The German Roach as a Laboratory Test Animal[1]

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The German cockroach, a common pest of kitchens, makes an excellent "guinea pig" for the preliminary testing of new insecticides in the laboratory. It can be reared in large numbers in the laboratory and is easily handled in the testing procedures. The use of this small insect provides a rapid screening method for chemicals whereby an estimate of their insecticidal value can be determined in the laboratory before more extensive tests are made in the field.

The present stock of roaches in our laboratory has been maintained with no difficulty for 12 years. The procedure followed in rearing roaches is to select 300 young females and 150 males about May 1. Approximately 20 females and 10 males are started in each of 15 glass battery jars. In each jar are placed a cotton-stoppered vial of water and an inverted paper cup. The cup has two small pieces cut out of the rim to permit the roaches to crawl inside the cup for protection. Food, a pellet form of commercial dog food, is placed on top of the paper cup. Roaches are kept in the glass jar by an inch band of vaseline around the inside of the mouth and by cheese cloth held in place over the mouth by a rubber band.

During the summer months the roaches are allowed to live and reproduce in the jars. They require only such attention as supplying water and food once a week and cleaning out the debris every two weeks. The trouble from mites killing roaches, experienced in some laboratories, has been avoided by cleanliness and by separating food and water to prevent the growth of mold. By early August each jar has a large population of about 200 adults and perhaps 1500 nymphs. When the jars become crowded, the entire lot is dumped into the large stock cage.

The stock cage consists of an outer wooden box 24 by 30 inches and an inner cage 19 by 24 inches. The outer box has 8 inch sides which are lined with a 7 inch continuous piece of celluloid. The inner cage consists of a wooden frame of 1 by 2 inch lumber with a screen-wire bottom and 6 inch celluloid sides. The celluloid attached to the wooden box is coated on the inside with a thin film of vaseline, while both sides of the inner celluloid walls are coated. Roaches are placed in the inner cage, where the adults are retained by the vaseline-coated walls, while the young can go through the screen-wire into the larger wooden box. Although the top is open, adults cannot fly out. The small

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nymphs occasionally crawl over the vaseline and so a band of sodium fluoride is kept on the table around the cage. Ground dog food is supplied by inverting a four-ounce glass jar on two small pieces of wood set in a petri dish. Water is supplied in the same manner except that a piece of screen-wire is placed under the jar openings to prevent entrance and subsequent drowning of roaches.

Roaches from the 15 battery jars are placed in the stock cage about August 1 and in two weeks a number of newly-hatched nymphs has accumulated. To remove the young the inner cage is tapped several times and then set out on a piece of paper previously prepared with a two inch band of sodium fluoride slightly larger than the bottom of the cage. The young are now exposed in the outer box and are picked up with a "suck" bottle for transferring to the glass battery jars. Subsequent lots are transferred to the jars at weekly intervals and thus give roach populations of known ages.

The length of time required for nymphal development varies with the temperature of their environment. According to previous studies (6) this period ranges from 103 days at 70 to 75°F to 74 days at 83 to 86°F. As the roaches mature, they are removed from the stock jars and are separated according to their sex. These roaches are ready for use in the insecticide tests on the following morning. The roaches not used in the tests are placed in the large stock cage in order to replenish that supply.

This rearing procedure has been followed for the past eight years and has provided an ample supply for testing by about October 15. The settling dust method of testing insecticides has also been followed for eight years and has been described in previous articles (2, 3, 4, 5). It consists, in brief, of blowing the insecticide up in a tower and permitting it to settle for seven minutes on a 5 by 5 inch wooden tray. Then the tray is taken out of the cylinder and 10 or 15 roaches introduced. The roaches run through the film for seven minutes and then are transferred to clean jars for observation for 96 hours.

The rapidity of roach development is of interest not only to the housewife but also to the laboratory worker. Previous studies by Gould and Deay (6) indicate that at a room temperature of about 76°F incubation of eggs requires 28 days and nymphal development about 95 days. In order to estimate the possible number of offspring in one year from a pair of roaches, the figures of the earlier studies (6) have been used. Suppose we assume the start of this colony May 1 with one female and one male and that half the offspring produced are females. The German female produces five capsules during her adult life of about six months. If the capsules contain an average of 32 individuals and all survive and reproduce, then the population the following May would consist of 3200 adults and 37,376 nymphs and a year later of 3,000,000 or more.

