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THE QUANTITATIVE TOXICITY OF FIVE TYPICAL CONTACT INSECTICIDES TO SEVERAL SPECIES OF INSECTS

By

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Major Subject Entomology

Approved

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Head of Major Department

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Dean of Graduate College

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1955
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I. INTRODUCTION

I.
A series of experiments was conducted by the writer in an attempt to account for the discrepancies of concentration-mortality dosages by basing the median lethal dosage on the quantity of insecticide in contact with the insect. The quantitative toxicity has been determined for five chemical types of contact insecticides to three widely different species. Dosages have been expressed in three ways, namely: as concentration in the contact liquid, as milligrams of insecticide adhering per gram of body weight, and as milligrams of insecticide adhering per square centimeter of body surface of the treated insects. The secondary purpose of these experiments was to contribute to the knowledge of insecticidal action and to a possible classification of contact poisons on that basis.
II. PREVIOUS RELATED INVESTIGATIONS

Prior to 1920 insecticide investigations were confined largely to field tests of sprays and spraying methods for the control of economic insect pests. McIndoo (1916) studied the effects of nicotine on honey bees and several other insects. He made microscopic examinations of the treated insects and concluded that nicotine caused death by paralysis. Moore and Graham (1918), and others undertook a study of the physical properties of insecticides as related to toxicity. Richardson and Smith (1923) tested a large number of organic compounds for toxicity to Apis rumicis L., employing a laboratory method of application. Tattersfield and Morris (1924) constructed an apparatus to apply known concentrations of insecticides under controlled conditions. They were the first to term the concentration producing fifty per cent mortality the insecticidal index. Trevan (1927) studied the action of poisons on mice and explained the terms, median lethal dosage, minimum lethal dosage, and maximum effective dosage.

O’Kane et al. (1930) (1931) (1932) performed a series of experiments to measure the surface forces affecting contact action. Hartnell and Wilcoxon (1931) conducted a well planned research to determine the relation of surface tension and wetting ability to the tracheal penetration of an insecticide.

Campbell (1930) and Richardson and Haas (1931) determined mean dosages of several common stomach poison insecticides and expressed the median lethal dose in terms of milligrams of poison per gram of body weight. Tattersfield, Hobson, and Gimmingham (1929) in the course of
pyrethrum investigations determined the actual quantity of pyrethrins I acting upon *Aphis rumicis*. Simanton (1932) studied the relative toxicity of phenol, nicotine, and sodium laureate to *Aphis rumicis* and *Blattella germanica* L., and expressed the median lethal dosage in terms of concentration, milligrams of poison per gram of body weight, and milligrams of poison per square centimeter of body surface.
III. MATERIALS

A. Protocols
Lycaena kalmii, a common insect of milkweed, was reared in lamp chimney cages supplied with milkweed seeds and water. Because of the immense number of individuals required, it was necessary to place the cages under optimum conditions in a constant temperature and humidity cabinet. This insect was reared according to the detailed procedure described by Simanton and Andre (1934). Fourth instar nymphs only were used in the insecticide tests.

B. Chemical

Five insecticides were tested against the three experimental insects. These were nicotine, sodium laureate, phenol, rotenone and a petroleum oil emulsion. The five insecticides were selected because they represent, in general, five chemical types commonly recommended for the control of injurious arthropods.

Nicotine was applied at several concentrations known to cover the range of toxicity. The sample used was freshly distilled, straw colored alkaloid that on analysis showed 99.2 per cent of nicotine. As low concentrations of nicotine have a high surface tension, practically the same as that of water alone, O’Kane et al (1930), the nicotine was diluted with a 1 per cent solution of saponin. The glucoside saponin in 1 per cent concentration has spreading properties superior to water alone, and exhibited no toxicity to the three experimental insects.

Sodium Laureate was an opaque, white, solid soap containing 29.5 per cent of soap solids. Sodium laureate has a high toxicity for a soap and exhibits excellent spreading properties.

The sample of phenol supplied for toxicity tests was colorless, crystalline, U.S.P. quality and contained 99 per cent phenol. As the
phenol concentrations were fairly high and showed nearly the same toxicity to the three insects, no additional spreader was deemed necessary.

Rotenone was obtained in the pure form as a white crystalline powder having a pleasant sweetish odor. A fresh acetone stock solution was prepared every three days from which the final concentrations were diluted with 1 per cent saponin in water.

