Laboratory Tests on the Toxicity of Technical DDT, DDT Isomers and Analogues, and DDD[1]

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Object of Investigation

Technical DDT and some of its more common isomers and analogues were to be compared by laboratory tests as to toxicity to four common species of insects, the pomace fly, *Drosophila melanogaster* Meig., the acrobat ant, *Crematogaster lineolata* Say, the spotted ladybird beetle, *Megilla fuscilabris* Muls., and the striped blister beetle, *Epicauta vittata* Fab.

Chemicals

The chemicals used, together with information as to purity, were generously supplied by the Geigy Company, Inc. of New York and E. I. Du Pont de Nemours Company, Inc. of Wilmington, Delaware. Most of the chemicals were supplied from laboratory stocks and were not commercially available. The ten chemicals used were:

1. DTolT Geigy. Ditolyltrichloroethane, about 90 per cent p,p' isomer of the methyl analogue of DDT.

2. DBrDT Geigy. Dibromodiphenyltrichloroethane, 90 to 95 per cent bromine analogue of DDT.

3. DFDT Du Pont. Technical grade fluorine analogue of DDT. Bradlow and VanderWerf (1) found a similar material to be composed of practically all p,p' isomer.

4. DMDT Geigy. Dimethoxydiphenyltrichloroethane, 99 to 100 per cent methoxy analogue of DDT.

5. DMDT Du Pont. Dimethoxydiphenyltrichloroethane, about 99 per cent p,p' isomer of methoxy analogue of DDT.

6. DDT. Geigy's technical grade dichlorodiphenyltrichloroethane, 90 degrees centigrade set point. Analyses by Gunther (7) and Haller *et al* (8) showed about 70 per cent of p,p' isomer, about 18 per cent of o,p' isomer, and about six per cent o,o' isomer DDT to be the major constituents, with a variety of minor constituents also present.

7. o,p'-DDT Geigy. 1-trichloro-2- (o.chlorophenyl) -2- (p.'chlorophenyl)ethane, 90 to 95 per cent pure.

8. p,p'-DDT Geigy. 1-trichloro-2-bis(p.chlorophenyl)ethane, 99 to 100 per cent pure.

[1] This paper is based on a Master's thesis submitted at Purdue University in August, 1948.

9. p,p'-DDT Du Pont. 1-trichloro-2-bis(p.chlorophenyl)ethane, about 97 per cent pure.

10. DDD Geigy. Dichlorodiphenyldichloroethane, 99 to 100 per cent pure.

The DFDT was a viscous, amber fluid at room temperature; all the other chemicals were white, crystalline powders.

Preparation of Deposits of the Chemicals

It was desired that a thin, uniform deposit be obtained for exposing the insects.

In preliminary experiments when acetone solutions of the chemicals were allowed to dry on glass surfaces, even high concentrations of material did not give uniform deposits. More satisfactory deposits were obtained when acetone solutions were used to impregnate small rectangles of paper.

Acetone solutions were prepared to contain 2.5 grams of chemical per 100 milliliters of solution; and one milliliter portions were pipetted out onto 1½ x 4 inch rectangles of an absorbent, heavy, white paper. The paper slips were supported on solvent-washed glass plates and air dried for 12 to 16 hours before use.

A series of weighings was carried out and it was found that 21 to 24 milligrams of material was retained in each slip of paper for an average of 3.5 to 4.0 milligrams per square inch.

Because of an anticipated higher sensitivity of the acrobat ant to the chemicals used, half milliliter portions of the solutions were used in preparing papers for those tests.

The Test Insects

The pomace flies used were bred from a culture of Swedish B strain maintained in the Department of Biological Sciences of Purdue University. They were cultured at room temperature in wide-mouth, four ounce, glass bottles containing a medium prepared from cornmeal, agar, molasses, and water, with a small amount of mold-inhibiting chemical added. A few drops of yeast suspension were added to each bottle 12 to 24 hours before flies were to be introduced.

The acrobat ants were secured by field collection from colonies in a wooded area near Lafayette. An inspirator bottle was used.

The spotted ladybird beetles were collected with the aid of an inspirator bottle from sweet corn in the vicinity of Lafayette.

The striped blister beetles were collected from an infestation on potatoes at a Purdue experimental farm.

Manner of Testing

Four ounce, wide-mouth bottles served as test chambers for each of the four species. The dried, impregnated papers were bent and inserted into these bottles; they were stiff enough to maintain their positions near the tops of the bottles.

For all four species five replications of tests were made with each replication including the ten chemically impregnated papers, one control with acetone treated paper, and one control with untreated paper.

