Degradation of 2,4,5-T by South Vietnamese Soils Incubated in the Laboratory

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INTRODUCTION

During the preliminary investigations of the National Academy of Sciences Committee on the Effects of Herbicides in Vietnam, soil and mud samples were taken from areas some of which were thought to have received applications of Agent Orange (a 50:50 mixture of the n-butyl esters of 2,4-D and 2,4,5-T) during military defoliation operations (N.A.S. Report 1974). These samples were sent to this laboratory for the determination of possible residues. However, the quantities were greater than necessary for this purpose, so the opportunity was taken to use the surplus to obtain an indication of the capacity of these soils to degrade 2,4,5-T.

MATERIALS AND METHODS

Soils

Four soils were used. Two, (1) and (2), from an agricultural area near Dong-Hiep village, Di-an District in Bien Hoa Province were grey podzolic soils (MOORMAN 1961) with pH values of 4.9 and 5.0 respectively. The others were samples of mangrove swamp soil, one from the Rung Sat (3) and one from the Cau Mau peninsula near Nam Can (4). Both the mangrove samples were saline undifferentiated alluvial soils (MOORMAN 1961) with pH values of 6.6 and 7.9 respectively. The samples were taken in October 1971. It was thought that sites (1) and (3) had received applications of Agent Orange in the period 1965-1971 but that sites (2) and (4) had not. Determinations of 2,4,5-T by the method of MCKONE and HANCE 1972 had shown a residue of about 0.01 ppm in soil (3) but none could be detected in the other soils (limit of detection 0.006 ppm).

Preparation of the soils

Soils (3) and (4) when received contained 35% and 47% of water respectively. They were made friable by air drying to a moisture content of about 15%. Soils (1) and (2), which contained about 15% water, did not require further drying. All the soils were passed through a 2.5 mm sieve. Duplicate samples of each were spread on polyethylene sheets and 1.0 ml of an acetone solution of ^{14}C-carboxyl labelled 2,4,5-T butyl ester was applied by pipette. In the first experiment 100 g of soils (1) and (2) and

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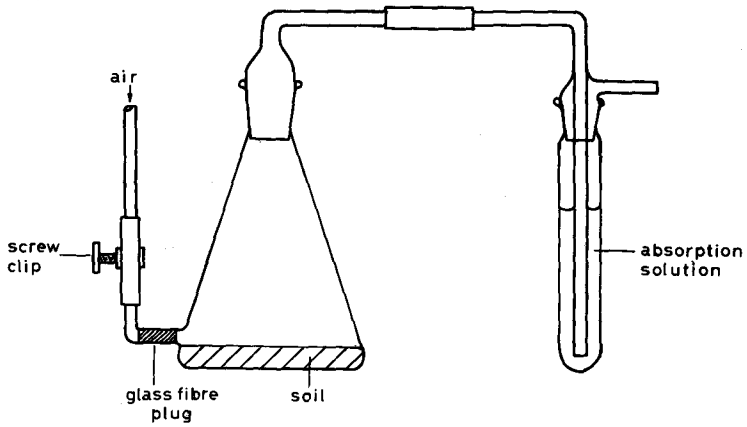
an amount of the partially dried mangrove soils calculated to give 100 g of mud of 35% and 47% water content respectively were treated with 100 µg a.e. 2,4,5-T butyl ester to give a concentration of 1 ppm w/w. In the second experiment the available soil was limited to 50 g quantities which were treated with 750 µg a.e. 2,4,5-T butyl ester (15 ppm w/w) in 1 ml acetone. The ratios of labelled to unlabelled 2,4,5-T were different in the two cases so that the total activity in the 1 ml of acetone solution was constant at 0.5 µCi. Controls were prepared with acetone alone.

After treatment the acetone was allowed to evaporate and the samples transferred to 500 ml respiration flasks. Water was added to the mangrove soils to restore them to their original moisture content. The weight of flask plus soil was noted in each case.

Incubation

The flasks were connected to the gas flow system illustrated in Figure 1.

FIG. 1



Gas flow incubation apparatus

The air supply was passed successively through a Union Carbide 5A molecular sieve, "Carbosorb", and water before entering the flask of soil through a glass wool filter in the side arm. The flow rate to each flask was controlled with the screw clip to give a rate of about 1 bubble/sec through a 4 mm id tube entering the absorption solution. The absorption solution, which was ethanol, ethanolamine (2:1), was changed every 7 days. The flasks were incubated at 25°C ± 2°C until about 70% of the applied ^{14}C had appeared as $^{14}CO_2$ from all samples. The flasks were weighed weekly and water was added if necessary to maintain the soil water content.

