

The Deposition and Retention of certain Plant Pest Control Materials in relation to their Biological Performance

*I. The Influence of the Mode of deposition and other factors on the recovery of 1 : 1 : 1 trichloro-2 : 2-bis (p-chlorophenyl)-ethane (D. D. T.) from leaf surfaces**

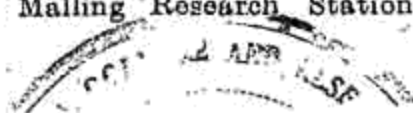
By

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Introduction: The release of 1 : 1 : 1 trichloro-2 : 2 bis-(p.-chlorophenyl)-ethane (p-p' DDT) for civilian consumption with the cessation of hostilities and its extensive use in agriculture and horticulture has given rise to a number of problems, many of which are still a matter of speculation and await solution. The voluminous literature available on the subject, is sometimes vague and contradictory. For example, the great stability and extraordinary persistence of DDT are well known (Fleck, 1944, Fleck and Haller, 1945, Balban and Sutcliffe, 1945). Cages treated with solution of DDT in kerosene killed flies even after a period of 8½ months (Lindquist, 1944), and one application of DDT was found sufficient to prevent an infestation of plums by the Japanese beetle for one full season (Fleming, 1944). Despite this accredited stability Gunther (1945) reported that deposits of DDT lost their toxicity in less than 2½ months under the climatic conditions of California where the shade temperature reached 125°F. Whether this loss is due to volatilization of the insecticide or to some other factors like absorption by the tissues or drying up of the films and dropping off is not known with any degree of certainty. Apparent contradictions of this nature may be attributed to the suddenness with which DDT has risen to prominence and to the fact that there is as yet no satisfactory method of estimating the p-p' DDT content (the insecticidally important isomer) of DDT samples.

To enable DDT to be applied in a variety of ways, such as dusts, suspensions, solutions, emulsions, aerosols etc., it is compounded with solvents like Freon and Tetralin which not only differ in their physical constants, but also in their chemical properties. This wide variety was not confined to the solvents alone, but extended to the emulsifiers as well. Applied in these multifarious forms it is but natural to expect variations in the biological performances of these preparations, and even the

*The investigations presented in this article formed part of a thesis submitted for the PH. D. degree of the University of London, and were conducted at the East Malling Research Station, Kent, during the years 1946-1947.



same preparation is likely to react differently depending upon the nature of the surface, the prevailing climatic and weather conditions, and the test insect. For example, when a preparation is tried on a non-absorbent surface like glass its response will be something different from that obtained when the same preparation is made on a porous and absorbent surface like a mud wall, especially if the solvents used in the compounding of the insecticide possess such strong penetrative powers that the solvent penetrates the tissue, carrying the insecticide with it. Thus the amount of the insecticide that is rendered unavailable and ineffective depends upon the mode of its deposition, the nature of the solvents used in the preparation of the insecticide, and the nature of the surfaces used.

The ability of non-agricultural materials like clothes, woodwork and many articles used in building construction to absorb DDT preparations, is well known. Stiff and Castillo (1946), studying the absorption of DDT by building materials like wood, canvas, rubber, etc., found that a greater portion of the insecticide penetrated into the materials when deposited in the form of a straight solution. Estimating by a rough qualitative test, they demonstrated the differential absorption capacities of the several materials, and concluded that similar results might be obtained with vegetation. Parkin and Hewlett (1946), working on the absorption of DDT by building materials (especially stones), demonstrated by biological tests the large-scale penetration of the insecticide. They showed that this could be effectively checked by pre-treating the surfaces with material like starch, size, etc., which are impenetrable and non-absorbent.

Ebeling (1944) was the first to demonstrate the absorption of DDT by agricultural crops. Leaves and twigs of Citrus when painted with DDT in kerosene were found to have absorbed considerable quantities of the insecticide, but this could be retarded to a great extent by preparing emulsions using aluminium stearate. Gunther (1946) sprayed orange and lemon trees with preparations of DDT made from kerosene and mixtures of kerosene with Tetralin and Velsicol A. R. 60, and found that the amount left as a residue on the surface varied with the nature of the solvent. He also found that with certain of the solvents used, the insecticide first penetrated the tissue and came up to the surface subsequently. Symes (1946), and Barlow and Hadaway (1946, 1947), working on the control of tsetse fly and mosquito in Uganda, reported considerable loss of the insecticide by absorption by the leaves and mud walls. The amount absorbed varied with the nature of the vegetation. A portion of the insecticide that was sprayed was found to be easily recovered, whereas the rest was not. This sort of penetration was not only confined to leaves which have stomata to facilitate the process, but was found to occur, by Wichmann (1946), in the case of apples which were hard and smooth.