In preparing the pomace fly tests, flies were used one week after emergence began. Carbon dioxide was used to immobilize the flies in the culture bottles. Then flies from four or five bottles were shaken out into a shallow pan loosely covered with a glass plate and the flies were mixed by shaking them about. Carbon dioxide anaesthesia was maintained in the pan as the flies were counted out into lots of 50 each. A small camel's hair brush was convenient for counting the flies and sweeping them onto small paper slips by means of which they were easily transferred into the test bottles. The test bottles were actually culture bottles complete with medium, seeded with yeast, and fitted with cellucotton plugs. The bottles were placed on their sides so that the flies rested on the clean glass wall above the medium while they recovered from the anaesthesia. This prevented them from resting on the impregnated paper and from becoming mired in the medium (which liquefied somewhat) while recovering from the anaesthetic.

The acrobat ants used in any one replication were all from the same nest. They were counted out in lots of 20 each for the tests and were handled under carbon dioxide anaesthesia in about the same way as the pomace flies. A weak solution of sugar was supplied in each test bottle in a small vial loosely stoppered with cotton. The tests were closed with several layers of cheesecloth and stored on their sides.

The spotted ladybird beetles were also handled and counted with the aid of carbon dioxide anaesthesia. Ten beetles were used per test; plain water was supplied in each test bottle in a loosely stoppered vial; and the tests were closed with cheesecloth and stored on their sides.

The striped blister beetles were handled, counted into lots of ten, and introduced into the test bottles without the use of anaesthesia. In each test chamber a water vial was supplied and, in deference to the voracious appetite of the beetles, two small, fresh cucumber leaves were supplied. The tests were stored on their sides.

Records

Throughout these experiments no less than a complete replication (ten chemical tests and two controls) was set up at any one time. In order that records could be taken conveniently every 12 hours it was convenient to start the tests either in the morning or evening.

Records taken were of the numbers of insects "down", "dead", or "alive". "Dead" was taken as meaning completely devoid of reaction to stimuli or of any signs of life. "Down" was taken to mean incapable of locomotion or of the righting reaction. "Alive" was taken to mean capable of locomotion and the righting reaction even though, perhaps, displaying toxic symptoms such as incoordination.

In no case in any of the tests was there any indication of recovery by insects recorded as "down".

Observations of the surplus insects remaining after tests were set up showed no mortality due to the anaesthetic.

Results

For evaluating the results of these experiments the records for the last readings before the control insects started to die off rapidly were used. For the pomace fly that is the 60 hour reading; for the acrobat ant, the 36 hour reading; for the spotted ladybird beetle, the 84 hour reading; and for the striped blister beetle, the 36 hour reading.

An analysis of variance was worked out on these records.

Table I summarises the data and presents the required differences for significance at odds of 19 to one.

TABLE I. Mean Survival of Pomace Fly, Acrobat Ant, Spotted Lady Bird Beetle, and Striped Blister Beetle; and Differences Required for Significance at Odds of 19 to 1.

Chemical	Purity	Pomace fly	Acrobat Ant	Lady Bird Beetle	Striped Blister Beetle
DTolT Geigy	90% p,p'	45.0	16.0	1.0	9.4
DBrDT Geigy	90-95%	36.2	17.2	5.6	5.8
DFDT Du Pont	Tech.	0.0	0.0	0.0	2.4
DMDT Geigy	99-100%	39.8	17.6	4.6	7.6
DMDT Du Pont	97% p,p'	45.0	17.3	4.6	4.8
DDT	Tech.	2.2	3.2	2.0	3.2
o,p'-DDT Geigy	90-95%	45.6	15.8	6.4	8.4
p,p'-DDT Geigy	99-100%	4.6	2.3	4.2	2.4
p,p'-DDT Du Pont	97%	5.6	6.6	3.0	2.6
DDD Geigy	99-100%	39.8	16.8	6.4	7.8
Acetone (control)	.....	46.2	19.0	8.2	8.8
Untreated (control)	.....	48.2	17.8	8.4	8.2
Difference required at odds of 19 to 1		15.54	5.30	2.51	2.42

In connection with the information given in Table I it should be noted that with the pomace fly DFDT Du Pont produced complete mortality in four replicates at 12 hours and at 24 hours in one replicate; and DDT produced complete mortality in one replicate at 24 hours, in one replicate at 36 hours, and in one replicate at 48 hours. With the acrobat ant DFDT Du Pont produced complete mortality in 12 hours in two replicates, and in 24 hours in two replicates. With the spotted ladybird beetle DFDT Du Pont produced complete mortality in one replicate at 36 hours, in one replicate at 48 hours, and in three replicates at 60 hours.

