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The Evaluation of Residual Deposits of DDT.

[2, 2, – bis (P – Chlorophenyl) 1, 1, 1 – trichloro – ethane] by chemical and biological methods** and the correlation existing between them. Part III

By

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Introductory: Investigations reported in the earlier articles (Satyanarayana, 1951), showed that the amount of DDT recovered from the surfaces of apple leaves varied with the mode of deposition, the nature of the solvent used, and the concentration of the insecticide in the preparation. The nature of the emulsifer used had but little effect, the percentage of the solvent in the preparation and the nature of the surface (upper or lower) though having some slight effect did not appear to be of much consequence. Though under the experimental procedure adopted in the present investigation it has not been possible to recover all the DDT initially deposited, it was shown that either by prolonging the period of shaking or by continued extraction in a soxhlet cent percent recovery was possible. It was, therefore, clear that the material that was not recovered was not lost by any other process like the catalytic decomposition by leaf tissue, etc., but could be recovered.

Absorption of insecticides into the plant tissue is not a new idea. Swain (1933), working with petroleum oils observed this and suggested a differentiation between the 'available' i.e. the insecticidally useful, and the 'non-available' or the locked up portions. Probably, a similar differentation needs to be made in the case of DDT also. In a way this fact seems to have been realized by Barlow and Hadaway (1949), when they designated the two fractions of DDT as 'inner' and 'outer'.

Object and scope of the present investigations: In view of the foregoing considerations further elucidation of the following points is clearly necessary :—

1. If one is dealing with a material treated with DDT., what is the most suitable method of extracting all the insecticidally useful portion ?

2. What is the insecticidal value of that portion which is not recovered by the method which is considered most appropriate ? and

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3. Is it possible to establish any relationship between values obtained by chemical estimation and those obtained by biological tests?

Methods and Materials: In reviewing the literature on extraction procedure (Satyanarayana 1951), it was shown that the methods adopted varied a lot from mere washing with a jet of benzene to a shaking period of 15–30 minutes. Naturally, the amounts of DDT recovered by these two methods cannot be the same, and the results presented in the earlier article also confirmed it. Considering all aspects of the problem it was decided to confine attention to preparations made with a low boiling solvent like benzene and a high boiling solvent like dekalin in the preparation of emulsions and solutions, and estimate the relative toxicities of deposits both by chemical and biological tests. A few trials with a suspension were also included for the sake of comparison. Since the effect of the surface (upper or lower) on the recovery of the insecticides was found to be insignificant, and as it also appeard to be more practicable to spread the deposits uniformly on the upper than on the lower surface due to the absence of veins and midrib, the experiments were restricted to the upper surface only. With the idea of confirming the absorption of the insecticide by the leaf tissue a non-absorbent surface like glass was also included in the tests for comparative purposes.

In the conduct of biological tests, when glass surfaces were employed, the technique described in the earlier communication was followed (Satyanarayana, P. 1951, 2). But in cases were leaf surfaces were used, squares 8 sq. cm. in area were cut from fresh and clean apple leaves (Variety, Cox's Orange Pippin) and after placing them flat in petri dishes, measured amounts of the insecticide were deposited as usual with a micropipette and spread uniformly without allowing them to overflow the edges. After the deposits on leaves had dried, which usually happened in 1–2 hours, the squares were cut into circles and fitted into the caps of "Universal" sample bottles (18–19 mm. internal diameter) fitted with rubber washers. The required number of insects were transferred to each bottle and the cap containing the leaf circle gently screwed on. The bottle was finally inverted thereby causing the insects to fall on to the leaf surface, and then left in the incubator at 25°C till taken out for counting after 144 hours.

Regarding the period that should lapse between the deposition of the insecticide and the transference of insects on the leaf surface, no hard and fast rule is available. Gunther (1946), in his spraying experiments with kerosene solutions and emulsions on fruit trees observed that the insecticide first penetrated the tissue along with the solvent but subsequently reappeared on the surface during the next 24 hours. He therefore, suggested that when samples are drawn for analysis it is better to do so 24 hours after spraying by which time the insecticide would have had ample time to stabilize itself. A few preliminary experiments were conducted on this aspect to ascertain the minimum period that should lapse between the deposition of the insecticide and the transference of insects. DDT emulsions of 0·04% and 0·02% strength prepared by using benzene as solvent were deposited on exactly measured leaf surfaces and the insects transferred at 2, 4, 8, 16 and 24 hours after the deposition of the insecticide (Table I)

TABLE I

The influence of time interval between the deposition of the insecticide and the transference of insects on the percentage kill.

Benzene emulsion on leaf — counted after 7 days.