Apart from the few references cited above, no other strict experimental evidence seems to be available in literature to indicate the result of depositing a known quantity of DDT on any specific surface and attempting to recover it. Previous investigators like Fathey (1945), Gunther (1945), and Wichmann (1946), who studied the accuracy and suitability of the methods available for the estimation of DDT sprayed their experimental materials by the ordinary orchard practice. In all such cases it may be possible to spray uniformly, but it is not possible to deposit any desired amount with exactitude. This limitation was well recognised by Symes (1946), who aptly observed that an attempt to spray 100 mgm. per square foot might result in a deposit varying from 50-300 mgm. By the ordinary spray practice it may be possible to obtain samples which are uniformly sprayed, and analysis of duplicates drawn from such samples are bound to agree since the absorption and loss of the insecticide, if any, would be common as the spraying is done under a standard set of conditions. The agreement between duplicates does not, therefore, prove the existence or non-existence of any factor. Unless one knows exactly how much is deposited, one cannot be sure how much is recoverable. Especially, when straight solutions are dealt with, the loss by penetration and absorption is maximum, and the time lag that intervenes between deposition of the insecticide and its estimation is sufficient to allow most of the insecticide to disappear. A balance sheet must, therefore, be drawn up before the loss of insecticide either by absorption or from other causes can be established.

Object and scope of the present investigations: In view of the insufficient and conflicting information available in the literature, it was proposed to study the absorption of DDT by vegetation under a variety of conditions and obtain information on:

- (i) The influence of the nature of solvent used in the preparation, on the recovery of DDT.
- (ii) The amount of insecticide recovered when equal quantities are deposited in the form of solutions and emulsions.
- (iii) The effect of the nature of the emulsifier used in the preparation of the emulsion and the nature of the emulsion on the recovery of insecticide.
- (iv) The concentration of the insecticide in the preparation, the concentration of the solvent used in the preparation of the emulsion and the nature of the surface on the amount of DDT recovered.

Methods and Materials: A perusal of literature showed that in many cases the exact details were not available, and even the few that were available differed widely from deposition to estimation of the insecticide.

Ebling deposited the insecticide by painting with camel hair brush ; Stiff and Castillo distributed it in the form of fine drops and then spread it into a thin film ; Gunther, Symes, and Barlow and Hadaway adopted the ordinary spray practice. The last two workers also deposited with a micropipette exact amounts in some cases. After deposition, the materials were allowed to remain for 48 hours before being extracted in the case of Stiff and Castillo, whereas Gunther analysed them from the time the drip ceased to 86 days after spraying. The quantities of material used for extraction also were widely varying. Fathey, Gunther, and Wichmann used 25 apples in every trial. Ebeling extracted 60 citrus leaves having an area of 2,000 sq. cms. ; and Gunther (1946) employed 75 leaves of orange or lemon.

Benzene appears to have been uniformly selected as the solvent for extraction, and the extraction procedure ranged from mere washing with a jet of benzene by Gunther, to an extraction for a period of 15-30 minutes as suggested by Carter and Hubanks (1946). Symes, and Barlow and Hadaway removed the surface deposits by washing every square foot of leaf sample with two 20.0 cc. portions of benzene. The extracted material was then dried, powdered and re-extracted in a Soxhlet to recover the insecticide which penetrated the tissue.

The hydrolysable chlorine method of estimating DDT was chosen by Gunther, Barlow and Hadaway, Carter and Hubanks, and Wichmann *et al.* The latter two also used the total chlorine method by reducing with sodium and iso-propyl alcohol. Fahey (1945), on the other hand adopted the combustion method of Winter. When the hydrolysable chlorine method was adopted, hydrolysis was carried out for 30 minutes by Wichmann *et al.*

In view of the above - mentioned variations, it was found necessary to evolve a suitable technique which would be practicable, reproducible and capable of extracting the insecticide which would normally be considered useful for insecticidal purposes. It was also intended to allow maximum contact between the insecticide and the material under test so that the absorption factor could be investigated. The solvents used for the preparations of solutions and emulsions were chosen on a broad basis so that they differed not only in their physical characteristics but also in their chemical nature. Similarly, the emulsifiers used were chosen so as to represent different ionic activities.

Apple leaves (Cox's Orange Pippin) were used in all the tests. After wiping off the moisture with a clean towel, the freshly picked leaves were cut to the desired area, either 16.0 sq. cm. or 20.0 sq. cm. by superimposing a cardboard of known area over them, and trimming off the edges. The cut leaves were weighted down to flatten them and then spread in petri dishes for the deposition of the insecticide. 200.0 sq. cms. of leaf area was used for each test.

