CROSS RESISTANCE TO ORGANOPHOSPHORUS COMPOUNDS IN MALATHION- AND DIAZINON-RESISTANT STRAINS OF THE GERMAN COCKROACH.

by

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Table of Contents

List of Tables.

I. INTRODUCTION.

The organophosphorus group of insecticides are increasingly being used to control insects which have developed resistance to the chlorinated hydrocarbon insecticides. At the time these compounds were developed, it was hoped, and laboratory experiments seemed to confirm, that insects would be unable to develop high resistance to them. However, further laboratory studies forced considerable modification of these original assumptions, and in the last decade reports of field resistance to certain organophosphorus insecticides have begun to accummulate (Keiding 1956, Labrecque & Wilson 1957, Hansens 1958, Kilpatrick & Schoof 1958, Lewallen & Nicholson 1959). Most of these reports concerned houseflies, but other groups, particularly mosquitoes, were included.

In 1959 and 1960 reports were made of the development of laboratory strains of Blattella germanica (L.) resistant to malathion (Burden, Lofgren & Eastin 1959; Grayson 1960); and in the following year, the occurrence of apparent resistance to Diazinon in a field population of this species (Grayson 1961). The serious consternation following these reports can perhaps be best understood when the high resistance to the chlorinated hydrocarbon insecticides already exhibited by this insect in most countries of the World is taken into account. Added to this was the disturbing feature that resistance to the organophosphorus compounds frequently induces and maintains cross resistance to the chlorinated hydrocarbons (March 1959). Cross resistance to the carbamate insecticides is also induced in some cases (Moorefield 1960, Eldefrawi et al, 1959).

The occurrence of resistance in any insect species poses certain questions, the answers to which are required if successful control by chemical methods is to be achieved. It is, for example, necessary to discover the range of cross resistance developed by the particular insect species (both within and between groups of insecticides) and also, if possible, the means which the insect has developed to detoxify or prevent entry of the chemical compounds involved in the resistance phenomena. Busvine (1959) has pointed out that one method of attack on the problem of insecticide resistance is to work out the "resistance spectrum" for each strain involved. This is done by determining, for any particular strain, the median lethal doses of a range of insecticidal compounds. The ratio of these LD50's to those obtained for a normally susceptible colony of the same species to the same compounds results in a series of tolerance levels for the strain, that is, a "resistance spectrum". The importance of such a "spectrum" is that it indicates the various insecticides which are involved in any given case of resistance, and may give information of more fundamental interest, since the pattern of resistance towards a group of related poisons presumably reflects the efficiency of the protective mechanism against the various compounds. Similarly, if a certain pattern is common to a number of different strains, the inference is that the same mechanism is common to all.

The primary purpose of the work reported here was to establish a "resistance spectrum" for two strains of Blattella germanica to a range of organophosphorus compounds. Both of these strains had been selected in the laboratory for resistance but malathion was used as the selecting agent in one case and diazinon in the other. It was hoped that this study would enable conclusions to be made as to whether more than one type of resistance to these compounds can be developed by this species, and which compounds can be grouped together in any one type of resistance found. It was thought possible also that by examination of the structure and chemical formulae of the various compounds, some indication of the resistance mechanism involved, or, conversely the reason for the greater toxicity of some compounds, would be forthcoming.

II. LITERATURE REVIEW.

Cross resistance within the Organophosphates.

An examination of the literature on cross resistance within the organophosphates reveals a complex situation. Jeppson *et al.* (1958) showed that when mites developed resistance in the field to Systox, an astonishing range of resistances to related compounds accompanied the phenomenon. Their resistance to E.P.N. was 1400 times (at LC_{50}) and showed decreasing resistance to parathion (x 883), diazinon (x 12), and malathion (x 8).

An extensive study by Busvine (1959) of cross-resistance in houseflies showed that the insecticide used for inducing resistance was by no means necessarily the insecticide to which greatest resistance was observed. This was also true in some cases studied by March (1959) where a parathion-selected strain was found to be six times resistant to parathion, but 74 times resistant to malathion; and a diazinon-selected strain was nine times resistant to diazinon, but 57 times to malathion. However, in March's study, selection with malathion did induce the most resistance to the selecting compound.

Another extensive study of cross-resistance in houseflies was made by Forgash and Hansens (1959), who found that selection with diazinon induced a 38 times resistance to diazinon, with cross-resistance to malathion (x 5), parathion (x 16) and ronnel (x 18).

Oppenoorth (1959) examined cross-resistance patterns of 13 organophosphorus compounds in seven strains of houseflies. In this study, unlike the results found by Busvine, the cross-resistance pattern was similar in all cases, regardless of selecting insecticide, with a decreasing order of resistance from malathion, diazinon, parathion and DDVP.

Other workers have found that resistance to one organophosphorus compound does not always impart cross-resistance to another. For example, Brown & Abedi (1960) found that malathion resistance in a strain of *Aedes aegypti* (L.) did not extend to parathion. A similar effect was found for *Culex tarsalis* Coquillett, (Gjullin and Isaak, 1957), and parathion resistance in *Aedes nigromaculis* (Ludl.) did not extend to malathion (Lewallen and Nicholson, 1959).

It is quite apparent therefore that organophosphorus insecticides must not be considered, for resistance purposes, as a single group of insecticides in the same general way, for example, that it has been possible to consider the Group II chlorinated hydrocarbon insecticides.

Structure and toxicity.

Literature on the relationship between structure of organophosphorus compounds and their toxicity to insects is extremely sparse. Some work has been done on the relationship between structure and anticholinesterase activity. O'Brien (1960) has pointed out that it is reasonable to expect that those substituents which when attached to the phosphorus make it a better electrophilic reagent (i.e. more positive) will improve its anticholinesterase activity. Therefore, in general, it is possible to say that the more electrophilic the substituents, the better the anticholinesterase activity will be. So in a compound $(RO)_2P(O)OX$ -

(a) the anticholinesterase activity would fall off in the order R=methyl> ethyl> propyl, etc.;

(b) replacing RO by R should reduce activity;

(c) the activity would increase with the electrophilic character of X.

Busvine (1959) and Oppenoorth (1959) both noted that resistant strains of houseflies tended to be more tolerant to diethoxy organophosphorus insecticides than to di-methoxy compounds, although exceptions occurred, and it is apparent that organophosphorus resistance is affected by the alkoxy structure of the insecticides.

These studies agree in principle with (a) above, and that of Fukuto and Metcalf (1956) with (c) above, but precisely opposite findings have been reported (Mackworth and Webb 1948, Spencer and O'Brien 1953, Ooms et al. 1958, Fukuto and Metcalf 1959), and the situation is by no means yet resolved.

Mechanism of Resistance.

Factors which have been studied in an attempt to explain resistance to the organophosphates are cuticular penetration, rates of degradation, storage, and cholinesterase susceptibility.

Krueger <u>et al</u>. (1960) found that in two strains of housefly - one x 40 resistant to diazinon and the other susceptible - there was only a 7% difference in rate of diazinon penetration, regardless of dose. Injection of parathion into resistant houseflies did not destroy the resistance (Oppenoorth, 1958). These two results suggest that cuticular penetration may not be an important factor in resistance, but this evidence needs to be balanced by the fact that with malathion there has been found to be a 15-fold difference between the median lethal doses required for topical application and injection (Krueger and O'Brien 1959).

Various attempts have been made to find differences in degradation rates of organophosphorus compounds. Lord and Solly (1956) found that resistant and susceptible strains of houseflies degraded topically applied paraoxon at precisely the same rate. Substantially similar conclusions were reached by Krueger <u>et al</u>. (1960) and by Mengle <u>et al</u>. (1959) as a result of studies with radioactive methyl parathion, malathion and diazinon. Oppenoorth (1958), however, found exactly contrary effects, but his method was not good and his results are therefore suspect. There is slight evidence that in resistant insects degradation of organophosphates is somewhat quicker than in susceptible strains (O'Brien, 1960), although the differences and the amounts involved do not seem to be large enough to offer a convincing hypothesis of the resistance mechanism. More recent work, has, however, produced some indication that the ability to enzymatically degrade organophosphates is perhaps the main cause of resistance in insects (Van Asperen 1959, Forgash, Cook & Riley 1962, Lovell 1963). Added to this is evidence that in Culex tarsalis, resistance to malathion is associated with a gene allele that causes more carboxyesterase to be synthesised (Matsumura and Brown, 1963).

