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THE EVIDENCE REQUIRED TO SHOW SYNERGISTIC ACTION OF INSECTICIDES
AND A SHORT CUT IN ANALYSIS

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The purpose of this paper is threefold: (1) To restate definitions of joint action of insecticides, (2) to show what is required for clear-cut recognition of synergism, and (3) to indicate a workable short cut in analysis.

Definitions

Synergism between insecticides may be defined as a joint action of two materials, such that the total effect is greater than the sum of the two effects when each is used alone. Cox (2) restricts the application of the term "synergism" to mixtures of insecticidal materials, each of which has some toxicity when used alone. He thus excludes the case where a substance without toxicity of its own improves the action of an insecticide. Such a substance is called simply an activator. The proof of activation is less complex than that of synergism; a significant added percentage of kill shown in replicated trials should be sufficient.

To understand synergism it is necessary to consider possible types of action of mixtures of poisons. The subject has been discussed clearly by Bliss (1) and Finney (3) and touched upon by the writer (5). Bliss discusses three types of action of two poisons in a mixture-- independent joint action, similar joint action, and synergistic action. Both Bliss and Finney mention the possibility of negative synergism, or antagonism, and both suggest methods for analysis of data on toxic action. The methods discussed may be extended to mixtures of more than two poisons.

Independent action can be defined as action in a different way by each poison, a different physiological activity or vital system being affected. There may be more or less correlation in susceptibility, since the individuals susceptible to one action may tend also to be susceptible to the other. If there is a marked positive correlation of this sort, both poisons will tend to work on the same group of insects, and we will get little or no increase in kill by the mixture over the mortality that would be caused by the stronger insecticide used alone.

If there is little or no correlation, there will be some kill by each substance and some overlapping, of the sort indicated by Abbott's formula for one mortality in the presence of another. In that case the total kill will be higher than it would be with correlation. The possibility of negative correlation, that is, the individuals susceptible to one poison being resistant to the other, might also be considered. Each poison would kill the group susceptible to it with little or no overlapping, and the total kill would be still higher. This condition seems unlikely. Finney defines expected independent action without allowance for correlation. This is equivalent to the concept of Abbott's formula. Suppose, for instance, that a certain concentration of poison A kills 80 percent and one of B 60 percent. If the two poisons are independent in action, we would expect a kill of 80 percent + 60 percent - (60 percent of 80 percent), or 92 percent. It seems probable that some positive correlation often occurs. Action significantly less than independent action indicates antagonism.

Similar joint effect is produced by two or more poisons acting similarly, and affecting the same organs or processes in the individual. It implies that one insecticide could be substituted for the other at a constant rate. If this rate is known, equivalence can also be determined. It is recognized that the soundest method of comparing two insecticides is to compare concentrations needed for a given effect, and this method has a special application here.

Suppose, for instance, that a concentration of 1 unit of insecticide A is required to give 50 percent kill of an insect species, and that 2 units of insecticide B are required for the same result. Then A is twice as effective as B. A mixture of $1/2$ unit of A and 1 unit of B will produce the same result as either 1 unit of A or 2 of B, if similar joint effect occurs. If action is independent, there will be some overlapping, and the mixture will have somewhat less effect. On the basis of similar joint effect, 1 unit of A and 1 of B will be equivalent to $1\ 1/2$ units of A alone.

It can readily be shown, by the nature of dosage-mortality curves involved and the slopes they usually show, that similar joint effect is greater than independent effect, either with or without correlation. The greatest effect of a mixture, which could be predicted from individual action of its ingredients, would be similar joint effect. If the effect of the combination can be shown to exceed significantly the action expected from similar joint effect, synergism is strongly indicated.

The Determination of Synergism

The determination of synergism will require (1) some estimate of similar joint effect inferred from the action of each ingredient used alone, and (2) the determination of significance of superiority in results, if any, over this similar effect.

To estimate the similar joint effect we need dosage-mortality curves for each poison alone, so that equivalence at a given mortality can be calculated by interpolation. The log-probit transformation is

convenient for this purpose, since it usually gives linearity between 40 and 95 percent mortality, and since special log-probability paper can be obtained for rapid graphic interpolation. As a matter of fact, between 25 and 70 percent the untransformed dosage-mortality curve is near enough linearity for rough interpolation, but above 70 percent the transformation is an improvement. When the estimate of equivalence has been obtained, the concentration of the mixture can be calculated in terms of either constituent, and expected similar joint effect can be read off the dosage-mortality curve for the constituent chosen. This predicted effect can be compared with the actual. It is desirable also to have a dosage-mortality curve for different concentrations of a mixture of given proportions. This is not essential, however, for preliminary determination, if one concentration that will cause mortality between 50 and 90 percent is available. Results with this one concentration of a mixture, if adequately replicated, may be compared with calculated joint effect derived from well-determined dosage-mortality curves of individual materials. The comparison involves the determination of significance, the second step mentioned. Significance is basically determined from consistency. Finney(3) discusses methods of determination of significance; the author, in the next section, suggests a simple method.