Concentration of DDT in the preparation %	Micrograms of DDT per sq. cm.	Interval between deposition of insecticide and transference of insects hours		Number of insects used	Number dead	% dead
0·04	5·0	2	(1)	91	63	69·2
		4		90	66	73·3
		8		89	49	55·0
		16		90	68	75·5
		24		89	61	68·5
0·02	2·5	2	(2)	91	18	20·0
		4		99	33	33·3
		8		90	24	26·6
		16		90	32	35·5
		24		90	32	35·5

Statistical examination of data in Table 1 :—

(1) The data do not appear to be homogeneous since p = 0·05 — 0·02; for p = 00·5 = 9·488, but the value actually obtained is 10·606 (4 df) which is very close to the expected value.

(2) p = 0·01 — 0·5, i.e., the data are homogeneous.

Counts taken after 7 days showed that the percentage kills with both the concentrations were independent of the interval that lapsed between the deposition of the insecticide and the transference of insects. In the present investigations, therefore, the insects were transferred to the leaf surfaces as soon as they appeared dry, and this generally happened in 1½ to 2 hours.

Having decided on the technique of the biological experiments the following experiments were conducted to ascertain :—

(1) The optimum quantity of benzene required for stripping all the insecticidally useful part of DDT from the leaf surfaces and test the insecticidal value of washed leaves,

(2) having ascertained the optimum quantity of benzene required for stripping, estimate the amount of DDT recovered when deposited in different forms, and,

(3) Attempt to evaluate the toxic action of deposits when deposited on different surfaces by biological methods, and correlate them with the chemical values.

The p—p' DDT obtained by recrystallising thrice the commercial product with alcohol and having a m. p. of 108—109°C, was used in all these investigations.

Estimation of the optimum quantity of benzene required for stripping the insecticidally useful part of DDT and the toxic action of the washed leaves: One percent solutions and emulsions using benzene as the solvent

were deposited on the upper surfaces of apple leaves at the rate of 5.0 micrograms per 100 sq. cm. of leaf area, and after allowing them to remain for 24 hours, the leaves were washed individually with 3, 6, and 9 cc. of benzene per leaf with a wash bottle jet and the extracts collected and analysed by determining the total chlorine content after reduction with sodium and ethyl alcohol (Satyanarayana, 1951). The washed leaves were thoroughly dried and their insecticidal value determined by confining *Tribolium confusum* on them.

TABLE II

Amount of DDT recovered from the surfaces of apple leaves when washed with varying quantities of benzene, and the insecticidal value of washed leaves.

	Chemical estimations				Biological tests		
Leaf area deposited Sq. cm.	Benzene used for washing c. c.	DDT deposited mgm.	DDT recovered mgm.*	Percentage recovered	No. of insects tested	Number dead	Percentage dead
			Solutions				
100	3·0	5·0	3 26	65·3	55	3	5·6
100	6·0	5·0	2 96	58·9	59	4	6·8
100	9 0	5·0	3 43	68·2	47	3	6·5
Average.				64·1			
			Emulsions				
100	3·0	5·0	3·35	66·71	59	1	2 0
100	6·0	5 0	2 98	59·60	48	1	2·1
100	9·0	5·0	3·23	65 60	60	3	4 9
Average				64.00			

* each value represents the average of four estimations.

The result of chemical and biological tests presented in Table II show that, (1) the amount of DDT recovered is independent of the amount of benzene, and (2) the residual toxic effect of washed leaves is insignificant. In other words, the amount of DDT recovered is the same whether the quantity of benzene used for washing each leaf is 3, 6, or 9 cc. Only 64%, of the material originally deposited is recovered. It, therefore, appears that the effective portion of the insecticide is completely removed even by mere washing and no elaborate shaking or extraction procedure is called for. The unrecovered portion of the insecticide which constitutes nearly one-third of that originally deposited, i. e., 1.7 mgm per 100 sq. cm. of leaf area, or 17 micrograms per 1.0 sq. cm. is practically ineffective as the percentage kill is insignificant. As will be evident from the results presented in the earlier communication (Satyanarayana. II, 1951) 10 micrograms of DDT is ample to give a 100% kill with *Tribolium* in an experimental period of six days. The inability of this unrecovered portion which though of the order of 17 micrograms per 1 sq. cm. to show any effect proves that it is not present in an easily available form to deal with insects of *Tribolium* type that are killed by contact action.

Amount of DDT recovered from the surfaces of leaves by washing following application of different preparations: As a corollary to the foregoing, further experiments were conducted to investigate in detail the

amount of DDT recovered from the surfaces of apple leaves when deposited in the form of solutions, emulsions and suspensions. As representatives of low and high boiling solvents benzene and dekalin were chosen in the preparation of solutions and emulsions, and in the case of suspensions diacetone alcohol at the rate of 25% was used as the solvent. Triton-X-100 at the rate of 1% was used for stabilising in the case of suspensions, and 0.5% sodium oleate as emulsifier in the case of dekalin and benzene emulsions. Solvent at the rate of 10% was used in the preparation of the emulsions. All the preparations were as usual spread on known areas of leaf surfaces to give a concentration of 5 mgm per 100 sq. cm. and after allowing to stand for 24 hours, each leaf was individually washed with 6 cc. of benzene, and the extract obtained analysed as usual. The residual toxicities of the washed leaves were also determined by confining insects on them. The results obtained for chemical and biological tests are presented in Table III. Results obtained for similar preparations when recovery was made by continuous shaking for 15 minutes are also presented side by side for the sake of comparison.

