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GAS CHROMATOGRAPHIC AND RADIOMETRIC STUDY OF THE BEHAVIOUR OF C^{14}-DDT ON MUSTARD PLANTS UNDER TROPICAL CONDITIONS

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Abstract. The behaviour of a DDT emulsifiable concentrate sprayed onto mustard plants was examined under tropical conditions using chemical and radiochemical techniques. The steady loss of DDT from the surface of leaves, measured as surface radioactivity, was closely similar to the loss assessed by washing the insecticide from leaves with hexane and measuring it by gas chromatography and radiometry. About one quarter remained on leaf surfaces 2 days after spraying and less than one tenth remained after 10 days.

After 2–4 days about one fourth of the DDT had penetrated into leaves, as shown both by chemical and radiochemical measurements of insecticide which could be extracted from leaves after washing the surfaces with hexane. The amount of DDT in the plant diminished with time although radiometric assay indicated a faster loss than chemical assay.

Most of the radioactivity found appeared to be present as DDT although there was gas chromatographic evidence of some slight degradation of DDT. It is concluded that loss was mainly by volatilization.

There is much current concern about the affects of traces of pesticides which remain in the foodstuffs at the time of consumption.[1–7] Ideally, food should be free from insecticide or other foreign biologically active materials, but to protect crops from insect attack it is often necessary to treat them with insecticides and traces of these chemicals may remain in the crop. However, to some degree at least, persistence of insecticides is a desirable property, for the longer an insecticide persists the fewer treatments that will be necessary to protect a crop from reinfestation by outside sources.[1,2]

Thus there are two reasons to measure persistence of insecticides on plants. The first is to determine when the amount of insecticide remaining on crops is too small to be a risk to the consumer or, put in another way, to determine the minimum time between treatment and harvesting. The second reason is to discover how long the chemical remains to give protection against insect attack so that the maximum interval between treatments may be used in any programme of insecticide treatment and only the minimum amounts of insecticides are used, such economy in the use of insecticides saves money and effort while at the same time diminishing the potential for leaving high residues.

Chemical studies of the loss of insecticides from plants will assist in achieving the best compromise between the two contradictory requirements for long-term protection of a crop and small final residues. Of the many causes of loss of insecticides from plants, evaporation or being washed away with rain obviously leave the plant free from risk, but if the chemicals are decomposed or metabolised it is important to be sure that the decomposition products are nontoxic. Chemical detection of decomposition[8] products of unknown identity is uncertain and perhaps the most satisfactory way of following the decomposition or metabolism of traces of insecticides is to use compounds labelled with radioactive isotopes, which can be followed and used to trace the decomposition of metabolic products from the labelled chemicals. Because persistence of pesticide is dependent on climate which varies from place to place, it is necessary to make such studies wherever an insecticide is used. As far as is known little or no work on the persistence and residues of pesticides has been done in Pakistan.

This paper describes an exercise undertaken to discover how long DDT persists on mustard plants and to check chromatographic methods of residue determination by comparing them with radiochemical methods. The synthetic organochlorine insecticide DDT was chosen because a sample of C^{14} labelled DDT was available and because it is a widely used insecticide that has received much attention as a persistent insecticide with a stable decomposition product DDE.

Mustard crop was used because it is quick and easy to grow and young plants are of a convenient size and shape for experimental work. It is also an important source of high quality edible oil in Pakistan.

Experimental

Mustard plants were grown in sterile sand in polythene bags which were destroyed at the end of the test to avoid hazards arising from contamination with C^{14} labelled DDT.

Plants, about 1 month old, 18–20 cm high were sprayed individually using a paint spray gun and C^{14} labelled DDT formulated as an aqueous emulsion. To ensure that all parts of the plants were treated, they were placed on a rotating platform for spraying.