To determine the equivalence, similar slopes in both dosage-mortality curves must be assumed. This is a fairly safe assumption for poisons of similar joint effect. In limited experiments slopes are not likely to differ significantly, and a common slope may be derived and used. If slopes really differ, equivalence will vary at different points. This condition may occur and add to complexity in some cases where action is independent; it seems unlikely that synergism will be found under such conditions.

Finney (3) gives a good exposition of methods of calculation, using data from an article by Martin (4) in the same journal. Finney illustrates the calculation of equivalence from log-probit dosage-mortality curves for a mixture for each ingredient, with common slope, and the statement of concentration of a mixture in terms of one of the substances involved. A dosage-mortality equation can then be written for the mixture, using the equation for that ingredient in terms of which the mixture is stated. The dosage giving 50 percent mortality (L.D. 50), or any other mortality level desired for comparison, can be readily calculated. Finney (3) outlines a chi-square test to compare the actual effect of a mixture with that expected from either independent or similar joint effect. He also cites a rather complex formula for standard error of difference of L. D. 50 of a mixture from predicted L. D. 50 on a joint-action basis. Calculations are based on statistical methods developed for probit analysis.

Short-Cut Procedures

Much time may be saved in getting a preliminary determination of equivalence by short-cut methods, using log-probability paper and graphic determinations. For example, we may take the data of Martin used by Finney on the effects of rotenone and deguelin on an aphid, *Macrosiphoniella sanborni* (Gill). Some results obtained over a range of toxicity suited to the problems are tabulated as follows:

<u>Rotenone</u>			<u>Deguelin</u>	
<u>Concentration</u>	<u>Mortality</u>	:	<u>Concentration</u>	<u>Mortality</u>
<u>Mg./liter</u>	<u>Percent</u>	:	<u>Mg./liter</u>	<u>Percent</u>
3.8	33.3	:	10.1	37.5
5.1	52.2	:	20.2	70.8
7.7	85.7	:	30.3	95.9
10.2	88.0	:	40.4	94.0

The data given above are plotted on log-probability paper (fig. 1), and eye-fitted lines are drawn. A reading taken from these lines at 50 percent shows that 13.2 units of deguelin are required to equal 4.8 of rotenone, or that deguelin is about 0.36 as toxic as rotenone. At the 90 - percent level 9.7 units of rotenone appear to equal 28.0 of deguelin, giving deguelin an equivalence of 0.35. The average is 0.355 (Finney's computed value is 0.37).

According to Martin, the mixture of rotenone and deguelin gave the results shown in table 1.

Table 1.--The actual and the interpolated mortality obtained with a mixture of rotenone and deguelin of given concentrations

<u>Mg./liter</u>	<u>Mg./liter</u>	:	<u>Rotenone</u>	:	<u>Mortality</u>	
					<u>concentration</u>	<u>concentration</u>
		:		:	<u>Percent</u>	<u>Percent</u>
1.0	4.1	:	2.5	:	47.8 ± 11.2	---
2.0	8.1	:	4.9	:	58.7 ± 5.0	51
3.0	12.2	:	7.3	:	79.2 ± 10.2	78
4.0	16.3	:	9.8	:	93.5 ± 2.9	90

^{1/} Rotenone + 0.355 deguelin.
^{2/} See fig. 1.

By using the "rotenone equivalent" as concentration, the expected joint effect can be read off from the eye-fitted rotenone line. For instance, with equivalent of 7.3 the expected kill is read as 78 per cent. It will be shown at each point that the actual is somewhat greater than the estimated effect, but the estimated effect comes within 1 or 2 standard errors of the actual. More exact calculation will give a little better results. The estimated, as well as the actual, values have calculable standard errors, which decreases the tendency to significant differences; on the other hand, the fact that all differences are in the same direction will increase this tendency. The conclusions of Finney are the same as have been reached by the shorter method in a few minutes work. The mixture tends to produce an effect exceeding joint action, but this tendency does not reach significance.