TABLE III

Amount of DDT recovered from the surfaces of apple leaves after depositing in various forms by washing and shaking procedures, and the residual toxicity of the washed leaves.

Solvent used and percentage	Chemical estimation					Biological test			
	Washing Technique				Shaking Technique				
	% DDT in the preparation	DDT Deposited mgm.	DDT Recovered mgm.	% Recovered	% DDT Recovered	Number of insects tested	Number Dead	% Dead	Remarks
Solutions									
Benzene	1·0	5·0	2 587 (1)	51·74	74·75	81	2·0	2·4	Washed leaves
						75	0	0	Controls
Dekalin	1 0	5·0	1·181 (1)	23·62	73·75	77	3	4	Washed leaves
						75	0	0	Controls
Emulsions									
Benzene (10%)	1·0	5·0	3·420 (1)	68·50	90·18	75	0	0	Washed leaves
						73	0	0	Controls
Dekalin (10%)	1·0	5·0	3 345 (1)	66·00	85·48	74	0	0	Washed leaves
						74	0	0	Controls
Suspensions									
Diacetone-alcohol (25%)	0 9034	4 517	3 710 (2)	82·14	91·04	...	...	...	...

(1) Each figure represents average of 4 estimations.
(2) do. do. do. 6 do.

The amounts of DDT recovered by the washing technique are in all cases lower than those obtained by the shaking procedure. The suspensions give the maximum recovery and are followed by emulsions and solutions. The order of recovery either by the washing or shaking technique is the same, but only the actual amounts recovered are

different. The difference in percentage recovery between the washing (82.14%) and shaking procedures (91.04%) is nearly 9% in the case of suspensions which shows that most of the insecticide being in a suspended state is only on the surface and is easily removed by washing. The extra 9% obtained on shaking is probably due to the small quantity of insecticide which penetrated the tissue along with the large quantity of the solvent used in the preparation of the suspension. In the case of emulsions and solutions the differences in recoveries by the two procedures are considerably large, the emulsions occupying an intermediate position between solutions and suspensions. These results are as expected and fit in with theoretical considerations.

TABLE IV

Average percentage recovery values and their relative proportions when DDT is deposited, in different preparations

Name of surface and preparation tested	Extraction procedure adopted		% excess recovery over the washing method	Ratio of shaking to washing procedure	Relative recoveries	
	Washing	Shaking			Washing	Shaking
Benzene solution leaf ...	51·74	74 75	23 01	1·445	1·00	1·00
Benzene emulsion leaf ...	69·50	90·18	21·68	1·310	1·325	1·21
Diacetone-alcohol suspension on leaf ...	82·14	91·04	8·90	1·109	1·590	1·22
Benzene emulsion on glass ...	100·00	100·00	0·00	1·00	1·93	1·34
Dekalin solution on leaf ...	23·62	73·75	50·13	3·10	1·00	1·00
Dekalin emulsion on leaf ...	66·50	85·48	18·58	1·16	2·83	1·16
Diacetone-alcohol suspension on leaf ...	82·14	91·04	8·90	1·109	3·47	1·23

The large percentage difference in recovery found in the case of solutions (Col. 4, Table VI) clearly shows that the insecticide penetrated the tissue and is recoverable only on continued shaking. It is the maximum in the case of dekalin (50·13%) and as already suggested is to be attributed to its greater non-volatility. Considering the relative recovery values, estimating by the washing procedure (cols. 2 and 6 of Table IV) for every one part of DDT recovered when deposited in the form of solution in benzene, 1·325 and 1·590 parts are recovered when deposited as emulsion and suspension respectively. Similarly, considering the relative quantities recovered by the shaking procedure (cols. 3 and 7 of Table IV), they are 1·0, 1·21 and 1·22 respectively. The same sort of relationship is exhibited when the recoveries with dekalin solution, dekalin emulson and diacetone alcohol suspension are considered. The

lower relative recovery values by the shaking procedure suggest, that as a result of prolonged shaking the inequalities due to the nature of preparations i.e., solvent etc., have been levelled up, and more or less uniform conditions establised. Values obtained by the washing technique seem to reveal better the differences existing between the different preparations and also fit in very well with theoretical considerations. Dekalin being a solvent with low volatility penctrates deeper and leaves less of the insecticide on the surface to be extracted. Either the washing or the shaking technique is capable of revealing the relative performances of the various preparations, but which of these two is better remains to be decided.

The residual toxicities of the washed leaves were tested in all cases (Table III), and in no case was any significant till recorded, including leaves treated with dekalin solution where only 23% recovery was obtained and a residue of 77%, equal to about 38·2 microgrammes of DDT per sq. cm. was left over. This clearly shows that even quantities of insecticide of this magnitude not recovered by washing are not toxic to insects like *Tribolium* which are killed by contact action and confirms the earlier observations.

