Toxicity of DDT to the American Cockroach When Lipid Content and Temperature are Varied

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When two groups of American cockroaches are similarly exposed to DDT and then placed, one at a high and the other at a low post-treatment temperature, the resultant per cent mortality at the lower temperature is found to be much greater than that at the higher temperature (Vinson & Kearns 1952). This phenomenon is referred to as the "negative temperature coefficient" of DDT (Richards & Cutkomp 1946, Fan et al. 1948). The negative temperature coefficient of DDT has been demonstrated with several different insects (Lindquist et al. 1945, Fan et al. 1948, Vinson & Kearns 1952, Roth et al. 1953, Hüffiger 1954a and b) where temperatures have been varied only at treatment and post-treatment periods.

Fan et al. (1948) found in their studies with mosquito larvae of Aedes aegypti that the negative temperature coefficient of DDT occurred with immersion of the larvae in dilute suspensions of the insecticide. A positive temperature coefficient occurred if DDT emulsions were injected into the larvae or if they were immersed in more concentrated suspensions of DDT. Therefore, they proposed that the effect could be due, in part, to the DDT-concentrating properties of the cuticle at lower temperatures. Following the concentrating mechanism, the DDT would be eluted at the cuticle-cell interface to act at some vulnerable site. However, with the American cockroach, Vinson & Kearns (1952) found that the negative temperature coefficient still persisted after solutions of DDT in dioxane were injected, a technique which presumably minimizes the effect of the cuticle. They also presented data to show that the effect could not be accounted for as a result of differences in rates or magnitude of detoxification of DDT at the two temperatures.

The studies in this paper were undertaken in order to (a) test a possible explanation of the negative temperature coefficient as the effect might be related to injected doses of DDT in relation to quantity of lipids in the cockroach at different post-treatment temperatures and (b) to determine more about the relation of different quantities of insect lipids to the toxicity of DDT in general.

Very little is known about the DDT-lipid relationship in insects although a large volume of literature has developed pertaining to the storage of DDT in the fat of vertebrates (Brown 1951, pp. 504–518). Lipids on insect cuticle have been studied in relation to DDT solubility and toxicity (Pradhan et al. 1952). There have been some studies relating DDT toxicity to internal lipids, however. Munson and Gottlieb (1953) showed a high positive correlation between the resistance of American cockroaches to DDT and their lipid content. Munson et al. (1954) have reported on preconditioning temperatures as they affect American cockroach resistance to DDT and have related this resistance to lipid content. Their study has some bearing on relations of total lipid content to resistance to DDT but differs from the present work in that it deals with preconditioning while we are more concerned with post-treatment temperature effects. Berin & Edelman (1949) stated that generally the fat content of two beetles, Agelastia alni (Family Galerucidae) and Pseudophonus pubescens (Family Carabidae) was directly related to their resistance to DDT; however, they found that even though the fat content was higher before overwintering and during egg development in the females, the resistance of these insects to DDT was lower. Increased metabolism was shown to occur during these periods. This would indicate a more rapid turnover of the fat and presumably a release of more DDT in a shorter time. In contrast to these conclusions, Reiser et al. (1953) concluded that there was no direct relationship between the seasonal increase in the lipid content of the boll weevil, Anthonomus grandis, and its resistance to chlorinated hydrocarbon insecticides. Their conclusion was supported by the fact that the boll weevils containing large amounts of lipids were also more resistant to non-fat soluble insecticides. A different interpretation of these data has been suggested, however (Munson et al. 1954).

METHODS AND MATERIALS.—The cockroaches used during the course of the experiments were obtained from two sources. One group was supplied by the Public Health Department of the University of Minnesota and the other group by the Department of Entomology at Rutgers University. The cockroaches from these sources had no previous contact with insecticides. During the rearing and testing procedures the two groups were treated separately. Since the two strains did not differ appreciably in their susceptibility to DDT, the data from both groups were pooled for analysis.

The rearing method was essentially the same as that described by Heal (1948). Egg capsules were collected from the refuse under the adult cages and placed in a 6x8 inch battery jar. The young nymphs were removed at 10-day intervals, placed in clean battery jars provided with fibre-board platforms, and given dog food and water.

The total lipid content of a given group of cockroaches was modified by using different diets. This dietary modification was made more than 2 months before any insecticide exposure. Special diets were used in preference to other possible methods such as starvation. Groups of cockroaches were randomly divided and placed on one of three diets at the time a few cockroaches in the colony had reached the adult stage. The cockroaches were left on the diets for approximately 80 days before testing. The diets used were suggested by Haydak (1953) and consisted of dietary extremes of protein and carbohydrate

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Table 1.—Composition of diets used to vary total lipid content.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Casein</th>
<th>Dextrose</th>
<th>Brewer's Yeast</th>
<th>Salt</th>
<th>Mixtures</th>
<th>Per Cent Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Protein</td>
<td>0</td>
<td>91</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>8.5</td>
</tr>
<tr>
<td>High Protein</td>
<td>86</td>
<td>10</td>
<td>10</td>
<td>4</td>
<td>4</td>
<td>91.0</td>
</tr>
<tr>
<td>Dog Fooda</td>
<td>84</td>
<td>10</td>
<td>10</td>
<td>4</td>
<td>4</td>
<td>84.0</td>
</tr>
</tbody>
</table>

a Ralston Purina product (Checkers). Composition: Crude protein, more than 94%; fat, more than 5%; fiber, less than 5%; and nitrogen free extract, more than 46%. Complete nutrient composition available from Ralston Purina Company.

