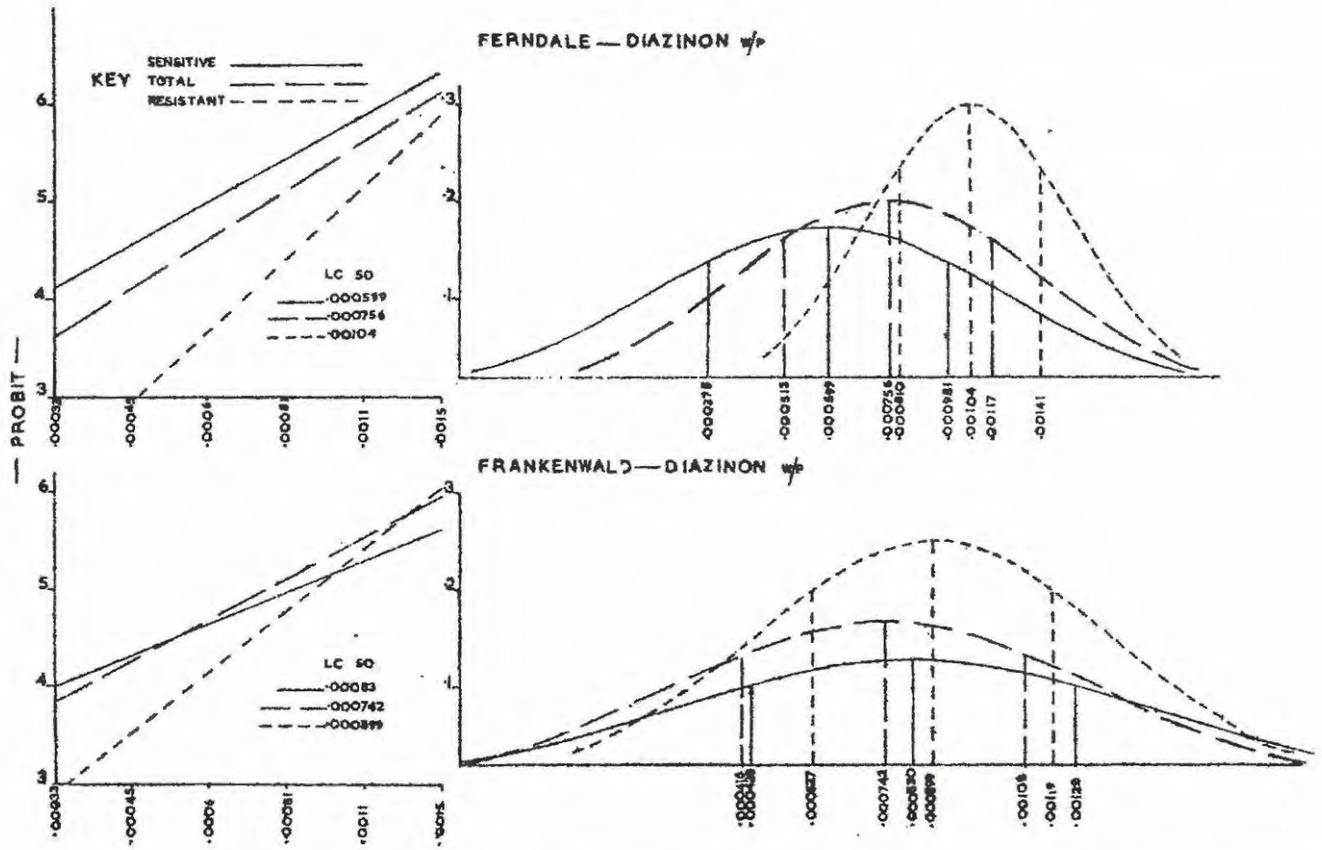


Fig. 48 The separation of tick larvae from Ferndale and Frankenwald into different populations in respect of their response to diazinon.

resolved into three parallel curves equivalent to three populations differing in response to dieldrin. By superimposing the results of tests with the F2 generation composed of theoretical 25 per cent susceptibles, 50 per cent hybrids and 25 per cent resistants separated by the discriminating dose technique, on the original dosage - mortality curve, a tripartite segregation of the original population, was indicated. From this evidence it was suggested that in the original dieldrin resistant population there existed three distinct populations i.e. resistant, hybrids and sensitive.

In tick larvae evidence of the existence of populations differing in response to insecticides occurring within the natural populations has already been presented. Unfortunately the blue tick cannot be bred satisfactorily and proof of the existence of populations of sensitive, hybrid and resistant in the natural population could not be obtained. In most of the graphs of response to insecticides in tick larvae there was evidence of only two populations, namely, sensitive and resistant. However, as Wilson-Jones and Davis have pointed out in the case of dieldrin resistance in A. gambiae the degree of dieldrin resistance in the resistant portion of the population is so high that it could not be measured. In fact, all that could be measured was the response of the sensitive and hybrid portions of the populations. The same explanation might also apply in respect of tick larvae although without a homogeneous sensitive population or a means of breeding the blue tick, speculation on the existence of a mixture of populations with a difference in distribution of response within the naturally-occurring populations would be folly.

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