INHERITANCE OF RESISTANCE TO LINDANE IN THE GERMAN COCKROACH, *Blattella germanica* (L.)

by

John G. Barker, B. S. M. S.

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APPROVED:

James McD. Grayson

Chairman, Advisory Committee

Wilson

Norby

Elwood M. Raffensperger

E. C. Turner Jr.

Robert D. Ross

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Blacksburg, Virginia

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INTRODUCTION

The World Health Organization's Expert Committee on Insecticides defines insecticide resistance as "the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species," (1956).

Acknowledged insecticide resistance antedates the present by some sixty years, but this phenomenon has not been accorded great emphasis until recent years. New heights of insect control were achieved in the immediate post World War II years through the use of the then recently discovered organic insecticides. These gains were threatened, however, by the rapid appearance of resistance in many insects. One may recall the praise with which DDT was hailed as it came into widespread use. Some observers rashly predicted that because of the effectiveness of DDT our insect problems were at an end. That this was not to be realized soon became apparent, for some insect strains began to show an ability to survive dosages of this insecticide which formerly would have killed the majority of them. This experience has since been repeated widely with many of our most effective insecticides. Seemingly, insecticide resistance resulted principally from artifical selection (the incompletely effective control measures), and it was inheritable. The genetic mechanisms have not been fully explored, yet it is presumable that an understanding of them will aid in mitigating the difficulties encountered in control of resistant insects.

The gamma isomer of benzene hexachloride, now known as lindane, was discovered to have outstanding insecticidal properties in 1945. It has been in extensive use as an insecticide for only ten years, yet numerous insect species have developed resistance to it. Through laboratory selection the German cockroach, <u>Blattella germanica</u> (L.), seemingly has developed resistance to lindane (unpublished data, Virginia Agricultural Experiment Station). Groups of roaches were treated with dosages of lindane which killed 75 to 90 percent of them. By inbreeding the survivors of such treatment through approximately 25 successive generations (during a period of eight years) there was developed a strain of roaches 8.8 to 17.7 times as resistant as the original stock. Several questions were formulated at the inception of this investigation, the answers to which it was thought would reveal the nature of the inheritance mechanism. These were as follows:

a) Is lindane resistance sex-linked, or autosomal?

b) If lindane resistance is autosomal, is it dependent upon a single gene or a small group of major genes, or is it dependent upon a system of multiple genes having minor effect individually?

c) Is the resistant strain homozygous for resistance?

d) Will lindane resistance decrease rapidly in the absence of selection?

The experiments were designed to supply answers to these questions.

The study to be reported here was a continuation of similar research in the Entomology Section of the Agricultural Experiment Station, Virginia Polytechnic Institute, Blacksburg. Previous investigators have interpreted the inheritance mechanisms of DDT resistance, and of chlordane resistance in the German cockroach. Appropriate comparisons will be drawn among the conclusions of the earlier investigators and the results of this study.

REVIEW OF LITERATURE

Although Smith (1897) observed some evidence of resistance to an insecticide, the inheritance of such resistance was first considered by Melander (1914) who reported positive evidence for this phenomenon in the San Jose scale, <u>Aspidiotus perniciosus</u> Comstock. It is significant that Melander found no cause for alarm in speculations that "totally immune" insects would result ultimately from control operations. He concluded that although there were resistant survivors of annual spraying operations their subsequent inbreeding with non-resistant survivors would serve so to dilute the strains as to make them at least partially susceptible.

The existence of certain conditions apparently favors development of resistance of an insect to a toxicant. These are as follows:

a) A portion of the insect population must survive the insecticidal treatment.

b) Diminution of insecticide residues to the point where they are sublethal to insect populations; this permits the selection process to become operative.

c) Sustained exposure to the insecticide through successive generations of the insect.

d) Increased concentrations of insecticide to correspond with the rise of resistance in an insect population.

e) High reproductive potential and/or a rapid rate of development in the insect species.

Quayle (1943) suggested that the genes responsible for the inheritance of resistance either were present in the insect population at the inception of treatment, or originated by mutation during treatment. The former explanation is generally accepted, there being little or no evidence of insecticides possessing mutagenic properties (Pielou, 1952; WHO, 1956; Crow, 1957). Yust _et al._ (1943) have also published findings in accord with this; a non-resistant strain of California red scale, _Aonidiella aurantii_ (Maskell), was found by these investigators to possess the hereditary potentiality for resistance. Results which agree with these have since been reported with respect to many other insect species, and in widely separated localities.

The rate at which insecticide resistance increases after its appearance is important to the entomologist. Melander's report (1914) of resistant San Jose' scales indicated that the use of lime-sulphur as a control measure had been in effect for at least six years at the time unsatisfactory results first were noted. According to Quayle (1943), as early as 1912 citrus growers in the vicinity of Corona, California, obtained unsatisfactory results from fumigation of _Aonidiella aurantii_ with hydrogen cyanide. From 1915 to 1922 Quayle fumigated scale-infested fruit from this and other localities simultaneously, and he found the Corona red scales to be especially resistant. Woglum (1925) reported that satisfactory control of red scale was not obtained in 1918 with twice the dosage which had thoroughly controlled the insect in 1907. In other areas, however, the scale was controlled by fumigation almost as easily in 1924 as it had been in 1909. Bruce and Decker (1950) found the acquisition of

DDT resistance in the house fly, *Musca domestica* L., to be gradual through the first few generations of selection. Provided selection was continued, however, the resistance increased more rapidly in later generations. Roadhouse (1953) discovered that house fly resistance to DDT in the field was always traceable to several seasons' use of the insecticide. Resistance of a strain of *Blattella germanica* to DDT was reported by Grayson (1954) to have increased as much as 7.8 times at LD-90 after seven generations of selection; whereas a strain of *B. germanica* simultaneously undergoing selection with lindane showed little increase in resistance. Two distinct strains of highly resistant *Drosophila melanogaster* Meigen were developed by King (1954) who had treated them for twenty generations with a DDT aerosol.

The inheritance mechanisms for insecticide resistance have long been the subject of investigation. Early in this work the possibility of autosomal inheritance, as opposed to sex-linked inheritance, came under scrutiny. Dickson (1941) concluded that the resistance of *A. aurantii* to hydrogen cyanide was inherited as a simple sex-linked characteristic. Quayle (1943), and Yust *et al.* (1943) agreed with this analysis. Cochran *et al.* (1952) found that resistance to DDT in the German cockroach is not inherited as a simple sex-linked factor, although it was thought that some sex linkage was involved in the chromosomal component of the inheritance mechanism. Pimentel *et al.* (1954), working with a DDT-resistant strain of house fly, reported that although the female parent seemingly influenced the resistance of the progeny more than did the male parent, there was no involvement of sex-linkage.

The involvement of cytoplasmic factors for inheritance of resistance have been reported by Cochran et al. (1952) in the German cockroach, and Johnston et al. (1954) in the house fly. Crow (1957) has pointed out that the results in the German cockroach could also be explained as due to maternal factors, and he found that the conclusions reported by Johnston et al. could not be correlated with their data. Therefore, there seems to be no proven instance of cytoplasmic inheritance of insecticide resistance.

In contrast to these few reports of sex-linked resistance, and possible maternally affected resistance, there stand the many accounts of resistance explainable upon the basis of autosomal inheritance. Investigators of autosomally inherited insecticide resistance have been most concerned with determining whether such inheritance is mono-factorial or multi-factorial. Hough (1928) made reciprocal crosses between a Colorado strain of codling moth, Carpocapsa pomonella (L.) resistant to lead arsenate, and a Virginia strain susceptible to this toxicant. The F_1 progeny of the crosses were intermediate to their parents with respect to their ability to enter sprayed fruit. Brown (1951) interpreted this to indicate that inheritance of the trait was autosomal and multi-factorial. Harrison (1951) postulated that the resistance to DDT shown by house flies with which she worked was recessive and mono-factorial. These conclusions were based upon the survival after treatment of 1/4 of the F_2 progeny of reciprocal crosses, and upon a survival-mortality ratio of 1:1 among the progeny of backcrosses. Harrison later (1953) altered these conclusions upon discovering that the resistance originally reported was with respect to temporary paralysis, not mortality; the true resistance was found to be multifactorial. An investigation by King (1954), of D. melanogaster resistant to DDT showed that the inheritance of this characteristic was expressed in a complex polygenic, or multi-factorial, system. As evidence of this, King cited his finding that selection of the flies at a low level of intensity, i.e. LD-50, was as effective as selection at the high level of LD-95. He adduced that this indicated the necessity of maintaining a high degree of genetic variability during selection. In his opinion selection would have been much more rapid at an LD-95 had there been present one or two genes of major effect. Even more indicative of the presence of numerous genes affecting resistance were the results King (1954) obtained upon crossing two resistant lines. The resistance of the F_1 progeny was not significantly different from that of the parents, however, the F_2 showed a sharp drop in resistance and a much greater range of variation. Openoorth and Dresden (1953), Bochnig (1954), and Crow (1954), also found the inheritance of DDT-resistance in Drosophila to be vested in a polygenic system. In the German cockroach resistance to chlordane has been shown to be multi-factorial and autosomal (Grayson et al., 1956; Jarvis et al., 1957), while resistance to DDT is more complex but is largely autosomal and perhaps multi-factorial (Cochran et al., 1953).

Maelzer and Kirk (1953) found DDT-resistance in the "Illinois" strain of the house fly to be dependent upon two genetic mechanisms. The "weak" sub-strain possessed a comparatively low resistance which was multifactorial. Flies of this group were found to be not nearly as resistant as those of the "strong" sub-strain. The latter seemingly possessed a single dominant gene for resistance superimposed upon the constitution of the "weak" type. Lichtwardt *et al.* (1956), investigating the inheritance of DDT resistance in the house fly, demonstrated the presence of a single autosomal dominant gene. Their results agreed essentially with those of Maelzer and Kirk. The greatest refinements in analyses of resistance have been possible with *D. melanogaster* because of the extensive knowledge of its genetics. Tsukamoto and Ogaki (1953), and Ogaki and Tsukamoto (1953) found that DDT-resistance in *Drosophila* is assignable to a single gene, or group of closely linked genes on chromosome II. Further, the gene or genes were found to be located near the vestigial gene, and to be responsible for BHC-resistance. Later Tsukamoto (1955), reported that nicotine sulfate resistance in *Drosophila* may be controlled by a main factor on the third chromosome modified by genes (enhancers) on other chromosomes. More recently Tsukamoto and Hiroyoshi (1956) found that nicotine sulfate resistance was dependent upon incompletely dominant genes. Further, these workers reported the loci for such resistance genes in two strains of *Drosophila*.

Thus there are numerous records of autosomal inheritance of resistance, and although in most instances a complex of genes is concerned, there have been reports of resistance determined by relatively few genes or even a single gene.

The entomologist is interested in determining the length of time necessary to establish resistance under normal control procedures, and in determining the hereditary mechanisms of resistance. He is interested also in the rate at which such resistance will decline when the selective agent is withdrawn. According to King (1954) a directional change in the frequency of a genetic element requires compensatory changes in the frequencies of others. Thereby a new equilibrium is established, but quite often the new equilibrium is unstable; thus it tends to return to the old one when the force producing the directional change is removed. Lerner (1954) calls this phenomenon "genetic homeostasis", whereas, Waddington (1953) prefers to call it "normalizing selection." In this respect Bruce and Decker (1950) found a DDT-resistant strain of house fly developed in the laboratory to be capable of retaining its level of resistance for 30 generations in the absence of selection. However, another DDT-resistant strain of house fly was reported by Pimentel _et al._ (1954) to have declined to normal susceptibility in 20 generations, and Keiding (1953) records the loss of resistance in a DDT-resistant strain in one year. Crow (1954) noted no decrease in resistance in DDT-resistant _Drosophila_ after three years during which there was no contact with the toxicant. The California red scale maintains resistance to hydrogen cyanide after more than 150 generations; this period is equivalent to more than 40 years in the natural state (Metcalf, 1955). Johnston _et al._ (1954) have attributed to "Dauer modification" the loss of resistance to DDT in house flies after nine generations. Dauer modification is defined as a cytoplasmic change induced by an environmental factor which decreases in penetrance and expressivity in succeeding generations in the absence of the inducing stimulus. This interpretation has been questioned by Jarvis (1956a), by Grayson and Cochran (1955), and by Crow (1957).

These results indicate that the ability to retain resistance in the absence of selection may vary within broad limits. Not only is this found to be true when comparisons are made among the several species of resistant insects, but it is true of the various resistant strains developed within a given insect species. Such variations are perhaps attributable in laboratory-developed resistance to the variety of techniques used in culturing and selecting, as well as testing the resistance of strains. In the field wide differences might emanate from variations in insecticidal treatment, particularly with respect to concentration of toxicant applied, and number of applications prior to investigation of developed resistance. The World Health Organization (1956) urges as essential to investigations of this problem the adoption of standards for evaluating resistance.

THE INVESTIGATION

Materials and Methods

I. Culturing of Cockroaches

Mass matings: In each of the one-gallon glass battery jars used as a rearing chamber there were placed approximately 60 pairs of adult cockroaches. In order to accommodate such a large number of insects in each jar eight tiers of 1/8-inch masonite were arranged. The individual squares of masonite were supported and separated by staples partially imbedded in their undersurfaces. Escape of the roaches was prevented by the application of a thin film of petroleum jelly about two inches wide on the inside rim of the jar. Finally, the jar was capped with a square of cheesecloth secured by two rubber bands. Usually two jars of breeders were employed in each strain to produce the next generation.

Dry dog food was strewn in the bottom of each jar to give the insects free access to it. Water was continuously available in a Petri dish placed upon the uppermost tier of masonite.

While yet in the nymphal stages the progeny in each jar were transferred to four-gallon aquaria. In these chambers additional surface was provided by the inclusion of six to eight pieces of masonite measuring 7 x 8 inches, and supported by staples. Food and water were placed upon the uppermost tier. Each aquarium was closed with a close-fitting wooden cover in which there was a screened opening 4 3/4 x 8 3/4 inches.

