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FINAL REPORT

ANALYSIS OF HERBICIDE ORANGE COMPONENTS IN SELECTED SOIL SAMPLES

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SUBMITTED TO

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REPORT OF ANALYSIS, HERBICIDE ORANGE COMPONENTS IN SOIL SAMPLES

by

E.L. Arnold, Lt Co, USAF

INSTRUMENTATION METHODOLOGY:

A Varian model 3700 automatic gas chromatograph was used for all quantitative analysis. This chromatograph was equipped with a CDS 111 microprocessor and model 8040 auto sampler. Eight ft x 2 mm I.D. glass columns packed with 10% SP-2330 in Chromosorb W/AW 100/120 mash were employed. Nitrogen was used as the carrier gas, flow = 300 ml/min with the column over operated isothermally at 170^{o}C. Injector and detector oven temperatures were 210 and 240^{o}C respectively. NI^{63} electron capture detection was used throughout.

REAGENTS

For quantitation of the free acid forms of 2,4.D and 2,4,5-T, ρ-bromo phenyl acetic acid was used as an internal standard. PBPA was dissolved in methanol to obtain a final concentration of 222 μg/ml. This internal standard solution was buffered by addition of sufficient 0.1 triethanolamine buffer to obtain a pH of 8.0 (∿ 2ml/100 ml) so that different soil pH would not effect the extraction (1.S.1)

For quantition of ester forms of 2,4,D and 2,4,5T, the isopropyl ester of PBPA was employed. This ester was formed by reaction of PBPA with 6% HCl in isopropanol (10 ml acetyl chloride + 90 ml isopropanol; acetyl chloride + isopropanol $\longrightarrow$ Isopropyl acetate + HCl). Following

Calculations and results of analysis are shown in Table IV.

Table IV

View Graphs 1 & 2

From these data, the percentage of each component remaining after 990 and 2,165 days was determined and is displayed in Table V. Values given in this table have been adjusted by use of the concentration of the non degrading octyl ethers of 245 trichlorophenol as an "internal standard." In other words, if the ether concentration determined differed from the mean values determined in the simulated initial samples, the values for the other components present were adjusted accordingly. While this practice may lead to calculated rates which are slightly reduced from reality (due to slight degradation of the ethers),the removal of one of the field variables yields more precise results. It should be noted in table V, that hydrolysis of isooctyl esters is much slower than that for n-butyl esters and is apparently far more dependent on initial concentration. This fact may have been responsible for some of our earlier observations that herbicide degradation reached a "plateau" level in the 4000 lb/A Utah plots.

Analytical results - selected J1 and NCBC site samples. Four samples, J16-1, J135-1, GP10-1 and GP24-1 were obtained which had been previously analyzed by the Flammability Research Lab and suspected to contain Orange II. The results of these analyses as well as the original FRC analyses are contained in Table VI. It should be noted that the NGP analysis reports all esters in terms of "free herbicide" while FRL does

reaction, the product was extracted into hexane and diluted wtih additional hexane to a concentration of 40 µg/ml. (I.S.1)

Free acids were converted to methyl esters for chromatography by heating with a methylating reagent prepared by reaction of 10 ml acetyl chloride with 90 ml methanol (6% HCl in methanol).

SAMPLE PROCESSING

Soil samples of 0.1, 0.2, 0.5, 1.0 and 5.0 grams were weighed into 16 x 125 mm glass screw cap tubes with teflon liners. Ten ml of internal standard I solution are added, the tubes shaken and heated at 60°C for 30 minutes with occasional shaking. After cooling, the tubes are centrifuged and two ml removed and transferred to a clean tube. This two ml aliquot is doubled in volume with hexane and analyzed for herbicide esters and pehnolic ethers without further treatment. To the material remaining in the soil tube, add 2 ml hexane and two ml internal standard solution II_s shake to loosen soil pellet and mix for 15 min on a rotary mixer. This tube is recentrifuged and 9.5 ml of the top layer (hexane) is removed and discarded. An additional 10 ml hexane is added and mixed for 15 minutes. A portion (5-6 ml) of the upper hexane layer is again removed and discarded to facilitate the removal of 0.5 ml of the bottom methanol layer with a long period pipette. This aliquot containing the acidic herbicide components is reacted with 1.5 ml methylating reagent by heating in a water bath for 15 minutes at 60°C. After cooling, the herbicide methyl esters are extracted into 10 ml hexane and the hexane layer analyzed by GLC (Figure 1).

Several standard solutions were needed for instrument calibration due to the wide range of herbicide concentrations experienced. Ester standards were prepared by addition of chloroform solutions to control soil, removal of chloroform under vacuum and extraction as described previously. Acid standards were prepared in a like manner.

To ascertain the contribution of transesterification to the amount of herbicide acids determined, a series of ester standards presumably containing little free acid were carried through the entire procedure and acids determines in the methanol layer. Results of these analysis are shown in table 1. From these analyses, it can be calculated that the $\bar{x}$ transesterification of 2,4-D esters is 8.9% while that for 2,4,5T is 5.6%. Butyl esters alone yield greater values than isooctyl esters which may reflect a small amount of acid present in the Orange formulation used to prepare betyl ester solutions rather than greater isooctyl ester stability. In samples containing significant amounts of herbicide esters, acid values were reduced by a factor equal to the amount of ester determined x .089 for 2,4.D esters or .056 for 2,4,5-T esters.