Toxicity of DDT when deposited in different forms and on different surfaces: Having found by chemical estimations that the recovery values for DDT when deposited in various forms were different, it was next intended to see whether they could be correlated with their biological performances. In the chemical estimation the concentration of DDT was invariably 1%, and this was purposely chosen in view of the limitations placed on the available analytical methods. As this high percentage would be quite unsuitable for biological tests, from a knowledge gained from preliminary experiments 0·08, 0·04, 0·02, 0·01 and 0·005 percent concentrations were chosen as the most suitable, the test insects being *Tribolium*.

Two sets of experiments were conducted. In the first one the relative biological performances of suspensions, emulsions and solutions were compared, and in the other the effect of the nature of solvent used in the preparation of the emulsion and the nature of the surface tested were compared. The results were statistically examined where-ever necessary and practicable.

The concentration of the solvent used in all these cases (excepting solutions) was 1% and that of the emulsifier 0·05% if it was product M. B. 320, and 0·02 cc. if it was Trition X — 100 (V/V). The required quantities of preparations (0·1 cc. for every 8 sq. cm.) were accurately measured and uniformly spread. The insects were transferred as usual and counted after 144 hours. In Tables V and VI and Chart 1 the results of tests conducted with suspensions, solutions and emulsions on leaf are presented, and in Tables VIII and IX and Chart 2 those with dekalin and benzene emulsions on leaf are presented. With the idea of confirming the absorption of the insecticide by the leaf surface, a non-absorbent surface like glass was included for comparison. The results are considered first on their face value and then statistically examined.

TABLE V

Relative Toxicities of DDT Solutions, Emulsions and Suspensions to *Tribolium confusum*

Age of insects ... 0 to 60 days.
Amount of solvent used in the preparation of emulsions and suspensions 1·0%.
Emulsifier used—Product M. B. 320 at 0·05%.

Nature of preparation	Concentration of DDT in the preparation %	Micro grams of DDT per sq. cm.	Test 1			Test 2		
			No. of insects used	No. dead	Percentage dead	No. of insects used	No. dead	Percentage dead
Glass Surface								
Benzene emulsion	0 08	10·0	89	84	94·4	...	...	...
	0 04	5·0	89	64	80·9	...	...	...
	0·02	2·5	90	62	68·9	...	...	...
	0 01	1 25	90	24	26 7	...	...	...
	0·005	0 625	89	3	3·4	...	...	...
Leaf Surface								
Benzene solution	0·08	10 0	90	53	58·9	91	35	38 4
	0·04	5·0	90	49	54·4	90	6	6·7
	0 02	2·5	92	1	1·1	90	0	0
	0·01	1·25	91	0	0	90	0	0
	0·005	0 625	90	0	0	90	0	0
Benzene emulsion	0·08	10·0	91	87	95·6	85	84	98·9
	0·04	5·0	88	60	68·2	90	64	71·1
	0·02	2·0	91	20	22·0	87	14	16 1
	0·01	1·25	90	1	1·1	90	0	0·
	0 005	0·625	90	0	0	90	0	0
Pyridine suspension	0 08	10·0	90	88	97 8	...	...	...
	0·04	5·00	90	78	86·7	...	...	...
	0 02	2·5	90	24	26 7	...	...	...
	0 01	1·25	90	1	6·7	...	...	...
	0 005	0·625	90	1	1·1	...	...	...
Controls			75	0	0	75	0	0

Considering first the relative performances of suspensions, emulsions and solutions on leaf amongst themselves (Table VI and Chart 1), and of all these against the performance of a benzene emulsion on glass, it is found that L. D. 50 is the lowest on glass (0·0170% or 2·21 micrograms per sq. cm.) which establishes the superiority of non-absorbent glass surface over that of leaf. On leaf, 6·56 micrograms per sq. cm. are needed if it is deposited in form of a solution, 3·91 micrograms in the form of an emulsion, and 2·83 micrograms in form of a suspension. The results, therefore, agree very well with theoretical considerations, and support the conclusions drawn from the chemical estimations. The percentage recovery values in the chemical estimations and their relative proportions, along with the results obtained in the biological tests (L. D. 50'5) are presented in Table VI. It is seen that the percentage recoveries or the relative values obtained in the chemical estimations by the washing technique are more closely related to the biological values than those obtained by the shaking technique. A high percentage recovery by the chemical method gives a low L. D. 50, and vice versa.

TABLE VI

Correlation between chemical estimations and biological tests obtained with suspensions, emulsions and solutions of DDT.