Except for the time devoted to caging and treating the cockroaches, the cultures were maintained in a walk-in cabinet at a temperature of

30° C. and a relative humidity of 55 per cent. Humidity was controlled by a commercial humidifier operating on a humidistat. An electrical heater under thermostatic control was employed for temperature regulation; a cooling device was found unnecessary because the cabinet operated at a temperature considerably above the surrounding room temperature. Circulation of air within the cabinet was accomplished with a continuously operating fan.

Pair matings: In each one-quart glass jar used as a rearing chamber was placed a single pair of adult cockroaches. Copper screen wire was folded and placed in each jar to provide greater surface, and a small quantity of dried dog ration was scattered in the bottom of the rearing jar. Water was provided in a metal salve box held within folds at the top of the screenwire. The roaches were prevented from escaping by the application of a thin film of petroleum jelly within the inner rim of the jar. Each jar was then capped with a square of cheesecloth fastened with rubber bands.

To facilitate handling the jars, they were placed in wooden trays with slatted bottoms. Each of the trays had a capacity of twelve jars, and the dimensions were such that each fitted readily the shelves in the constant temperature-humidity room. The slatted bottoms of the trays allowed free circulation of air about the jars.

Soon after nymphs were noted in a given jar the adults were removed to a new jar. By this measure it was possible to rear for separate testing the progeny from successive egg cases produced by the females.

II. Toxicological Procedure:

Adult cockroaches not more than ten days in age were used in the toxicity tests. These were aspirated into screen wire cages which were then stoppered and laid aside until time for treatment. Usually groups of twenty to thirty roaches were so treated from the mass matings, whereas groups of five to fourteen were treated from the pair matings. Males and females were treated separately.

A stock solution of one gram of lindane dissolved in 250 ml. of acetone was prepared for treating the cockroaches. At the time of testing the cockroaches the desired concentrations of insecticide were formulated by volumetric measurement from the stock solution. For each concentration additional acetone according to a pre-determined schedule was added to facilitate suspension of the lindane; the percentage of acetone in the formulations ranged from 3 to 13 per cent in accord with an increase in concentration of the toxicant.

Application of the insecticide was achieved by immersing the cages of roaches in the proper dosage of insecticide at 30° C. for twenty seconds. The caged roaches were returned to the constant temperature-humidity room where they remained for one hour. The insects were then released in recovery jars in which food and water were available. Mortality counts were made on the fourth day following treatment.

A roach incapable of normal locomotion was considered dead, for it was found that this condition preceded death.

III. Genetic Technique:

At the outset determinations were made of the toxicity of lindane to the resistant and non-resistant strains of cockroaches.

Reciprocal crosses between resistant and non-resistant strain were then made. Virgin females were isolated for these crosses. Both the F_1 and the F_2 progeny of these pairings were treated to determine their order of resistance to lindane.

For purposes of identification the progeny of the resistant females crossed with the non-resistant males were designated "A" strain; whereas, the progeny of the resistant males crossed with non-resistant females were designated "B" strain. Backcrosses were made between F_1 male progeny of the reciprocal crosses, and virgin females of the resistant and the non-resistant strains as follows:

a) "A" males x Resistant females

b) "A" males x Non-resistant females

c) "B" males x Resistant females

d) "B" males x Non-resistant females

Although it would have been desirable to make backcrosses using female as well as male progeny of the reciprocal crosses, limitations of time made this impossible.

Immediately following the determination of level of resistance in the lindane-resistant strain, a group of breeders was segregated from that strain. This strain, designated "Selection Discontinued", was then reared in the absence of exposure to lindane. As each generation of this stock matured the members were treated to determine its level of resistance.

For the pair matings, individual pairs of males and virgin females were segregated from resistant and non-resistant strains. As the progeny from each egg case matured they were removed for treatment.

IV. Statistical Procedure:

The data obtained by treating the various strains of cockroaches at various concentrations of lindane were plotted on logarithmic-probability paper, and regression lines were fitted to the points by the method of least squares (Bliss, 1935). Four to six points were plotted to establish each line. For each dosage of insecticide 100 to 200 insects were used, and the tests were replicated three to six times.

Ninety-five per cent fiducial limits were determined for any lines with positions and slopes which indicated that they may have been derived from genetically similar populations.

These methods were not feasible for the data obtained from the treatment of progeny of pair matings, however, so an "index of resistance" was calculated for the male and female progeny of each parental pair. The index figure is the sum of the products obtained by multiplying the reciprocal of each concentration of insecticide by the corresponding mortality in decimal form. As a result, the greater the resistance the smaller the index numeral, and, conversely, the lower the resistance the larger the index value.

RESULTS

Figures 1 through 15 present, in the form of regression lines, the results from testing the progeny of the various strains and crosses. The regression lines for the parental strains are shown in figure 1.

The F_1 progeny of reciprocal crosses between resistant and non-resistant cockroaches were intermediate to their parents with respect to resistance (figures 2 and 3).

The F_2 progeny of the cross between the resistant females and non-resistant males (A strain) were much more resistant than were their parents (figure 4). Surprisingly, the resistance of these F_2 progeny approaches closely that of the resistant parents. Differing significantly from the foregoing were the results (figure 5) obtained by testing the F_2 progeny of the cross between non-resistant females and resistant males (B strain). The males showed a wider range of resistance than did the parent males; whereas, the females showed a slightly greater range of resistance than did their female parents, but this did not approach that shown in the males.

A backcross of A strain males to resistant females (hereinafter designated as A-R) produced F_1 progeny which were intermediate to the parents (figure 6). The males and females of the F_1 demonstrate a slightly greater range of variation than the parents.

When the F_1 progeny of A-R were inbred to produce an F_2, the males of this generation were found to be less resistant than were the F_1 (figure 7). The decreased slope indicated that they were slightly less homogeneous in this respect. In contrast to this, the females were more homogeneous in the F_2 in spite of having become slightly less resistant than the F_1 females.

Figure 8 presents the results of crossing A strain males to non-resistant females (A-NR). The male and female progeny were much less homogeneous than the parents. The F_2 progeny obtained from inbreeding the A-NR strain showed no significant difference from the F_1 (figure 9).

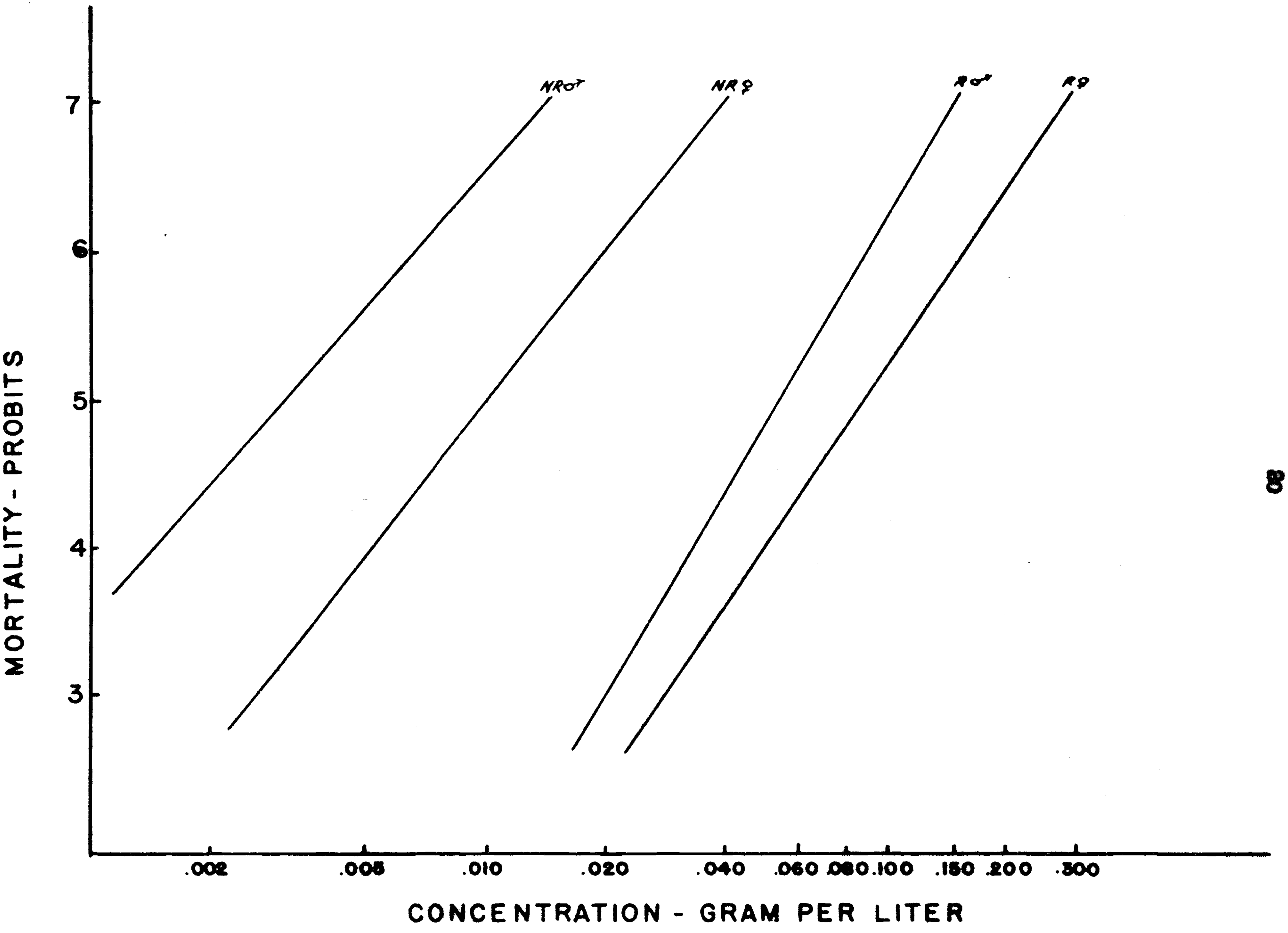
Backcrossing of B strain males to resistant females produced F_1 males and females which were intermediate to, but slightly more heterogeneous than, the parents (figure 10). Inbreeding of these B-R progeny produced males which were essentially the same as their male parents with respect to resistance. However, the female progeny were a much more homogeneous group in this respect while showing much the same LD-50 value as that of the female parent (figure 11).

Figure 12 presents the results of backcrossing B strain F_1 males to non-resistant females (B-NR). F_1 males from this cross were considerably less resistant and more variable in response than were their male parents. The F_1 females, however, were more resistant than either parent and the regression line had about the same slope as that of the female parent. Inbreeding this strain produced males with a slightly greater range of variation and females with a much greater range of variation than that demonstrated by the parents (figure 13).

In figures 14 and 15 are shown the responses of eight generations of resistant strain roaches following the discontinuance of selection. As can be seen there was no appreciable loss of resistance either in the females (figure 14), or in the males (figure 15), in the absence of selective pressure. Though fiducial limits were calculated for the parent strains, in the interest of simplicity they were not plotted on the figures.

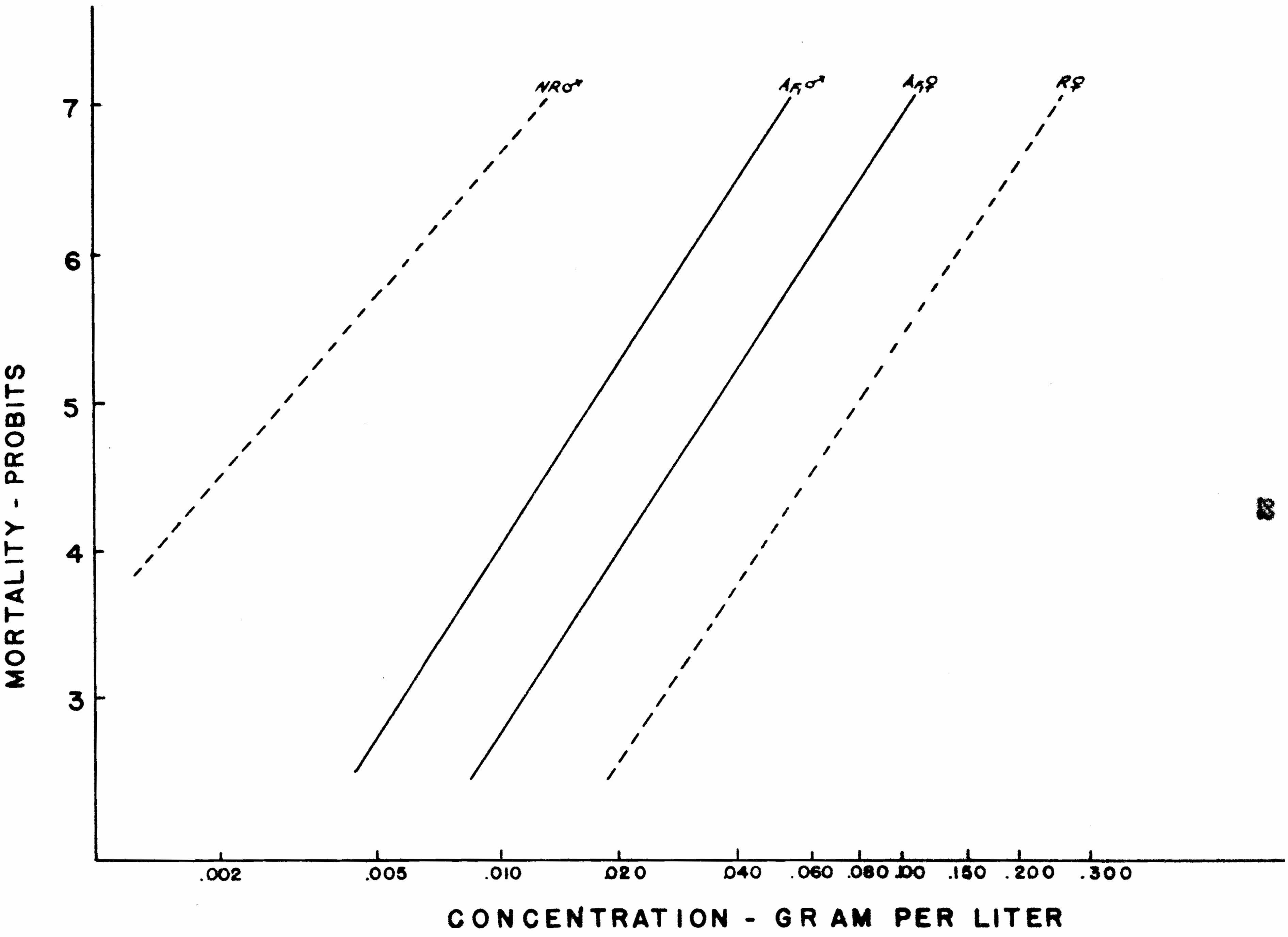
EXPLANATION OF FIGURE I

Figure 1 - Toxicity of lindane to the parental strains. Non-resistant strain, NR. Lindane-resistant strain, R.