The remarkable induction of cross resistance to the chlorinated hydrocarbons by selection with organophosphates suggests that numerous changes occur during development of organophosphorus resistance, and it is therefore necessary to take great care not to attribute resistance to any one property which may be found to differ in susceptible and resistant strains.

The metabolism of organophosphorus compounds *in vivo* is usually relatively rapid, and whilst the possibility that storage in body lipids cannot presently be completely discounted or ignored, it seems unlikely that it is a primary cause of organophosphorus resistance (March 1959). Similarly it has been shown that the susceptibility of cholinesterase to inhibition is not a primary cause of resistance (Lord and Solly 1956, Oppenoorth 1958, March 1959, Van Aspenen and Oppenoorth 1959).

III. MATERIALS

Entomological.

Three strains of *Blattella germanica* were chosen for this study.

(a) Check Strain S.-- For some fifteen years, a strain of *Blattella* known as the VPI Normal Strain, has been in culture in the Department of Entomology of the Virginia Polytechnic Institute, and it is the strain most commonly used in resistance studies where a normal, susceptible colony of *Blattella* is required for comparison purposes.

In 1959 some selection for susceptibility within the strain was made and the insects resulting from this selection were cultured. In order to distinguish this strain from the VPI Normal Strain, it was called "Check Strain S", and it is at present the most susceptible strain in culture at the Virginia Polytechnic Institute.

For purposes of this study, it was necessary to have a susceptible strain to enable the levels of resistance of other strains of cockroaches to be determined, and the strain chosen for this role was the Check Strain S described above.

(b) R-malathion Strain.-- This strain was developed by laboratory selection to malathion, from a colony the individuals of which were highly resistant to the Group II chlorinated hydrocarbon insecticides. The selections had been conducted in each generation for 23 generations, and the adult females of the F16 generation had been shown to have a resistance of better than x 130 at LC_{50} when tested by the tarsal contact method (Grayson 1963).

(c) R-diazinon Strain.-- This strain is similar to the R-malathion Strain, in that it also was developed by laboratory selection to diazinon from cockroaches highly resistant to the Group II chlorinated hydrocarbon insecticides. Selection had been conducted for 21 generations. The adult females of the 16th generation had been shown to have no appreciable resistance at LC_{50} when tested by the tarsal contact method (Grayson 1963), but further selection resulted in the F21 generation having a resistance of approximately times six.

Only the adult females of the three strains described were used, as usually the females exhibit an equal or higher level of resistance than the males of the same population.

Age variations in susceptibility were avoided by using cockroaches which were between 15 and 25 days old. A small proportion of the insects used in the tests, due to the method of rearing, may have been slightly younger or older, but the close correllation between replicates seemed to indicate that control of this variable was adequate.

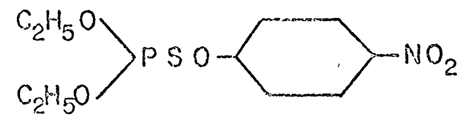
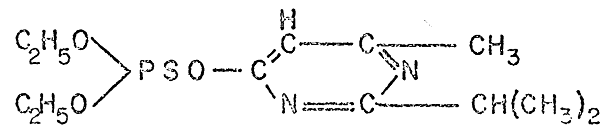
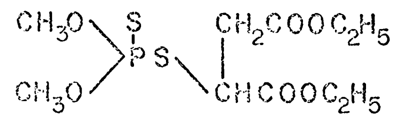
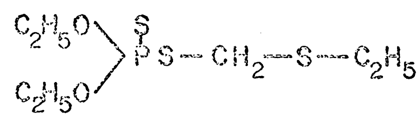
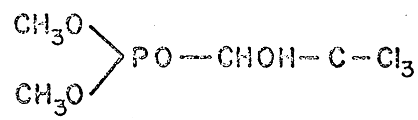
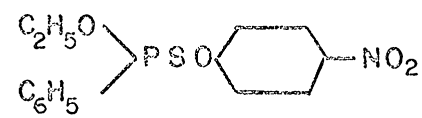
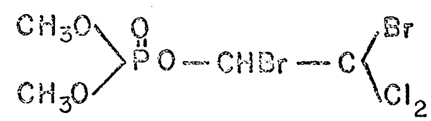
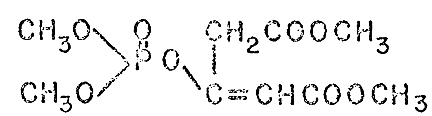
Chemical.

The organophosphorus compounds which were evaluated against the two resistant strains of Blattella germanica, were as follows:

Parathion	:	O,O-diethyl O-p-nitrophenyl phosphorothioate (American Cyanamid.)
Diazinon	:	O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate (Geigy Co.)
Malathion	:	O,O-dimethyl dithiophosphate of diethyl mercapto-succinate (American Cyanamid.)
Thimet	:	O,O-diethyl S-ethylthiomethyl phosphorodithioate (American Cyanamid.)
Dipterex	:	Dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate (Chemagro.)
E.P.N.	:	Ethyl p-nitrophenyl benzene thiophosphonate (Du Pont.)
Dibrom	:	1,2-dibromo-2,2-dichloroethyl dimethyl phosphate (California Chemical Co.)
GC 3707	:	Dimethyl 3-(dimethoxyphosphinyloxy) glutaconate (Allied Chemical Corp.)

The chemical names are those listed by Kenaga (1963) as being in accordance with the principles of Chemical Abstracts nomenclature. The names underlined are the common names of the compounds and are the names used to designate these compounds throughout this thesis. The chemical formulae and structure of these compounds, together with the purity of the sample used, are indicated in Table 1.

Table 1 : The structural formulae and chemical grouping of the organo-phosphorous compounds evaluated.

Common Name	Chemical Group	Sample Purity	Structure
Parathion	Orthothio-phosphate	99.6%	
Diazinon	Orthothio-phosphate	93.5%	
Malathion	Dithio-phosphate	95.0%	
Thimet	Dithio-phosphate	98.4%	
Dipterex	Phosphonate	100.0%	
E.P.N.	Phosphonate	90.0%	
Dibrom	Phosphate	92.8%	
GC 3707	Vinyl phosphate	95.0%	

IV. METHODS.

Dosing.

Busvine (1957) has reviewed, in some detail, the various methods by which insects may be exposed to various insecticidal compounds in toxicological studies. Whilst in a comparative type of study, such as projected here, there was little advantage in using any one way over another, it was felt that the"reproducibility" which is inherent in the more exact topical application or injection of insecticides, was a factor which should not be overlooked. Of these two methods, injection has the disadvantages of a more time-consuming technique and of a higher probability of mortality due solely to the method. Furthermore, resistance studies by Krueger and O'Brien (1959), and by Beard (1949), have shown that levels of resistance determined by this method are of a much lower order than when determined by most other methods, a fact of importance here since the R-diazinon strain of Blattella,which was to be used, was known to have a relatively low level of resistance to diazinon - that is, a resistance of only about times six.

In view of the above considerations, it was decided to use topical application, that is, individual cockroaches would be treated by depositing a known weight of each O.P. compound on a precise area of the cuticle, namely the ventral surface of the thorax at the base of the mesothoracic coxae.

The choice of topical application for the treatment of the cockroaches automatically necessitated the choice of some universal solvent in which each organophosphorus compound could be dissolved and diluted, and which itself was non-toxic at the quantities to be employed. The obvious choice was acetone, and that used was always redistilled from commercial grade prior to use.

A quantity of each O.P. compound was weighed out, and dissolved in acetone to make up a master solution of known strength. Measured proportions of this master solution were then diluted to make up a series of solutions for the dosage of each strain. The actual strengths of these solutions were determined by preliminary experimental dosing of each strain. The master solution and its diluted fractions were always made up as shortly as possible before use, and were never kept longer than 21 days - all necessary tests being completed in this time. The solutions were kept in tightly stoppered bottles and it was considered that no significant breakdown of the O.P. compounds, or evaporation of the solvent, would occur under these conditions. Here again, the small variation between replicates tended to confirm these views.