$^{14}CO_2$ estimation

The absorption solution was made up to its original volume (10 ml). A 2 ml aliquot was added to 3 ml of 1,4-dioxan and 5 ml of scintillation cocktail (33.4 g 2-ethoxy ethanol, 2.0 g PPO, 0.1 g POPOP, 10 g naphthalene made up to 100 ml with 1,4-dioxan) in a counting vial. Samples were counted on a Beckman scintillation counter using an external standard. The possibility that volatilisation of 2,4,5-T ester and its subsequent trapping in the absorption solution might give rise to a spurious count was checked by g.l.c. 5 ml aliquots of used absorbent were diluted with 20 ml of water and extracted with 2,2,4-trimethylpentane (2 x 5 ml). The solvent was evaporated and the residue taken up in 1 ml of trimethylpentane. 5 µl injections were made into the g.l.c. using the conditions established by MCKONE and HANCE for the determination of 2,4,5-T butyl ester. No ester was found (limit of detection 0.005 ppm).

Estimation of residual ^{14}C

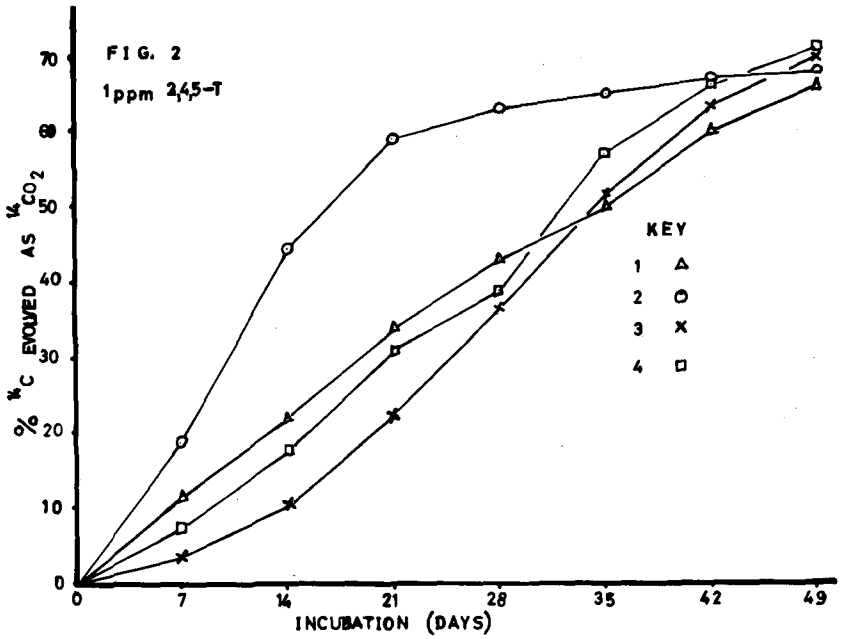
At the end of the incubation 5 g samples of soil were air dried, reweighed and transferred to a 50 ml flask. The soil was oxidised by a method similar to that of ALLISON et al. (1965) modified in that the evolved CO_2 was trapped in 10 ml of ethanol ethanolamine solution. As Cl^- was present in the mangrove soils it was necessary to include traps containing 50% KI and saturated Ag_2SO_4 in order to remove CrO_2Cl_2, formed by the oxidation of Cl_2 with $K_2Cr_2O_7$. Aliquotes (2 ml) of the absorption solution were counted as before.