(see Table 1). Dog food was used as the normal diet for comparison.

After completion of the conditioning period, the male and female cockroaches from each of the diets were separated, and each group was randomly divided into two smaller groups. One group was used for testing at 15° C. and one group for testing at 30° C. Solutions of DDT in acetone were injected into the abdomen of the cockroach (method similar to Heal & Menusan 1948). An ultramicrotube fitted with a No. 27 hypodermic needle was used. Volumes of acetone containing DDT ranged from 0.8 cubic millimeters to 2.0 cubic millimeters per cockroach. This was done to get a range of doses of DDT. No mortality occurred with these volumes of acetone when used alone with the exception of some cockroaches on the high protein diet. An occasional death occurred in cockroaches on the high protein diet when injected acetone exceeded 1.0 cubic millimeter. Because of this, the doses of acetone were kept below 1.0 cubic millimeter to this particular group of cockroaches. The group of cockroaches kept on the high protein diet were more sensitive to high temperatures (35° C.) and were the most susceptible to DDT at both post-treatment temperatures. However, the toxicity data from this group were homogeneous to DDT at both post-treatment temperatures. How-

ever, the toxicity data from this group were homogeneous (see Table 2 for confidence limits).

Results.—The data presented include toxicity responses pooled from a series of five different toxicity experiments in which male and female cockroaches from each of the three diets were tested. The statistical method of Litchfield & Wilcoxon (1949) was used to make the analysis. The average dose and per cent mortality for each group of four or five cockroaches was calculated. The data are presented in Figure 1.

Both sexes of cockroaches were more susceptible to DDT when they were exposed to a low post-treatment temperature (15° C.). This was consistent with all three diets (see Figures 1 and 2). Using the dog food diet as an example, the male cockroaches had an LD_{50} of 1.7 micrograms of DDT per gram of body weight at 15° C., in contrast to an LD_{50} of 10.5 micrograms per gram at 30° C. This represents a dose six times as great at the 30° C. temperature. A similar comparison with female cockroaches shows a difference of more than 10 times at the two temperatures. The LD_{50} values in this instance are 5.3 and 58.0 micrograms of DDT per gram of body weight at post-treatment temperatures of 15° and 30° C., respectively.

Additional observations revealed that cockroaches injected with DDT and showing no symptoms at 30° or 39° C. would elicit typical DDT tremors within minutes.
when the temperature was lowered by as little as 5°. Furthermore, the symptoms could be made to disappear at 80° C, and return (at 20° and 25° C.) more than a dozen times in some individuals. In most cases these observations were carried out over a period of 2 to 3 weeks.

The greater susceptibility of male cockroaches in comparison to female cockroaches as indicated in the example above (1.7 vs. 5.3 and 10.5 vs. 58.0 micrograms of DDT per cockroach) was evident on all diets and at both temperatures (Table 2).

The high protein diet produced cockroaches with a greater susceptibility to DDT than those reared on the low protein or the dog food diet. The cockroaches reared on a dog food diet were the least susceptible. These results were consistent at both post-treatment temperatures.

The lipid content resulting from the three diets showed considerable variation (Table 2). Furthermore the variable susceptibility of cockroaches fed on different diets could be due to the diets or the lipid content. The lipid contents were studied relative to responses to DDT by graphically presenting dosages of DDT per milligram of lipid per cockroach. The data from all three diets were pooled for this analysis (Figure 3). Using the same individuals, the data were analyzed from the standpoint of DDT per gram of body weight (Figure 4). This provided a comparison between the two methods of expressing dosage. In those diet, sex and temperature groups where an analysis of the data was possible, regression lines were plotted both on the basis of DDT per unit of body weight and DDT per milligram of lipid per cockroach (Figure 5).

In general, the range of dosages was greater when plotted...
against body weight than when plotted against lipid values. This is apparent by differences in slope of the regression lines.

The results show that when lipid content is high, more DDT is required to produce a given per cent mortality. A similar trend is apparent when the data are expressed on a body weight basis. Thus, a close relationship between the body weight of the cockroach and its lipid content is indicated. By plotting the body weights of the cockroaches versus lipid contents, a positive correlation appears to exist (consult Figure 6). However, the variation was so great that a precise analysis could not be made.