Application of the various compounds was by means of a hypodermic syringe, fitted with a size 27 needle (0.4 mm. dia.). The end of the needle was ground off square, and the tip bent to facilitate formation of droplets. The syringe was calibrated in one hundredths of a milliliter, and was placed in a clamp so that the plunger could be operated by a micrometer screw. Rapid multiple application was effected by means of a rachet and spring-loaded lever, each depression of the lever advancing the plunger by precisely the same amount. Calibration revealed that each depression of the lever expressed exactly 1.6 microleters of liquid, and this was the quantity of organophosphorus compound plus solvent (or of solvent alone in the case of "control" experiments) which each treated cockroach received. It was observed that the droplets were small enough to rapidly spread over the ventral surface of the thorax, reducing subsequent risk of loss.

To avoid deaths due to extraneous causes, it was decided not to use carbon dioxide to subdue the insects during treatment, and instead they were placed for a short period in the freezing compartment of a refrigerator, and held in a beaker surrounded by ice prior to dosing. The total time cockroaches were kept at low temperatures was from 10 to 40 minutes. In all the experiments carried out only two out of over four hundred cockroaches died in the control jars. This was considered highly satisfactory.

The treated cockroaches were placed in observation jars and the percentage mortality at each dosage level noted 48 hours after treatment, this percentage being used in the analysis of data. That forty-eight hours would be an adequate time interval was based primarily on the experience of several years of work by the Department of Entomology at the Virginia Polytechnic Institute on the effect of organophosphorus materials on Blattella germanica, and was supported by results obtained by Chamberlain and Hoskins (1951) on other species of insects.

The cockroaches were given ample food and water before and during the observation period, and were kept at constant temperature at all times except during the actual dosing. Overcrowding, both in culture and during recovery was avoided.

Analysis of Data.

The common method of determining the level of resistance of a given population of an insect species, is to obtain, for any one compound, the ratio of the dosage required to kill a certain percentage of the given population, to the dosage required to kill the same percentage of a normal, susceptible colony of the same species.

The percentage chosen for this work was 50%, and the dosages required to kill this percentage of the two resistant strains and the Check Strain S for each of the O.P. compounds listed in Table 1, were determined in the following way:-

Three hundred or more female B. germanica cockroaches of each strain were divided into three replicates of approximately one hundred cockroaches. Each hundred was treated at intervals of at least two days to the range of concentrations of the particular O.P. compound being evaluated (the method of preparation of the concentrations of the O.P. compound has been described in the previous section). The mortality at each dosage level was noted, and a final figure obtained by combining the results of the three replicates.

Examination of variation between replicates revealed that reasonably consistent results were being obtained in all cases, and further evaluation of the variation was not considered necessary. Furthermore, the extremely low control mortality - under 0.5% - was considered so satisfactory that no correction was made.

The data were then plotted on logarithmic-probability paper, and a provisional line fitted by eye. A regression analysis of the data was then made by the method of least squares (Bliss, 1935), and the dosage level for a 50% mortality calculated. Calculations of the 90% confidence limits of slope and intercept for the lines were also made, and the accuracy of the calculated dosage level for 50% mortality was estimated by using Fieller's Theorem (Fieller, 1944). A 95% fiducal limit was chosen.

It was previously stated, without explanation, that a 50% mortality level was chosen. The choice was made because it was known that the main feature of the confidence limits of the calculated regression line is the curvature they exhibit. This curvature results in estimates being most precise at the average dosage value, and may be almost useless at dosage values far removed from the mean dosage level. By "almost useless" is meant that the confidence and prediction intervals may become so wide as to render them of little value. Since the experiments carried out were designed to give a range of dosages from 0% mortality to 100% mortality, it was expected that the LD_{50} - i.e. the dose which killed 50% of the cockroaches - would be close to the mean of the doses employed.

The result of this manipulation of the data was therefore the determination of the LD_{50}, with a measure of its accuracy, this in turn permitting the calculation of the level of resistance of the strain to the particular O.P. compound being considered, from the equation:-

$$\text{Level of Resistance} = \frac{LD_{50} \text{ of R-malathion Strain (or R-diazinon Strain}}{LD_{50} \text{ of Check Strain S.}}$$

Also obtained was a measure of the slope or "b" value of the regression line, again with an indication of its variation. The importance of the slope lies in its ability to indicate latent resistance within a strain, and possibly also its genetic purity.

V. RESULTS.

The results of the treatment of the three chosen strains of Blattella germanica with eight different organophosphorus compounds are summarised in Tables 2, 3 and 4, and the actual regression lines have been included in the Appendices. Comparison in the sections below is always between results found for the strain being discussed with those of the control population, Check Strain S, for the particular compound or compounds. For example, the statement that "the LD_{50} of the R-diazinon strain to diazinon is significantly different" means that there is statistical evidence that the LD_{50}'s of the R-diazinon and Check Strains to the compound diazinon are different.

Examination of these results reveals certain very obvious trends.

(a) R-malathion Strain (Table 3).-- The resistance to malathion in the R-malathion strain is highly specific, no certain indication of resistance appearing to any of the other compounds evaluated. There are indications that the slopes of the regression lines, resulting from exposure of this strain to parathion, diazinon and dibrom, have been steepened by the selection pressure of malathion. Conversely, the line for GC 3707 has been flattened. Selection pressure seems to have had no effect on the slope of the regression lines resulting from exposure of this strain to thimet, dipterex or E.P.N. The LD_{50} for thimet has been statistically significantly affected.

Table 2 : The slope and LD_{50} values of the regression lines calculated for Check Strain S for eight organophosphates.

Insecticide	Check Strain S: Slope [a]		LD_{50} (μ gm) [b]	
Parathion	3.36	±0.74	0.2031	±0.02
Diazinon	2.29	±0.75	0.9210	±0.19
Malathion	7.19	±1.70	0.9007	±0.06
Thimet	4.14	±0.93	0.6836	±0.06
Dipterex	3.73	±0.77	3.9630	±0.48
E.P.N.	4.15	±0.73	0.5662	±0.05
Dibrom	4.92	±1.42	0.7001	±0.06
GC 3707	11.77	±3.08	0.1848	±0.009

a/ 90% Confidence Intervals

b/ 95% Confidence Intervals

Table 3 : The slope and LD_{50} values of the regression lines calculated for the R-malathion Strain for eight organophosphates, and a measure of the resistance of this strain to each compound.

Insecticide	R-Malathion Strain: Slope a/		LD_{50} (μ gm) b/		x Resistant
Parathion	5.91	±1.91	0.3101	±0.02*	1.5
Diazinon	4.58	±0.94*	0.9122	±0.07	1.0
Malathion	2.19	±0.60*	150.4000	±37.30*	167.0
Thimet	4.77	±1.00	1.0700	±0.084*	1.5
Dipterex	3.86	±0.80	4.7700	±0.50	1.2
E.P.N.	3.31	±0.63	0.7263	±0.12	1.1
Dibrom	10.92	±2.81*	0.8093	±0.03*	1.1
GC 3707	4.17	±0.94*	0.2515	±0.02*	1.3

a/ 90% Confidence Intervals

b/ 95% Confidence Intervals

* Indicates that the figure is statistically different at the chosen level to the corresponding figure for the Check Strain.

Table 4 : The slope and LD_{50} values of the regression lines calculated for the R-diazinon Strain for eight organophosphates, and a measure of the resistance of this strain to each compound.

	R-diazinon Strain		
Insecticide	Slope a/	LD_{50} (μ gm) b/	x Resistant
Parathion	5.16 ±0.88*	0.7716 ±0.05*	3.8
Diazinon	4.35 ±1.38	5.0710 ±0.45*	5.5
Malathion	3.00 ±0.85*	13.0100 ±2.30*	14.4
Thimet	3.84 ±0.84	3.7410 ±0.605*	5.4
Dipterex	2.84 ±0.88	11.9900 ±1.75*	3.0
E.P.N.	3.33 ±0.71	1.3690 ±0.16*	2.4
Dibrom	6.76 ±2.60	1.6390 ±0.13*	2.3
GC 3707	3.11 ±0.67*	0.5023 ±0.06*	2.7

a/ 90% Confidence Intervals

b/ 95% Confidence Intervals

* Indicates that the figure is statistically different at the chosen level to the corresponding figure for the Check Strain.

(b) R-diazinon Strain (Table 4).-- The results here are of perhaps greater interest. The strain is obviously markedly resistant to malathion where a significant change in both LD_{50} and slope of the regression line has resulted from the selection pressure of diazinon.