NR♂
NR♀
R♂
R♀
7
6
5
4
3
MORTALITY - PROBITS
.002
.005
.010
.020
.040
.060
.080
.100
.150
.200
.300
CONCENTRATION - GRAM PER LITER

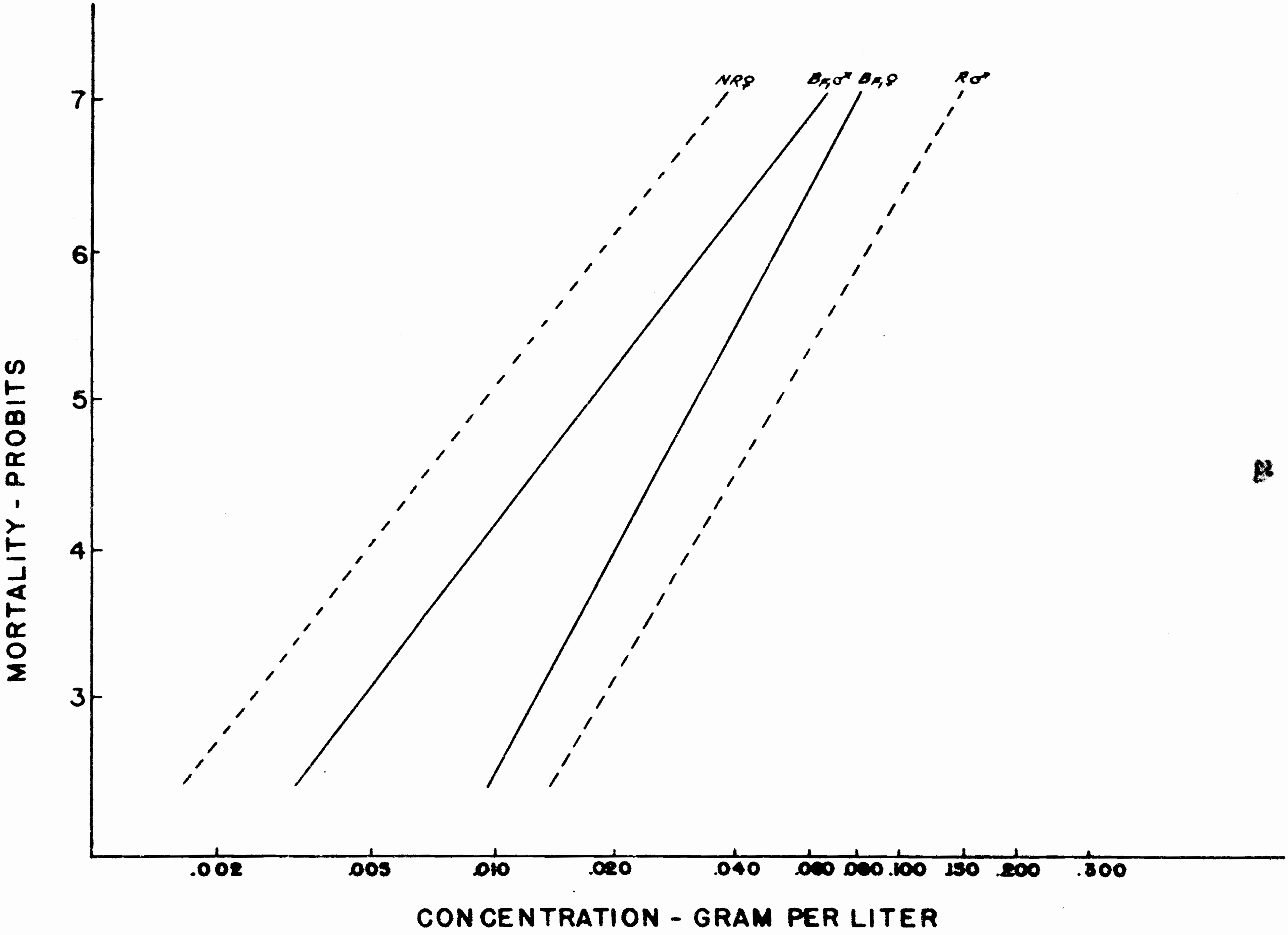
EXPLANATION OF FIGURE II

Figure 2 - Toxicity of lindane to the progeny of a cross between resistant females, R, and non-resistant males, NR. Progeny of the cross are designated, A.


NR♂
R♀
7
6
5
4
3
MORTALITY - PROBITS
.002
.005
.010
.020
.040
.060
.080
.100
.150
.200
.300
CONCENTRATION - GRAM PER LITER

EXPLANATION OF FIGURE III

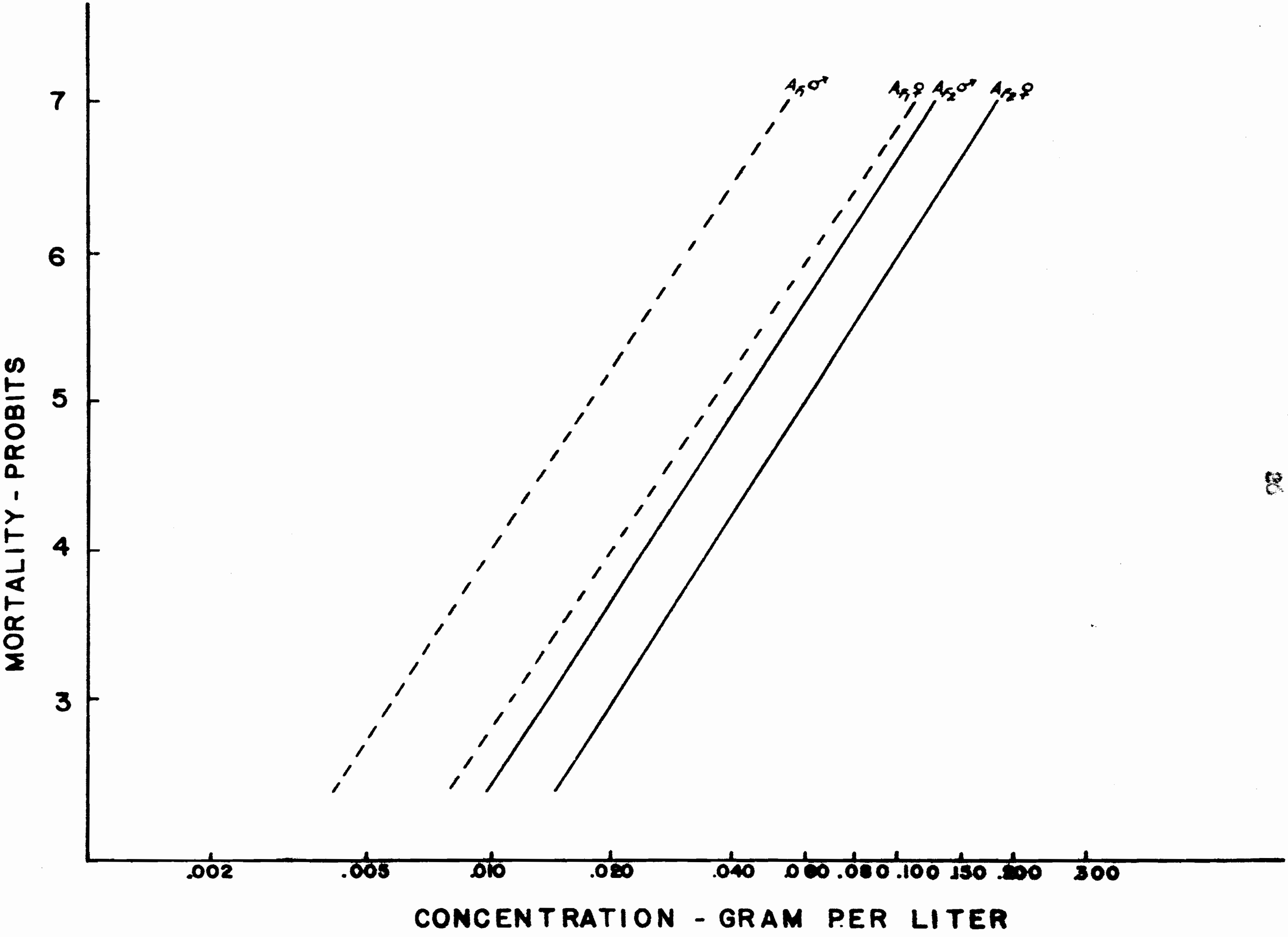
Figure 3 - Toxicity of lindane to the progeny of a cross between resistant males and non-resistant females. Progeny are designated, B.



MORTALITY - PROBITS
7
6
5
4
3
.002
.005
.010
.020
.040
.100
.150
.200
.300
CONCENTRATION - GRAM PER LITER

EXPLANATION OF FIGURE IV

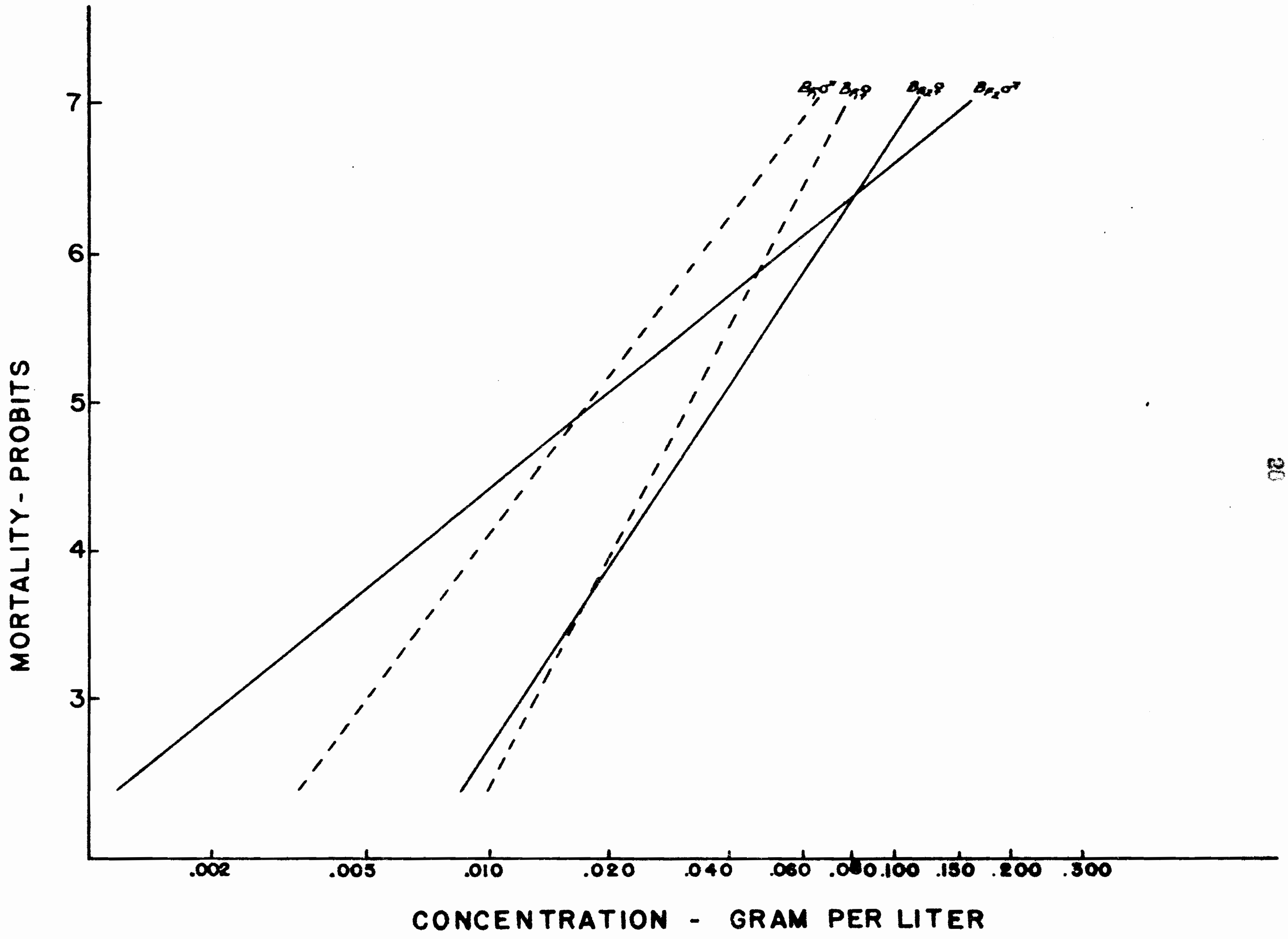
Figure 4 - Toxicity of lindane to the first and second generations of A strain.