A fumigant action by DFDT Du Pont was suspected and was subsequently verified in a supplementary experiment with the pomace flies. It should be noted, however, that cheese cloth covers were used on the ants and ladybird beetles and ventilation of these tests would be expected to be adequate.

From Table I it is apparent that the results obtained with one species in general parallel those obtained with the others. For all four species four materials, DFDT Du Pont, DDT, p,p'-DDT Geigy, and p,p'-DDT Du Pont, were significantly effective. If the rapidity of action is considered, DFDT Du Pont would be indicated to be first and DDT would be second.

Although Table I is self-explanatory, one should point out a few further items: (1) Ordinary technical DDT compared remarkably well with the more highly refined p,p'-DDT samples. (2) The sample of methyl analogue was significantly effective only against the spotted ladybird beetle. (3) The samples of methoxy analogue and of bromine analogue were definitely inferior to DDT in insecticidal efficiency. (4) The samples of DDD and o,p'-DDT were practically ineffective.

Comparative Data in the Literature

The author attempted to examine all literature reports having bearing on comparisons among the isomers and analogues of DDT. Of this literature only a rather small fraction is particularly pertinent.

One of the species used here has been previously used in similar tests. In 1945 Morrison (12) reported on the use of pomace flies for exposure to deposits of chemicals impregnated into paper. In 1947 Proverbs and Morrison (16) reported on the use of this technique in testing eighteen chemicals including technical DDT, fluorine, bromine, and methyl analogues, and DDD, among others. They found the halogen analogues to be, in order of decreasing toxicity, fluorine, chlorine, bromine, and iodine. All analogues except the fluorine analogue were less toxic than DDT. Further, the fluorine analogue was found to be somewhat volatile and less persistent but about nine times as effective as the technical DDT at twenty hours exposure.

In 1946 Kirkwood and Dacey (9) reported on tests of three fluorine analogues against the pomace fly. The p,p' analogue was the most effective, but only about two fifths as effective as DDT.

In 1947 Metcalf and Gunther (13) reported p,p'-DDT ten times as effective as the methyl analogue and a hundred times as effective as the promine analogue against the pomace fly.

Considerable comparative work has been done on houseflies and mosquitoes. In 1943 Prill *et al* (14) ran tests on houseflies by means of the Peet-Grady technique. They found the ethoxy, and methoxy analogues (among others) to be inferior to DDT. Others of their tests rated the methoxy analogue equal to DDT against mosquito larvae. In 1946 Gersdorf (6) compared o,p'- and p,p'-DDT against houseflies and found the p,p' isomer forty-four times as effective at the ten per cent L. D. Neither gave fast knockdown.

In 1946 Prill *et al* (15) reported on a three year investigation of the comparative toxicities of thirty-two chemical relatives of DDT as tested against *Culex quinquefasciatus* and the housefly. They found the methoxy analogue gave a more rapid knockdown but less total toxicity than DDT. From the results of these and other investigations the authors concluded that substitution of radicals larger spatially than methoxy or ethoxy radicals into the p,p' positions, and any variation from the p,p' positions greatly reduced toxicity.

In 1947 Fay *et al* (5) tested several organic compounds for residual action against houseflies and mosquitoes over a twenty-six week period. DDT proved superior to DDD in these tests.

Comparative work has also been done on lice and bedbugs. In 1946 Busvine (2) summarized the results of tests of forty-one analogues of DDT against bedbugs and human lice with the general conclusion that DDT and the chemicals closest to it in chemical structure were the most effective. In another article Busvine (3) rated DDT superior to DDD and both superior to the fluorine analogue when used against the same two species.

In 1946 Cristol *et al* (4) reported the o,p' isomer of DDT ineffective and the o,o' isomer only slightly effective against lice. In 1947 Riemschneider (17) reported making some fluorine analogues of DDT and claimed one of them was the most effective insecticide yet made. Against lice one per cent of this fluorine analogue was as effective as ten per cent DDT.

In 1944 Martin and Wain (11) reported on tests of various DDT analogues as contact poisons against several species. They concluded that the fluorine analogue had the highest insecticidal effectiveness, that technical DDT was of intermediate effectiveness, that the bromine and iodine analogues were of low effectiveness, and that organic-substituent analogues were still less effective.

Knipling in 1947 (10) suggested that although the methoxy analogue and DDD were generally less effective than DDT as insecticides, they had promise of being useful because of a lesser toxicity to warm blooded animals.

Conclusion

The results of this work appear to be largely in accord with results obtained by others with the same and other species of insects. Technical DDT, p,p'-DDT, and the fluorine analogue of DDT are shown to be more effective against the pomace fly, the acrobat ant, the spotted ladybird beetle, and the striped blister beetle, than are others of the more common isomers and analogues.

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