Some steepening of the regression line, coupled with an increase of the LD_{50} value, is indicated for parathion, dibrom, thimet and diazinon. This steepening of slope is very comparable with the effect found in the R-malathion strain due to malathion selection pressure, when that strain was evaluated against these same compounds, but in this case is statistically significant only for parathion. On the other hand, all the LD_{50} values for these four compounds are significantly different, indicating a tolerance to them in this strain.

The slope of the regression lines for dipterex, E.P.N. and GC 3707 have flattened, but this flattening is statistically significant at the chosen level only for GC 3707.

VI. DISCUSSION.

Before discussing the results of the experiments given in the previous section, it is necessary to outline the information which data of the type obtained is capable of producing.

As has been stated previously, the slope of the regression line is important in its ability to indicate genetic homogeneity and also latent possibilities of resistance. The latter point is of considerable interest in this study. Hoskins and Gordon (1956) have proposed that strains on the way to true resistance show an initial flattening of the dosage-mortality regression line, whereas with vigor tolerance these lines stay parallel. It is also generally accepted that in vigor tolerance the highest level of resistance which can be achieved is approximately times ten. A pivoting of the line about the highest mortality presumably indicates that the more susceptible genotypes have been eliminated without any more tolerant genotypes appearing.

In interpreting the results obtained in this study on the basis of such considerations, however, it is important to remember that the susceptible strain chosen as a base for comparison with the two resistant strains was not genetically identical with these strains prior to their being put under selection pressure. As a result, we are dealing with two gene "pools" - one for the check strain and another for the two resistant strains (both the R-diazinon and R-malathion strains were developed from one common strain).

Undoubtedly the most outstanding and uncontroversial result is the apparently high degree of specificity which is exhibited by the R-malathion strain in its resistance pattern. No cross-resistance to any of the other organophosphates evaluated has occurred, in spite of selection in each generation for twenty-three generations. This is in close agreement with results obtained by Brown and Abedi (1960) for a strain of *Aedes aegypti*, and by Gjullin and Isaak (1957) for a strain of *Culex tarsalis*. The only indication of resistance to any other compound -- i.e. a significantly higher LD_{50} -- was a resistance of times 1.5 to thimet. Thimet, like malathion, is a dithiophosphate and this similarity could be of significance; however, the lack of significant change of slope of the regression line probably indicates that this is, at best, a case of vigor tolerance, or may reflect only that the two genetic stocks react slightly differently to thimet.

Significant differences in slopes of the regression lines were found in the R-malathion strain for diazinon, dibrom and GC 3707, the line steepening for the first two compounds and flattening for the last. Comparison of the two steeper lines of this strain with those of the check strain reveals that the lines are pivoting about the higher mortalities, and it may be that selection with malathion has eliminated some genotypes which are susceptible to these compounds. Before accepting this hypothesis, however, it should be borne in mind that the R-malathion and check strains are not of identical ancestry. However, the lines for these two strains to dipterex and E.P.N. are almost identical, so it is possible to argue that in one or the other case selection pressure with malathion has affected the population. It seems logical to suppose, therefore, that the significant steepening of the regression lines for diazinon and dibrom is in fact due to the selection pressure.

The flattening of the regression line for GC 3707 against the R-malathion strain, compared to the very steep line given by the check strain, probably indicates that selection with this compound would rapidly induce resistance. The close structural similarity between GC 3707 and malathion possibly accounts for this indication of latent resistance. It would certainly appear difficult to dismiss the difference in regression line slope as a pure coincidence.

Evaluation of the same organophosphorus compounds against the R-diazinon strain produces some interesting similarities. In this strain selection with diazinon has induced the highest level of resistance to malathion. Before this can be labelled cross resistance, however, it is necessary to examine the resistance of this strain to its selecting insecticide, diazinon. The regression lines for the check strain and for the R-diazinon strain resulting from exposure to diazinon seem to indicate that the R-diazinon strain is not in fact resistant to diazinon, but is exhibiting vigor tolerance, combined probably with some elimination of susceptible genotypes. This conclusion is based on the steepening of the regression line for the R-diazinon strain, and the pivoting of this line about the highest mortality level. Confirmation for this view possibly lies also in two unrelated facts. One is that selection with diazinon for 21 generations has resulted in only a times 5.5 resistance level; the other is that, in the case of evaluation of this strain against malathion, where true resistance of times 14 was found, the regression line was significantly flattened, in agreement with the criterion proposed by Hoskins and Gordon (1956). In these circumstances, therefore, it would appear that rather than cross resistance to malathion due to diazinon selection, the selection procedure has resulted in the accummulation of factors required for resistance to malathion, whilst developing only vigor tolerance to diazinon. This indicates that either the tolerance mechanism developed against diazinon is more effective against malathion, or that two genetically closely linked mechanisms exist. This latter possibility seems unlikely, however, in view of the lack of any indication of cross tolerance to diazinon in the malathion-selected and resistant strain. In any event, there is an indication that the genes necessary for true resistance to diazinon may have been absent in the chlorinated hydrocarbon resistant strains originally chosen for diazinon selection experiments. If this interpretation is correct, it would be of great interest to determine whether the necessary genes for the development of true resistance to diazinon exist in any strain of Blattella, or whether they are absent in this species.

As in the case of the R-malathion strain, the regression lines for dipterex and E.P.N. for the R-diazinon strain show an insignificant difference in slope, when compared with the check strain lines for these two compounds. The LD_{50}s are, however, significantly different so that the apparent resistance exhibited here can only be attributed to vigor tolerance. Exactly the same arguments apply to dibrom, where a times 2.3 tolerance was found, and to thimet (times 5.4).

Comparison of the regression lines for the R-diazinon and check strains resulting from exposure to parathion reveals a significant steepening of the line due to the selection pressure of diazinon. The line has, however, pivoted about the highest mortality, so that the position is similar to that of diazinon, and the times 3.8 tolerance found must be attributed to vigor tolerance, rather than to true resistance.

As was the case with the R-malathion strain, evaluation of GC 3707 against the R-diazinon strain produced a significantly flatter regression line than is found in the check strain, together with a tolerance of times 2.7. These differences indicate incipient resistance to this compound within the R-diazinon strain.

Examination of the tolerance levels found to the various organophosphates evaluated, does not indicate a greater toxicity for compounds with dimethoxy substituents as compared with those having diethoxy groups, as was found by Busvine (1959) and

Oppenoorth (1959) and as postulated by O'Brien (1960). The only other conclusion which can be drawn from this study as to the relationship between structure and toxicity is with reference to the vinyl group in the compound GC 3707. The very close structural similarity between this compound and malathion would lead one to expect that a strain showing resistance to malathion must also exhibit resistance to it, particularly if this resistance was due to carboxyesterase enzyme activity. The activity of this enzyme has been cited several times as being the most probable cause of resistance to malathion (Krueger and O'Brien 1959, Matsumura and Brown 1963). The fact that the R-malathion strain was not at all resistant to GC 3707 obviously indicates that the presence of unsaturated carbon atoms in the vinyl group is of over-riding importance in preventing the action of the resistance mechanism.

The cross tolerance exhibited by diazinon-selected cockroaches to malathion presumably indicates that some of the genes which confer vigor tolerance to diazinon must be closely linked with resistance to malathion.

VII. SUMMARY.

1. Resistance to malathion in the strains of the German cockroach examined has been shown to be highly specific, no cross tolerance to other organophosphorus compounds occurring.

2. There are indications that the tolerance exhibited to diazinon by the diazinon-selected strain is not in fact resistance but vigor tolerance.

3. It would appear that selection with diazinon induces some tolerance to all organophosphates. This tolerance may in some cases rise to a high level and amount to true resistance. This is particularly true of cross tolerance to malathion.

4. The presence of the vinyl group in GC 3707 apparently enhances its toxicity. No structural characteristic could be shown to specifically affect the toxicity of any of the other compounds.