MORTALITY - PROBITS
7
6
5
4
3
.002
.005
.010
.020
.040
.060
.080
.100
.150
.200
.300
CONCENTRATION - GRAM PER LITER
A_{F_1}♂
A_{F_1}♀
A_{F_2}♂
A_{F_2}♀

EXPLANATION OF FIGURE V

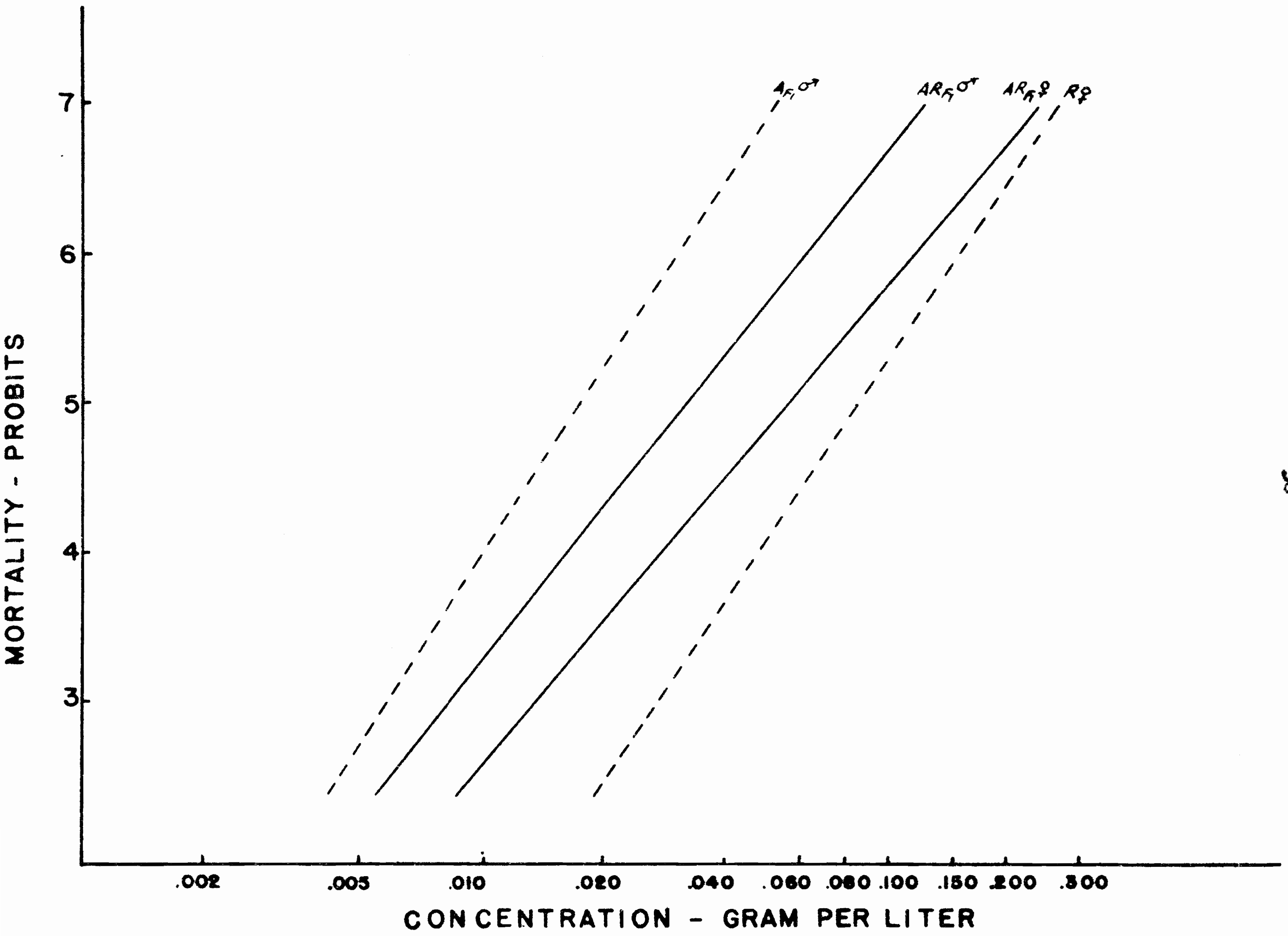
Figure 5 - Toxicity of lindane to the first and second generations of B strain.



MORTALITY - PROBITS
7
6
5
4
3
.002
.005
.010
.020
.040
.060
.080
.100
.150
.200
.300
CONCENTRATION - GRAM PER LITER
B_{F_1}♂
B_{F_1}♀
B_{F_2}♀
B_{F_2}♂

EXPLANATION OF FIGURE VI

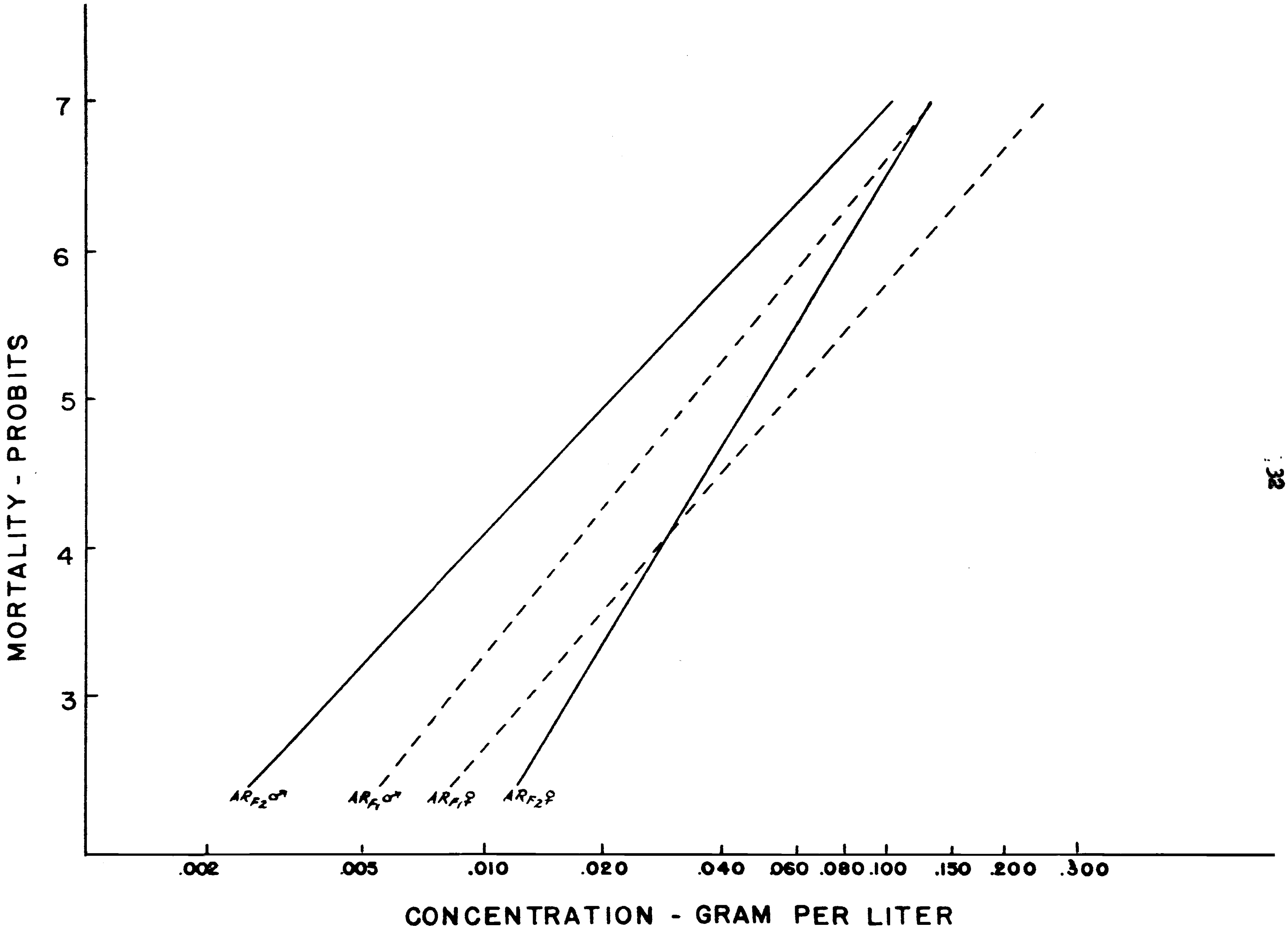
Figure 6 - Toxicity of lindane to the progeny, A-R, of a backcross between A strain males, and resistant strain females.



MORTALITY - PROBITS
7
6
5
4
3
CONCENTRATION - GRAM PER LITER
.002
.005
.010
.020
.040
.060
.080
.100
.150
.200
.300
A_{F_1} ♂
AR_{F_1} ♂
AR_{F_1} ♀
R♀

EXPLANATION OF FIGURE VII

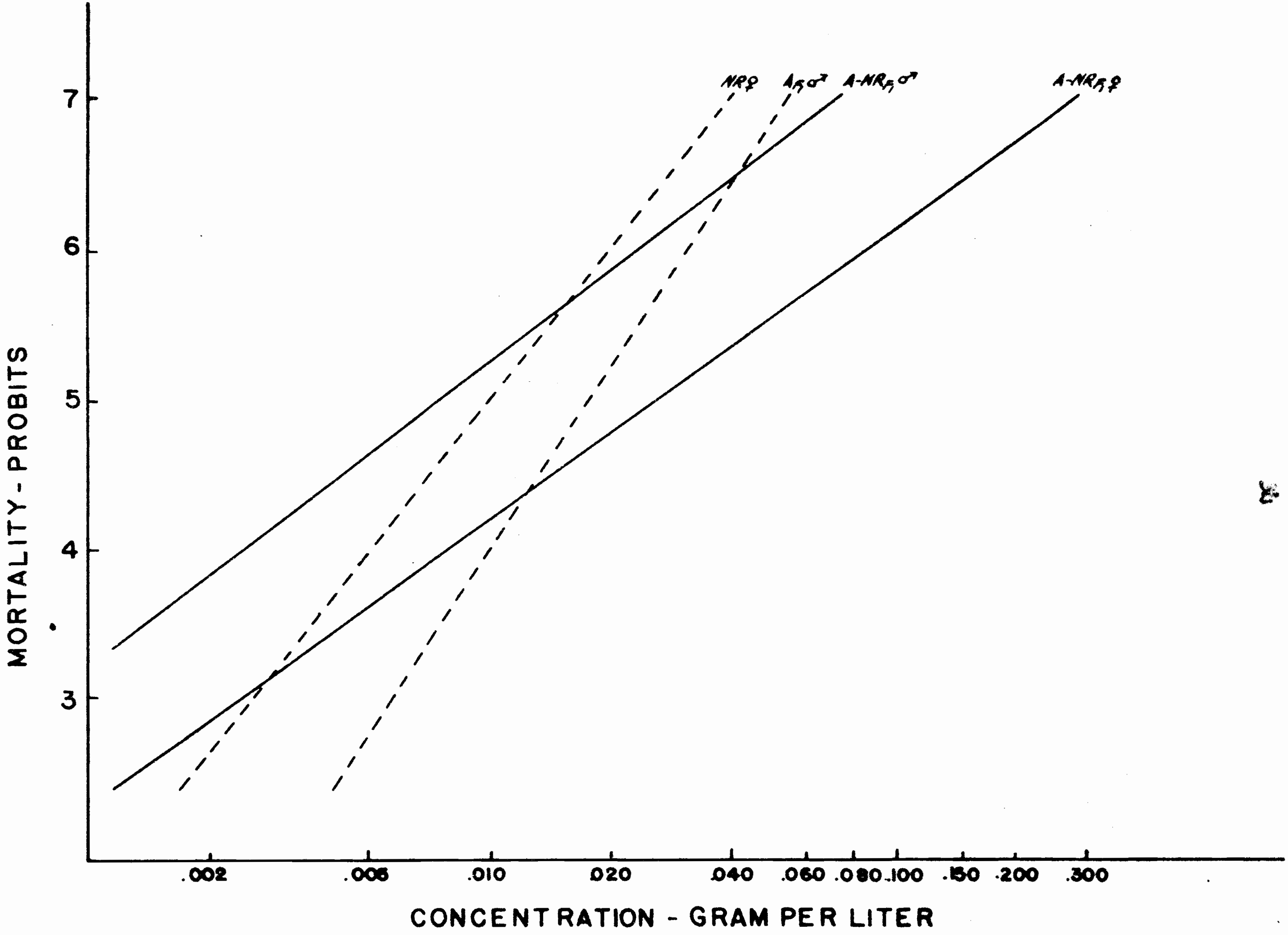
Figure 7 - Toxicity of lindane to the first and second generation progeny of a cross between A strain males, and resistant strain females.



MORTALITY - PROBITS
7
6
5
4
3
AR_{F_2}♂
AR_{F_1}♂
AR_{F_1}♀
AR_{F_2}♀
.002
.005
.010
.020
.040
.060
.080
.100
.150
.200
.300
CONCENTRATION - GRAM PER LITER

EXPLANATION OF FIGURE VIII

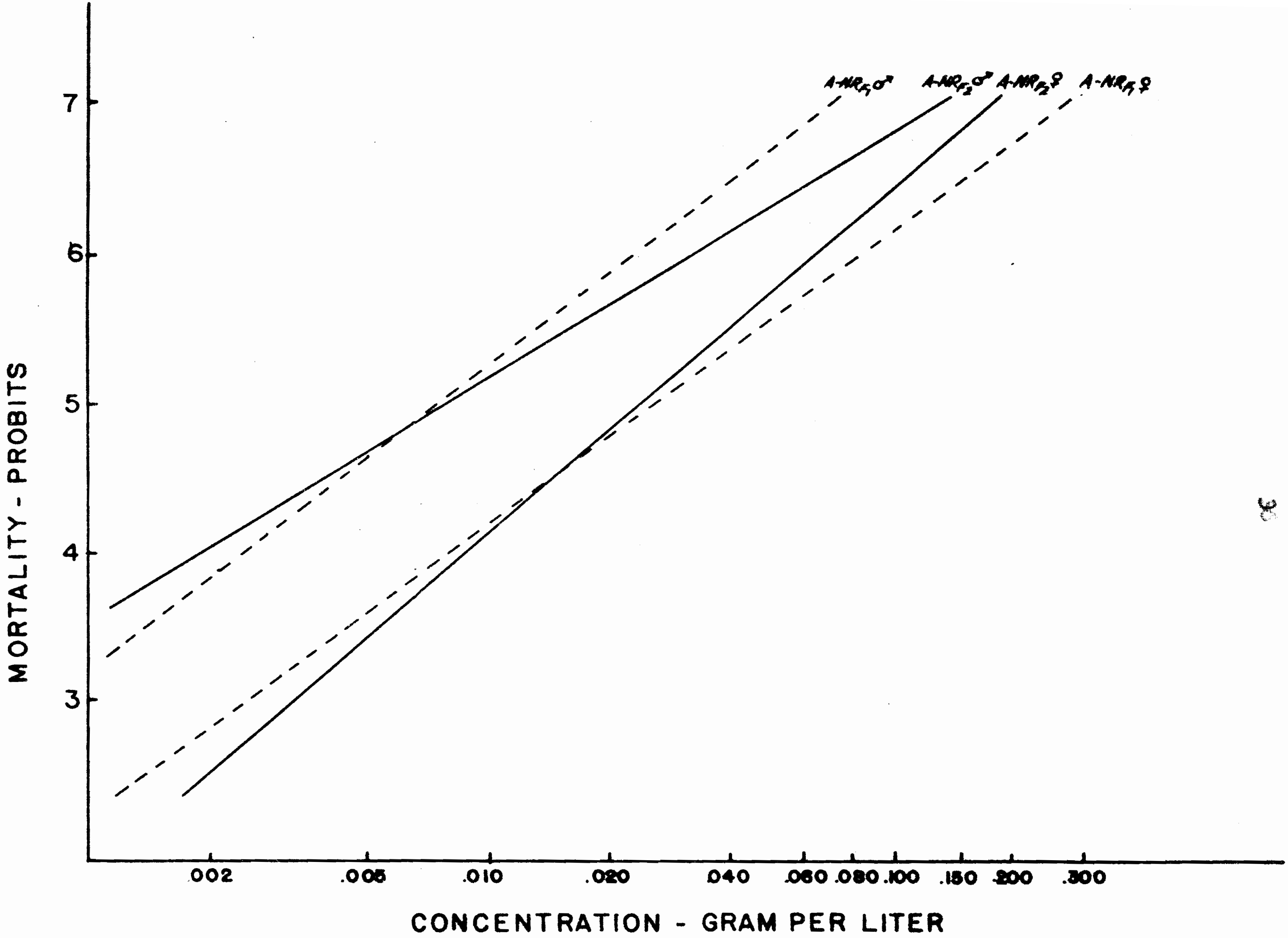
Figure 8 - Toxicity of lindane to the progeny, A-NR, of a backcross between A strain males and non-resistant strain females.