The American roach has also been used as a test insect but is not so well adapted for rearing. It requires 15 to 18 months to mature and being larger needs more space and food. Usually when this roach is used, it is trapped as needed. Burnett (1) and others have stated that the American roach is more prolific, as a female produces 1440 eggs as compared to 160 for the German Roach. This is true but the American has a much longer developmental period and would have about 1200 offspring, all nymphs, in a 12 months period as compared with the 40,000 for the German.

The advantages of using the German roach for laboratory testing are many and include ease of culture, low cost of rearing, rapid rate of reproduction, small rearing space, species readily available, species representative of a group, species economically important in itself, and the results are applicable to other species. According to a survey of test insects used by entomological laboratories, the National Research Council (Mimeograph report, October, 1949) found the German roach second to the housefly in a list of 192 insects. The American roach was used frequently, while a few laboratories listed the Oriental roach, the brown-banded roach and the smoky brown roach.

Variation in susceptibility to insecticides of different insects and by different stages of the same insect has been noted by many workers. Among roaches the fifth instar is considered the most difficult to kill, followed by the adult female, the fourth nymphal instar and the adult male. In our tests both males and females were used until 1948 when females alone were used. In table I the effectiveness of some common

TABLE I. Comparison of the kill of male and female German cockroaches treated with certain insecticides, 1947.

Insecticide and per cent of active ingredients		Males		Females	
		Per cent kill	Survival time in hours	Per cent kill	Survival time in hours
Sodium	5	52	73	14	90
fluoride	10	86	64	32	74
	25	92	53	74	68
D.D.T.	0.75	34	59	28	76
	1.0	85	51	46	63
	2.5	100	41	88	71
Benzene	0.1	50	58	20	74
hexachloride	0.2	100	31	94	42
	0.3	100	35	100	56
Lindane	0.1	11	72	5	..
	0.3	67	54	35	62
	0.5	100	22	86	55
Toxaphene	0.5	82	78	33	80
	0.75	97	68	42	86
	1.0	100	61	80	81
Parathion	0.03	42	45	20	52
	0.05	92	37	70	44
	0.1	100	25	98	38
Chlordane	0.5	56	78	22	90
	1.0	98	52	83	83
	2.0	100	57	89	74

chemicals against the two sexes is compared. The averages given in the table are based on 10 tests of five roaches each. The kill of males was from 20 to 270 per cent better than that of females. An average of the 13 comparisons in the table show a 70 per cent kill of males and a 40 per cent kill of females. The response of the two sexes to the action of some insecticides shows a high degree of correlation. Since the females in these tests and in most others made between 1941 and 1947 were nearly twice as difficult to kill as the males, females alone have been used in most tests in the past two years.

Several hundred mixtures have been tested in the laboratory during the past seven years and results of 1943 to 1947 have been published (2, 3, 4, 5). In the 1948 and 1949 tests two changes were made in procedure: first, females were used in most tests and, second, dilutions of the active ingredient were reduced to a point where kills were less than 100 per cent. In the past there has been no method of comparison where two materials killed all roaches. By the use of lower dilutions three or more values giving less than 100 per cent kills can be plotted on logarithmic probability paper (Wadley, 7). When the three or more points are located, a straight line can be fitted to them.

The data on females for 1947 (Table I) were the first to be plotted on log probability paper. For 1948 (Table II) and 1949 the complete series of 10 tests for each material was run only on the females. The straight lines for these materials are shown on the accompanying graph (Fig. 1). Where these lines intersect the line for a 50 per cent kill, a value for each in terms of percentage of active ingredients can be ascertained. If the quantity for the best material is assigned a value of 1, then the comparative values of the other materials can be established. These values based on the per cent active ingredients necessary for a 50 per cent kill are as follows:

Material	Per Cent Active Ingredient	Value
Compound 497	.031	1.0
Parathion	.042	1.3
Compound 118	.0715	2.3
Pyrethrins in pyrethrum powder	.107	3.5
Benzene hexachloride (gamma)	.123	4.0
Lindane (gamma)	.283	9.1
Chlordane	.425	13.7
Toxaphene	.71	22.9
DDT	1.1	35.5
DDD	3.0	97.0
DMDT	4.7	151.6
Sodium fluoride (males)	4.8	154.8
Fluoro DDT	8.6	277.4
Sodium fluoride (females)	13.5	435.5

TABLE II. The percentage of kill and the survival time in hours of female German roaches treated in the laboratory in a settling dust chamber with various strengths of insecticides. Prophyllite was used as diluent in all mixtures. 1948.