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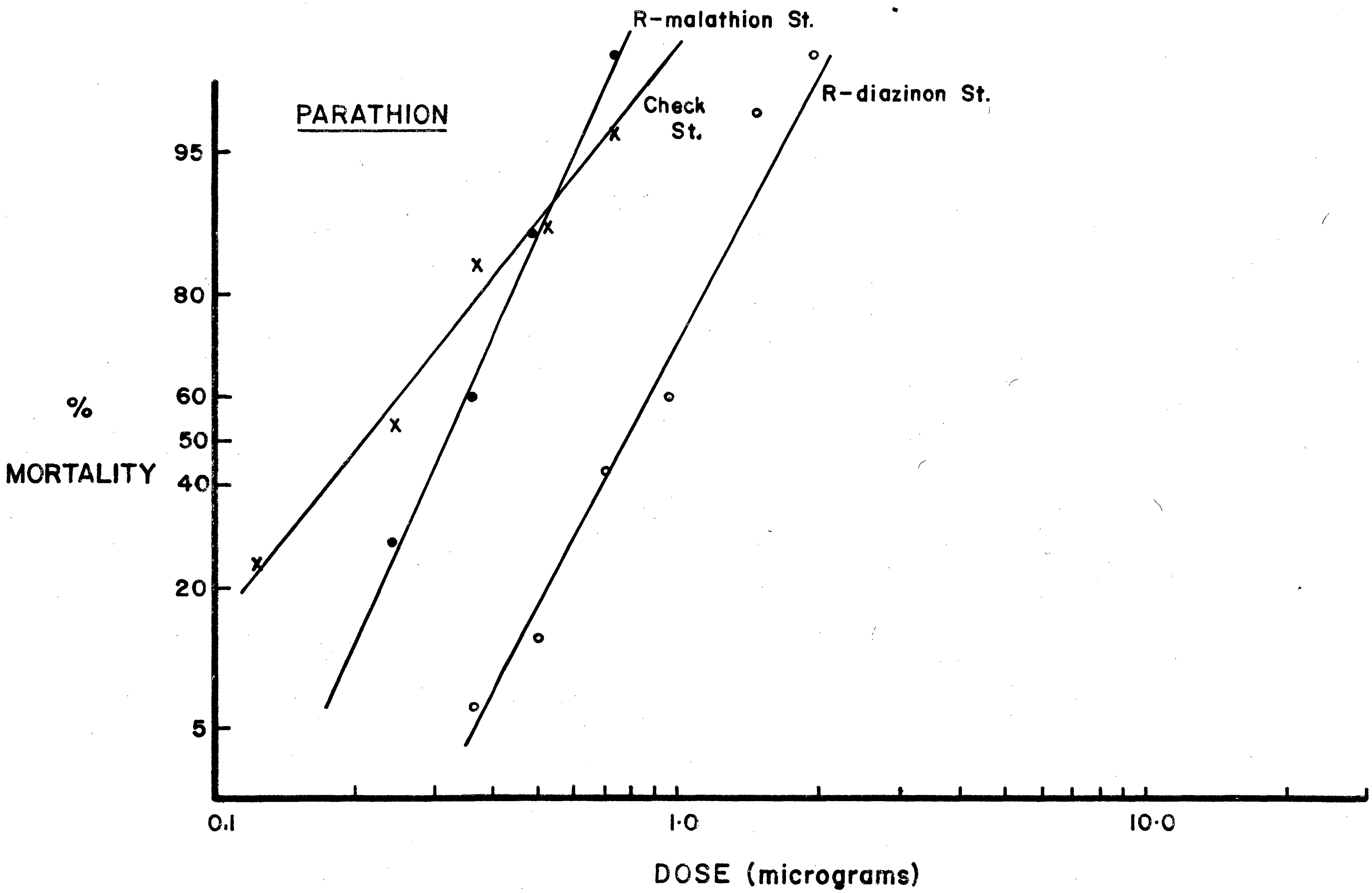
XI. APPENDICES

On the following pages will be found the calculated regression lines for the three strains of *Blattella germanica* chosen for evaluation against eight organophosphorus compounds.

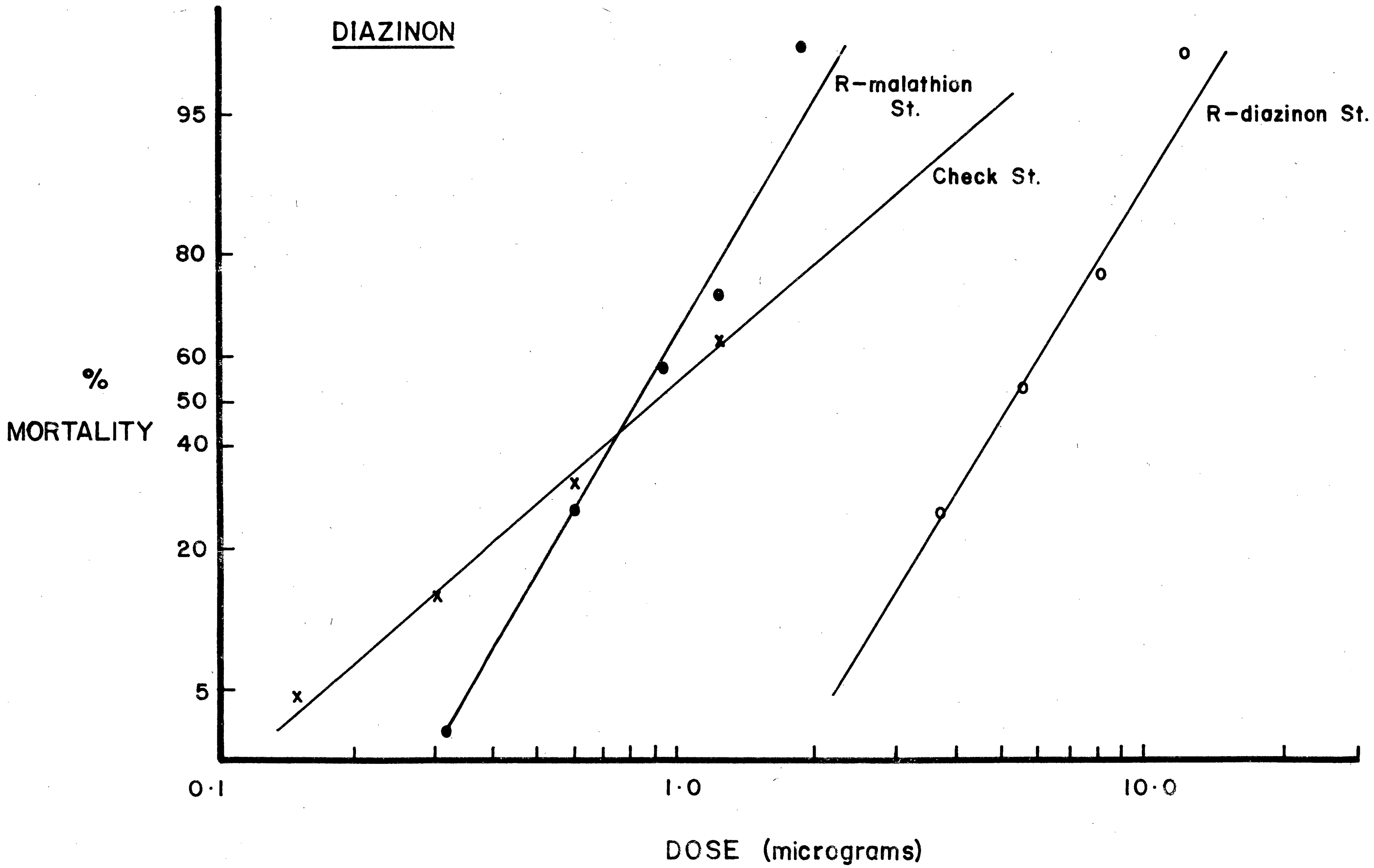
APPENDIX A.

Dosage-mortality lines for parathion shown by three strains of the German cockroach.