NR♀
A_{F_1}♂
A-NR_{F_1}♂
A-NR_{F_1}♀
7
6
5
4
3
MORTALITY - PROBITS
.002
.006
.010
.020
.040
.060
.080
.100
.150
.200
.300
CONCENTRATION - GRAM PER LITER

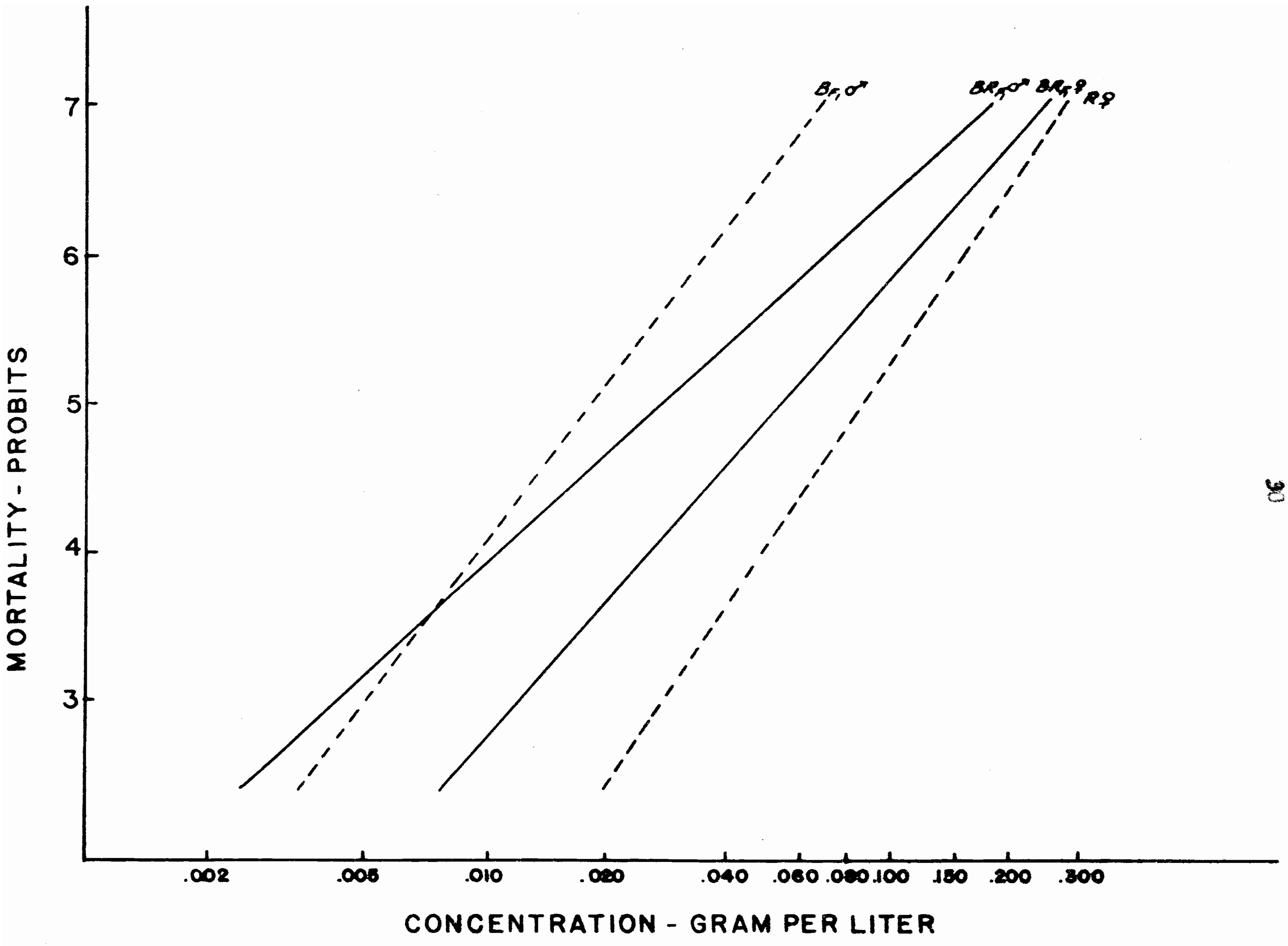
EXPLANATION OF FIGURE IX

Figure 9 - Toxicity of lindane to the first and second generation progeny of a cross between A strain males, and non-resistant strain females.


A-NR$_{F_1}$ ♂
A-NR$_{F_2}$ ♂
A-NR$_{F_2}$ ♀
A-NR$_{F_1}$ ♀
7
6
5
4
3
MORTALITY - PROBITS
.002
.005
.010
.020
.040
.060
.080
.100
.150
.200
.300
CONCENTRATION - GRAM PER LITER

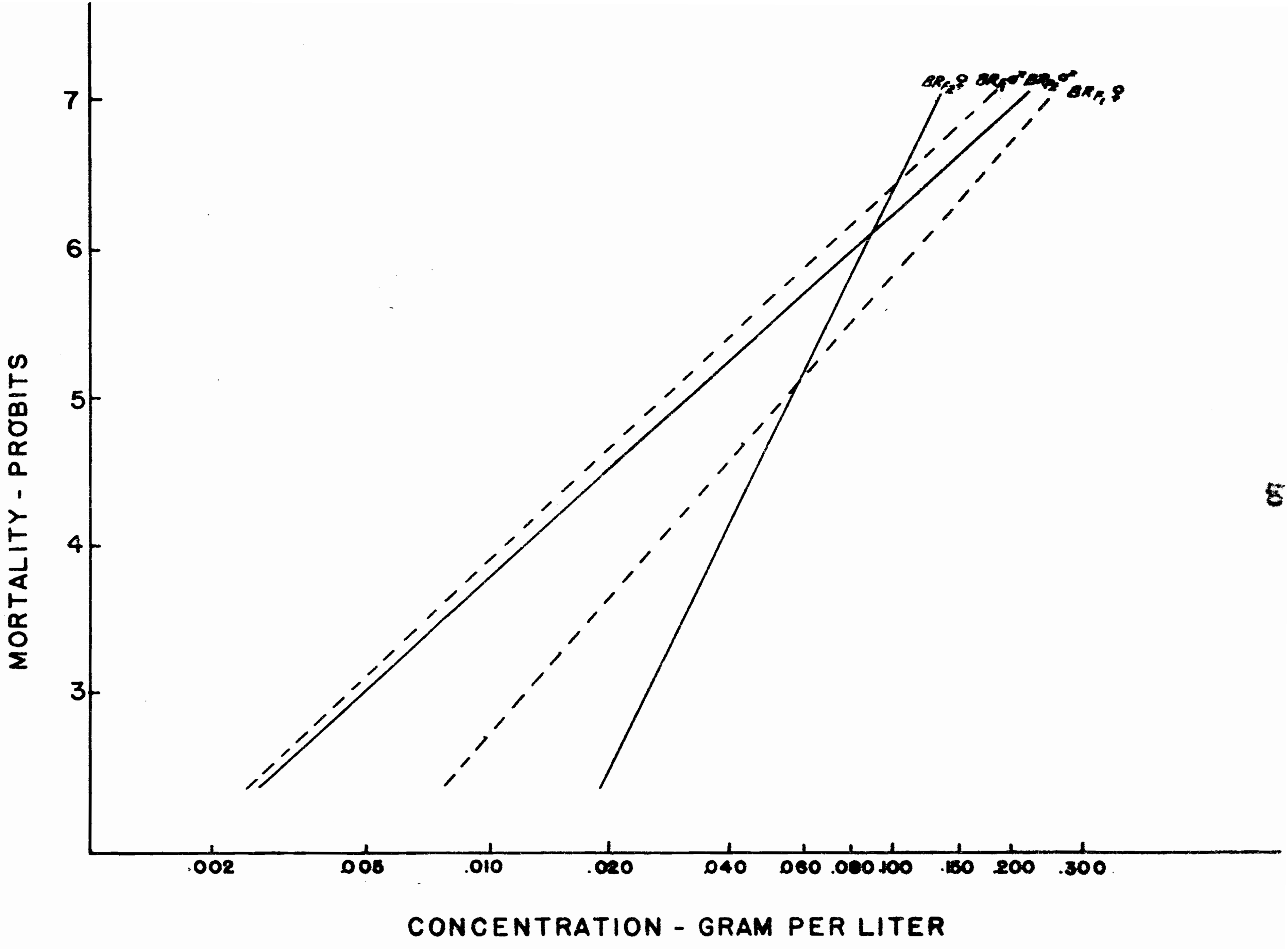
EXPLANATION OF FIGURE X

Figure 10 - Toxicity of lindane to the progeny, B-R, of a backcross between B strain males, and resistant strain females, R.


MORTALITY - PROBITS
7
6
5
4
3
CONCENTRATION - GRAM PER LITER
.002
.005
.010
.020
.040
.060
.080
.100
.150
.200
.300
BF_1 ♂
BRF_1 ♂
BRF_1 ♀
R♀

EXPLANATION OF FIGURE XI

Figure 11 - Toxicity of lindane to the first and second generation progeny of a cross between B strain males and resistant strain females.

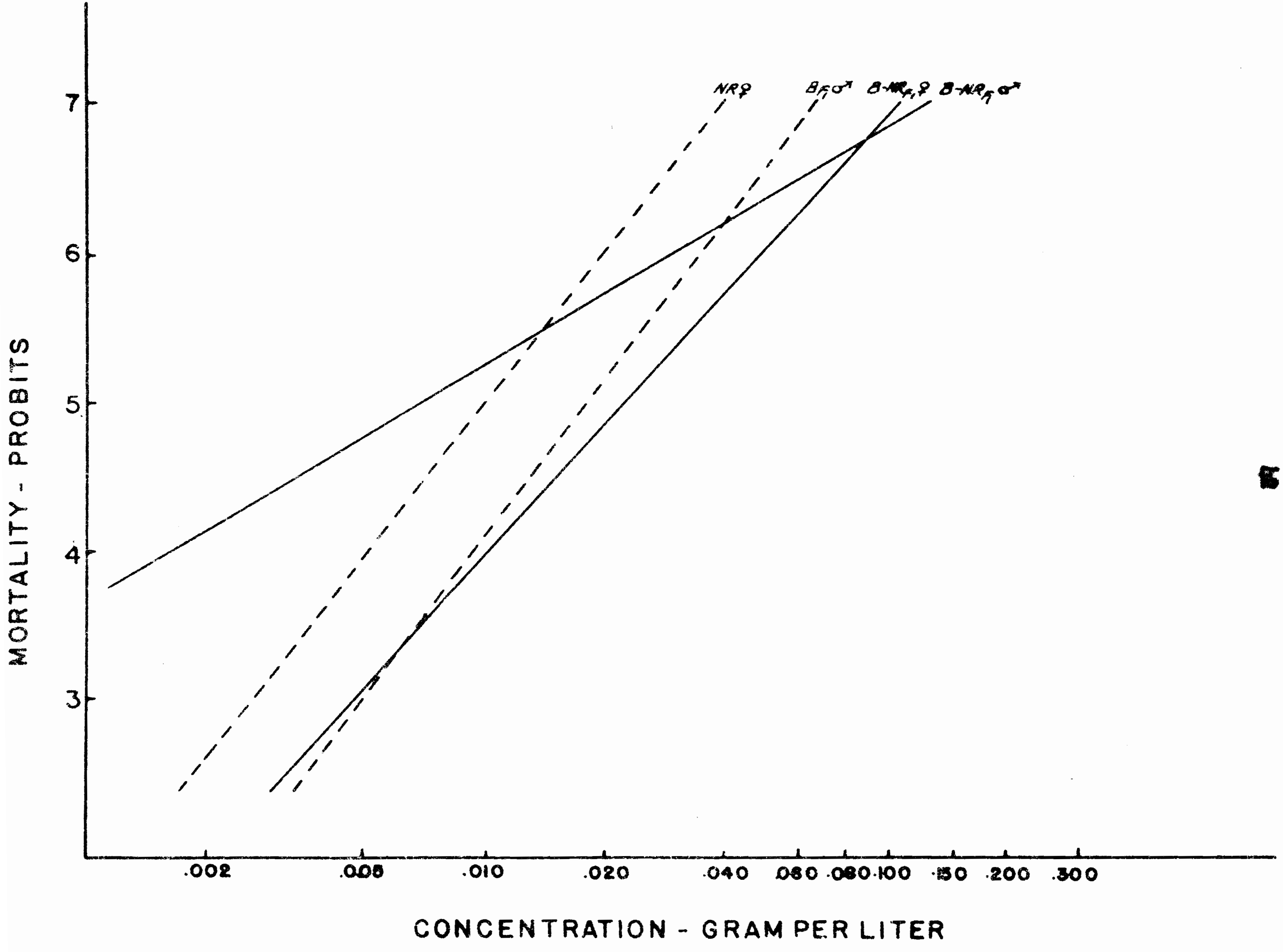


MORTALITY - PROBITS
7
6
5
4
3
.002
.005
.010
.020
.040
.060
.080
.100
.150
.200
.300
CONCENTRATION - GRAM PER LITER
BRF_2 ♀
BRF_1 ♂
BRF_2 ♂
BRF_1 ♀