To check uniformity of treatment of individual leaves and of plants, the amount of radioactivity on indi-

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vidual leaves was measured immediately after spraying using a thin end window Geiger–Muller counter. Loss of radioactivity was then followed by making further measurements at intervals until the plants were taken for chemical assay. DDT and decomposition products washed from the surface of plants or extracted from the plants were assayed and identified by gas or thin layer chromatography in conjunction with autoradiography. Analysis was checked by comparison with the amounts of radioactivity measured in the washes and extracts.

Materials. Pure *p,p'*-DDT was prepared from a sample of DDT provided by Messrs Geigy by repeated crystallisation from ethanol until the m.p. was constant at 107–108°C. Chromatography on Whatman S.G. 81 silica-loaded paper with hexane as solvent and gas chromatography confirmed the purity of the insecticide.

p,p'-DDE was a pure sample provided by Messrs Geigy, Switzerland.

Purification of C^{14}*-Labelled* p,p'*-DDT.* Ring C^{14} labelled DDT, specific activity 19.1 mc/mm, supplied by the Radiochemical Centre, Amersham, was purified by chromatography on silica-gel-loaded paper (Whatman S.G. 81).

74.4 μg DDT in 1 ml hexane was applied as a 5-cm strip on silica-loaded paper and the chromatograms (descending) developed with hexane.

The chromatogram was autoradiographed to locate radioactivity and the area corresponding to *p,p'*-DDT was cut from the chromatogram and eluted with acetone. The solution of C^{14} *p,p'*-DDT was concentrated to 0.4 ml. Purity was checked by paper chromatography, autoradiography and by gas chromatography.

Mustard Plants. Mustard plants were grown in polythene bags 10 cm high, 15 cm dia, in formalin sterilised soil. Polythene bags were chosen as containers because they are inexpensive and may be easily disposed off by burning at the end of the test. This avoids problems of contamination either with insecticide or radioactivity. Seeds were germinated on filter paper in petri dishes and after 3 days, the seedlings were transplanted singly into the polythene bags, which were perforated to allow the drainage necessary for the health of the plants. Each bag contained 400 ml sandy soil moistened with 100 ml water. Plants were watered as required, but once a week, each seedling was given 20 ml a 1:10 dilution of a nutrient solution[10] prepared by mixing 20 ml 20% KNO_3, 33% $Ca(NO_3)_2$, 18% $MgSO_4.7\,H_2O$, 21% $NaH_2PO_4.2H_2O$ and making the mixture to 1 litre.

DDT Emulsion for Spraying Mustard Plants. Mustard plants were sprayed with an emulsion containing 0.1% DDT prepared from an emulsifiable concentrate made by dissolving 25 g pure *p,p'*-DDT and 3 g emulsifier CM 753* and 1 g oil-soluble dye in 100 ml xylene.

Radioactive emulsions were made by evaporating 0.1 ml of the purified C^{14} DDT solution equivalent to 16 μg DDT, emulsifying in 80 ml water.

*Manufactured by Chemical Products Ltd., N.S.W., Australia.

Methods

Treatment of Plants. A rigidly fixed paint spray gun Model No. 1, China National Import Export Corporation, Shanghai Branch, People's Republic of China was used to spray growing plants centrally placed on a turn table rotating at 20 rev/min. In this way each side of the plant was presented to the spafy at least twice during the time (7–8 sec) needed to discharge 1.5 ml insecticide emulsion.

The turn table, driven by a small electric motor through a chain of gears and belt and pulley, was made from the case and one bearing of a motor car generator.

The spray gun was mounted so that the nozzle pointed down at 45° to the vertical and directly at the plant. The nozzle was placed at a horizontal distance of 25 cm from the centre and 32 cm above the base of the plant. This position was chosen to ensure complete spray cover of the plants after determining the size and shape of the jet at spray by measuring the wetting of the sheets of paper placed horizontally at different heights below the nozzle and vertically at different distances from the nozzle. To check that these relative positions of the nozzle and plants were suitable two sheets of paper—one horizontal, the other vertical—were placed on the turn table and the area of the sheets wetted by spraying as the table turned observed. A final check of distribution of spray was made by spraying a paper cone height 20 cm and base 15 cm, which corresponded approximately to the size of the plants and ensuring that it was completely wetted.