Material	Per cent active ingredient	Per cent kill	Survival time
Rotenone (5% cube)	3.0	0	..
Rotenone extract (34%)	2.5	12	75
Rotenone extract	5.0	62	65
Rotenone extract	7.5	42	46
Rotenone extract plus 1% piperonyl butoxide	1.0	8	
Pyrethrum powder (0.3%)	0.075	18	52
Pyrethrum powder	0.1	28	61
Pyrethrum powder	0.15	86	43
Pyrethrum extract (20%) impregnated	0.25	20	62
Pyrethrum extract	0.375	38	60
Pyrethrum extract	0.5	86	46
Pyrethrins plus .25% piperonyl cyclonene impregnated separately and mixed	0.25	6	..
Same mixed as liquids and impregnated	0.25	40	67
Pyrethrins plus .50% piperonyl cyclonene	0.50	86	37
Pyrethrins plus .50% piperonyl cyclonene	0.2	84	58
Pyrethrins plus .25% piperonyl butoxide	0.25	70	46
Pyrethrins plus .125% piperonyl butoxide	0.125	8	
Pyrethrins plus .50% piperonyl butoxide	0.5	96	37
Pyrethrins plus .20% piperonyl butoxide	0.2	68	57
Pyrenone T127 (pyrethrins and p. cyclonene)	0.02	0	
Pyrenone T127	0.04	20	48
Pyrenone T127	0.06	38	38
Pyrenone T127	0.1	70	52
Pyrenone T166 (pyrethrins and p. butoxide)	0.02	4	
Pyrenone T166	0.03	28	46
Pyrenone T166	0.04	67	49
Pyrenone T166	0.05	76	46
D.D.T. 50% wettable powder	2.5	80	80
D.D.T. 100% impregnated on powder	2.5	71	81
D.D.T. 50% wettable aerosol grade	2.5	88	78
pp'D.D.T. 100% powder	2.5	16	72
D.D.T. 100% powder	5.0	33	84
Ditolyl trichloroethane 25% powder	2.5	6	..
pp' isomer of above	10.0	0	..
Fluoro D.D.T. 100% impregnated in powder	2.5	2	..
Fluoro D.D.T.	5.0	26	90
Fluoro D.D.T.	7.5	40	69
Fluoro D.D.T.	10.0	58	91
D.D.D. 50% wettable powder	2.0	22	85
D.D.D.	4.0	78	79
D.D.D.	6.0	86	74
Methoxychlor 50% wettable powder	2.5	10	
Methoxychlor	5.0	60	58
Methoxychlor	7.5	78	63
pp' isomer of above 50% wettable powder	2.5	0	
pp' isomer	5.0	10	74
pp' isomer	10.0	12	75
Chlordane 5% dust	0.3	21	90
Chlordane	0.5	62	71
Chlordane	0.75	93	71
Chlordane	1.0	100	66
Toxaphene 50% powder	1.0	82	77
Sodium fluoride	17.5	66	74

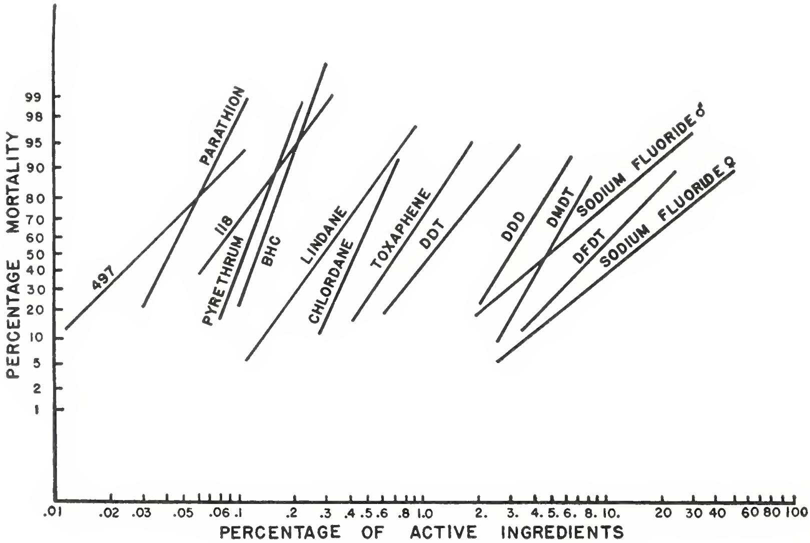

Figure 1. The percentage mortality of the female German roach exposed to different concentrations of insecticides.