Except where the effect of the concentration of the insecticide on its recovery was studied, all the preparations contained 1.0% of p-p' DDT. (Pure p-p' DDT was obtained by crystallising the commercial product thrice from 98% alcohol and had a m. p. 107-108°C.). No special procedure was involved in the preparation of the solutions.

In the case of emulsions, where soap was used as the emulsifying agent, little difficulty was experienced for the emulsions could be easily prepared by mere shaking by the hand, but with other emulsions, however, the emulsions were not easily formed, and they had to be put through an emulsifying mill. Thus, in all cases where the relative efficiencies of the different emulsifiers were compared; the requisite quantity of the insecticide, solvent, emulsifier and water were mixed and the mixture put through the mill four times. Good stable emulsions were thus obtained and their final DDT content determined by analysis. In all cases the amount of solvent used was the minimum required to dissolve the insecticide.

In a few preliminary experiments conducted with the idea of depositing uniform quantities by spraying with Tattersfield's apparatus, it was found that the amount of insecticide deposited varied with the solvent in the preparation of the emulsion. (Table I)

TABLE I

Amount of DDT deposited when emulsions prepared with different solvents are sprayed with Tattersfield's apparatus.

Sprayed in petri dishes
of uniform diameter

Pressure used :— 35 cms.
Vol. of emulsion
used in each case } 2.0 c.c.

Volume of solvent in emulsion :— 12.0%

Emulsifier :— Sodium oleate 1.0 %.

Solvent used for preparing the emulsion.	% of DDT in emulsion	Amount of DDT deposited (mgm.)
Benzene	0.9647	4.627
Dekalin	1.0070	6.151
Cotton seed oil	1.0420	5.832

Depositing by this method was, therefore given up, and the following technique adopted.

Portions either of solution or emulsion 1 c. c. in volume, containing about 10.0 mgm. of DDT, were measured accurately with a micropipette and deposited as uniformly as possible by gently leading the end of the pipette over the entire leaf area. In the case of solutions the liquid penetrated quickly, but in the case of emulsions, as the penetration was not so quick the liquid was uniformly deposited in the form of fine drops over the entire area, and then spread by means of a bent needle. Whereas no particular difficulty was experienced in the case of the upper smooth surface, the presence of midrib and veins on the under surface made the uniform distribution rather difficult.

After deposition the materials were left in a room at a fairly constant temperature near 15°C (there being no direct sunlight) for a period of 24 hours. At the end of that period the leaves were carefully transferred to a 500 c.c. conical flask without touching the deposits on them, (the petri dishes in which they were kept were wiped with a swab of cotton wool dipped in benzene and this was also put in the flask) and were shaken successively three times with 50.0, 30.0 and 25.0 c. c. portions of benzene. The flasks were well stoppered, and the shaking done by hand each time for a period of five minutes. The whole shaking operation, therefore, took fifteen minutes to complete and this was assumed to be sufficient to recover all the insecticide that would normally be considered useful. At the end of each shaking period the solutions were decanted into a 100.0 c. c. volumetric flask, finally made up to volume, and aliquots of 50.0 c. c. taken for analysis.

Since pure p - p' DDT was employed throughout, the estimation of DDT was done by an estimation of its total chlorine content by reducing with sodium and ethyl alcohol. After removing benzene the reduction was effected by using 2 grams of sodium and 25.0 c. c. of ethyl alcohol (98%) at 80° for 30 minutes. The contents of the flask were diluted with water, neutralised with nitric acid and after adding nitrobenzene and ferric nitrate the chloride was estimated by the usual Volhard procedure.

Discussion: *The effect of the nature of solvent used in the preparation of the solution on the recovery of DDT (Table 2).*

TABLE 2
The effect of the nature of solvent used in the preparation of the solution on the recovery of DDT.
 1.0 c.c. of 1.0% solution containing 10.0 mgm. of DDT,
 distributed over a leaf area of 200 sq. cms.

	Solvent used	Specific gravity	Boiling point °C	DDT % Recovered		Increase or decrease of the upper surface over the lower.
				Upper surface	Lower surface	
1.	N-hexane	0.6603	69	78.02	69.45	+8.57
2.	Benzene	0.8794	80	74.75	70.91	+3.84
3.	Petroleum ether	...	80-100	69.50	60.99	+8.51
4.	Pyridine	0.9029	115.3	74.99	74.30	+0.69
5.	Diacetone alcohol	0.9306	164-166	52.49	52.49	0.0
6.	Dekalin	0.934	188-190	73.75	57.89	+15.86
7.	Cotton seed oil	0.917	...	70.43	85.09	-5.66

The boiling points of the solvents tested ranged from 70° - 100°C and their specific gravities from 0.66 - 0.93. They differed too in their chemical properties : two were aromatic and two aliphatic hydrocarbons, one alcohol, one base and one vegetable oil. The differential property of the last solvent, cottonseed oil, from the rest of the solvents was exhibited throughout with both solutions and emulsions. It had neither the penetrative power, nor the scorching effect, produced by the rest.