For spraying, an air pressure of 5 lb/in² was used. No other pressures were tested because of limitations of the air pumps available.

Gas Chromatography. A Phillips gas chromatograph model PV 4000 series was used with two stainless steel columns and electron capture detectors. One column was packed with 5% DC-200 on Celite (80–100 mesh), the other with 5% SE 30 on Phasesep W. Both the columns were pretreated with hexamethyl disilazine and operated at 170°C. The carrier gas was pure, oxygen-free nitrogen at 25 lb/in², giving a flow rate of 40 ml/min.

The response of the gas chromatograph was standardised using a solution of pure *p,p'*-DDT in hexane 5 μg/ml prepared from a solution of 1 mg DDT per ml in hexane. The peak height was proportional to the amount of DDT injected into the instrument over a range of 5–20 nanograms.

Chromatography on Silica-Loaded Paper. Samples of extracts and washings of leaves were spotted on silica-loaded paper (Whatman SG 81) and chromatographed (8–9) in the ascending fashion using hexane as solvent. Marker spots of pure unlabelled DDT and DDE were run for comparison. Nonradioactive chlorinated compounds were detected using the silver nitrate chromogenic reagent of kovacs.[11] Radioactive substances were detected by autoradiography as dark areas on X-ray films.

Autoradiography. Autoradiographs were made by clamping the specimens next to Agfa-Gevaert X-ray film Curix F.W. between sheets of glass. Time of exposure was adjusted according to the amount of

radioactivity present and ranged up to 20 weeks. After exposure the film was developed for 5 min in Johnson X-ray developer, immersed in 2% acetic acid stop bath and finally fixed using Johnson X-ray Fixadon.

Measurement of Radioactivity. Radioactivity on the surface of leaves was measured by counting the activity on the area of each leaf coverd by a thin end window Geiger–Muller tube, pressed against the experimental leaf spread flat on a 1-cm thick sheet of polyurethene foam, backed by a tile. This supported the leaves and protected them from damage by the rim of the G.M. tube mount. The radioactivity in washings and extracts of leaves was determined by evaporation of known amounts of washings and extracts of leaves onto small (2×2 cm) glass microscope cover slips which were mounted at a standard distance below a G.M. tube.

Washing and Extraction of Mustard Plants. Single leaves were placed in 100 ml beakers and washed by three successive portions each of 2 ml n-hexane from a 5-ml measuring cylinder. The n-hexane was passed through a Na_2SO_4 plug to dry the wash and to remove dirt and dust. The solution was then concentrated. As the tests progressed the concentrations of DDT diminished and it became necessary to use more than one leaf for an analysis. When two leaves were used they were washed with 3 lots of 3 ml of solvent, batches of four leaves were washed with 3 lots of 5 ml of hexane.

After washing the leaves were ground in an all-glass macerator with a mixture of hexane and acetone (2:1). Samples of one or two leaves were extracted with 5 ml and 4 leaf samples with 10 ml of the solvents mixture. The extract was filtered through a cotton-wool plug into a separating funnel and washed with 2 ml 2% Na_2SO_4 and then with two more lots of 2 ml 2% Na_2SO_4 solution. The volume of hexane layer was measured and the extract was dried (Na_2SO_4) and used to correct the total amount of insecticide found. After decanting off the extract the sodium sulphate was washed with 0.5 ml hexane, 3 times to completely remove solutes. The combined extracts and washings were taken just to dryness and dissolved in 0.5 ml hexane. Extracts of leaves contained substances which interfered with the assay of DDT by gas chromatography. The interfering substances were removed by passing the extract through a silica gel column as follows: silica gel (2 g) was slurried in hexane and poured into a glass-stoppered chromatographic column of 1.5 cm dia giving a column of 3.5 cm height. 0.3 ml concentrated extract was carefully placed on the top of the column and then eluted with 12 ml of hexane. Three fractions 3, 6 and 3 ml were collected. Preliminary tests, showed that the second fraction of 6 ml contained all the DDT. This fraction was taken just to dryness and made to 0.5 ml before assaying for DDT by gas chromatography.