The petroleum oil was applied in the form of a dilute soap emulsion. The oil used was a highly refined, straight cut fraction having a viscosity of 62 seconds Saybolt at 100 degrees F., and an unsulphonated residue value greater than 90. Such an oil is commonly termed a summer spray oil. A stock emulsion was prepared by the cold stir method according to the formula:

<table>
<thead>
<tr>
<th>Water</th>
<th>15 grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium oleate</td>
<td>2 grams</td>
</tr>
<tr>
<td>Oil</td>
<td>85 grams</td>
</tr>
</tbody>
</table>

All concentrations were made from the stock on the basis of actual oil content and were diluted with 0.1 per cent sodium oleate to maintain constant spreading properties. Use was made of the soap emulsion in preference to protein or gum emulsions, because it gave small oil droplets that did not separate readily upon contact with the insect. A quick breaking emulsion (droplet size greater than 12) would have interfered with the determination of the actual amount of oil adhering to the individual. No dilutions were used later than eight hours after preparation.

C. Apparatus

Many types of spraying apparatus have been employed by research workers for the application of liquid contact insecticides. Richardson and Smith (1926) used two methods of application, one in which aphids on leaves were sprayed by a small hand atomizer and another method in
which the insects were submerged for a definite period. The latter method has been described more completely by Richardson and Shepard (1931). Tattersfield and Morris (1924) devised and constructed an apparatus for applying a spray under controlled conditions. They calibrated their apparatus to determine distribution of spray and amount of liquid falling per second on the bottom of the spraying chamber. By this method, pressure, rate of application, concentration of spray, size of atomized particles and distribution of insecticide were the factors controlled.

O’Kane et al (1930) used a apparatus of somewhat similar design in studying the physical factors affecting the toxicity of contact insecticides. Their apparatus consisted of a spraying chamber equipped with a revolving base, into which the atomized liquid was directed at an angle of 45 degrees. Peet and Grady (1928) developed a six foot cubical spraying chamber for testing household insecticides of the kerosene-pyrethrum type against houseflies under controlled conditions. The Peet-Grady method has been accepted as a standard test for commercial household insecticides. Recently Campbell (1934) devised a multiple spraying chamber that enabled the operator to run six tests in rapid succession using the same atomizing equipment.

For a quantitative study of the type herein described, it was obvious that an accurate method for applying the insecticide must be used. Likewise, because of the great number of tests necessary for a sound comparative investigation the method chosen was capable of moderately rapid operation.

The apparatus used was in general similar to that of Tattersfield
and Morris. It consisted essentially of the following parts:

1. A spraying chamber devised from a five gallon carboy with bottom removed.

2. An atomizing nozzle modified from a DeVilbiss No. 15 atomizer. The nozzle was adjusted to give a fine uniform spray.

3. A graduated glass cylinder serving as a reservoir for the material to be sprayed. The tube was made from a portion of burette by sealing one end.

4. An accurate reducing valve connected to a compressed air line. The valve was equipped to supply dry air at constant pressure to the atomizer.

This apparatus except for the absence of the manometer was identical with that illustrated by Simanton (1932). In operation nozzle pressure was maintained a 5 lbs.; temperature at 78°-85°F, and exactly 10 cc. of the liquid sprayed for each test.
IV. METHODS

A. Application of the insecticide

The experimental procedure for applying the spray to aphids was as follows: Twenty aperous agamic female aphids of approximately the same size were placed in a tared weighing bottle and weighed. The insects were then removed from the weighing bottle and placed on a double thickness of filter paper fitted to the inside of a standard half Petri dish. The Petri dish was then placed in the center of the bottom of the spraying chamber and 10 cc. of insecticide were applied. After the application, the aphids were picked up by a leg with a very fine forceps and again transferred to a weighing bottle and weighed. Care was taken not to remove any of adhering spray during the transferring operation. After the second weighing, the aphids were carefully removed from the bottle to a fresh nasturtium plant. The plant was placed in the center of a dish of water to trap any individuals that attempted to desert the nasturtium.

The numbers of dead and living aphids were recorded at the end of twenty-four hours. Those aphids not able to walk were recorded as dead. Aphids which fell in the water were recorded as alive.

The procedure in the case of the roaches was similar to that for aphids. Ten roaches in the fourth nymphal instar and of approximately the same size were used as the test unit. The roaches were weighed and then transferred from the weighing bottle to a six inch Petri dish with 1 inch sides, the inner margins of which had previously been coated with a thin film of petroleum jelly that served to confine the roaches. The dish was placed in the center of the spraying chamber and the spray was
applied as before. After the application, the roaches were transferred from the dish to the weighing bottle by the same method used for aphids and again weighed. The insects were then removed from the bottle to a large beaker containing food. Record of the dead and living roaches was taken at the end of twenty-four hours.

Fourth instar nymphs of Lygaeus kalmii were handled in identically the same manner as the roaches.

With Elattella germanica and Lygaeus kalmii counts were also made at the end of forty-eight hours to supply additional information which has not been presented in this paper. It was not possible to obtain forty-eight hour counts on aphids.