In all cases when DDT was deposited in the form of a solution, cotton seed oil excepted, there was severe scorching action on both the surfaces, and at the end of the 24 hour period crystalline deposits were noticed when the leaves were taken up for analysis. Cotton seed oil being non-volatile, did not leave any visible white deposit, but only oily patches were present. The appearance of the insecticide on the outside, therefore, did not appear to be an indication of the amount recoverable, since the values obtained in the case of cotton seed oil where no deposits were visible were higher than those obtained with dekalin, etc., where deposits were clearly visible on the surface. Working with 1% solutions, and under the experimental conditions where the insecticide was given an opportunity of remaining in contact with the surface for a sufficiently long time, 100 per cent recovery was not possible in any case, even after shaking for 15 minutes.

Excluding cotton seed oil, it appears as though there is a slight correlation between boiling point of the solvent and the recovery of DDT. If a generalisation is permitted, preparations made with solvents having low boiling points give higher recoveries, whereas those made with solvents having higher boiling points give lower ones.

It does not, however, appear to be easy to establish any relationship between the chemical nature of the solvent and recovery of the insecticide. Gunther (1946) working with preparations made from kerosene and other auxiliary solvents, also met with a similar experience. When preparations made either with kerosene or with 90% kerosene and 10% tetralin as auxiliary solvent were sprayed, the insecticide first penetrated the tissue along with the solvent, and was subsequently brought to the surface during the next 24 hours. But no penetration and emergence was found to take place when the solvent used was a mixture of 90 parts of kerosene and 10 parts of Velsicol A. R. 60. No explanation for this differential behaviour was given, but it was suggested that when such preparations are employed it is advisable to draw the sample 24 hours after spraying, by which time the insecticide would have had enough time to stabilize itself. Symes (1946), and Barlow and Hadaway (1946) sprayed scrub vegetation with 5% DDT dissolved in 50% kerosene and 50% cotton seed oil and found that of a total of 104 mgm. recovered 54 mgm. were obtained by mere washing of the surface, and the rest (50 mgm.) from inside after continued extraction in a Soxhlet. They suggested a

a differentiation of DDT into two portions, one the "inner" which is recovered with difficulty by extraction and the other "outer" which is easily recovered by washing of the surface.

The amount recovered from the lower surface is usually slightly less than that recovered from the upper and this difference might be due to the ease with which the solvent penetrates the tissue through the stomata as indicated by Rohrbaugh (1934).

The effect of the nature of solvent used in the preparation of the emulsion on the recovery of the insecticide. (Table 3). As usual cotton seed oil behaved differently from the rest. In all cases when emulsions were deposited on the lower surfaces there was scorching (browning) of the tissue. No such effect was noticed when they were deposited on the upper surface, not even when the emulsion contained 95% benzene. (Table 6). In the case of cotton seed oil there was no scorching on any side, but only oily patches were visible. There seems to be little relationship between this scorching effect and the recovery of the insecticide.

As the emulsions dried, with all excepting cotton seed oil, white deposits of DDT became visible on the surface. As in the case of solutions this was again found to have no direct correlation with the amount recovered. The recovery values from both the upper and lower surfaces were considerably higher than the corresponding values obtained with solutions, but still in no case was 100% recovery possible. As in the case of solutions, emulsions prepared by using solvents having a low boiling point gave slightly higher values which again suggests the existence of a correlation between volatilisation and recovery. The large differences in recovery noticed between the low and high boiling solvents when used in the form of solutions were considerably levelled up in this series and were attributed to the physical states of the two sets of preparations.

TABLE 3

The effect of the nature of the solvent and the emulsifier used in the preparation of the emulsion on the recovery of DDT. 1.0 c.c. of emulsion spread over 200 sq. cms. of leaf area in each case.