Results

Radioactivity on the Surface of Leaves. On the day of spraying the radioactivity on the surface of each of the 4 marked leaves, of all treated plants, was measured using a thin-end window counter which measures radioactivity over a fixed area so that the counts recorded are a measure of radioactivity or insecticide per unit area. Measurement of activity on the surface of the individual leaves, which were identified by coloured threads, was repeated at intervals until the plants were taken for chemical analysis. In this way it was possible to follow the loss of radioactivity from single leaves until they were destroyed. On the day of spraying the radioactivity on individual leaves was widely variable and counts ranged from 29 to 216 per minute (eight-fold) (Table 1). The average number of counts per minute for the 4 leaves on each plant was less variable than for individual leaves and ranged from 74 to 145 (two-fold). This shows that despite the precautions taken with the spraying technique deposits on individual leaves were not uniform, and although the average amounts on plants was not constant, it varied much less than the amounts on individual leaves. Radioactivity on individual leaves decreassed progressively with time and showed similar trends on all leaves, although some leaves seemed to loose insecticide relatively faster than others (Table 1, Figs. 1. and 2). To facilitate comparisions, results at different times after spraying have been expressed as a percentage of the amount of radio-

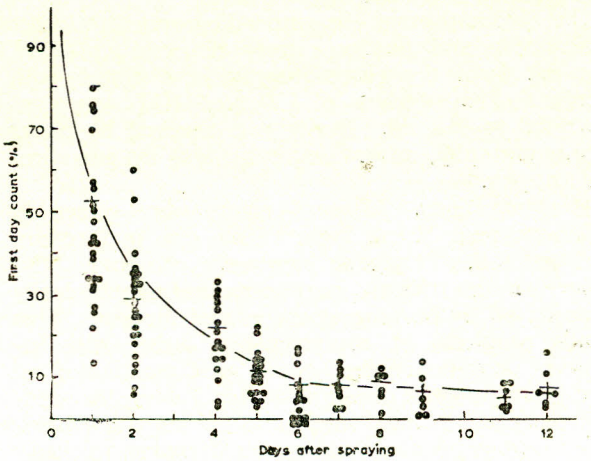

Fig. 1. Loss of C^{14} DDT from leaves of mustard plants. Collected results of radioactivity measured on surface of individual leaves, ● single leaves, × daily mean of all leaves.

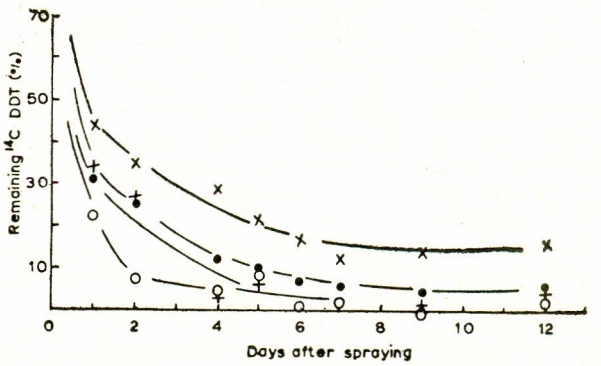

Fig. 2. Loss of C^{14} DDT from the surface of four individual leaves of a mustard plant.

TABLE 1. RADIOACTIVITY COUNTED ON THE SURFACE OF INDIVIDUAL LEAVES AT THE TIME OF SPRAYING AND AT THE TIME OF CHEMICAL ANALYSIS.