Nature of surface and preparation	Chemical estimations		Biological tests		
	Washing technique and relative values	Shaking technique relative values	L. D. 50 as % DDT in preparation	L. D. 50 as micrograms DDT per sq. cm.	Relative values
Benzene emulsion on glass	1·93, 1·0	1·34, 1·00	0·0178	2·21	1·0, 0·34, ...
Pyridine suspension on leaf	1·59, 0·82	1·22, 0·91	0 0226	2·83	1·28, 0·43, 1 0
Benzene emulsion on leaf	1,33, 0·69	1·21, 0 90	0·0313	3·91	1·77, 0·60, 0·38
Benzene solution on leaf	1·00, 0·52	1·00, 0·75	0·0525	6·56	2·97, 1 0, 2·32

It may, therefore, be stated that the chemical recovery values are roughly inversely proportional to the L. D. 50'5, or in other words, the product of chemical recovery values and L. D. 50 is a fairly constant quantity. Applying this criterion in the present investigations it will be seen that the product of recovery by washing technique and L. D. 50 (excepting the case of solutions) varies from 2·0 — 2·3 (Col. 5, Table VII). On the other hand the product obtained by multiplying the recovery value obtained by the shaking method by L. D. 50 is not so very consistent (Col. 6).

TABLE VII

Relative potencies of the several preparations as judged by the chemical and biological tests and the product of both values.

Nature of preparation and surface	Relative recoveries by chemical estimation		L. D. 50	Product of recovery by chemical estimation X L. D. 50	
	Washing technique	Shaking technique		Washing technique	Shaking technique
(1)	(2)	(3)	(4)	(5)	(6)
Emulsion on glass ...	1·93	1·34	1·00	1·93	1·34
Suspension on leaf ...	1·59	1·22	1·28	2 04	1·56
Emulsion on leaf ...	1·33	1·21	1·77	2·30	2·14
Solution on leaf ...	1·00	1·00	2 97	2·97	2·97

A statistical examination of the data using the results of the first test (Table V), gave the following information.

Comparison :	The regression lines are :	P	L. D. 50%
Emulsion on leaf ...	$\gamma = 4{\cdot}278\ X - 1{\cdot}385$	< 0·9	0·0311 * (0·0313)
Solution on leaf ...	$\gamma = 3{\cdot}244\ X - 0{\cdot}637$	> 0 001	* (0·0546) * (0 0525)
Suspension on leaf ...	$\gamma = 4{\cdot}079\ X - 0{\cdot}678$	0·01–0·001	0·0247 * (0·0226)
Emulsion on glass ...	$\gamma = 2\ 686\ X - 1{\cdot}695$	0· –0·05	0·0170 * (0·0178)

* These are values actually obtained from the graphs and agree very closely with the calculated figures.

Comparison of treatment on glass and leaf (emulsions) gave $\chi^2_a = 44{\cdot}399$ ($P < {\cdot}001$) and $\chi^2_b = 16{\cdot}035$ ($P < {\cdot}001$) indicating the reduced toxicity on the leaf surface. The fact that the two lines depart significantly from parallelism, however, shows that the reduced toxicity of the deposit on the leaf cannot be wholly ascribed to the absorption by the leaf of a constant proportion of DDT applied, — either there is greater absorption from the less concentrated solutions or some other unidentified factors are involved.

Further examination of the results to determine which difference may be regarded as significant gave the following data.

Comparison		χ^2_a	P	χ^2_b	P
Emulsion on glass	Emulsion on leaf	44·339	<·001	16·035	<·001
Emulsion on leaf	Solution on leaf	0·572	0·7 — 0·6	0·526	0·7 — 0·6
Emulsion on leaf	Suspension on leaf	1·76	0·3 — 0·2	0·207	0·8 — 0·9
Suspension on leaf	Solution on leaf	2·093	0·2 — 0·1	0·301	0·8 — 0·7

These data again confirm the greater toxicity of a deposit on glass when compared with the same type of deposit on leaf. The apparently greater toxicity of suspension as compared with emulsion and solution agrees with the evidence obtained from the absorption of DDT by the leaf which is greater for the solution and least for the suspension, but the differences shown in the biological test are not significant at a probability level of $P = 0{\cdot}05$. This is largely due to the marked variability of insects in susceptibility, and the deviation of the determined mortality figures from the theoretical linear relationship between log. concentration and probit.

TABLE VIII

The effect of surface and nature of solvent used in the preparation of the emulsion on the toxicity of DDT.

Insect used and age :— *Tribolium confusum* ... 0 — 60 days
Benzene concentration in emulsion ... 1·0%
Emulsifier used ; — Sodium oleate ... 0·05%

Solvent used	Concentration of DDT in emulsion %	Micrograms of DDT per cm	Number of insects used	Number dead	% Dead
Glass Surface					
Benzene	0·08	10·0	60	53	88·3
	0·04	5·0	60	21	51·7
	0·02	5·0	60	19	31·7
	0 01	1·25	60	8	13·3
	0·005	0·625	60	0	0·0
Leaf Surface					
Dekalin	9·08	10·0	59	53	86·0
	0·04	5·0	59	13	86·0
	0·02	2·5	48	3	6·2
	0·01	1·95	50	3	0·0
	0·005	0·625	33	9	0·0