PARATHION
R-malathion St.
Check St.
R-diazinon St.
95
80
60
50
40
20
5
%
MORTALITY
0.1
1·0
10·0
DOSE (micrograms)

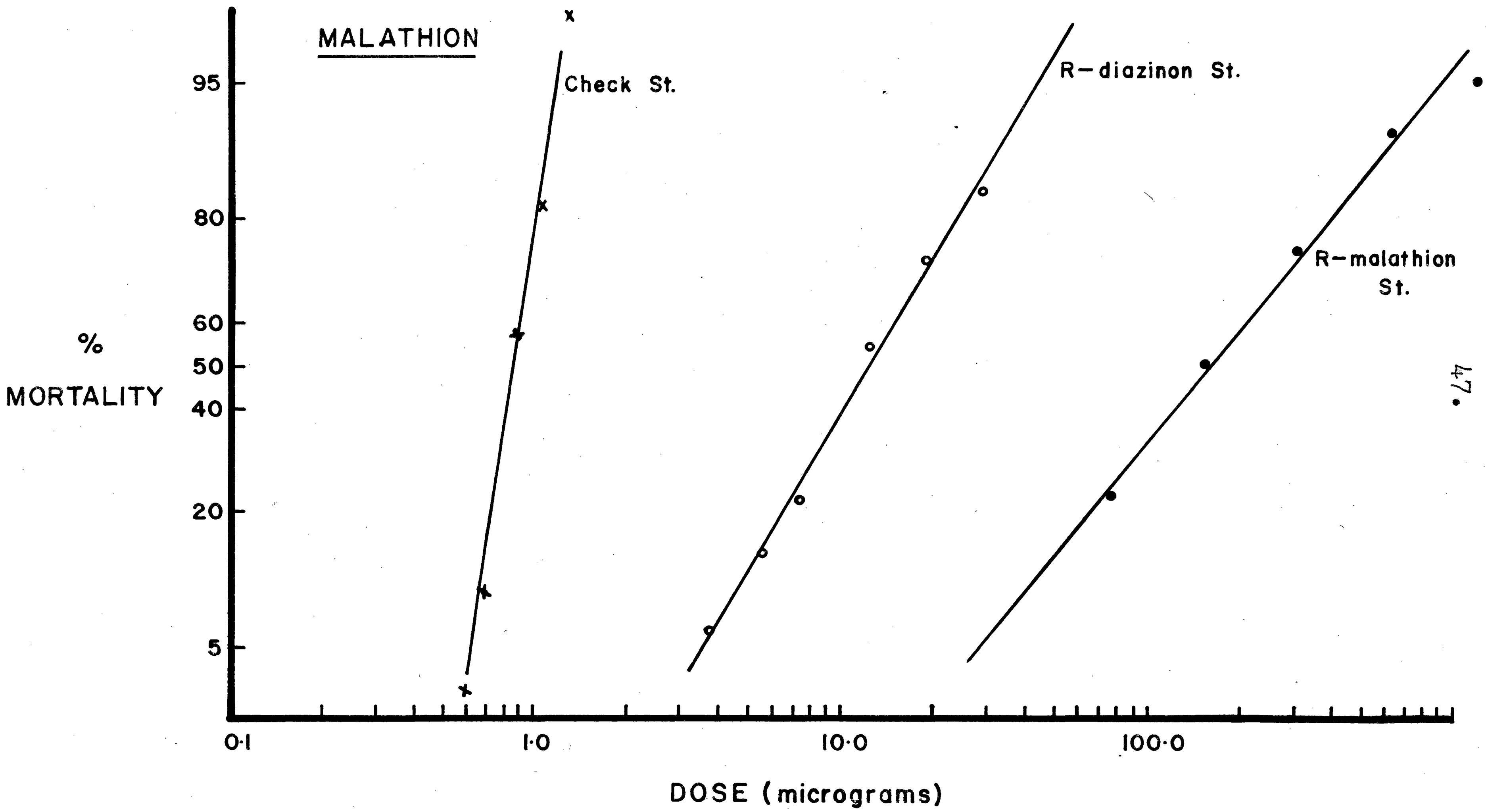
APPENDIX B.

Dosage-mortality lines for diazinon shown by three strains of the German cockroach.


DIAZINON
R–malathion St.
Check St.
R–diazinon St.
95
80
60
50
40
20
5
% MORTALITY
0·1
1·0
10·0
DOSE (micrograms)

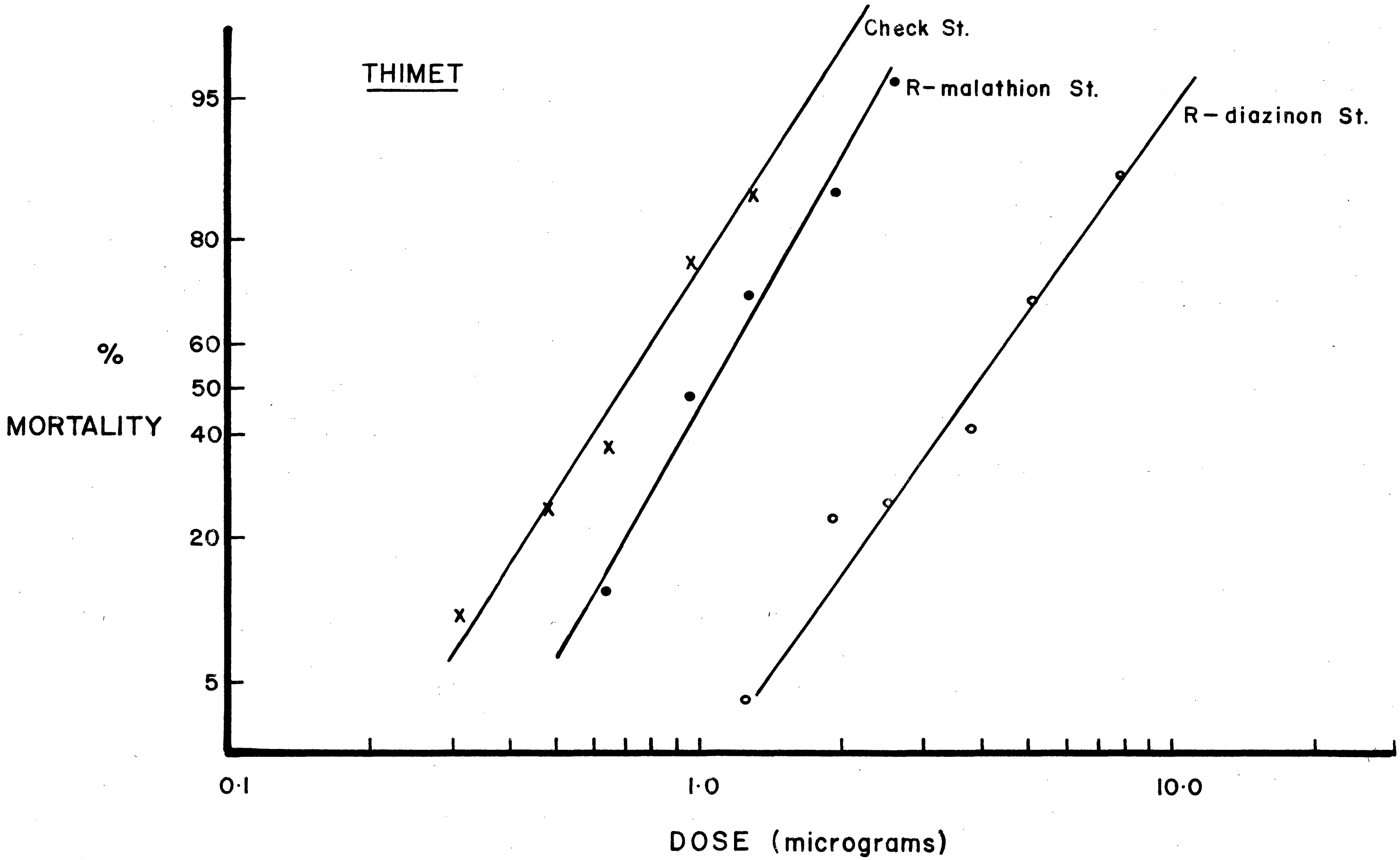
APPENDIX C.

Dosage-mortality lines for malathion shown by three strains of the German cockroach.