Chemical assay days after treatment	Leaf	At spraying (counts/min) Leaves	Mean per plant	At analysis (counts/min) Leaves	Mean per plant
0	A	63	82		
	B	78			
	C	119			
	D	66			
2	A	216		53	
	B	47	106	3	22
	C	54		12	
	D	109		22	
4	A	123		33	
	B	155	145	27	35
	C	199		66	
	D	103		14	
6	A–1	174		3	
	B–1	105	135	6	12
	C–1	99		32	
	D–1	163		7	
	A–2	66		—	
	B–2	85	79	—	4
	C–2	103		16	
	D–2	63		—	
9 *	A–1	133		6	
	B–1	68	135	1	10
	C–1	146		14	
	D–1	192		19	
	A–2	133		12	
	B–2	200	(168)	23	18
	C–2	—		25	
	D–2	170		12	
12 *	A–1	83		6	
	B–1	107	118	2	6
	C–1	147		8	
	D–1	134		10	
	A–2	128		4	
	B–2	114	137	3	6
	C–2	139		11	
	D–2	166		7	
13 *	A–1	121		7	
	B–1	45	74	1	7
	C–1	29		4	
	D–1	99		16	
	A–2	56		3	
	B–2	175	138	11	8
	C–2	196		12	
	D–2	123		5	

*Last count of activity are one day before analysis

activity on the day of spraying. The collected results for all leaves and the average amount of activity remaining on all the leaves counted on any one day show a progressive loss with time (Figs. 1 and 2). The loss continued throughout the test period as illustrated by the radioactivity measured at intervals on the 4 individual leaves of one of the two plants kept until the end of the experiment (Fig. 2). The general pattern of loss of individual leaves of the two plants was similar and resembled that of the collected results for all leaves tested (Figs. 1 and 2).

After 4 days about four fifths of the activity was lost from the surface of all the plants and after the seventh day the activity remaining was so little above background that reliable estimates could not be made.

DDT Washed from Leaf Surfaces. Leaves of sprayed plants were washed with hexane and the DDT removed assayed by gas chromatography. The amount of DDT washed from individual leaves varied even on the day the plants were sprayed (Table 2). It might be expected that larger leaves would retain more DDT but the amount of DDT per unit weight of leaf also varied though less than the amount on individual leaves. If weight is taken as an approximate measure of leaf area this indicates that the leaves were not uniformly sprayed. As a result reliable estimates of loss of insecticide from leaf surfaces cannot be made from assays of a few leaves washed on different days because insecticide can only be washed from the leaves once, and the amount of DDT on individual leaves is variable. However, these variations are insufficient to obscure the loss of insecticides from the surface of leaves with the passage of time (Table 2). Gas chromatography suggested that there were decompsition products, including DDE on the leaf washes after 6 days but the amounts were too small to measure.

Not all the samples washed from leaf surfaces were assayed for radioactivity but there are sufficient tests to show loss of activity with the passage of time (Table 2).

Paper chromatography and autoradiography of the washes only showed radioactivity present as *p,p'*- DDT. It diminished with time. At first autoradiographs were made in two weeks but samples from leaves washed after 6–13 days needed 20 weeks for autoradiography.

Substances Extracted from Leaves after Washing. After leaves had been washed with hexane they were ground with a mixture of hexane–acetone (2:1) to extract DDT and any decomposition products. A part of the extract was examined by gas chromatography (Tables 1 and 2) after treatment to remove plant materials which interferred with the assay. In preliminary tests no DDT was extracted from leaves which had been washed immediately after spraying. These extracts were lost in the main test. Some DDT was found in all leaves which were not analysed immediately after spraying. The amounts increased to a maximum of 12 p.p.m. by 4–6 days after treatment and then diminished slowly. After 2 days small gas chromatographic peaks with retention time of DDE were observed which increased with time and were greatest at the end of the test. However, amounts were too small to be measure. In addition traces

TABLE 2. MATERIALS FOUND IN WASHES AND EXTRACTS OF LEAVES.