Solvent used	Concentration of DDT in emulsion %	Micrograms of DDT per cm	Number of insects used	Number dead	% Dead
Benzene	0 08	10·0	50	41	82·0
	0·04	5·0	50	18	36 0
	0·02	2·5	30	8	16·0
	0 01	1·25	50	0	0·0
	9·005	0·625	40	0	0·0
Benzene	0·08	10·0	85	64	75·3
	0 04	5·0	90	39	33·3
	0 02	1·5	88	4	4·5
	0 01	1 25	99	1	1·1
	0 005	0 625	99	0	0 0
Dekalin	0 08	10·0	90	69	76·7
	9·94	5·0	90	14	15·6
	0 02	2·5	88	2	2·3
	0·01	1·25	90	0	·0
	0·005	0·625	90	2	2 2
Controls			45	0	0 0
			50	0	0 0

Test — On leaves fixed to cells and counted after 7 days,

Test — In bottles as usual and counted after 6 days.

TABLE IX

Correlation between chemical estimations and biological tests
Benzene and dekalin emulsions on leaf and glass

Nature of surface and preparation	Chemical estimations				Biological tests		
	Washing technique		Shaking technique		D.D. 50 as % DDT in the prepa-ration.	L.D. 50 as micro-grams per sq. cm.	Relative values.
	Percen-tage reco-very	Rela. tive values	Percen-tage reco-very	Rela-tive values			
Test I							
Benzene emulsion on leaf ...	68·5	1·02	90 18	1·06	0.0468	5 85	1·0, —
Dekalin emulsion on leaf ...	66·9	1·00	85·48	1·00	0·0507	6·34	1·08, —
Test II							
Benzene emulsion on glass ...	100·0	1·50	100·0	1·17	0 0329	4·05	1·0, —
Benzene emulsion on leaf ...	68 5	1·32	90·2	1·057	0·0525	6·56	1·62, 1·0
Dekalin emulsion on leaf ...	66·9	1·00	85·5	1·0	0.0610	7 63	1·88, 1·16

In the other set of experiments where benzene and dekalin were chosen as solvents in the preparation of emulsions (Table VIII and IX, and Chart 2), and compared on glass and leaf surfaces, the L. D. 50's with benzene and dekalin emulsions on leaf were 0·0468% and 0·0507% (i.e. 1 : 1·08) respectively in the first test and 0·052% and 0·0610% (i. e., 1 : 1·16) respectively in the second test, and these bear a close inverse relationship to the chemical recovery values which were obtained as 68·5% for benzene emulsion and 66·9% for dekalin emulsion (Table IX).

That means, for every one unit of DDT deposited in the form of benzene emulsion the amounts required for giving the same percentage kill if dekalin is substituted are 1·08 and 1·16 units respectively as obtained in the two tests. Comparing the relative efficiencies of these two preparations (benzene and dekalin) on leaf against a similar preparation on glass, the latter surface is definitely superior to the former. The L. D. 50 in the case of glass and benzene emulsion is 0·329% (i. e., 4·05 micrograms per sq. cm.), and in the case of leaf and benzene emulsion 0 0525% (6·56 micrograms per sq. cm.). These values are in the ratio of 1·0 : 1·62, which again compares very favourably with the values obtained in the earlier experiments (Table VII), 1·0 : 1·77). That is, for every unit of DDT applied on glass surface in the form of benzene emulsion nearly 1·6 or 1·7 units must be deposited on the leaf surface to produce a similar effect. This is again in close agreement with the chemical recovery values where only 68·5% of DDT was recovered from the leaf surfaces when deposited in the form of benzene emulsion by washing technique (Tables III and IX). In other words for every 100 parts deposited on glass 150 parts of DDT must be deposited on leaf to give an equal effect, a result which is in close agreement with the values obtained in the biological tests (1·6 and 1·7). The chemical recovery values by the washing technique again appear to be related very closely to the biological values but in the inverse proportion. The product of these two factors as obtained in the present investigations are as follows :—

Relative potencies of the several preparations as determined by the chemical and biological tests and the product of both the values

TABLE X

Nature of Preparation	Relative recoveries by chemical estimation		L. D. 50	Product of chemical recovery value and L. D. 50	
	Washing	Shaking		Washing	Shaking
(1)	(2)	(3)	(4)	(5)	(6)
Benzene emulsion on glass ...	1·50	1·17	1·00	1·50	1·17
Benzene emulsion on leaf ...	1·02	1 06	1·62	1·65	1·72
Dekalin emulsion on leaf ...	1·00	1·00	1·88	1·88	1·88

Examining statistically the above results (Table VIII) we have :

Comparison	Regression lines are :–	P	L. D. 50%
Benzene emulsion on glass	$Y = 2·582 \times + 1·109$	1·2 — 0·1	0·0321 *(0·0329)
Benzene emulsion on leaf	$Y = 3·675 \times - 1·323$	> 0·9	0·0526 *(0·0525)
Dekalin emulsion on leaf	$Y = 4·938 \times - 3·748$	0·2 — 0·1	0·0591 *(0·0610)

*These are the values actually obtained from the chart by plotting probits against log. concentration.