While the need to adjust values was regretable, it was preferred given the advantages of the developed methodology for the analysis of complex herbicide contaminated soil samples. Most current GC methodology for chlorophenoxyherbicides use nor polar stationary phases (OV+1, SE30, OV101, DC200) nor polar stationary phases for the separation. While these phases given an acceptable separation of the methyl and n-butyl esters of 2,4-D and 2,4,5-T, certain compounds in complex mixtures are not satisfactorily separated. In our preliminary analysis

of samples for the Utah biodegradation plots, we noted that the size and shape of the peak with the correct R_t for the n-butyl 2,4,5-T was different than that observed in standard solutions. Large peaks of long retention time were also observed in these samples. On lowering column temperatures we found the retention time for the peak we originally thought to be 2,4,5-T butyl was slightly different but inseparable from n-butyl 2,4,5-T. Chromatography of these extracts on a more plar stationary phase SP-2330, revealed that relatively large amounts of two previously unobserved compounds were present and only a small amount of n-butyl 2,4,5-T was present in these soils. These compounds were later identified by mass spectrometry as the iso octyl ethers . The use of polar columns also allows use of p-bromeo phenylacetic acid and its isopropyl ester as internal standards. Since this compound is closely related chemically to the phenoxy herbicides it can be carried through the entire analytical procedure and therby compensate for any loss during extraction and/or methylation. Methyl PBPA cannot be separated from the solvent front when using non-polar columns.

Disadvantage of polar stationary phases are there inability to completely resolve 2,4-D n-butyl ester and 2,4,5-T methyl ester which necessitates the analysis of separate extracts for acid and ester determinations.

Analytical Results - AFLC Test Range -

Table III is the identification of the 45 samples analyzed from the AFLC test range while Table IV provides the results of these analyses.

In order to provide a baseline level for comparison and calculation of degradation rates for all compounds of interest, we calculated the initial soil concentrations of Orange II from application data and then prepared spiked soil samples at these approximate levels of 2,4,5 trichlorophanol. Synthesisk chromatography and mass spectral analysis of 2(2,4,5 trichlorophenoxy) octane further verified this conclusion - see below.

Further study of extracts from the Utah plots on SP-2330 columns revealed that the material originally incorporated in the Utah test plots was not Orange but another formulation known as Orange II. Analysis of this formulation (Orange II) from an archive sample revealed the following composition Table II.

Table II - Composition comparison, Orange I and II

I		II	
53.0%	2,4-D, η-butyl ester	32.8%	2,4,5T isooctyl esters
43.0%	2,4,5-T η-butyl ester	28.9%	2,4-D η-butyl esters
1.1%	2 methoxy - 4,5D η-butyl	16.8%	2,4,5-T η-butyl esters
1.3%	Di-and trichlorophenols	15.3%	2,4-D isooctyl esters
1.6%	other herbicides and esters	0.6%	2,4,5 - Trichlovephenol isoctyl ethers
	2,4,-D & 2,4,5-T acids		
		5.6%	other herbicide acids & testers. 2,4D and 2,4,5T isobutyl esters, MCPA η butylester

not make this conversion. With the exception of J16, most analyses correlate well between the two laboratories sample handling and storage may account for the reversal of ratio of n-butyl esters/acids seen between the two labs on sample J16. Isooctyl ethers were also found in all four samples. Approximately 2,000 ppm ether was found in J16 and GP24 while approximately 1,400 ppm ether was found in J135 and GP10. This likely indicates the initial extent of the spill. While the ratio of ether to ester herbicide provides a good estimation of the age of the spill.

Several other site monitoring samples have been analyzed, GP 9-1, GP10-1, GP9-2, GP 10-2, GP 24-2, GP10-2, with results in general agreement with those obtained by FRL.

Analytical Results - Eglin Test Plots

Table VII identifies the 33 samples analyzed from the Eglin test site while Table VIII gives the analytical results. Since most of these samples contained no esters (except charcoal plots) a modified procedure was used. One gram samples were extracted with 2.0 ml methanol/I.S., 1 ml of the extract methylated, extracted into hexane and chromatographed. A similar procedure was employed to estimate initial concentration as was used with the Utah samples. The calculations and analytical results are shown in Table IX. From these results, half lives were calculated based on remaining herbicides on three different sampling dates; 1640, 2005, and 2400 days after application. As can be seen in Table X, the half life of total 2,4,5T was not significantly different if any of the three sampling dates were chosen for the calculation in the plots without any ammendments. On the other hand, in plots with ammendments (lime, fertilizer, organic matter) the calculated half lives increased with

each succeeding date used for calculation. Half lives in ammended plots were also significantly lower than those in plots were herbicide only was incorporated ($\bar{x}$ = 237 days vs. $\bar{x}$ = 307 days. Half lives calculated from the single sampling of the charcoal plots were considerably longer and still contained low concentrations of herbicide esters even six years after application.

Since samples from plots 5,6,7 and 8 contained only the acid form of 2,4,5T on all sampling dates, half lives for this single component could be calculated by considering the concentration at day 1640 as being the "initial" concentration of this compounds. On this basis, a half life of approximately one year was calculated for both treatments (her. raly and herb and ammendments). As this value is higher than values calculated for total 2,4,5T over the six-year period, one might speculate that additional degradation mechanisms apply to 2,4,5T esters which result in accelerated breakdown. We have long postulated that the first step in degradation is hydrolysis of the ester forms followed by ether cleavage.

These data would seem to argue for another secondary initial step, possibly:

which would complement the hydrolysis mechanisms and result in accelerated breakdown of esters relative to acid forms.

It is anticipated that data contained in this report will be submitted for publication independent of the final report on brodegration. Specifically this will entail (1) those portions dealing with methodology and (2) those portions dealing with identification, synthesis and characterization of TCP isooctyl ethers.