5

EXPLANATION OF FIGURE XII

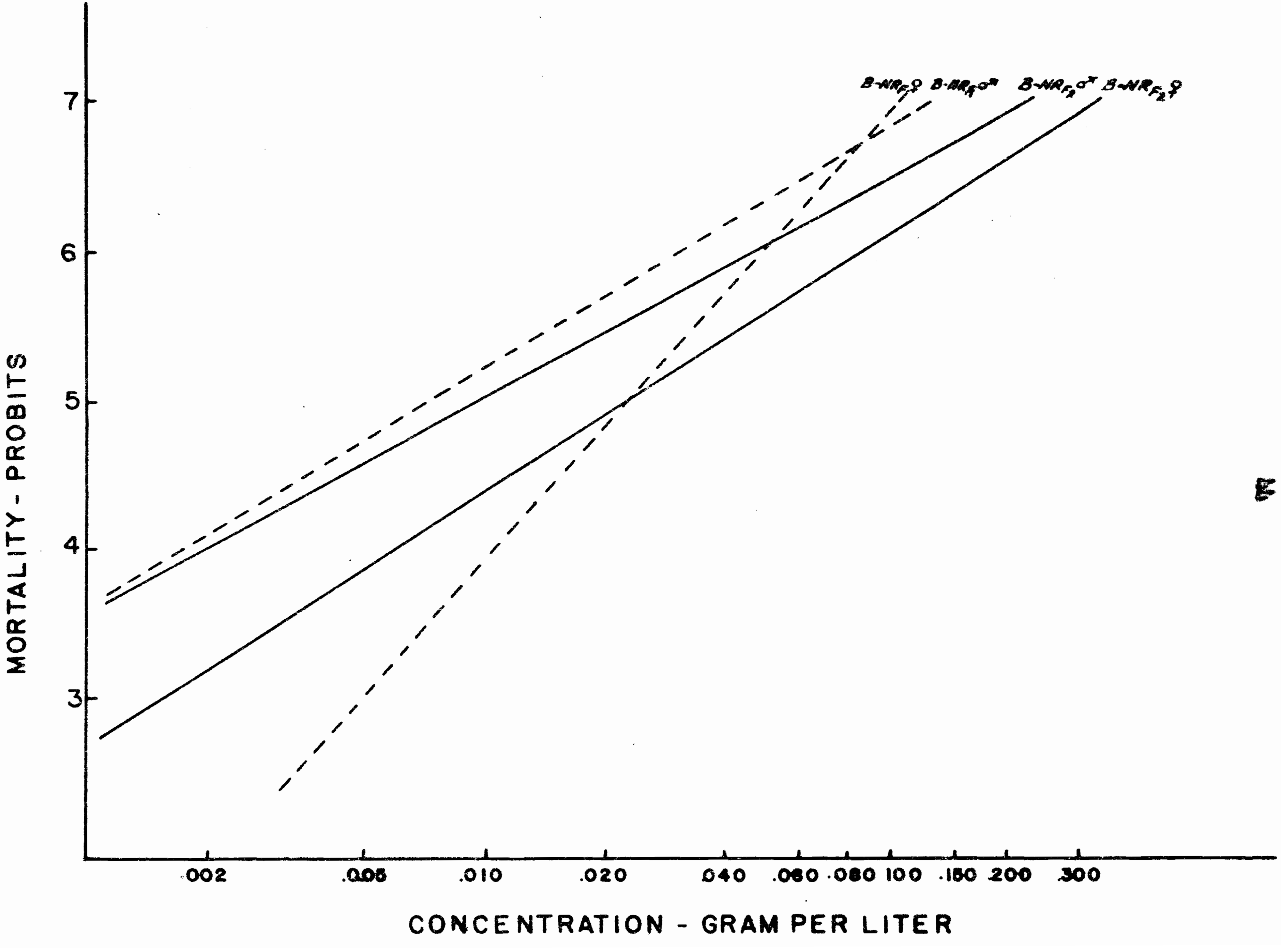
Figure 12 - Toxicity of lindane to the progeny, B-NR, of a backcross between B strain males, and non-resistant strain females.



MORTALITY - PROBITS
7
6
5
4
3
NR♀
B♂
.002
.006
.010
.020
.040
.060
.080
.100
.150
.200
.300
CONCENTRATION - GRAM PER LITER

EXPLANATION OF FIGURE XIII

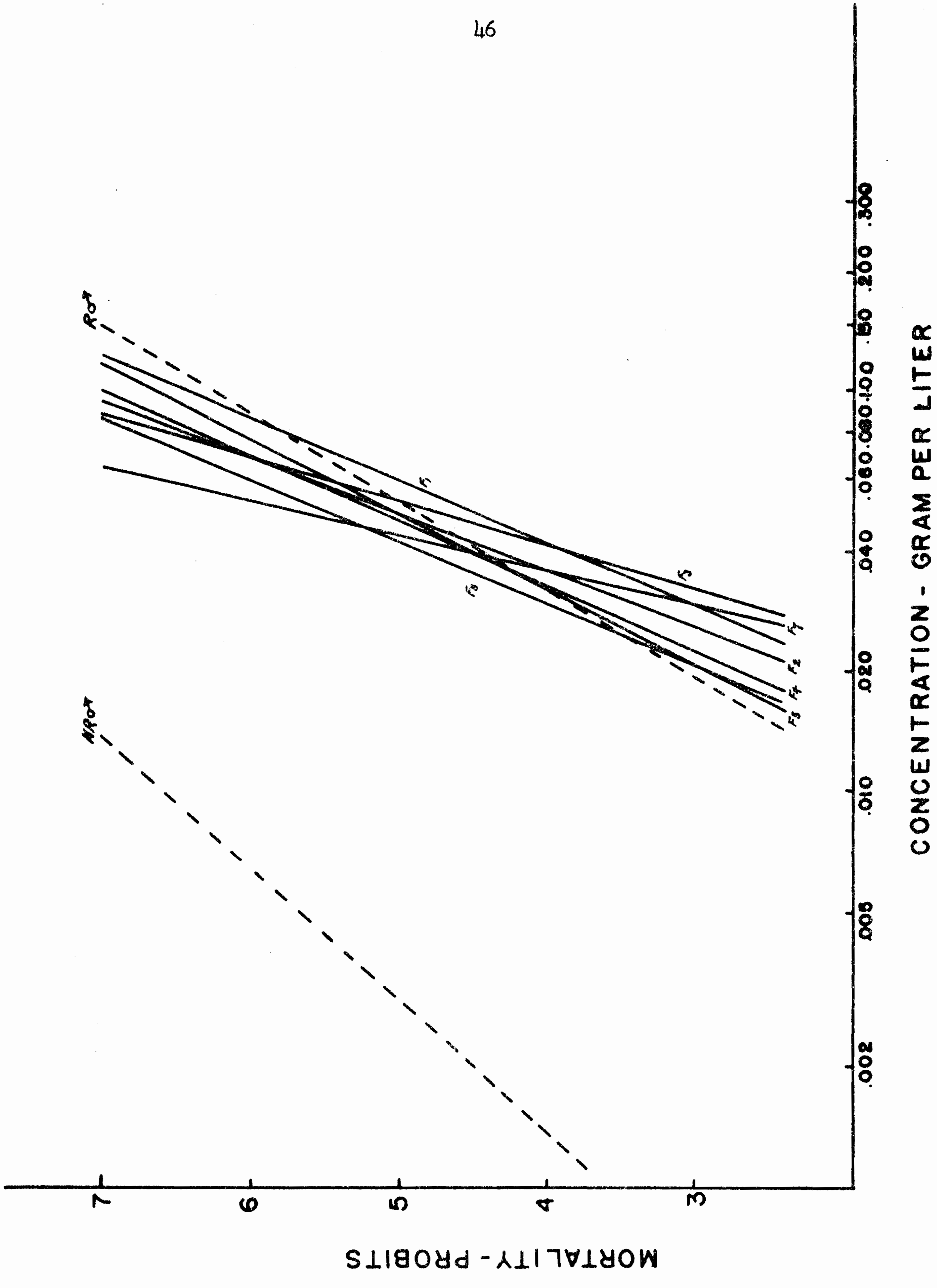
Figure 13 - Toxicity of lindane to the first and second generation progeny of a cross between B strain males, and non-resistant strain females.


B-NRF1 ♀
B-NRF1 ♂
B-NRF2 ♂
B-NRF2 ♀
7
6
5
4
3
MORTALITY - PROBITS
.002
.005
.010
.020
.040
.060
.080
100
.150
.200
.300
CONCENTRATION - GRAM PER LITER

E

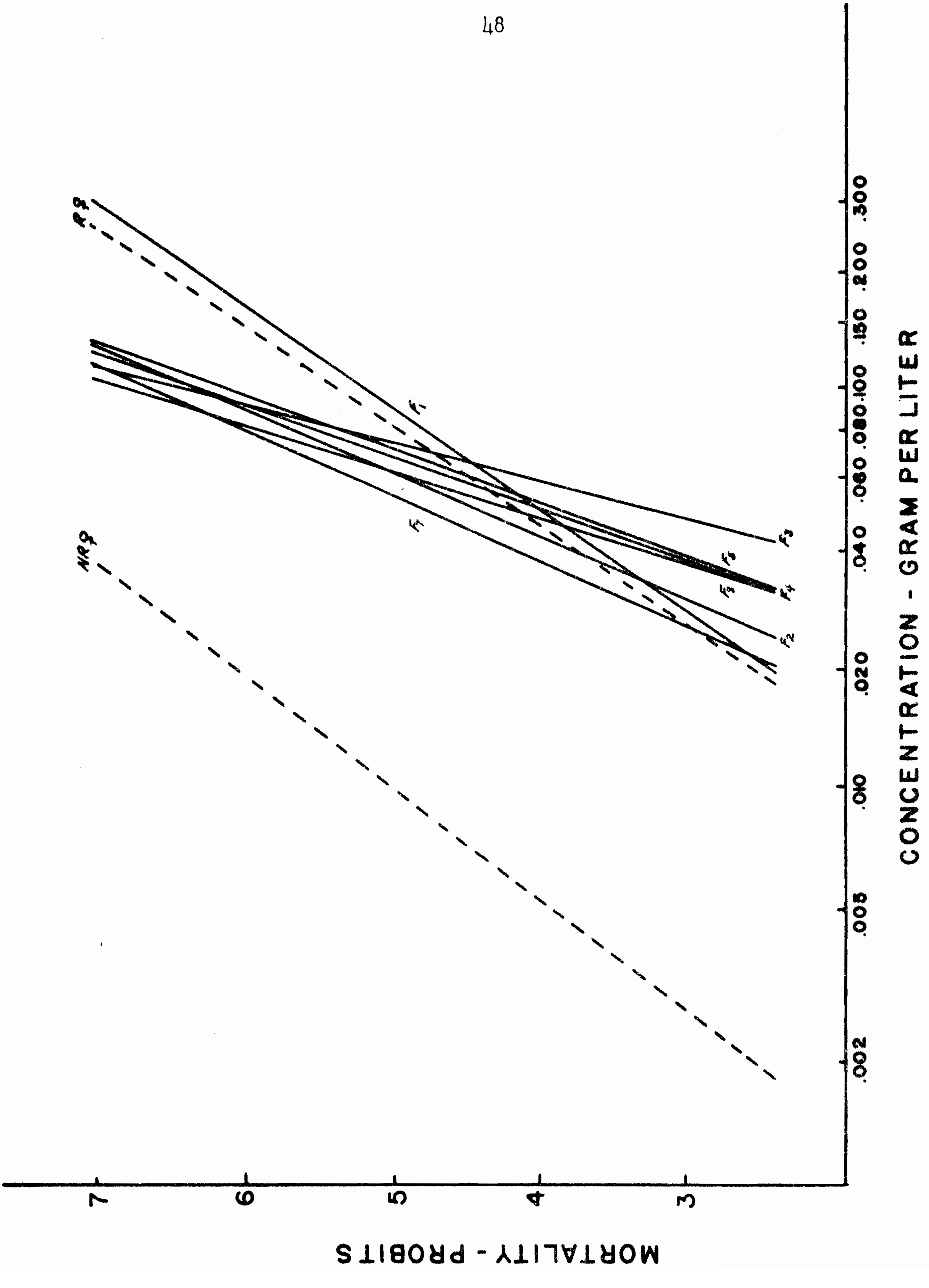
EXPLANATION OF FIGURE XIV

Figure 14 - Toxicity of lindane to the males of generations 1, 2, 3, 4, 5, 7, and 8 of a resistant strain of cockroaches no longer subjected to insecticidal selection.