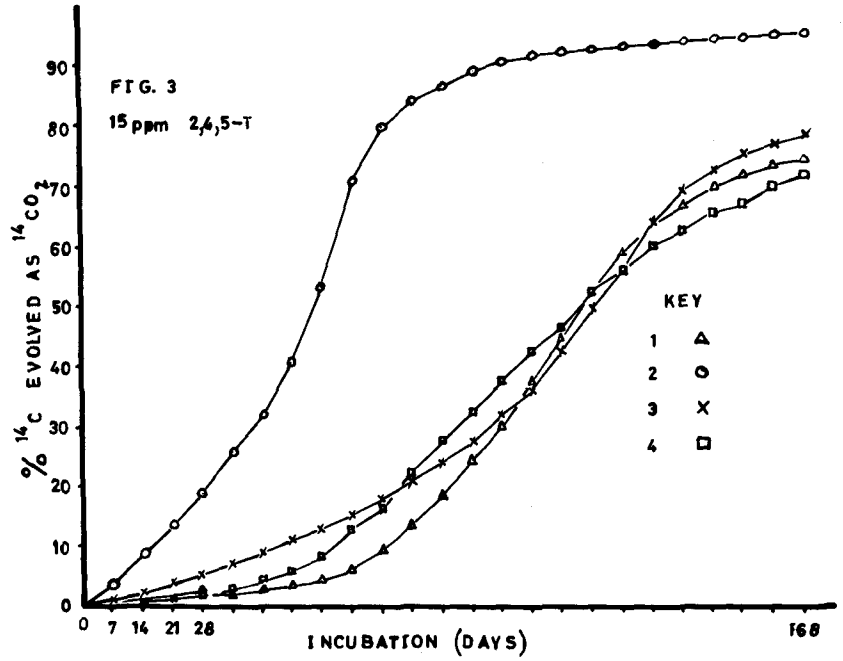
Estimation of residual 2,4,5-T

At the end of the incubations samples of soil from both experiments were analysed for 2,4,5-T by the method of MCKONE and HANCE (1972). At the same time samples from both experiments were subjected to a hydrolysis procedure based on that suggested by CHOW et al. (1971) for the release of bound 2,4,5-T residues in wheat. The soil (10 g) was refluxed with 60 ml 0.2N NaOH for 75 min. The mixture was cooled, filtered, and the filtrate acidified to pH 1 with dilute H_2SO_4 after which it was extracted with 3 x 40 ml portions of diethyl ether. The extracts were passed through a pad of anhydrous Na_2SO_4 and bulked. The ether was evaporated and the residue butylated for 2,4,5-T determination (MCKONE and HANCE 1972).

RESULTS AND DISCUSSION

Figs. 2 and 3 show the cumulative evolution of $^{14}CO_2$ with time. In both experiments the initial rate of $^{14}CO_2$ evolution was greatest with soil (2). There is no obvious explanation for this. With all soils at 15 ppm and soil (3) at 1 ppm the maximum rate occurred after an initial period of much slower $^{14}CO_2$ production.





Cumulative evolution of $^{14}CO_2$ with time

This pattern has frequently been observed with phenoxyalkanoic acids under a variety of conditions (AUDUS 1964) and it is thought that organisms capable of metabolising the molecule become adapted during the early period of slow degradation. However, in this experiment an additional factor with the mangrove soils was that they were dried to 15% water content during preparation and then rewetted to about 40% so part of the lag may simply have been the effect on soil organisms of these changes. This does not apply to the soils (1) and (2).

Following the 1 ppm treatment the soils had evolved 64-69% of the applied ^{14}C in 49 days while at the 15 ppm level, 74-96% of the ^{14}C had been evolved in 168 days. This is consistent with similar experiments with other compounds in which the higher the starting concentration the slower the rates of degradation (HANCE 1969, HANCE and MCKONE 1971). However, this observation must be treated with some reserve as the 1 ppm and 15 ppm experiments were run consecutively, not simultaneously so it is possible that the conditions of the 2 experiments were not identical.

The results of the 2,4,5-T and residual ^{14}C analyses are given in the table.

TABLE

2,4,5-T residues and ^{14}C distribution after incubation of 2,4,5-T butyl ester with soils

Treatment	Soil	% applied ^{14}C			2,4,5-T residue as % of applied	
		CO_2 evolved	Residue in soil	Total	McKone & Hance Extraction	Hydrolysis Extraction
1 ppm, 49 days incubation	1	64.5	22.3	86.8	1	2.8
	2	67.0	25.7	92.7	1	1.2
	3	69.0	19.3	88.3	3.6	2.2
	4	69.5	29.2	98.7	1	2.8
15 ppm, 168 days incubation	1	76.0	19.5	95.5	3.8	9.6
	2	95.9	13.3	109.2	1.0	2.1
	3	78.8	15.5	97.4	3.1	3.6
	4	74.5	33.9	108.4	16.0	14.3

Although appreciable quantities of ^{14}C were left in the soil little 2,4,5-T could be extracted with or without hydrolysis; less than 4% of the quantity applied at 1 ppm could be extracted by the 49th day and 1-16% of the 15 ppm application was extracted at 168 days. Presumably the unextractable ^{14}C had been incorporated into the soil organic matter in some way, probably after some degree of degradation of the parent molecule.

Care must be exercised in extrapolating these results to the field situation because of the great disparity of conditions. However, it appears that the 4 soils studied are inherently capable of degrading 2,4,5-T at levels at least as high as 15 ppm, which corresponds very roughly to 30 lb/ac 6 in. or about twice the rate of military applications in Vietnam.

Acknowledgements

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