

# The Estimation of Toxicity of Insecticidal Deposits by Biological Methods\*

## Part—II

By

DR. P. SATYANARAYANA, M. sc., PH. D. (Lond.), F. R. I. C.  
Agricultural College, Bapatla

**Introductory:** Investigations reported in the earlier communication (Satyanarayana, 1951) showed that the amount of D. D. T. (1 : 1 : 1) Trichloro - 2 : 2 - bis (P - Chlorophenyl - ethane), recovered from the surfaces of apple leaves and estimated chemically varied with the mode of deposition, the nature of solvent used in the preparation of the insecticide, and the concentration of the insecticide itself. As it was next intended to see how far these chemical recoveries could be correlated with their biological performances experiments were conducted in advance to test the suitability of the insect used for the test and the extent to which the results could be relied upon and reproduced.

In estimating toxicities of deposits by biological methods different insects were used by different workers. In cases where the insecticide tested is one which kills by contact action, it is preferable to choose an insect that does not fly. Symes (1946), Barlow and Hadaway (1946), using tsetse fly and mosquito as test insects experienced considerable difficulty in making them remain in contact with the treated surfaces and so no clear-cut relationship could be established between the chemical and biological tests. *Tribolium castaneum* was used by Parkin and Hewlett (1946), and McIntosh (1947), with satisfactory results. This species is not only stationary with the advantage of being easily handled, but occupies little space, is economical, and is convenient in several other ways. After a scrutiny of the available literature, *Tribolium confusum* (DUV), was selected as the test insect and experiments conducted with emulsions of D. D. T. on glass surfaces using the following technique.

**Experimental:** Glass slides (25 x 70 mm) used in microscopic work were taken and 3 squares each 4.0 sq. cm. in area were marked on one side of the slide with a diamond pencil. The slides were then cleaned with 1% Triton - X - 100 solution to free from grease, washed with alcohol and dried. Accurately measured quantities of the insecticidal preparation (usually 0.05 cc.) were deposited in the centre of each square with a micropipette and then uniformly spread and allowed to dry. After the deposit had dried, cylindrical glass cells 2.5 to 3.0 cm. in height and 1.8 cm. in diameter whose rims were finely ground down,

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were lightly painted with collodion solution and placed on each square so that the whole base of the cell was covered by the deposit. After an hour or two when the deposits had dried and the smell of the solvent disappeared, the insects which were previously drawn into specimen tubes and pre-conditioned by keeping in the incubator at 25°C, were transferred to each cell and the slides then transferred to an incubator kept at 25°C. Counts were taken after 6 days (144 hours), since 100% kill was usually obtained within that period with the maximum concentration of the insecticide used in the investigations, viz., 0.05 cc. of 0.08% preparation which gave a concentration of 10 micrograms of D. D. T. per sq. cm.

**Discussion:** In the conduct of biological tests strictly comparable conditions like the age of insect, temperature, humidity etc. are very necessary, and provided they are satisfied, reliable and reproducible results are obtained as seen from the results presented in Table 2. The variation of L. D. 50, with age of insects is illustrated by the results presented in Table 1, and confirm the observations made by Hunt (1947).

**Summary:** A method of estimating the biological performances of insecticidal deposits is described. Provided the essential conditions are satisfied, reliable and reproducible results are assured.

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TABLE — I.

The variation in L. D. 50 with age of insects.

Surface used: Glass. % Benzene in Emulsion: 2.0%  
Emulsifier used: Triton-X-100 at 0.02% (V/V).

Concentration of DDT in the emulsion.	Micrograms of DDT per sq. cm.	Age of insects 0-12 days.			Age of insects 0-19 days.			Age of insects 0-23 days.		
		Number used.	Dead.	% Dead.	Number used.	Dead.	% Dead.	Number used.	Dead.	% Dead.
0.04	5.0	60	59	98.3	59	57	96.6	59	46	78.0
0.02	2.5	60	59	98.3	61	48	82.0	60	38	63.3
0.01	1.25	59	54	91.3	59	29	49.2	59	25	42.4
0.005	0.625	62	31	50.0	60	18	30.0	60	3	5.0
0.0025	0.3125	61	10	16.4	62	5	8.1	59	0	0
Controls	—	50	1	2.0	50	1	2.0	48	0	0

For L. D. 50:—			
% D. D. T. in emulsion	0.00427	0.0088	0.0155
Micrograms per sq. cm.	0.523	0.10	1.94

TABLE — II.

Testing the reliability and reproducibility of biological tests.

Insect used: *Tribolium confusum*. 1% Benzene and 0.02% Triton-X-100 (V/V) were used in emulsions.

Surface used: Glass.

Concentration of D. D. T. in emulsion.	Micrograms D. D. T. per sq. cm.	Test 1.			Test 2.			Test 3.			Test 4.		
		Number used.	Dead.	% Dead.	Number used.	Dead.	% Dead.	Number used.	Dead.	% Dead.	Number used.	Dead.	% Dead.
0.08	10.0	56	56	100	60	60	100	60	60	100	60	60	100
0.04	5.0	58	58	100	60	60	100	60	59	98.3	60	60	100
0.02	2.5	60	59	98.3	60	57	95	60	59	98.3	60	60	100
0.01	1.25	58	27	46.6	60	26	43.3	60	21	35.0	60	24	40
0.005	0.625	60	6	10.0	30	4	13.3	60	9	15	60	4	6.7
Controls	—	51	1	2	—	—	—	—	—	—	—	—	—

**Statistical Analysis:** For 144 hours:— Analysis of variance after transferring percentages to angles of equal information ( $p = \sin^2 \theta$ ), showed differences between concentrations to be highly significant ( $P < .001$ ), whereas differences between trials were non-significant, ( $P > .0.2$ ).

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