MALATHION
Check St.
R-diazinon St.
R-malathion St.
% MORTALITY
95
80
60
50
40
20
5
0·1
1·0
10·0
100·0
DOSE (micrograms)

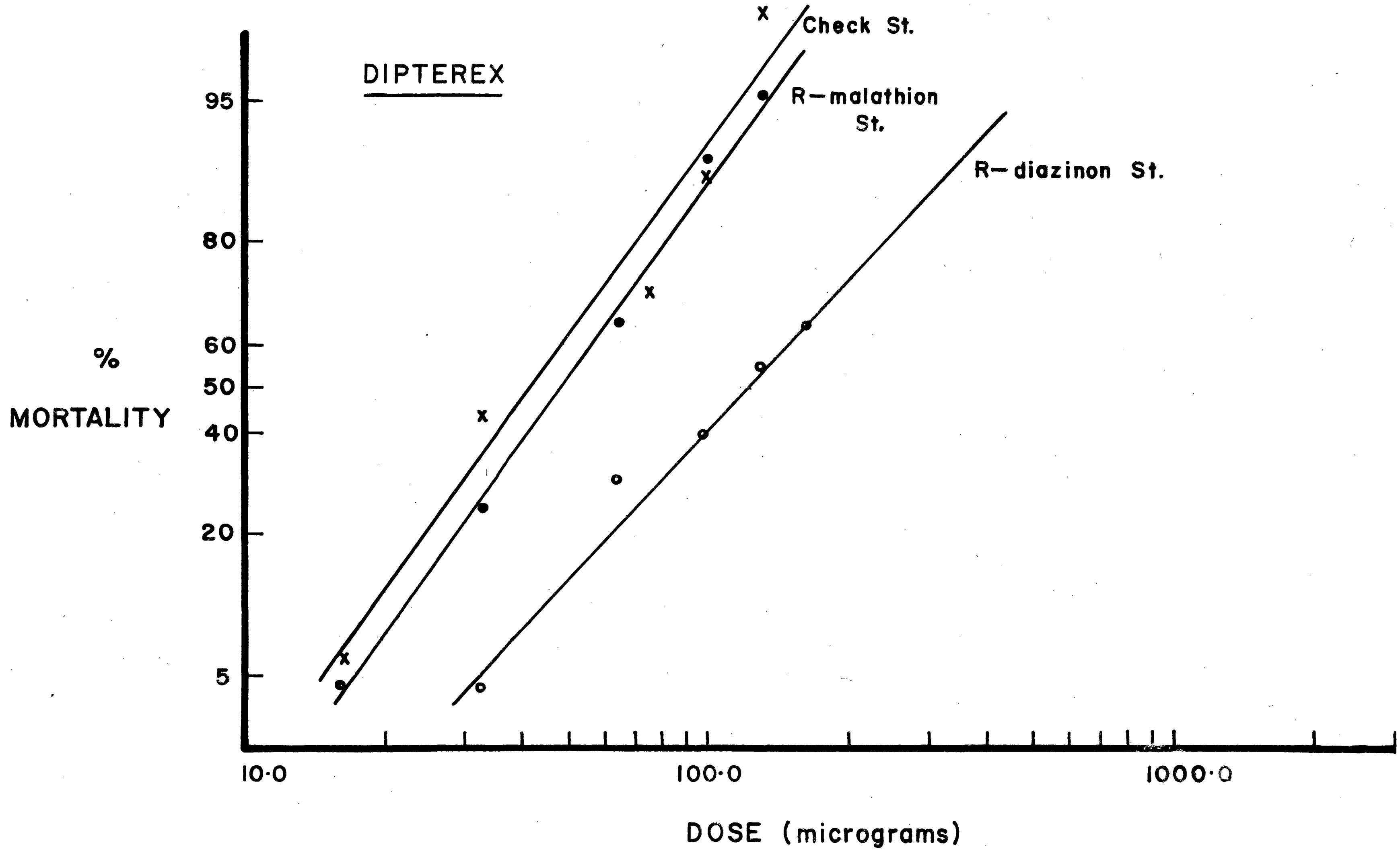
APPENDIX D.

Dosage-mortality lines for Thimet
shown by three strains of the
German cockroach.


THIMET
Check St.
R-malathion St.
R-diazinon St.
95
80
60
50
40
20
5
%
MORTALITY
0·1
1·0
10·0
DOSE (micrograms)

APPENDIX E.

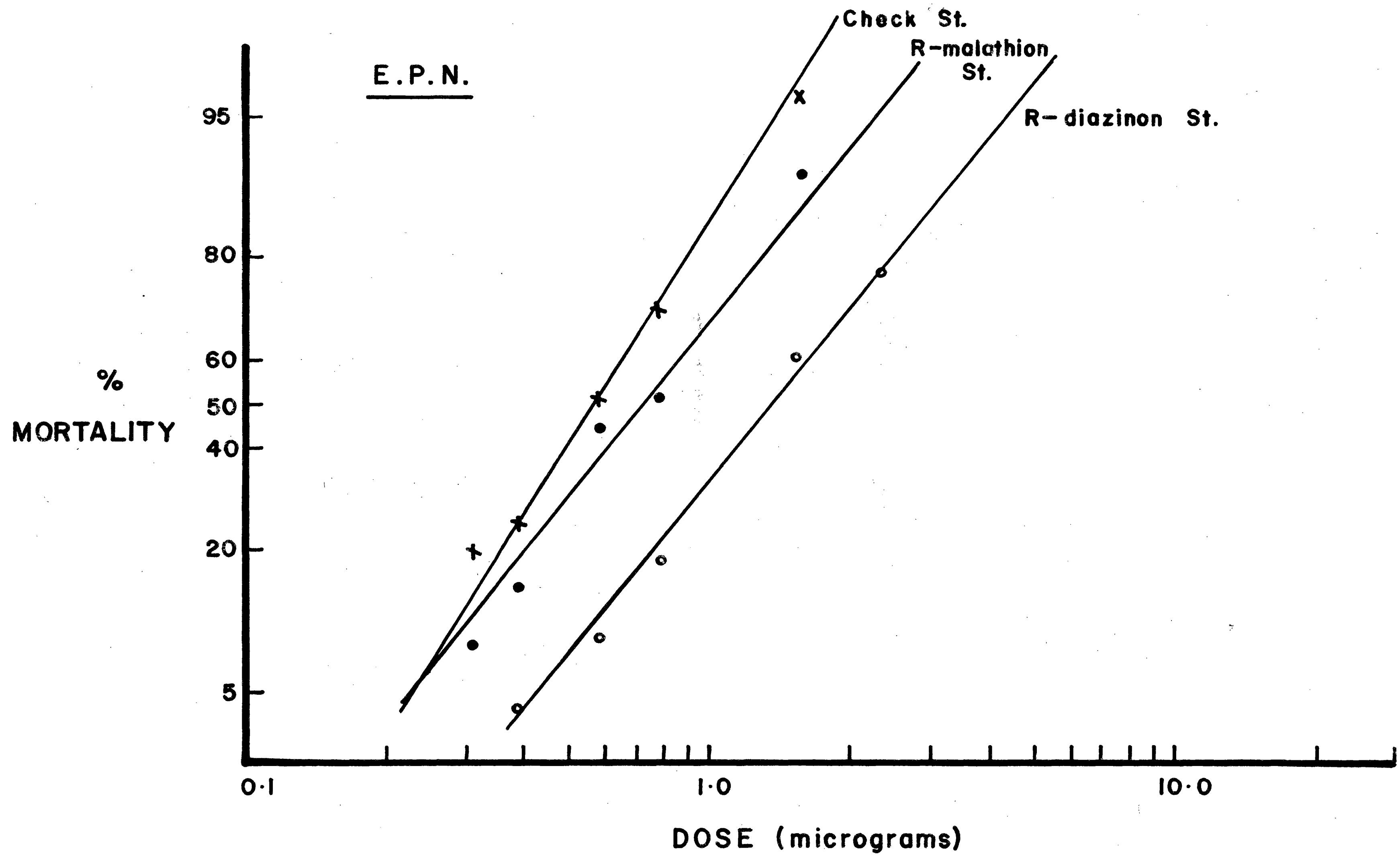
Dosage-mortality lines for dipterex shown by three strains of the German cockroach.