The difference in slope and position when tested gave :—

Treatment compared		χ^2_a	P	χ^2_b	P
Benzene emulsion on leaf	/ Dekalin emulsion on leaf	4.735	0 05 — 0·02*	4·291	0·05 — 0·02*
Benzene emulsion on glass	/ Benzene emulsion on leaf	27·630	< 0·001*	5·548	0·02 — 0·01*
Benzene emulsion on glass	/ Dekalin emulsion on leaf	42·690	< 0·001*	17·710	< 0·001*

* Significant.

All the lines therefore, differ in the slope and position which suggest that an estimate of their relative potencies is not possible. The line for dekalin cuts the benzene line, which indicates that the reactions of the deposits are different. The results for benzene and dekalin emulsion differ significantly though not widely (1 : 1·12). Also, it is evident that the deposit on glass is significantly more toxic than that for the same emulsion on leaf.

Discussion : Reviewing the foregoing results it is seen that the nature of the surface and the mode of deposition have great influence on the toxicity of the insecticide. Absorption of the insecticide by the leaf surface both by chemical and biological tests has been established. This absorption is maximum following deposition as a solution and least from a suspension. Comparing similar preparations on glass and leaf, the recoveries from leaf are roughly two-thirds (66%) of the original amount deposited, and their relative performances as revealed by L. D. 50's are inversely proportional to the percentage recoveries obtained by washing procedure. The nature of the solvent used in the preparation of the emulsion can influence toxicity to some extent. A solvent with high boiling point like dekalin gives a less toxic deposit than one prepared by using benzene which has a low boiling point. Broadly speaking, the chemical recoveries are in agreement with theoretical considerations, and it would also appear that they might enable one to predict their insecticidal performance. For example if one finds by analysis that there is a surface concentration of 10 micrograms of DDT per sq. cm., 100% kill of an insect like *Tribolium* which is killed by contact action is certain. Conversely, if a deposit gives a kill of 100% with *Tribolium* in a period of six days the surface concentration of the insecticide may be assumed to be 10 micrograms per sq. cm. and over.

When examined statistically some results were found to be significant and some not. Making due allowance for the limitations placed on biological tests, it was however found that all results obtained were in agreement with theoretical considerations. Different preparations showed different toxicities, but whether their slopes are strictly parallel or not, and whether any strict comparison could be made will have to be decided by conducting further work with a variety of preparations and

insects. The tests conducted in the present investigations were confined to *Tribolium* which is killed by contact action. Under such conditions a preparation made with a solvent having a high boiling point is less toxic than one made with a solvent having a low boiling point. The same preparations, however, when used in the case of caterpillars which are killed by stomack poisoning action also may give different results.

Summary: Comparing the relative merits of recovering DDT when deposited in various forms by washing and shaking techniques it was found that mere superficial washing was enough to remove all the insecticidally useful portion. The recoveries effected by such a method seem to be related to the results obtained in the biological tests but in an inverse proportion. The nature of the solvent used in the preparation of the insecticide and the nature of the surface on which the insecticide is tested largely influence the insecticidal value of deposits.

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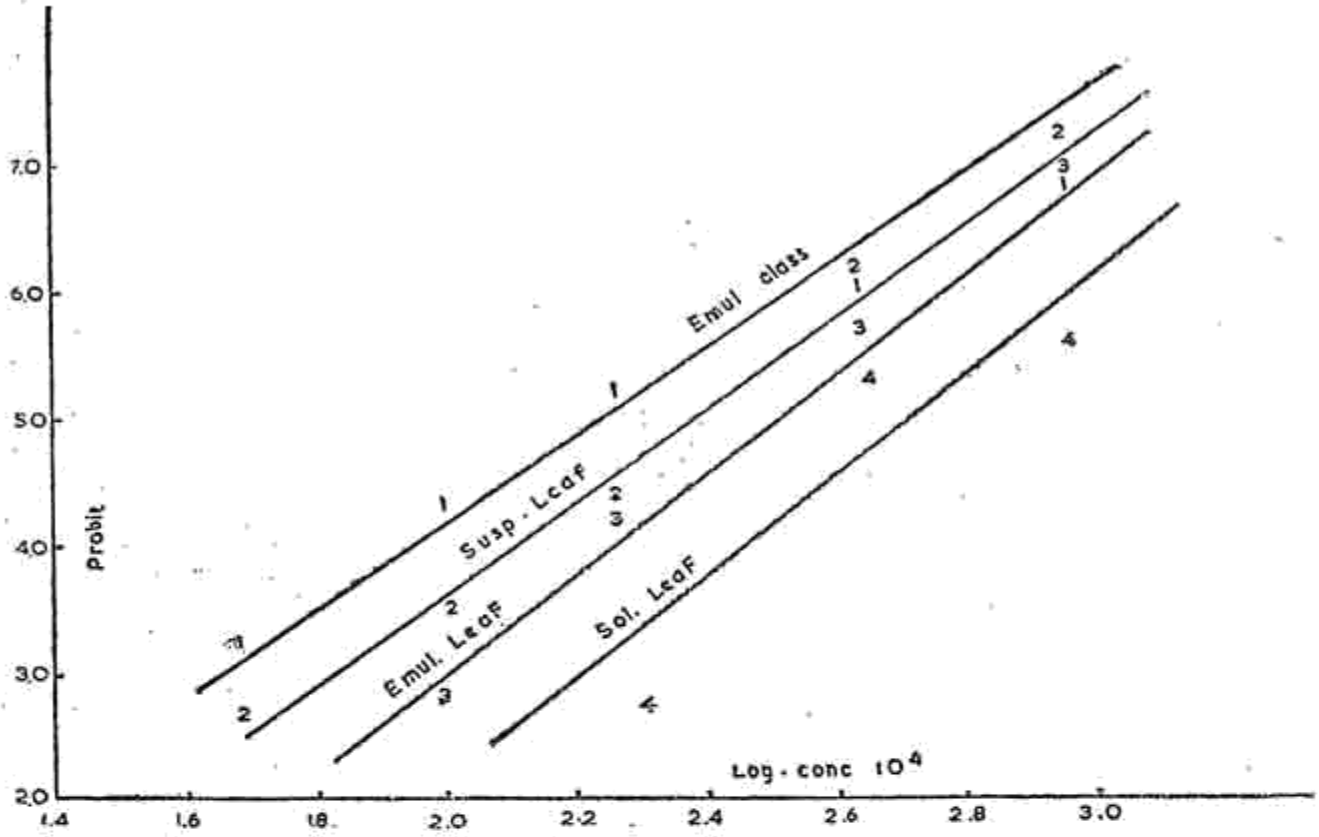