From the above procedure the following data were obtained:

1. The weight of the test unit before spraying.
2. The weight of the test unit after spraying.
3. The number of insects dead and the number of insects alive in each test group at the end of twenty-four hours.

With this information the following data were calculated:

4. Quantity of spray adhering to the test unit.
5. Average weight per insect for each test unit.
6. Average quantity of spray adhering to each insect of a given test unit.
Several concentrations of each liquid were applied in order to cause mortalities falling at various points within the toxic range. At least sixteen test units and in most cases twenty test units were considered sufficient to establish a toxicity curve for one insecticide against a single species of insect.

B. Computation of toxicity

Concentration - mortality curves were taken directly from the mortality data furnished by the applications of a given concentration of insecticide under the conditions previously stated.

Curves with milligrams of insecticide per gram of body weight (mg./g.) as abscissae and percentage of mortality as ordinates were drawn from data calculated from each test unit as shown in the following example:

Weight of spray adhering to 10 roaches = 0.1038 g.

Concentration of sodium laurate producing 60 per cent kill = 0.35 per cent.

\[
0.1038 \times 0.35 = 0.0003808 \text{ g. or } 0.3808 \text{ mg.}
\]

\[
\frac{0.3808 \text{ mg.}}{100}
\]

Therefore the ratio \( \frac{0.3808 \text{ mg.}}{0.4872 \text{ g.}} \), which on the basis of 1 gram of insect is 0.78 mg.

Curves with milligrams of insecticide per square centimeter of body surface (mg./sq.cm.) as abscissae and percentage of mortality as ordinates were drawn from data obtained in much the same manner as the mg./g. data. The surface areas of the individuals were obtained by formulae developed from a method described by Simanton (1933). The three formulae, by means
of which the surface area in square centimeters was obtained directly
from the weight in grams of the individual, were:

- **Aphis rumicis**, \( S = 3.28 \ W^{.60} \)
- **Blattella germanica**, \( S = 12.17 \ W^{.63} \)
- **Lygaeus kalmii**, \( S = 6.87 \ W^{.65} \)

From the example in the preceding paragraph the quantity of in-
secticide adhering to the test unit has been determined. The weight
of the test unit is also known. To proceed with the computation the
amount of spray adhering per insect and average weight per insect must
be determined since the above surface-weight formulae are adapted only
for use with single insects and will not apply to test units.
V. TOXICITY CURVES

The data obtained by the procedures described in preceding pages of this thesis are presented in Figures 1 to 15 and Table 1. Throughout the entire investigation records were carefully kept for each insecticide and every test unit to aid in interpretation and for checking results. Since the tabulated experimental records approximated ten thousand individual data, an analysis was undertaken to eliminate the routine and non-essential information and to preserve the major findings.

It has been shown by Tattersfield and Morris (1924) and by Trevan (1927) that the point of 50 per cent mortality is statistically the most acceptable point for the comparison of toxicity. However, it is also known that the portion of the toxicity curve lying between 20 and 80 per cent mortality is in most cases nearly straight. A study of existing toxicity curves disclosed a great variation in the slope of the curve, thus two insecticides having identical 50 per cent points may require widely different concentrations to produce a kill of 20 or 30 per cent.

It was also desirable, from the standpoint of relative toxicity, to place the toxicity curves for a given insecticide against the three species on a single graph. For this purpose the three-cycle semilogarithmic ruling was well adapted as it permitted a wide range on the abscissae with neither complicity of interpretation nor curvilinear distortion.

For the above reasons the tabulated records have been omitted and the major findings presented in forty-three toxicity curves and one table. The three figures for each insecticide show successively the toxic range
on three bases; concentration of the insecticide, milligrams of active insecticide per gram of body weight, and milligrams of active poison per square centimeter of body surface.
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<tr>
<th>Temperature (°C)</th>
<th>0%</th>
<th>10%</th>
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<th>40%</th>
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Table I. Quantitative Microbiological Procedures to Produce a 50% Per Cent Mortality of Each Species of Insectes.
Per cent concentration

Figure 1. Toxicity of nicotine with 1 per cent saponin as a spreader.
Figure 2. Toxicity of nicotine with 1 per cent saponin as a spreader.
Milligram per sq.cm.

Figure 3. Toxicity of nicotine with 1 per cent saponin as a spreader.
Figure 4. Toxicity of sodium laurate.
Figure 5. Toxicity of sodium laurate

Milligrams per gram

Per cent mortality
Figure 6. Toxicity of sodium laurate.
Figure 7. Toxicity of phenol
Figure 8. Toxicity of phenol
Figure 9. Toxicity of phenol
Figure 10. Toxicity of rotenone with 1 per cent saponin as a spreader.
Figure 11. Toxicity of rotenone with 1 per cent saponin as a spreader.
Milligrams per sq.cm.