DIPTEREX
Check St.
R—malathion St.
R—diazinon St.
% MORTALITY
95
80
60
50
40
20
5
10·0
100·0
1000·0
DOSE (micrograms)

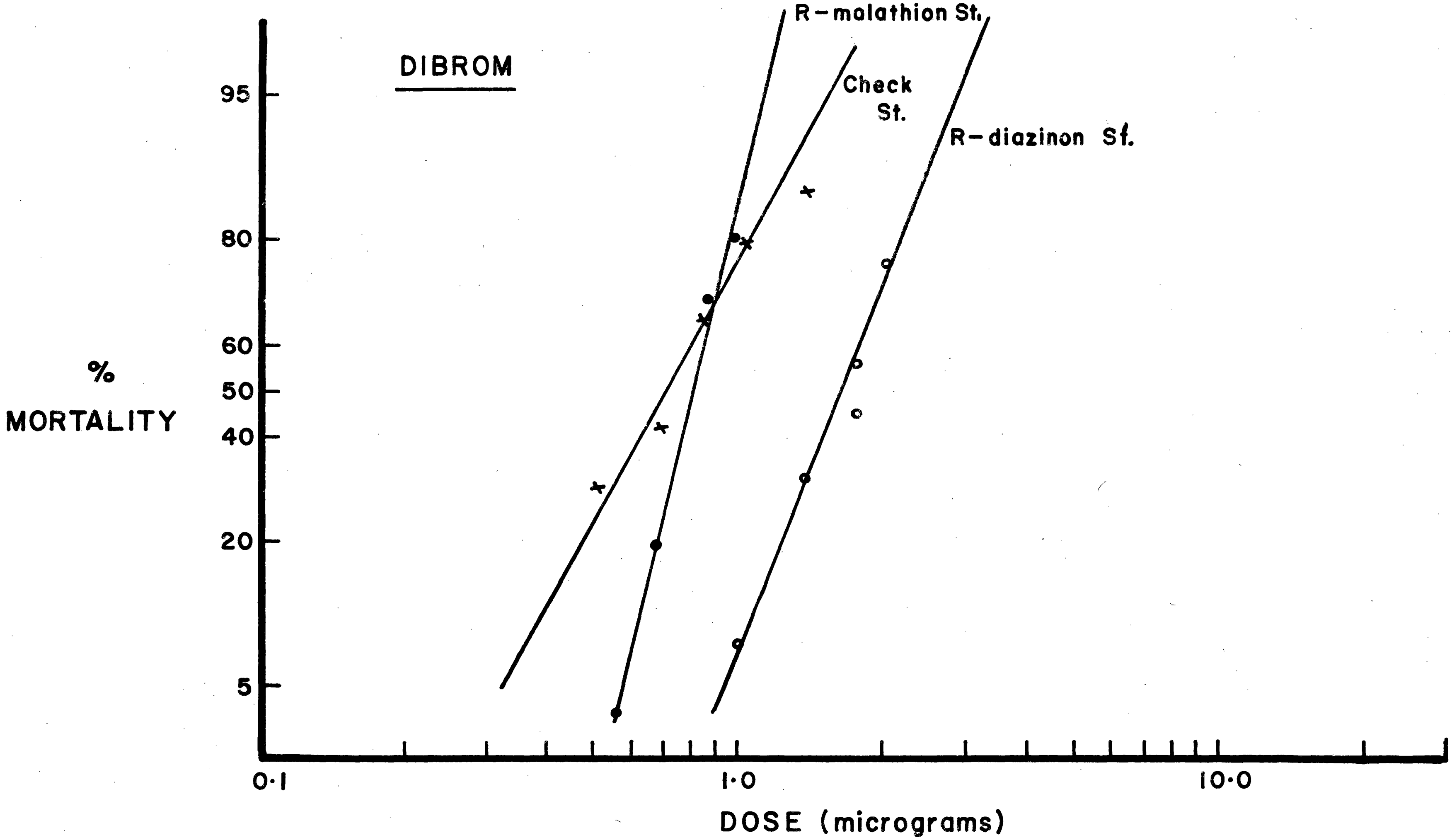
APPENDIX F.

Dosage-mortality lines for E.P.N.
shown by three strains of the
German cockroach.


E.P.N.
Check St.
R-malathion St.
R-diazinon St.
% MORTALITY
95
80
60
50
40
20
5
0·1
1·0
10·0
DOSE (micrograms)

APPENDIX G.

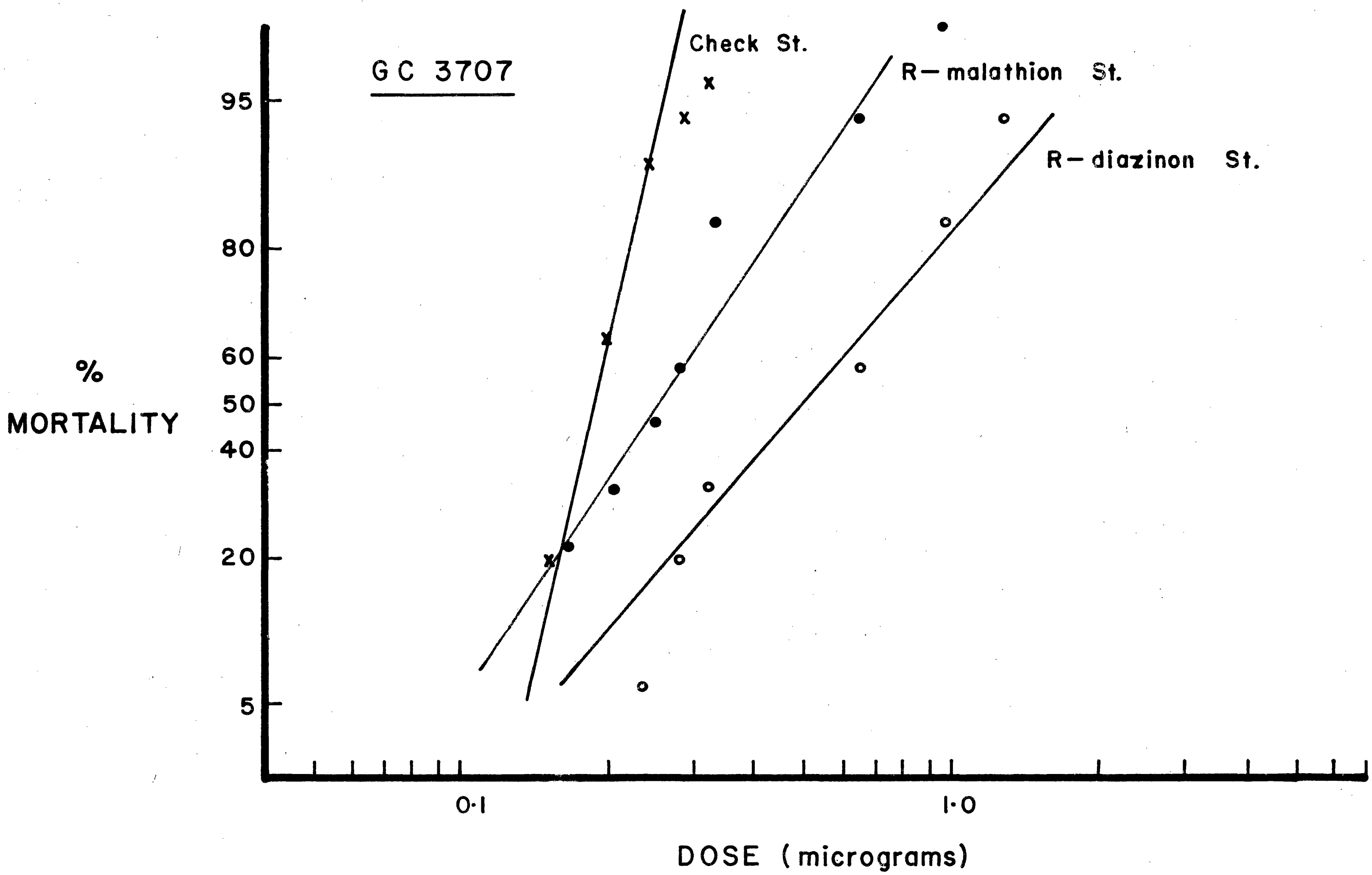
Dosage-mortality lines for dibrom shown by three strains of the German cockroach.



DIBROM
R – malathion St.
Check St.
R – diazinon St.
95
80
60
50
40
20
5
%
MORTALITY
0·1
1·0
10·0
DOSE (micrograms)

APPENDIX H.

Dosage-mortality lines for GC 3707
shown by three strains of the
German cockroach.


GC 3707
Check St.
R— malathion St.
R— diazinon St.
95
80
60
50
40
20
5
%
MORTALITY
0·1
1·0
DOSE (micrograms)

ABSTRACT.

Dosage mortality data by a topical application method for eight organophosphorus compounds are given for two laboratory-selected strains of *Blattella germanica* (L.), one resistant to malathion and the other resistant to diazinon. Comparison of this data with similar data for a normal susceptible strain of the same species reveals that development of resistance to malathion in this species is highly specific imparting no effective cross resistance to any of the other seven compounds evaluated. Selection with diazinon induces a low level of tolerance to all the organophosphates studied, but there are indications that in most cases this is due to vigor tolerance rather than to true resistance.

There is reason to believe that if carboxyesterase activity is responsible for the resistance phenomena to malathion exhibited by this insect, its action is blocked or its detoxifying ability rendered ineffective by the presence of a vinyl group in an organophosphorus compound, such as GC 3707.