CHART I

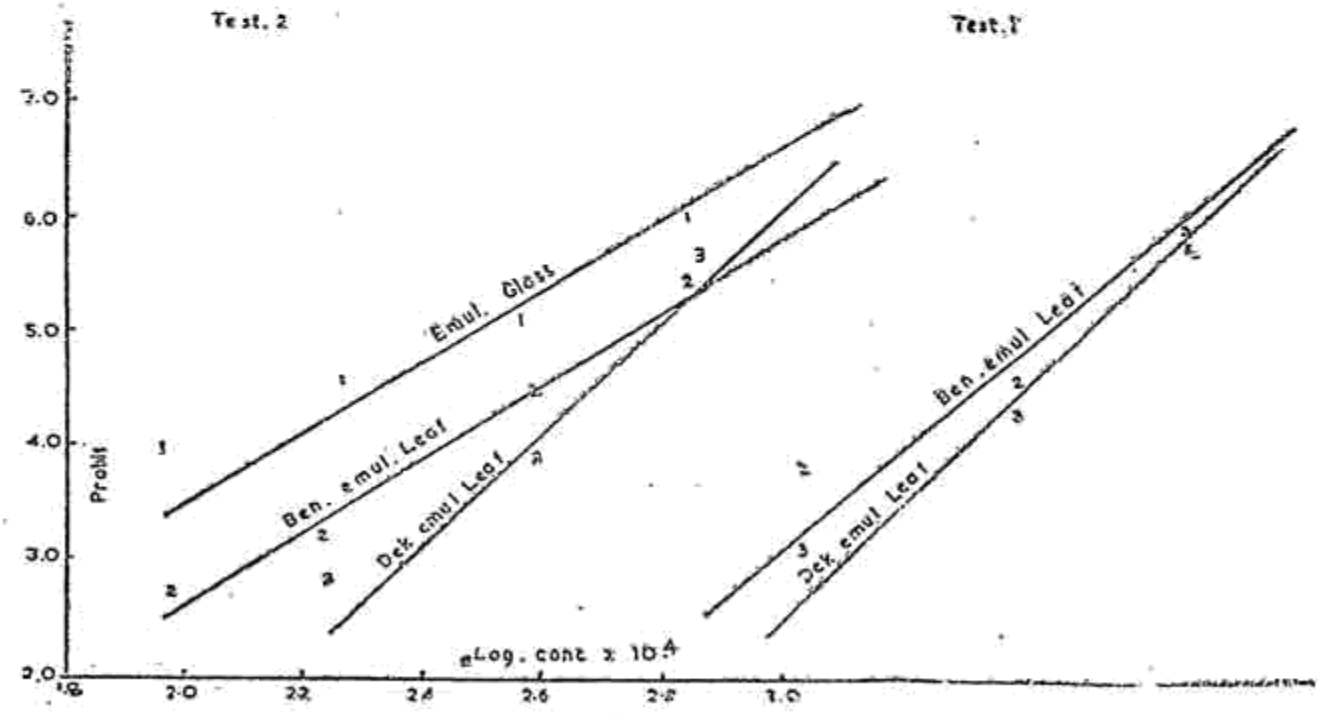

CHART II