Solvent used	% of solvent in the emulsion	% of DDT in the emulsion	Emulsifier used	% of emulsifier in the emulsion	% Recovery		Increase or decrease of the upper surface over the lower
					Upper Surface	Lower Surface	
Benzene	3.0	0.989	Sodium oleate	0.50	88.40	85.31	+3.09
	3.0	0.993	Product M.B. 220*	0.025	92.87	81.31	+11.56
	3.0	1.007	Fixanol	0.025	88.25	89.51	-1.33
	3.0	1.064	Triton-X-100†	0.500	90.70	83.99	+5.01
Average.					90.6	85.05	+5.01

Solvent used	% of solvent in the emulsion	% of DDT in the emulsion	Emulsifier used	% of emulsifier in the emulsion	% Recovery		Increase or decrease of the upper surface over the lower
					Upper Surface	Lower Surface	
Petroleum ether (80-100°) B. P.	30.0	1.091	Sodium oleate	0.50	92.31	94.91	-2.60
	30.0	0.993	Product M.E. 320	0.10	92.78	91.57	+1.21
	30.0	0.936	Fixanol	0.10	92.38	89.33	+3.05
	30.0	0.950	Triton-X-100	0.50	88.05	91.06	-3.01
Average.					91.38	91.72	-0.34
Dekalin	9.0	0.9175	Sodium oleate	0.50	85.02	86.53	-1.56
	9.0	0.9501	Product M.B. 320	0.025	83.62	77.64	+5.98
	9.0	1.077	Fixanol	0.025	85.60	84.28	+1.32
	9.0	1.035	Triton-X-100	0.50	97.68	93.17	-5.49
Average.					85.48	85.42	+0.06
Cotton seed oil	12.0	1.007	Sodium oleate	0.50	83.12	84.80	-1.68
	12.0	1.042	Product M.B. 320	0.10	88.46	83.03	+5.43
	12.0	1.085	Fixanol	0.10	86.28	86.28	0.0
	12.0	1.106	Triton-X-100	0.50	85.92	85.92	0.0
Average.					85.95	85.01	+0.58

* Cyclohexylamine salt of sulphonated lorol (anionic)

x Cetyl Pyridinium bromide (kationic)

† Alkylated, aryl, polyether alcohol.

Amount of DDT recovered when deposited in equal amounts in the form of solution and emulsion. (Table 4). As the physical states of solutions and emulsions are different, their reactions towards the surfaces on which they are deposited are also bound to be different. The resultant effect of all these will be reflected in the recovery values, and the results of the present investigations bear out this fact. The solutions being highly reactive penetrate the tissue quickly carrying the insecticide with them, and possibly bring it again to the surface as they evaporate. The emulsions, on the other hand, are not so reactive and do not penetrate so quickly, as the solvent used in the preparation of the emulsion is enmeshed by the layer of the emulsifier and the continuous phase. With all solvents, excepting cotton seed oil where it is apparently immaterial whether it is deposited as a solution or an emulsion, higher values were obtained when they were deposited as emulsions than in solutions. The differences in percentage recoveries vary from 6-40%. Ebeling, Gunther, Symes, Barlow and Hadaway, Stiff and Castillo, had similar experiences. Ebeling, working with 4% preparations obtained 15% recovery with solutions and 62% with emulsions.

TABLE 4
Average percentage recovery values of DDT obtained from the surfaces of apple leaves when deposited in the form of solutions and emulsions.

Solvent used	% DDT in preparation	Upper surface			Lower surface		
		Emulsions %	Solutions %	% difference between Emulsions & Solutions	Emulsions %	Solutions %	% difference between Emulsions & Solutions
N-Hexane	1.0	93.61	78.02	+15.59	75.45	69.45	+6.00
Benzene	1.0	90.18	74.75	+15.43	85.05	70.91	+14.14
Petroleum ether	1.0	91.38	69.50	+21.88	91.84	60.99	+30.85
Dekalin	1.0	85.48	73.75	+11.73	85.42	51.12	+27.30
Cotton seed oil	1.0	85.95	79.43	+6.52	85.01	85.09	-0.08
Benzene	0.7	85.80	64.75	+21.05	70.66	49.63	+21.03
"	0.4	71.33	49.66	+21.67	78.19	35.48	+42.69

The nature of the emulsifier used in the preparation on the recovery of DDT. (Table 5). The nature of the emulsifier, whether it be anionic, kationic or non-ionic, had no appreciable influence on the recovery. Whatever be the emulsifier used, cotton seed oil gave very poor emulsions. Using soap, the emulsions were easily prepared, even by mere shaking with hand. The remainder had to be put through the mill to obtain satisfactory emulsions. The emulsions prepared by using Fixanol as emulsifier seemed not to wet the glass surfaces in which they were stored.