MORTALITY - PROBITS
7
6
5
4
3
CONCENTRATION - GRAM PER LITER
.002
.005
.010
.020
.040
.060
.080
.100
.150
.200
.300
MRo♂
Ro♂
F1
F2
F3
F4
F5
F6
F7

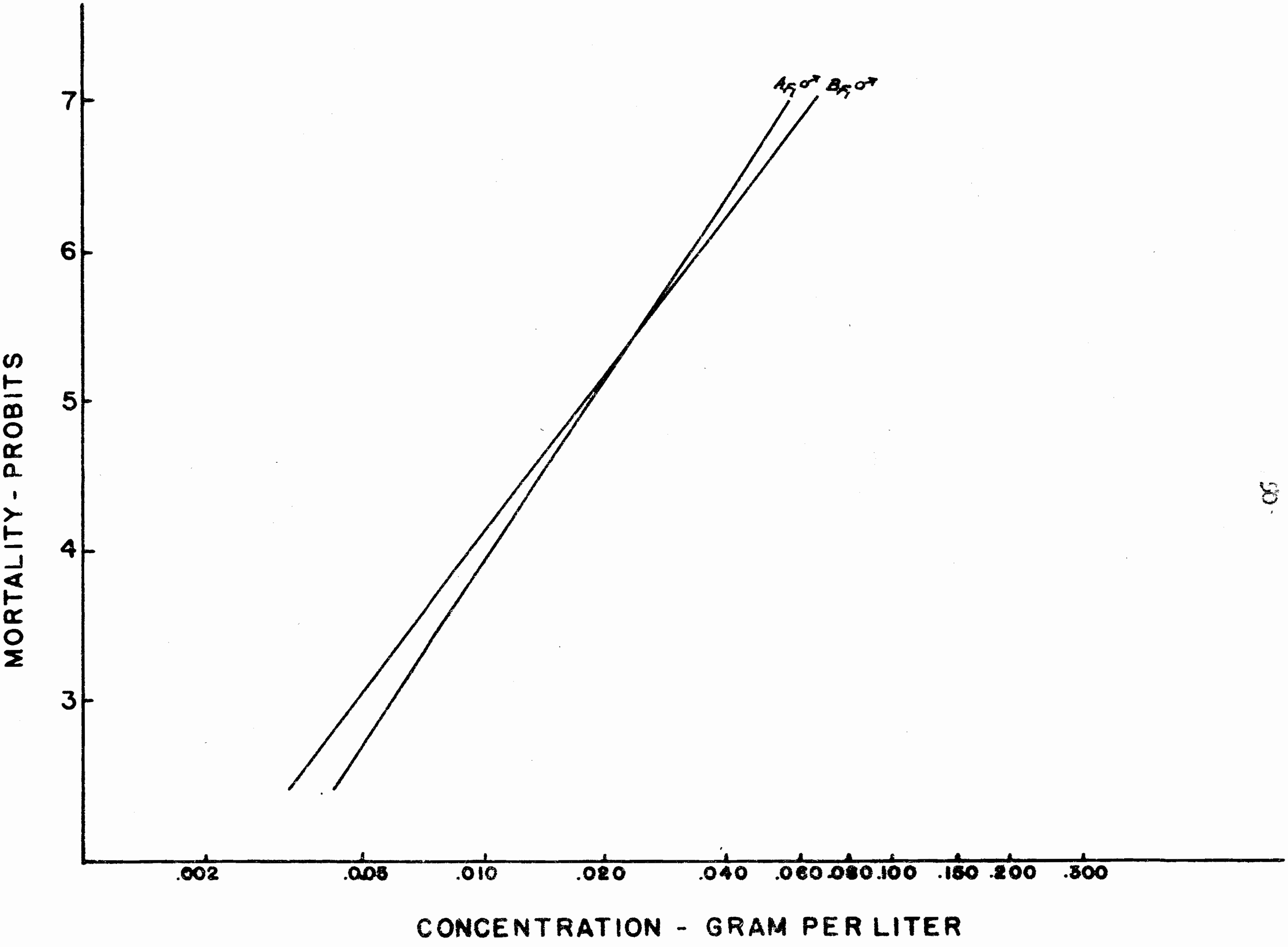
EXPLANATION OF FIGURE XV

Figure 15 - Toxicity of lindane to the females of generations 1, 2, 3, 4, 5, 7, and 8 of a resistant strain no longer subjected to insecticidal selection.


MORTALITY - PROBITS
7
6
5
4
3
CONCENTRATION - GRAM PER LITER
.002
.005
.010
.020
.040
.060
.080
.100
.150
.200
.300
R♀
NR♀
F1
F2
F3
F4
F5

EXPLANATION OF FIGURE XVI

Figure 16 - Toxicity of lindane to the first generation male progeny of reciprocal crosses between resistant and non-resistant strains. Cross between resistant females, and non-resistant males, A. Cross between non-resistant females, and resistant males, B.



MORTALITY - PROBITS
7
6
5
4
3
A_{F_1} ♂
B_{F_1} ♂
.002
.005
.010
.020
.040
.060
.080
.100
.150
.200
.300
CONCENTRATION - GRAM PER LITER

TABLE 1

Comparative resistance at LD-50 of progeny from reciprocal crosses and backcrosses.

Strain	Males	Females
Non-resistant	1.0	1.0
A-NR	2.5	2.6
A	6.0	3.7
A-R	10.7	5.8
Lindane-resistant	17.7	8.8
Non-resistant	1.0	1.0
B-NR	2.3	2.3
B	6.1	3.3
B-R	10.7	5.4
Lindane-resistant	17.7	8.8

TABLE 2

Toxicity of lindane at several concentrations to male progeny of pair matings within non-resistant strain.

Pair Number	Percent Mortality - 4 days .0040	.0060	Index* of Resistance
3	6	3	20.00
34	6	32	68.33
25	31	21	112.50
4	31	38	140.83
26	26	52	151.67
31	33	46	159.17
32	33	47	160.83
16	27	58	164.17
7	37	46	169.17
24	33	52	169.17
9	43	39	172.50
1	51	52	214.17
21	50	74	248.33
30	69	65	280.83
11	53	90	282.50
17	71	72	297.50
6	67	79	299.17
28	67	79	299.17
22	76	83	328.33
14	79	91	349.17
15	78	93	350.00

* Explanation is in the text.

TABLE 3

Toxicity of lindane at several concentrations to female progeny of pair matings within a non-resistant strain.

Pair Number	Percent Mortality - 4 days .012	.025	Index* of Resistance
3	0	14	5.6
34	12	32	22.80
31	0	70	28.00
30	14	70	39.67
9	20	60	40.66
25	25	67	47.63
4	28	50	53.33
32	36	61	54.40
7	40	60	57.33
1	27	91	58.9
22	38	74	61.27
15	36	83	63.20
21	39	86	66.90
24	38	94	69.29
26	52	65	69.33
14	40	95	71.33
17	47	88	74.37
16	57	76	77.90
28	59	83	82.37
11	70	93	95.53
6	82	93	105.53

* Explanation is in the text.

TABLE 4

Toxicity of lindane at several concentrations to male progeny of pair matings within a resistant strain.

Pair Number	Percent Mortality - 4 days .080	Percent Mortality - 4 days .150	Index* of Resistance
31	12	29	3.43
33	9	43	4.00
27	6	53	4.28
30	6	55	4.42
19	4	74	5.43
32	15	54	5.48
18	12	72	6.30
17	22	56	6.48
29	22	57	6.55
25	13	76	6.70
35	23	69	7.48
14	24	81	8.40
23	27	76	8.45
21	34	92	10.38
12	48	92	12.13
2	50	90	12.25
20	48	94	12.27
10	61	100	14.30
24	63	100	14.55
5	74	91	15.32
1	71	100	15.54
16	72	100	15.67
11	78	100	16.42
22	84	91	16.57
13	85	92	16.76

*Explanation is in the text.

TABLE 5

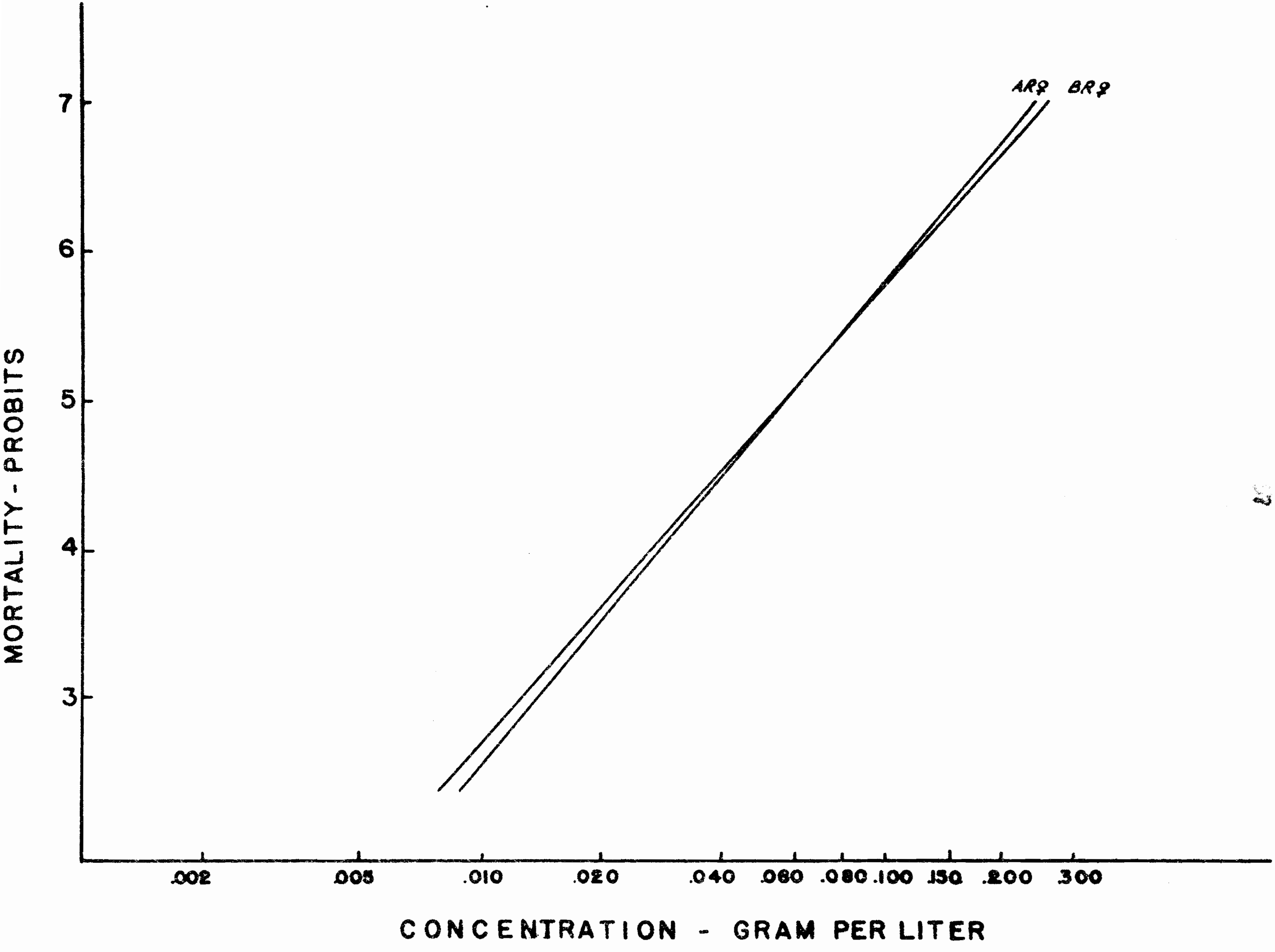
Toxicity of lindane at several concentrations to female progeny of pair matings within a resistant strain.

Pair Number	Percent Mortality - 4 days .100	.200	Index* of Resistance
19	0	11	0.56
31	3	20	1.30
25	0	38	1.90
24	5	33	2.15
30	8	29	2.25
2	13	22	2.40
21	15	47	3.85
29	23	33	3.95
17	24	53	5.05
23	29	68	6.30
20	33	70	6.80
27	32	85	7.45
18	38	78	7.70
5	43	69	7.75
16	56	50	8.10
11	44	79	8.35
14	37	96	8.50
1	51	100	10.10
13	58	91	10.35
10	59	100	10.90
22	69	86	11.20
12	70	100	17.10

* Explanation is in the text.

EXPLANATION OF FIGURE XVII

Figure 17 - Toxicity of lindane to the females of a cross between A strain males and resistant strain females, A-R; and a cross between B strain males and resistant strain females, B-R.


AR♀
BR♀
7
6
5
4
3
MORTALITY - PROBITS
.002
.005
.010
.020
.040
.060
.080
.100
.150
.200
.300
CONCENTRATION - GRAM PER LITER

DISCUSSION

Crow (1957) has indicated that because high levels of resistance occur only where there is a history of exposure to a toxicant, it raises the question of the role of the poison in the development of resistance. Two explanations have been given to define the importance of the toxicant. Postadaptive resistance may result from physiological or genetic changes induced directly by the poison. On the other hand, preadaptive resistance results when genetic differences already present in a population are acted upon by the poison in such a manner that genotypes for resistance are favored. Crow favors the view that most instances of resistance are preadaptive in nature. Some of the results of this research suggest that lindane resistance in the German cockroach is preadaptive. As can be seen by comparing the indices of resistance (tables 3 and 5) the non-resistant females can produce offspring as resistant as some of the progeny of resistant strain females. This seemingly indicates that the non-resistant strain cockroaches possessed potentiality for resistance which, when acted upon by the agent of selection (lindane), could have made possible the evolution of the resistant strain.

<u>Sex linkage and cytoplasmic considerations</u> - Let us consider first the evidence which has a bearing upon the possibility of sex-linkage as an explanation of lindane resistance. The German cockroach male is an XO type with respect to the sex-determining mechanism. By this it is meant that the male has in the diploid condition only one X chromosome. Conversely the female German cockroach has two X chromosomes in the diploid condition. Thus in each gamete which the female produces there will be

found an X chromosome and a full complement of autosomes, but male gametes are of two types. One type of male gamete bears an X chromosome and a full complement of autosomal chromosomes; the other type contains no X chromosome, only a set of autosomes.

If the genes for lindane resistance were a part of the X chromosome their existence should have been detectable in the responses of the F_1 males of the reciprocal crosses. Indicating an assumption of sex-linkage of resistance by a superscript R, the crosses could be diagrammed as follows:

Cross A (non-resistant males x resistant females)

$$XO \times X^R X^R$$

$$F_1 \quad X^R O \quad X X^R$$

Cross B (resistant males x non-resistant females)

$$X^R O \times XX$$

$$F_1 \quad XO \quad X^R X$$

This demonstrates that assuming sex-linkage of genes for lindane resistance one would expect the F_1 males of cross "A" to be distinctly more resistant than the F_1 males of cross "B". There is, however, no significant difference between the F_1 males of these two crosses with regard to resistance (figure 16). One may conclude therefore, that the principle genes for lindane resistance are not to be found on the X chromosome of this animal, nor is there any indication of the involvement of cytoplasmic factors for this, too, would have produced differences between the F_1 males of the reciprocal crosses. Further evidence in support of these conclusions is available from the backcrosses.