The other cases treated by Martin and Finney have been studied in the same way. Working as above, the author calculated a rotenone equivalent of 0.215 for elliptone, as compared with Finney's 0.20. For toxicarol the equivalent calculated is 0.175, as compared with Finney's 0.16. The conclusions as to synergism arrived at by the rapid method were the same as those reached by Finney by the more complex mathematical method.

Dosage-mortality curves from replicated experiments would afford opportunity for several independent determinations of equivalence, and of expected mortality from a mixture. The latter could be used in calculating a standard error. With error estimates for both calculated and actual effects, the error and significance of the difference could easily be calculated.

Summary

The author defines the types of joint action of insecticides combined in a mixture. He then shows that, in order to prove the existence of synergism, the effect of the mixture must be shown to be significantly greater than the maximum effect predictable from separate actions of the insecticides. This maximum is given by assumption of similar joint effect. Dosage-mortality curves for separate ingredients may be used to estimate equivalence and expected similar joint effect. Replicated trials with a mixture may be used for comparison with the estimated effect. A much-shortened graphic procedure will give results of practical value. In many cases the type of action produced by a mixture will not be exactly determinable from results, but a clear-cut superiority over a calculated similar joint effect will indicate synergism.

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LOG.-PROBABILITY INTERVALS (FOR DOSAGE -MORTALITY ETC.)