Table 2 shows the lipid content of cockroaches tested at 15°C to be consistently higher than that from cockroaches tested at 30°C. For example, the total lipid content for male cockroaches at 15°C is 34.0 milligrams per cockroach, while at 30°C cockroaches which were taken from the same colonies had an average lipid content of 14.8 milligrams per cockroach. Inasmuch as the cockroaches tested at the two temperatures were selected randomly from the same colonies prior to the experiment, one would expect similar lipid contents at the outset of the experiment. The lower lipid values occurring at 30°C might be accounted for by increased activity or metabolism at the higher post-treatment temperature; at present we have no experimental evidence to verify this point.

**DISCUSSION.**—Dosage-mortality curves for DDT at 15°C and 30°C were established for cockroaches reared on three different diets. The diets were used to produce three different levels of lipid content. The different average lipid levels are shown in table 2. For example, the male cockroaches tested at 15°C and reared on the low protein, high protein, and dog food diets averaged respectively, 18.0, 13.8, and 34.0 milligrams of lipid per cockroach. However, there was an overlapping of the individual lipid contents of the cockroaches on all three diets, especially on the low protein and high protein diets. Using the example of male cockroaches tested at 15°C again, the range of the lipid contents in milligrams of lipid per cockroach were as follows: low protein, 5.8 to 40.0; high protein, 3.7 to 23.6; dog food, 16.7 to 92.0.

The lipid extractions revealed that cockroaches of the same sex and fed the same diet had a consistently higher lipid level at 15°C than at 30°C. Since lipid variations occurred the customary method of plotting data, that is, mortality versus microgram of DDT per gram of cockroach, was not completely satisfactory for studying the effect of the total lipid content upon the toxicity of DDT. A better evaluation of the importance of lipids appeared possible by presenting the data as per cent mortality versus micrograms of DDT per milligram of lipid (see Figure 8).

A greater homogeneity of data is evident when plotted on the basis of DDT per milligram of lipid; that is, the range of dosages between dead and alive cockroaches is narrower. As a result, a large part of the data based upon body weight fell into the 100% and 0% mortality groups when based upon lipid content. Since there did not appear to be any large variations between the cockroaches fed the three different diets, all the data for each sex and temperature were pooled and analyzed. In addition, regression lines were plotted (Figure 5) for those diet and temperature groups in which enough data were available.

The influence of one particular dietary factor cannot be readily evaluated from the results obtained. For example, the cockroaches reared on a high protein diet weighed more on the average than the cockroaches reared on the other two diets. The factors causing this increase in weight may or may not be influential in relation to the responses to DDT.

Keeping in mind these comments pertaining to the analysis, the diets and the lipid content of the cockroaches, a discussion of the toxicity results will follow.

The toxicity data presented in figures 1 and 2 and table 2 show that DDT is consistently more toxic at post-treatment temperatures of 15°C than at 30°C. The magnitude of the difference in toxicity, however, varies from group to group. For example, using the LD<sub>50</sub> value...
DDT is shown to be 16 times as toxic at 15° C. as at 30° C. for male cockroaches on a high protein diet. This difference is only 6.5 times for male cockroaches fed the dog food diet. The other series of cockroaches are intermediate in response between these two figures.

The lipid content of the cockroaches on two different diets does not appear to be a factor which influenced the differences in toxic levels given above. In the above example the toxicity differences at the two temperatures are less on the dog food and this diet produced cockroaches with greater quantities of lipids. This may be due to nutritional differences but without supporting data does not lend itself to a ready interpretation.

Despite the variations which occurred in the lipid values, the results show that cockroaches having low levels of lipids gave no indication of reversing or even minimizing the negative temperature coefficient effect of DDT. Conversely, there was no trend indicating that cockroaches with higher lipid contents had a wider range of sensitivity to DDT between 15° C. and 30° C. This does not preclude the possibility that cockroaches with a negligible lipid content (below 10 mgs. lipid dry weight) might not respond to the temperature difference positively instead of negatively. However, the data do not indicate any such trend. The lack of such a trend is quite apparent in figure 3 where the dosage-response curves are nearly parallel. However, the results do not rule out the possibility that qualitatively, the type of lipid might be important in the negative temperature coefficient relationships.

Summary and Conclusions.—Dosage-mortality responses to injected DDT were determined for male and female American cockroaches subjected to post-treatment temperatures of 15° and 30° C. The cockroaches were selected from three different dietary groups, a high protein, a low protein and a "standard" dog food. The results are considered from the standpoint of dietary influence and total lipid content of individual cockroaches.

A higher per cent mortality resulted from DDT at the lower post-treatment temperature and in all cases males were more susceptible than females. These results were similar on all three diets. Cockroaches fed on the high protein diet were more susceptible to DDT than cockroaches from the other diets. These cockroaches also had a lower lipid content. There was no indication that the quantity of lipids present was a factor which might be responsible for the negative temperature effect of DDT which occurs when cockroaches are subjected to contrasting post-treatment temperatures. Furthermore, the differences in quantity of lipids between sexes is not sufficient to explain sex differences in response to the action of DDT.

References Cited