In general, the responses of the backcross progeny present a rather confusing picture, (figures 6 through 13). Some order may be drawn from this, however, by comparing the reactions of the strains at a single point. The dosage which produces fifty percent mortality (LD-50) has been selected for this purpose. Admittedly this single point does not convey any conception of the range of variation within a given population. It does, however, describe the central tendency of the group and as such may be used, with foreknowledge of its limitations, as a basis for comparison. In table 1 are presented the results of such comparisons. The similarities in responses among the several crosses are quite regular, there being, with one exception, no difference greater than four-tenths of a unit. It should be recalled that the A strain originated from a cross of lindane-resistant females with non-resistant males, and that the B strain resulted from the reciprocal of this cross. It is thus apparent that the degree of resistance was little affected by the sex of the parent contributing the complement of genes for resistance; i.e. the genes for resistance were equally transmissable in both sexes. Seemingly this evidence supports the contention that the inheritance of lindane-resistance is in no important manner bound to the sex-determining mechanism of the individual.

Having excluded sex-linkage and cytoplasmic inheritance as possible explanations of lindane resistance in this strain of cockroaches, we must conclude that the principle genes for resistance are part of the autosomes.

<u>Multifactorial inheritance</u> - In the course of his investigations of inherited characteristics Gregor Mendel dealt principally with distinct

differences between the plants he interbred. Some observers have indicated that this procedure was the key to his success, for whereas his predescessors in studies of inheritance attempted to analyse continuously intergrading traits, Mendel chose characteristics which could be sharply differentiated into alternative categories. Such categories were then open to the numerical analyses which led Mendel to postulate the principles upon which genetics was founded. It has since been found that many of the most important characteristics of organisms do not segregate into distinct classes, yet they are explainable on the basis of Mendelian genetics. Variations in qualities such as egg production, and yield of meat or milk in animals, and characteristics of man such as height, weight, and mental ability are of this nature. Analysis of these traits reveals the existence of numerous, continually intergrading classes. This absence of clear-cut segregation is the principle difference between characters determined by single genes, or relatively small numbers of genes, and those dependent upon combinations of large numbers of genes—e.g. systems of multiple factors.

It is generally accepted that if two organisms representing the opposite extremes of a quantitative characteristic are interbred the F_1 individuals will be intermediate between the parents, and the F_2 will show a wide array of variant types. The variant types in the F_2 may, and often do, include the parental extremes. The first two generations of progeny from cross B furnished evidence that lindane resistance may be dependent upon multiple factors (figures 3 and 5). The F_1 individuals were intermediate to the parent types, and may be considered to have represented the combination of extremes in factors for resistance or susceptibility received from the parents. The greater range of variant types in the F_2 may be considered to have resulted from gene recombinations which might as easily have produced individuals more resistant as individuals less resistant than the F_1. This may be presumed to indicate the presence of a number of independent genes with a cumulative effect for resistance.

In support of this hypothesis of multi-factorial inheritance of lindane resistance there may be cited the experience of Grayson in developing this strain (personal communication). The resistance of the lindane strain used in this study required 25 generations of selection, and at no time was there detected a rapid increase of resistance such as might have accompanied increased frequency of a major gene. Such a gradual accumulation of potency for a particular trait leads one to assume close relationship between the increase in resistance and the accumulation of a number of genes. Each gene may be postulated to add very little to the expression of the trait, but in toto their effect could give rise to an increased resistance. If, on the other hand, the resistance had been dependent upon the selection of one or a very few major genes, one might expect that their accumulation would have given rise to sudden increases in resistance.

Still further evidence of multi-factorial inheritance was found in the results obtained from testing the progeny of individual pairs within strains. The non-resistant strain cockroaches showed a considerable range, as evidenced by the indices of resistance (tables 2 and 3). As opposed to this, the progeny of resistant strain pairs showed a much narrower range of response, but not nearly so restricted a range as might have been expected if their resistance had been dependent upon a few genes of major effect (tables 4 and 5).

Thus, it is possible that there are no genes of major effect present. If this were true one could postulate that the entire resistance effect is founded upon the gradual accumulation of numerous genes for resistance, all of which are of minor effect.

The results obtained by testing the A strain F_2 are, at present, inexplicable. A marked increase in the resistance of these cockroaches actually returned them to the same level of resistance as the original resistant strain (figure 4). No explanation based upon sex-linkage, sex-limitation, sex-influence, cytoplasmic inheritance, maternal effect, or epistasis has been found to explain these results. The matings have been made again in an attempt to establish whether or not these results are reproducible. If results similar to those of the first series of matings are obtained in this second series, further matings will be made in an attempt to fathom this seemingly aberrant behavior. No light is thrown on this problem by the testing of backcross progeny. As is shown by figure 17, the responses of the female progeny of B strain males mated to resistant females are almost duplicates of the responses of the female progeny of A strain males mated to resistant females. These results suggest that the explanation of the unusual A strain F_2 reactions may be found in backcrosses of A strain females to resistant and to non-resistant strain males.

<u>Stability of resistance</u> - As previously mentioned, a question of great importance to the economic entomologist confronted with a resistant species of insect is, "How long will this resistance be maintained in the absence of chemical control measures?" Underlying this question is, perhaps, the hope that under these circumstances the insect will revert rapidly to susceptibility, and, therefore, may be controlled again. The results of this research give no cause to encourage such hopes with respect to lindane-resistant cockroaches. As can be seen from figures 14 and 15, there has been no significant loss of resistance in the resistant strain after 8 generations of breeding without exposure to an insecticide. At LD-50, the order of resistance in the males dropped only from 17.7 times in the resistant strain to 11 times in the eighth generation of the selection-discontinued strain; whereas the order of resistance in the females decreased from 8.8 times in the resistant strain to 6.1 times in the selection-discontinued strain. This relatively small loss of resistance occurred during eighteen months isolation from insecticidal treatment, so it is apparent that lindane resistance under these conditions is rather stable.

<u>Comparisons with DDT and chlordane resistance</u> - Certain comparisons may be drawn among the results of this experimentation and the results obtained by previous investigators of DDT and chlordane resistance in the German cockroach. Cochran <u>et al.</u> (1953) found DDT resistance in the German cockroach to be explicable upon the basis of autosomal and sex-linked factors, and these investigators indicated the possible involvement of cytoplasmic factors. Crow, however, wrote that these results may equally

well be explained as maternal influences attributable perhaps to delayed phenotypic expression. Inherited chlordane resistance was found to be multi-factorial, autosomal, and to show evidence of some dominance (Jarvis et al., 1957). Inherited lindane resistance in the German cockroach differs from DDT resistance in that there is little or no involvement of sex-linked factors, and there is no maternal effect demonstrable. Unlike inherited chlordane resistance, lindane resistance shows no evidence of dominance. Apparently, these three instances of inherited insecticide resistance are alike, however, in that all are principally dependent upon autosomal, multi-factorial systems.

It is an interesting feature of lindane resistance that during the period of selection the male cockroaches increased in resistance at a greater rate than the females. Whereas the females at LD-50 became 8.8 times resistant to lindane, the males became 17.7 times resistant. No explanation can be given for this difference, but it is extremely interesting that such a response was not apparent in either the chlordane- or the DDT-resistant strains. Chlordane resistant females and males, were, respectively, 470.5 and 200 times more resistant than the non-resistant strain. DDT-resistant females and males were, respectively, 333.3 and 14.8 times more resistant after selection. This matter appears promising for future investigation.

General considerations - The nature of lindane resistance in the German cockroach is such as not to lend itself to exact genetical analysis. Certain attributes of this animal seemingly fit it for genetic studies. It is easy to rear and culture in the laboratory, thus supplying large numbers of experimental subjects. Additionally, its life cycle is sufficiently long that one working with it on a part-time basis is not overwhelmed by the mechanics of handling the insect. Despite these advantages, grave disadvantages detract from the genetic usefulness of the German cockroach. First, the genetic mechanisms are almost entirely unexplored and lacking in critical analysis. Second, the German cockroach seems to be quite lacking in morphological mutants; visible markers as it were, which might be very useful in investigations of the animal's heredity. Thus, although a population of resistant cockroaches may be differentiated statistically or toxicologically, there is no reliable means whereby a single resistant cockroach can be distinquished from a non-resistant cockroach. Finally, although the lindane-resistant strain of cockroaches was selected with rigour equal to that used in selecting the DDT strain, and perhaps also the chlordane strain, the lindane strain still does not possess the degree of resistance that is characteristic of the other strains. This third defect introduces difficulty in interpreting the results of most of the crosses necessary to a genetic analysis.

Although the lindane-resistant strain of cockroaches is not as different from the non-resistant strain as would be desired for a study of this kind, it should be possible to increase the differential in resistance between these strains. In order to accomplish this one could make the non-resistant strain more homogeneous and more susceptible by controlled breeding techniques. This could be accomplished by mating pairs of cockroaches, and treating with insecticide one-half of the female progeny of the first egg-case. If the females so treated were susceptible to the toxicant it would be a strong indication that the male parent had supplied a genome characterized by susceptibility to the insecticide. The remaining females of the first egg-case then could be backcrossed to the susceptible male parent. The progeny of the backcross might then be dealt with as was the first pair of cockroaches. After a sufficiently susceptible strain had been developed one might introduce measures to produce mutants in the susceptible and resistant strains. It is suggested that treatment with mutagenic substances might produce viable morphological mutant types which might then be used in the desired crosses. Association between a visible mutant and its being susceptible or resistant to the insecticide would lend a degree of refinement to the genetic techniques heretofore unattained.

CONCLUSIONS

Under the stated conditions of this experiment it is concluded that:

1. Lindane resistance is not inherited as a simple, sex-linked factor.
2. The inheritance of lindane resistance is not attributable to maternal effects, cytoplasmic factors, or to sex-linked epistatic genes.
3. Lindane resistance is principally inherited through the autosomal chromosomes.
4. The genes for resistance comprise a multi-factorial system and individually are of minor effect.
5. Selection for resistance somewhat reduces the variation among individuals within the strain.
6. The resistant strain, though the product of lengthy selection, is still a heterogeneous population with respect to resistance to lindane.
7. Only a slight loss of resistance had occurred after eight successive generations without any selection to lindane.

SUMMARY

1. The inheritance mechanism of resistance to lindane in the German cockroach, Blattella germanica (L.), was studied by determining the toxicity of lindane to the following populations of cockroaches: a) lindane-resistant and non-resistant strains, b) the F_1 and F_2 progeny of reciprocal crosses between the two parental strains, c) the F_1 and F_2 progeny of backcrosses between the reciprocal cross F_1 males and resistant and non-resistant strain females, d) the progeny of pair matings within resistant and non-resistant strains, and e) eight successive generations of a resistant strain isolated from further insecticide selection.
2. The methods employed in rearing the cockroaches and obtaining toxicological data on the different populations, as well as the genetical and statistical procedures, are described.
3. The toxicological data for the mass matings are presented in the form of regression lines fitted by appropriate statistical analyses.
4. The toxicological data for the pair matings, transformed into "indices of resistance", are presented in tabular form.
5. Appropriate conclusions are drawn from the data to explain the inheritance mechanism of lindane resistance in the German cockroach.

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VITA

Name: John Grove Barker

Date of Birth: June 24, 1926

Place of Birth: Ocala, Florida

Parents: William Rolfe and Betty Cummings Barker

Secondary Education: Veterans' Educational Center,
Washington, D. C., 1946-1947

Colleges Attended and Degrees: Shenandoah College 1947-1948
Concord College 1948-1951, B. S.
University of Maryland 1951-1953, M. S.

Positions Held: Graduate Assistant in Zoology, University of Maryland 1952-1953
Instructor in Biology, Radford College, 1953-1954.
Assistant Professor of Biology, Radford College, 1954-1955
Fellow, National Science Foundation, 1955-1956
Fellow, Southern Fellowships Fund, 1956-1957

Honors: Chi Beta Phi Science Fraternity
Blue Key Society
Phi Sigma Society

Professional Affiliations: Entomological Society of America
Entomological Society of Washington

John G. Barker

INHERITANCE OF RESISTANCE TO LINDANE

IN THE GERMAN COCKROACH, _Blattella_ _germanica_ (L.)

Abstract submitted by John G. Barker

Candidate for the degree Doctor of Philosophy in Entomology

Approved: James McD. Grayson

Major Professor

Inheritance of Resistance to

Lindane in the German Cockroach, Blattella germanica, (L.)

The World Health Organization defines insecticide resistance as "the development of the ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species." Although resistant insects have been encountered for some sixty years it has been only since World War II that they have caused widespread concern. It is apparent that the spread of resistance in this period is a direct result of the more rigorous selection brought about by use of the organic insecticides acting through inheritance mechanisms about which little is known. Thus there is a need for studies of the genetic mechanisms of insecticide resistance.

A population of German cockroaches resistant to lindane was developed by laboratory selection for eight years at the Virginia Polytechnic Institute. It was decided to determine whether this trait was due to autosomal or sex-linked genes; the relative frequencies of the genes for resistance, i.e. whether the character was dependent upon a single gene, or a complex of genes; and the length of time necessary for the resistant population to revert to the non-resistant condition in the absence of selection.

The cockroaches were reared under conditions of controlled temperature and humidity. Adults under 10 days in age were exposed to the insecticide by immersion in aqueous suspensions of the insecticide at selected concentrations. After four days mortalities were determined. The resulting data were plotted on log-probit paper in the form of regression lines by the method of least squares. Four to six points were used to establish each line; and for each concentration the tests were replicated from 3 to 6 times and involved from 100 to 200 insects.

Reciprocal crosses were made between the resistant and non-resistant strains of cockroaches. Controlled pair matings were made within the resistant and non-resistant strains. Backcrosses were made between reciprocal cross male progeny and females of the two parental strains. Further, a population of resistant cockroaches was reared in isolation from exposure to lindane for eight successive generations.

The following conclusions were drawn from this study.

1. That lindane resistance is not inherited as a simple sex-linked factor.
2. That it is not attributable to maternal effects, cytoplasmic factors, or to sex-linked epistatic genes.
3. That it is principally inherited through the autosomal chromosomes.
4. That lindane resistance is embodied in a multi-factorial system of genes.
5. That the resistance is still in a heterogeneous condition although the variation in the resistant strain was not nearly so great as in the non-resistant strain.
6. That lindane resistance is relatively stable, there being a rather small loss of resistance through eight successive generations in absence of selection.