Figure 12. Toxicity of rotenone with 1 per cent saponin as a spreader.
Figure 13. Toxicity of oil emulsion with 0.1 per cent sodium oleate as a spreader.
Figure 14. Toxicity of oil emulsion with 0.1 per cent sodium oleate as a spreader.
Figure 15. Toxicity of oil emulsion with 0.1 per cent sodium oleate as a spreader.
VI. DISCUSSION

An examination of the toxicity curves for nicotine shows immediately the greater toxicity of the poison to *Aphio rumicis* than to either of the two larger insects, regardless of the basis of comparison. This observation may indicate that nicotine can penetrate the thin integument at a greater rate than it is capable of entering the more heavily sclerotized integument of the roach, or that the concentration gradient of the nicotine vapor on the integument is of such intensity that it can penetrate the short distance into the nervous system of the aphid at a lethal concentration, but cannot extend the greater like distance in the large insects without losing its lethal intensity. The wide range of poison tolerated by *Lygaeus kalmii* and the variable relative toxicity indicates a relationship to size and body shape of this insect as compared to the roach. A considerable interspecific biological variation appears evident, an assumption supported also by the curves for sodium laurate and oil emulsion. The curve for *A. rumicis* is nearly identical with nicotine curves obtained for this species by previous workers.

Sodium laurate toxicity curves presented in Figures 4, 5, and 6, indicate without doubt that the soap is a truly non-specific insecticide. Fleming and Baker (1934) in their work with the Japanese beetle gave the median lethal concentration of 0.37 per cent for sodium laurate, a figure which further supports the non-specific contention. The curves support the assumption that the soap has a uniform toxic effect, probably due to physical properties. There is a relative change in the curve for *A. rumicis*
on the mg./sq. cm. basis, but the degree of re-arrangement is not sufficiently great to be indicative. A test of significance showed *Lygaeus kalmii* to be the most susceptible, whereas the relative toxicity of the other two species was not significant for this insecticide.

Phenol also exhibits non-specific toxicological tendencies. *Blat-
tella germanica* is somewhat the less tolerant to this poison. The relationships of the phenol curves are similar to those for sodium laurate but show only one-tenth the toxicity. In general, the phenol curves on a single graph do not show a significant difference when 50 per cent points are compared. The fact that the relative phenol toxicities are reversed with the regard to the soap curves indicates a different type of physiological action. This reversal, however, is only significant for the curves of *Lygaeus kalmii*.

Rotenone toxicity curves are given for *Aphis rumicis* and *Lygaeus kalmii* only. *Blattella germanica* was not appreciably harmed by a 0.5 per cent concentration, the maximum strength that can be applied as a homogeneous aqueous suspension. It must be concluded, therefore, that rotenone is decidedly specific in action and of great toxicity to certain species. A comparison of the curves shows rotenone to be approximately 100 times as toxic as nicotine to the aphid, and 1000 times more toxic than nicotine to *Lygaeus kalmii*. The close uniformity of the toxic range and the constancy of the relative toxicity are remarkable.

The petroleum oil emulsion shows a concentration toxicity to the three species in the ratio: *Aphis rumicis*, 10, *Lygaeus kalmii*, 4, *Blattella germanica*, 1. The roach is decidedly more resistant on all
bases of comparison. Lygaeus kalmii is slightly more resistant to oil than the aphid except on the mg./g. basis. Size and body shape will account for the relative rearrangement of the curve for Lygaeus kalmii and the heavier integument may account for the increased resistance of the roach. It was noticed throughout this investigation that the complicated physical and colloidal properties of an oil emulsion enable only a rough quantitative measurement to be made. It is the writer's opinion that the relative toxicity figures are the only determinations of toxicological value.

A comparison of curves on the three bases of toxicity discloses a general relationship. Figures for mg./g. ratios are approximately five times greater than concentration figures, whereas values for mg./sq. cm. ratios are usually one-tenth of the concentration values.

The tabulated experimental data gathered during the course of these investigations showed quite definitely that variations in mortality resulting from identical applications of a given insecticide were due to the varying amounts of poison adhering to the insects.

In many cases in which different concentrations of the same insecticide were applied and approximately the same quantity of poison adhered to the test unit the occasional slight difference in mortality was correlated with the concentration in 90 per cent of the cases. This phenomenon may be expected as the higher concentration would tend to act the more rapidly.

The variation in mortality resulting from repeated identical applications was not excessive. In 80 per cent of the cases repeat tests did
not vary in mortality more than plus or minus 10 per cent on the basis of identical concentrations, and not more than plus or minus 5 per cent on the basis of identical mg./g. or mg./sq. cm. values.
II. SUMMARY AND CONCLUSIONS
VIII. LITERATURE CITED

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