TABLE 5
Effect of concentration of DDT in the preparation on its recovery

% of DDT in preparation	DDT Deposited mgm.	Upper Surface		Lower Surface	
		DDT recovered mgm.	% recovered	DDT recovered mgm.	% recovered
BENZENE SOLUTIONS					
1.000	10.0	7.475	74.75	7.091	70.91
0.700	7.0	4.537	64.75	3.475	49.63
0.3997	3.997	1.986	49.66	1.418	35.48
BENZENE EMULSIONS (Emulsifier — Sodium oleate at 0.5%)					
0.9891	9.891	8.739	88.40	8.437	85.31
0.7025	7.025	6.027	85.80	4.904	70.66
0.4076	4.076	2.907	71.33	3.187	78.19

Recovery as influenced by the concentration of the insecticide in the preparation. The amount of DDT recovered, expressed as a percentage of the amount deposited, decreased with the decrease in concentration both in the case of solutions and emulsions.

This decrease was greater in the case of solutions than with emulsions, and as usual the lower surface gave smaller values than the upper.

This lowered recovery with decrease in concentration may have an important practical bearing, especially when low concentration of the insecticide are applied. If the surfaces sprayed have great absorptive power most of the insecticide sprayed penetrates and gets locked up, while very little remains on the surface to act as a contact insecticide.

Effect of the concentration of the solvent used in the preparation of the emulsion on the recovery of the insecticide. (Table 6). Benzene was used as solvent with 0.5% sodium oleate as the emulsifier. The amount recovered decreased slightly with concentration of solvent in the initial stages, and remained fairly constant subsequently. The recovery values, even with 95% benzene in the emulsion, were much higher;—88.3% and 82.9% respectively from the upper and lower surfaces compared to values similarly obtained with solutions, viz., 75% and 71%, which clearly illustrates the differential nature of the emulsion from the solution. An emulsion containing even 95% benzene is, therefore, different in behaviour to a solution which contains 100% of it.

TABLE 6
Effect of the concentration of solvent in the emulsion on the recovery of DDT.

% solvent in emulsion	% recovered		Increase of decrease of the upper over lower surface.
	Upper Surface	Lower Surface	
2.0	95.72	95.72	0.0
6.0	78.72	79.43	-0.71
25.0	79.43	70.92	+8.51
50.0	87.23	85.10	+2.13
75.0	86.09	85.10	-0.01
95.0	88.30	82.96	+5.34

Influence of the surface on the amount of DDT recovered. (Tables 2, 3 and 6).

Whereas lower recoveries were usually obtained from the lower surfaces when DDT was applied in the form of a solution, the effect of the surface on the recovery was practically nil when deposited in the

form of an emulsion. The almost parallel recovery figures in the case of emulsions from both the upper and lower surfaces taken in conjunction with the absence of any injury when the emulsions are applied on the upper surfaces of leaves, strongly suggests that there is hardly any relationship between the amount of DDT recovered, and the scorching effect produced either by the emulsions or the solutions.

From a consideration of the several points discussed in the previous sections, it is evident that when the insecticide is allowed to remain in contact with the leaf surface for a sufficiently long time all the material originally deposited is not recovered. The amount recovered varies with the nature of the solvent used in the preparation; the mode of deposition, i. e. whether in the form of solution or emulsion; the concentration of the insecticide in the preparation, and the nature of the surface tested, etc. The recoveries in the case of solutions are always low, and though they are higher in the case of emulsions they are never complete. This failure to attain complete recovery may be attributed to a variety of factors such as the decomposition of the insecticide by catalytic action, absorption and retention of the insecticide by the tissues, the efficiency of the extraction methods, and lastly the methods employed in the estimation of the insecticide itself.

Loss due to weathering and climatic conditions are ruled out, since all tests were conducted under strictly controlled laboratory conditions. Of these, the method of estimation could not have been responsible, since it was selected as the most suitable after a detailed examination of the available methods. Of the rest, considering the catalytic decomposition first, a number of chemicals, chiefly the anhydrous chlorides of iron and aluminium dehydrohalogenate DDT with ease, giving rise to the ethylene compound which is non-insecticidal. Also, a number of solvents like nitrobenzene are also known to accelerate this change. Since in the present investigations the insecticide was in contact with the leaf surface for a considerable time, it was considered likely that either the leaf may have contained, or been contaminated with, substances which bring about such a decomposition. To throw light on this aspect, a few experiments were conducted adopting the procedure described by West and Campbell (1946). Using the same kind of apparatus, 2 grams of apple leaf powder was intimately mixed with 2 grams of DDT and introduced into the test tube fitted with a cork having two holes through which bent glass tubes were passed. The test tube was immersed in a paraffin bath kept at a constant temperature of 125°C, and a current of carbondioxide, free air was drawn through the apparatus for two hours. The dehydrohalogenation products, if any, were absorbed in a solution of deci-normal sodium hydroxide, and at the end of the operation, the solutions in the absorption tubes were back titrated with standard acid and the amount of alkali used during the process calculated. The results

presented in Table 7 indicate the absence of any catalytic action by the leaf material, and even the presence of nitrobenzene which is said to accelerate such a change did not affect it. The low recoveries obtained in these investigations cannot, therefore, be attributed to any decomposition of the insecticide involving loss of the chloride.