Time after spraying (days)	Leaf/ plant	Washes of leaves						Extracts of leaves					
		DDT (μg)				Radioactivity		DDT (μg)				Radioactivity	
		Per leaf	Mean	Per g	Mean	Per leaf	Mean	Per leaf	Mean	Per g	Mean	Per leaf	Mean
0	A	17		46									
	B	14	15	44	49								
	C	21		70									
	D	8		36		155							
2	A	10		15		109		2·0		4		140	
	B	7	5	12	10	23	48	0·5	1·0	1	2·5	18	72
	C	2		6		23							
	D	2		6		36		0·5		2		57	
4	A	2		6		32		2		7		20	
	B	5	3·5	12	11·5	43	32	5	3·5	13	12	82	36
	C	3		15		25		3		15		18	
	D	4		13		26		4		13		23	
6*	A1+2	3		7		26		4		10			
	B1+2	2	3·0	8	8	16	20	4	4	15	12		
	C1+2	4		10		15		3		8			
	D1+2	2		6		25		5		13			
9*	A1+2	2		6				2		6			
	B1+2	1	1·0	4	4			1	2	4	7	12	
	C1+2	0·5		2				2		8		5	18
	D1+2	1		3				3		9		36	
12†	(1)	0·5	0·5	1	1·0	8	8	3	2·5	8	8	15	15
	(2)	0·5		1		7		2		8		14	
13†	(1)	0·25		0·6		4		3		8		7	
	(2)	0·5	0·2	1·0	0·8	11	8	1	2·0	2	5	17	12

*Leaves from two plants combined for assay. †All the four leaves from a plant assayed together.

of an unidentified substances with a longer retention time than DDT were detected in gas chromatograms 4 days after spraying the plants.

A small amount of radioactivity was extracted from leaves (Table 2). The amount diminished slowly throughout the test and did not closely correspond to the amount of DDT found by gas chromatography, indicating the presence of radioactive substances other than DDT.

Autoradiographs of silica-gel-loaded paper chromatograms of the extracts showed that most of the radioactivity present was in the form of DDT but small amounts did not move from the point of application and traces had R_f about half that of DDT. Chromatograms of extracts made from leaves up to 4 days after spraying were autoradiographed for 2–4 weeks and those of extracts made 9–15 days after spraying required up to 20 weeks. It is possible that this radioactivity represents DDT which had penetrated into the plant and was metabolised, but it was not possible to separate and detect such substances. However, the relatively larger amount of DDT found in the plants 2 days after treatment compared with the radioactivity in later extracts does not support such metabolism. It is possible that radioactivity was masked by substances extracted from the plants and there is evidence of a small amount of metabolism or decomposition of DDT in later extracts which contained radioactive substances not DDT, as well as of traces of DDE and other electron-capturing substances not identified by gas chromatography. The amount of DDT extracted from the plant was greatest 4–6 days after spraying when it was about equal to the amount remaining on the surface and less than one fourth of the amount of DDT originally applied to the plants. So that by the fourth day the amounts of insecticide and radioactivity accounted for are about one half of the quantities applied to the leaves; by the sixth day less than one half was recovered (Table 2, Fig. 3). It is unlikely that there was much loss by metabolism or decomposition to products not detected either by chemical or radiochemical methods, although traces of substances, probably DDE, were found by gas chromatography in extracts (and washes) from plants 2 days after treatment and there were indications of other products by autoradiography of paper chromatograms. Because loss is not likely to be the result of undetected decomposition, other causes must be sought. During the period of the test there was no rain, and the plants were watered carefully through the soil so that DDT was not washed away from the plants and losses could only be caused by volatilisation of the insecticide, by wind errosion or by penetration into the plant.

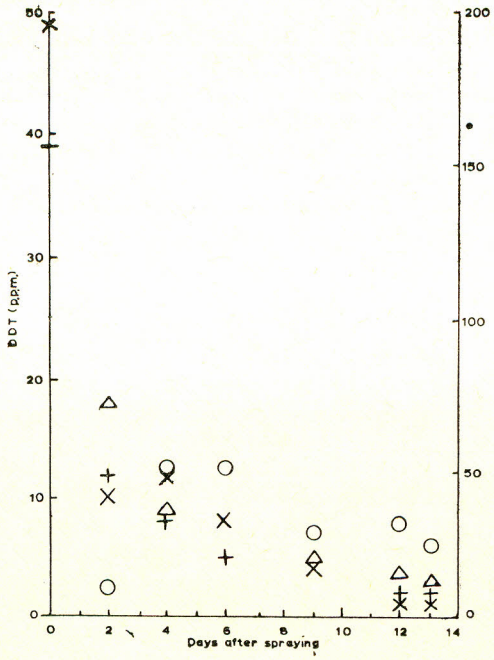

Fig. 3. Loss of DDT and radio activity from mustard plants. Average amounts of DDT in surface washes × and extracts o. Average amounts of radioactivity in surface washes + and extracts Δ of plants treated with C^{14}DDT.