In the tests of 1949 (Table III) considerable attention was given to the non-poisonous materials, pyrethrum and rotenone. These materials were used alone and in combinations with activators. Rotenone in the form of cube powder produced no mortality, while the extract gave a low kill. The addition of the activator, piperonyl butoxide, to the extract produced no measurable response. On the other hand the addition of either piperonyl cyclonene or piperonyl butoxide greatly increased the effectiveness of pyrethrum extract. The butoxide gave better results as a 50 per cent kill was obtained with .037 per cent pyrethrins while the cyclonene required .07 per cent of the pyrethrins for the same kill. Pyrethrum extract alone required .37 per cent active material for a 50 percent kill, while pyrethrum powder required .11 per cent. In the preparation of laboratory mixtures it was found that the liquid pyrethrum extract had to be combined with the liquid piperonyl material before they were impregnated on the dust. Of the many laboratory mixtures prepared with pyrethrum none were as effective as the commercial preparation.

In the second series several of the new chlorinated hydrocarbon materials were compared. A dust prepared from a standard DDT wettable powder was more effective than dusts prepared from the pp'isomer and the technical DDT, but was less effective than the dust made from the aerosol grade of DDT. DDT was better than the fluoro and tolyl analogs of DDT. It was also better than DDD and methoxychlor, but was less effective than chlordane and toxaphene.

In 1949 two new compounds were tested along with several of the older materials. Considerable variation was found in the results from

TABLE III. The percentage of kill and the survival time in hours of female German roaches treated in the laboratory in a settling dust chamber with various strengths of insecticides. Pyrophyllite was used as diluent in all mixtures. 1949.

Material	Per cent active ingredient	Per cent kill	Survival time
Compound 118-K-W	0.075	63	84
	0.1	66	72
	0.175	82	81
	0.25	98	63
Compound 497	0.01	14	92
	0.03	36	94
	0.05	75	81
DDT 50% wettable powder	1.0	23	92
	1.5	45	70
	2.0	65	75
DDT micronized 50% wettable powder	1.0	42	85
	1.5	54	67
	2.0	84	79
Fluoro DDT	2.5	31	67
	5.0	71	58
	10.0	94	51
Chlordane	0.5	62	76
Toxaphene	0.35	24	47
	0.4	34	86
	0.5	92	72
CS-645A	2.0	26	54
Sodium fluoride	10.0	28	86
	17.5	57	81
	25.0	70	51

different laboratory mixtures and between commercial mixtures. This variation was especially noticeable when a commercial mixture was compared with a laboratory mixture. The process of manufacture also influenced results as indicated in the difference between a micronized and a regular DDT. The finely ground material gave 10 to 20 per cent better kills at the various dilutions. A sample of fluoro DDT gave better kills this year than did the sample from another company used in 1948.

The two new compounds, No. 118 and No. 497, gave excellent results with roaches. Several samples formulated by different companies or at different times by one company showed variable results but in general were about as good as parathion. Another new chemical, 2-Nitro-1,1-bis(p-chlorophenyl) propane, (CS-645A) showed some toxicity to the roach, as it killed 26 percent at a 2 per cent dilution. Two mixtures prepared by a graduate student in chemistry and designated as Cl 12 and R103 showed no toxicity to roaches.

Summary

The German roach has been used in the laboratory for the past eight years as a test insect in the evaluation of insecticidal efficiency.

Because of its ease in rearing and its rapid rate of reproduction this insect has proven itself to be an excellent "guinea pig" in the study of insecticides. These preliminary tests in the laboratory have given a comparative rating of many new materials and have indicated those materials worthy of further tests in the field.

Of the materials used in the past eight years the compound 497 has given the best kill. Other materials in order of decreasing efficiency are parathion, No. 118, pyrethrins in pyrethrum powder, gamma-benzene hexachloride, lindane, chlordane, toxaphene, DDT, DDD, DMDT, DFDT, and sodium fluoride. Pyrethrum extract activated with one of the piperonyl compounds gave an excellent kill and on the basis of pyrethrins only would rate near 497.

A high rating in these tests does not necessarily indicate that the material would be of practical value in roach control. Many of these materials are highly toxic to humans and consequently would be of little value in the home and around food manufacturing plants or storage. Among the recommended treatments pyrethrum powder and activated pyrethrins are foremost in the contact materials and chlordane oil solution as a residual treatment. DDT, chlordane dust and even sodium fluoride are still of value against certain roaches and in certain locations.

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