TABLE 7
Studies in the catalytic decomposition of DDT by leaf powder:
The mixtures were heated at 125°C for 2 hours.

Composition of mixture	Amount of 0.1N NaOH used up cc.
1. 2.0 gm. leaf powder air-dried (control)	0.65
2. 2.0 gm. leaf powder air-dried + 2 gm. DDT	0.65, 0.75
3. 2.0 gm. leaf powder air-dried + 2 gm. DDT mixed with 5.0 cc of benzene and allowed to stand 30 minutes before heating.	0.60
4. 2.0 gm. leaf powder air-dried + 2 gm. DDT 5 cc. nitrobenzene.	0.50
5. 2.0 gm. leaf powder steam oven-dried + 2 gm. DDT + 5 cc. nitrobenzene.	0.70
6. 2.0 gm. leaf powder air-dried + 5 cc. nitrobenzene.	0.50

The other two factors, viz., the insufficiency of the method of extraction, and absorption and retention of the insecticide by the tissues, being closely related to each other, will be considered together. In reviewing the methods of extraction available in literature, it was pointed out that no standard procedure has as yet emerged. The published methods varied from a mere washing with a stream of benzene to a shaking period of half an hour, as suggested by Carter and Husbanks. In the present investigations shaking by hand for a period of 15 minutes was uniformly adopted in the hope that it would be possible to recover all the amount originally deposited. The insecticidal value of that part of the insecticide which is not extracted even after such vigorous shaking appears doubtful, at least in the case of insects that are killed by contact action. Further investigations were, however, conducted to see whether it would be possible to recover all the insecticide initially deposited either by prolonging the shaking period or by continued extraction in a Soxhlet.

Continued shaking by hand (experiments with emulsions.)

1% emulsions prepared using benzene (3%) and sodium oleate (0.5%) were spread as usual, and instead of the usual three shaking periods of five minutes each, six (each again of five minutes duration) were made, thus making a total extraction period of 30 minutes. The amounts of benzene used in the six shakings were 50.0, 35.0, 30.0, 30.0, 30.0 and 30.0 cc; which finally gave an extract of about 200 cc. After making the volume to 200 cc., 100 cc. aliquots were analysed, and the

results obtained (Table 8) showed that an additional shaking period of 15 minutes gave a higher recovery of 7% from the upper surface, and 10% from the lower surface. But how far this supplementary amount extracted, expending nearly double the amount of time and solvent, is really insecticidally useful remains to be decided.

TABLE 8
Effect of the period of shaking on the recovery of DDT.

% DDT in emulsion	% Benzene in emulsion	Emulsifier used	Duration of shaking	DDT deposited mg.	% DDT recovered	
					Upper surface	Lower surface
1.028	3.0	Soap 0.5%	30 min.	10.28	97.34	95.21
0.989	3.0	Soap 0.5%	15 min.	9.89	88.40	85.31
0.993	3.0	Product M.B. 320	15 min.	9.93	92.87	81.31
1.007	3.0	Fixanol	15 min.	10.77	81.25	89.58
1.064	3.0	Triton-X-100	15 min.	10.64	90.70	83.99
Average			15 min.....		90.06	85.05

Continued extraction in a Soxhlet (experiments with solutions). As the recoveries in the case of solutions were much less than in the case of emulsions, a more drastic method of extraction was adopted. 10.0 gms. samples of apple leaf powder were weighed into fat extraction thimbles, and they were allowed to stand for a period of 24 hours in Soxhlets containing benzene to which was added 25 mgm. of DDT. This procedure enabled the insecticide to remain in prolonged contact with the leaf material, thus permitting maximum absorption by the tissue. At the end of the 24 hour period the Soxhlets were connected to suitable flasks, and the materials extracted with benzene continuously for 36 hours. The extracts after removing benzene were reduced as usual with sodium and ethyl alcohol, neutralised with acid, and as they were densely coloured, extracted thrice with 50 cc. portions of a mixture (1:1) of ether and amyl alcohol. The united ether, and ether alcohol extracts were washed twice with water, added to the aqueous layers containing the chloride, and the estimation carried out as usual. Out of 25.0 mgm. of DDT originally taken 19.0 and 21.57 mgm. were recovered, thus representing a recovery of 76% and 86.28% respectively, or an average of 81.1%.