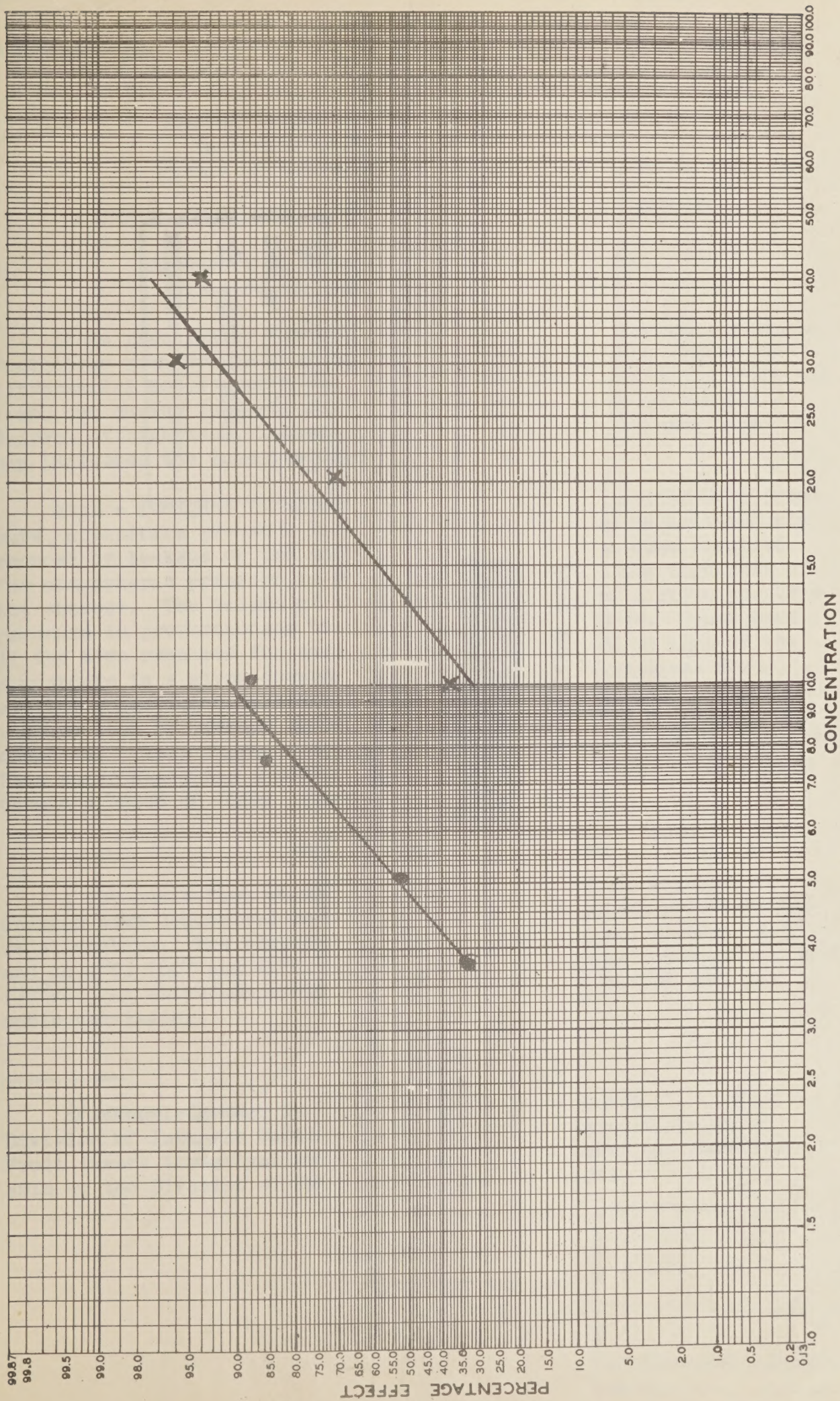
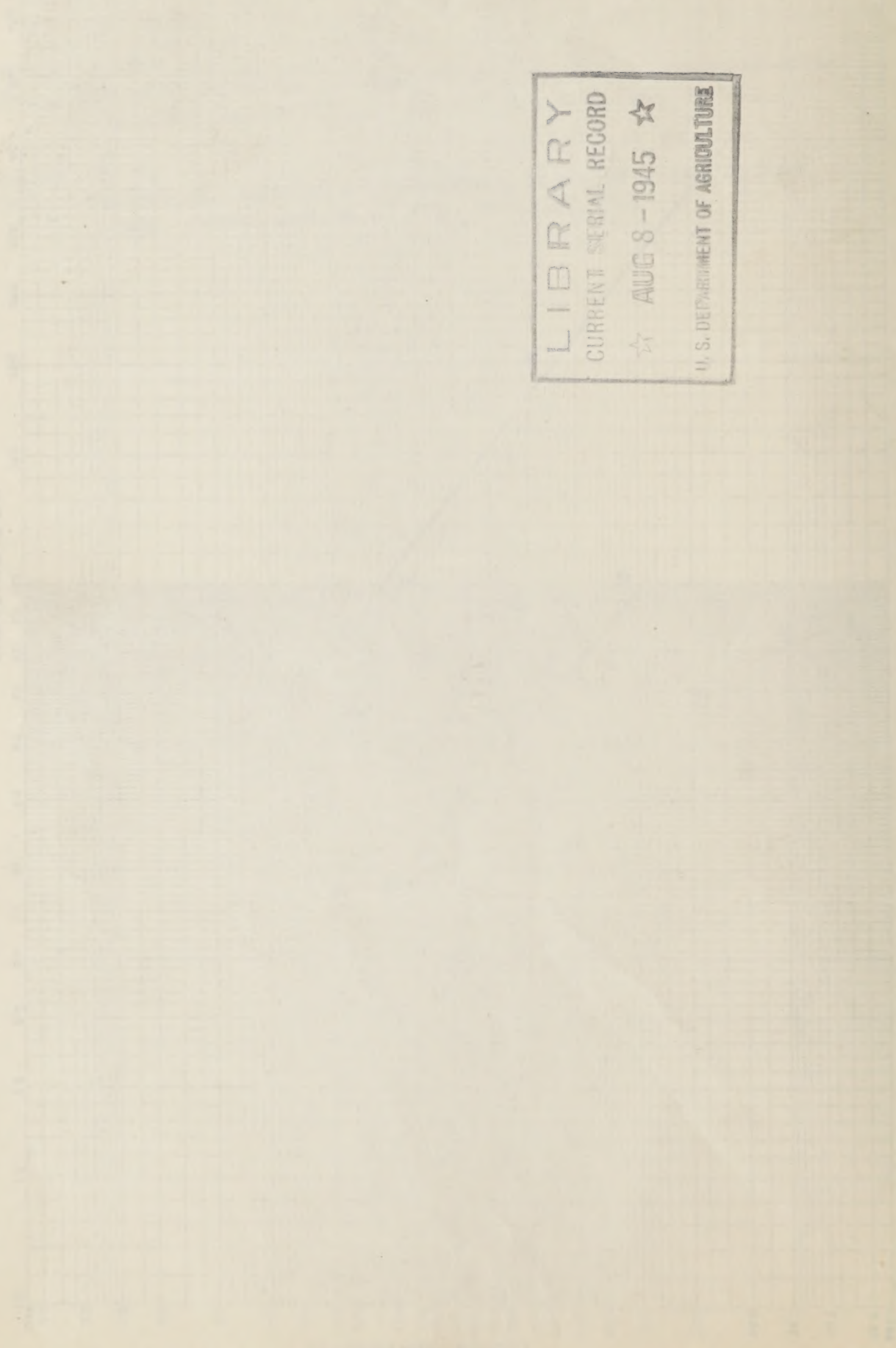


Figure 1.-The percentage of mortality obtained with several concentrations of rotenone and deguelin, plotted on log-probability paper. Dots indicate rotenone; crosses, deguelin.

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