Discussion

The amounts of insecticide found on the 4 leaves analysed chemically immediately after spraying (Table 2) differed; taking weight as a rough measure of area, the amounts per unit area (Table 2) were as variable as the amounts on individual leaves which must depend at least to some degree on leaf area, This variation was confirmed by measurement of radioactivity on the surface of the plants (Table 1) suggesting that the spray technique did not give a uniform deposit on individual leaves. A possible reason for the variation between individual leaves is the bending of leaves from their natural positions caused by the air flow from the spray gun even though the leaves were supported by a wire frame when sprayed. The variation of deposit may also have been increased by the tendency of leaves to present differing aspects to the spray despite rotation. The variable amounts of insecticide deposited on individual leaves makes it difficult to compare results unless they are obtained from the same leaves or are the average from a large number of leaves. Fortunately, the radioactivity on the surface of leaves is a measure of DDT and its measurement is nondestructive so that repeated determinations on a single leaf were possible until it was taken for chemical assay. The DDT as measured by the radioactivity on the surface of the leaves at first diminished rapidly and after 6 days about one tenth was left thereafter, the loss became slower and by the 12th day between one tenth and one fiftieth (mean one twentieth) remained on leaves (Table 1, Fig. 2) but the accuracy of individual measurements was poor because of the small amount of radioactivity present. Chemical analysis shows a similar rapid loss of DDT from the plants surface although no precise estimates can be made because the chemical assay is destructive and only 4 leaves were tested each day, a number too small to obtain a reliable average.

Chemical analysis showed that after 6 days the amount of DDT per unit area remaining on the surface of leaves was about one sixth of that on the leaves analysed immediately. But on the day of spraying the average radioactivity found on the surface of the plants analysed after 6 days was about one and a half times more than the radioactivity on those plants analysed immediately after spraying. Allowing for this difference about one ninth of the insecticide remained, a result in good agreement with surface measurements of radioactivity which indicates that at the time of analysis about one tenth of the DDT remained when the surface activity was last counted 6 days after spraying (Table 1). This agrees with the activity of DDT averaged for all plants (Fig. 1). Thus loss of DDT from the surface of plants shown by chemical assay is similar to that shown by radiochemical measurements and discrepancies between the two techniques are small.

There is also a close correspondence between the amount of DDT and radioactivity extracted from plants, except on the second day after treatment when an unexpectedly large amount of radioactivity was found (Table 2, Fig. 3).

Some insecticide was lost from the surface of the plant by penetrating into the plant but this is only a small fraction of the total loss from the surface. Thus there only remains volatilisation and wind erosion to account for the loss of insecticide. At the time of the tests the weather was hot, sunny and windy (Table 3) with temperatures at the nearby meterological station ranging between 66–105°F with average daily wind speeds of 4–8 knots. Although minimum temperatures recorded in among the plants were close to those in official records, the maximum temperatures were frequently 5–10° warmer, as may be expected because the thermometer was not kept in a screen. Under these conditions of high temperature it is likely that greatest part of the loss resulted from volatilisation, although some DDT penetrated into the plant and was possibly metabolised. Because some DDT is taken into the plant where it appears to persist longer than that on the surface, action as a stomach poison may last longer than as a contact poison. Both radioactivity measurements and analysis by gas chromatography show that at least half of the applied material was lost in 2 days and about 90% after 10 days. Thus DDT cannot be expected to give prolonged protection against insects, under the climatic conditions in Karachi, where it seems to evaporate rapidly.

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