In another experiment 10.0 gm. samples of leaf powder were weighed into thimbles as usual, and before keeping them in contact with the DDT solution, they were extracted with ether for 8 hours to remove the colouring matter, waxes, etc. The residues which were brown in colour were air-dried, and the procedure repeated as above by making them stand in Soxhlets containing solutions of DDT for a period of 24 hours, and extracting for 48 hours after that to recover the absorbed

material. 24.11 mgm. and 24.53 mgm. were recovered out of the 25.0 mgm. initially taken, representing a yield of 96.44% and 98.12%, or an average of 97.56%.

From the foregoing it is clear that either by prolonging the shaking period or by continuous extraction in a Soxhlet 100.0% recovery is possible. But, it remains to be seen how far a recovery thus effected after so much labour and expense represents the true insecticidal value of the material. Absorption of DDT by the vegetable tissues being an established fact, how is one to judge what proportion of the insecticide applied is available for insecticidal purposes, and how much is not? Also, when a material sprayed with DDT is taken up for analysis, for how long must extraction be carried out to be sure that all the insecticidally important portion is extracted?

To throw light on this point, the rate of extraction of DDT under the usual experimental procedure was studied, using 1% emulsions prepared with benzene and soap. The normal shaking and extraction procedure consisted of three shakings of five minutes each with 50.0, 30.0 and 25.0 cc portions of benzene. The first shaking period with 50 cc. was varied, and shaking for $\frac{1}{2}$, 1, 3 and 5 minutes was carried out on different batches of leaves deposited with known quantities DDT and the amounts recovered estimated. After the usual shaking with 50 cc. for 5 minutes, the residual leaf was extracted with 30 cc. and 25 cc. portions of benzene to finish off the extraction as usual, and these two extracts analysed separately.

TABLE 9
Study of the rate of extraction of DDT deposited as emulsions
(benzene 3.0%), 0.5% sodium oleate as emulsifier
on the upper surface of leaf.

Volume of Benzene used for shaking cc.	Duration of shaking Minutes	DDT deposited mgm.	DDT recovered mgm.	% recovered
50.0	$\frac{1}{2}$	10.25	8.083	78.08
50.0	1	10.25	8.155	78.77
50.0	3	10.25	8.224	79.45
50.0	5	10.25	8.792	84.94
30.0	5	...	0.4964	4.794
25.0	5	...	0.2127	2.059
Total recovered in 15 min.		10.25	9.501	91.79
Previous recovery figures obtained with benzene emulsions using different emulsifiers :—			Sodium oleate ...	88.4%
			Product MB 320 ...	92.19%
			Fixanol ...	88.25%
			Triton-X-100 ...	90.70%

The results of these tests presented in Table 9 show that, between half-minutes shaking and 3 minutes' shaking there is not much difference, and 80% of the material originally deposited is recovered. In 5 minutes' shaking, 85% is recovered and in the two subsequent ones with 30 and 25 ccs., 4.79% and 2.06% are recovered, thus making a total of 91.79%, which is in agreement with the values obtained previously. Extraction with 50 cc even for one minute appears to be more than ample for recovering all the insecticidally useful material. The insecticidal value of that portion which is not extracted by such a treatment seems doubtful at least in the case of insects which are killed by contact action. As in the case of petroleum oils where a differentiation is made between the available and the total (Swain, 1933), a differentiation may have to be made in the case of DDT also. Under normal field practice, and when sprayed in the form of emulsions, the loss by absorption may not be great, but when straight solutions are used there is scope for a great loss on this account. This is aggravated if the surfaces sprayed have also a porous and open structure. Probably a solvent with a low boiling point will be preferable in the case of sprays intended for dealing with insects that are killed by contact action, and one with high boiling range in cases where the stomach poison action of DDT is more important.

Summary: When known amounts of DDT are deposited on vegetable surfaces it is not possible to recover all the amount deposited by a simple extraction technique. The amount recovered varies with the mode of deposition, the nature of the solvent used in the preparation, the concentration of the insecticide in the spray, and the surface on which it is deposited. The nature of the emulsifier used has little effect on recovery and the concentration of the solvent (in emulsions), though depressing the recovery in the early stages, does not do so in the later stages.

It was experimentally proved that the failure to effect 100% recovery was not due to any loss of the insecticide by catalytic decomposition by leaf tissue. By adopting more drastic methods of extraction all the amount deposited could be recovered. The true insecticidal value of the material thus extracted appears doubtful, and the really useful portion seems to be capable of being recovered without much effort.

Acknowledgements: The writer gratefully acknowledges the help and encouragement given throughout the investigations by Dr. R. G. Hatton, Dr. H. Shaw and Dr. R. L. Wain. To Dr. J. K. Eaton and other members of the Plant Protective Chemistry Section who have helped in several ways, and to Dr. R. A. E. Galley for permitting me to refer to certain unpublished reports, I offer my sincere thanks.

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