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DETERMINATIONS OF THE EFFECT OF SEVERAL HERBICIDES
ON
SOIL MICROORGANISMS

by

Donald E. Kratochvil

A thesis submitted to the faculty of
South Dakota State College of Agriculture and Mechanic
Arts in partial fulfillment of the requirements for the
degree of Master of Science

December, 1950

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This is to certify that, in accordance with the requirements of South Dakota State College for the Master of Science Degree, Mr. Donald E. Kratochvil has presented to this committee three bound copies of an acceptable thesis, done in the major field; and has satisfactorily passed a two-hour oral examination on the thesis, the major field, Agronomy, and the minor field, Chemistry.

Jan. 5, 1951

Date

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INTRODUCTION

Millions of acres in the United States are being chemically treated each year for the control of weeds. Weed investigators have generally accepted the fact that certain herbicides affect certain plants to a greater degree than others. Because of this, numerous chemicals are being used. Many herbicides are yet in the experimental stage of development, and exact rates of application have not been determined. Older chemicals such as 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), TCA (trichloroacetic acid), PCP (pentachlorophenol), Endothal (disodium 3,6 endoxohexahydrophthalate), IPC (isopropyl-N-phenylcarbamate), E.H. No. 2 (dichloral urea), and E.H. No. 1 (sodium 2,4 dichlorophenyl "cellosolve" sulphate) have had rates established for their specific uses. These rates vary greatly and in a relative inverse proportion to the established phytotoxicity of the several herbicides respectively. We find recommended rates of application varying from a few ounces per acre for herbicides such as 2,4-D; to 100 pounds of the active ingredient per acre when using TCA.

Most of the work done so far, involving both recommended and experimental chemicals, pertains to their herbicidal properties on higher plants. Very little work has been done on the influence these chemicals may have on the soil microorganisms. The bacteriocidal and bacteriostatic properties of 2,4-D have been investigated rather extensively. TCA, while now having a well established use in the control of grass type weeds, has not been investigated as to its influence on micro-organic activity.

It is the purpose of this paper to report a study of the influence that several recommended and experimental herbicides have on the flora

and fauna of the soil. It is also being done to establish the usefulness of a simple procedure outlined by Nelson (8) in making preliminary determinations of this type. A procedure requiring a minimum of equipment and time should be of use in revealing this one phase of herbicidal action of chemicals before general recommendations for field use are made to the public.

LITERATURE REVIEW

The literature available to date on the bacteriocidal qualities of chemicals used in this study refer only to 2,4-D. Investigators have studied the influence of 2,4-D on microorganic activity by several methods. Crafts (2), Hanks (5), Hamner, Moulton, and Tukey (4), Taylor (12), Van Overbeek, and Velox (13) have reported their studies of the persistence of 2,4-D in the soil. Results indicate 2,4-D is relatively non-toxic to most plants after periods ranging from 2 to 7 weeks in most soils. This period may be prolonged in dry soils.

Payne and Fults (9) reported that 0.075 pound of 2,4-D acid per acre when mixed with the soil, was sufficient to depress nodulation of roots of common beans. They do not indicate whether this was due to the effect on the free bacteria, or the depressing effect of the 2,4-D on the bean plants. Lewis and Hamner (6) found no influence of 2,4-D on Rhizobium leguminosarium -- even at high concentrations. This work was carried on by using seeded plates of bacteria and treating these plates by placing a filter paper disc dipped in the 2,4-D over the media of the plate. Carlyle and Thorpe (1) studied the effect of 2,4-D on Rhizobium leguminosarium, R. phaseoli, R. trefolii, R. lupini, R. japonicum, and R. meliloti, using both live plant and plating methods. They concluded that the depressing effect on nitrogen fixing bacteria, if any, occurred as a result of the herbicidal response of the host plants;

rather than the direct response of rhizobium living free in the soil. Martin (7) showed that low concentrations (10 ppm) of 2,4-D did not inhibit soil organisms and fungi. Heavier concentrations inhibited some microorganisms but not others. He reported that 2,4-D was more toxic to soil organisms under acid conditions than under alkaline conditions. Smith, Dawson, and Wenzel (10) concluded that 2,4-D at 1 ppm to 100 ppm had no significant effect on total plate counts of Actinomyces, fungi, and protozoa in the silt loam soil used. Nitrite and nitrate formers were reported injured at 100 ppm but recovered in 10 to 14 days. Stevenson and Mitchell (11) reported fungus (Penicillium sp.) grew vigorously on 0.1 percent 2,4-D in 0.5 percent carbowax and potato dextrose agar at a pH of 5, 6, or 7, but was inhibited at a pH of 8. They also reported that 0.8 percent 2,4-D used on the same media entirely inhibited Bacillus subtilis. Aerobacter cloacae appeared more tolerant. Culler, Weiser and Witman (8) detected no physiological changes due to 2,4-D in cultures of several bacteria associated with food spoilage. Fungi treated were inhibited at very high concentrations of 2,4-D, -- 10,000 ppm or higher.

MATERIALS AND METHODS

Eight herbicidal chemicals were studied in this investigation. They were an amine salt of 2,4-D at rates equivalent to 1, 2, 3, or 4 pounds per acre, 2,4,5-T at rates of 1, 2, 3, 4, 8, or 16 pounds per acre, sodium salt of PCP at 2, 4, 8, or 16 pounds per acre, sodium salt of TCA at 10, 50, 100, or 150 pounds per acre, IPC at 4, 8, or 16 pounds per acre, E.H. No. 2 at 2, 4, or 8 pounds per acre, E.H. No. 1 at 1, 2, 4, or 8 pounds per acre, and Endothal at 1, 2, 4, or 8 pounds per acre. Rates of each chemical were selected to include levels within the range

of recommended field rates of application.

Samples of treated and untreated soil were run in quadruplicate for all chemicals except 2,4,5-T and E.H. No. 2. These latter were run in triplicate.

The technique used in this study is a modification of the one described and used by Nelson (8). Barnes silt loam soil was used for all determinations. The soil was taken from the surface six inches of a field on the South Dakota State College Agronomy farm at Brookings. The field had grown red clover during the preceding season. The soil was high in organic matter. Large roots and stems of the current crop were removed by hand. The soil was air dried by spreading it on a greenhouse bench and was then ground to pass through a 20 mesh sieve. It was thoroughly mixed so that uniform samples would be taken from the stock bin. The pH of the soil was determined to be 6.45 on a 1 to 5 water dilution basis.

One hundred grams of soil were used for each sample. Four grams of dextrose was added to each 100 gram soil sample and thoroughly mixed so that a high media level was obtained for microorganic activity. The samples were placed in 250 cc. Erlenmeyer flasks. Treatments were made using rates of the above named chemicals. The amount of chemicals required for each specific rate were mixed with sufficient water so that when added to the 100 gram soil sample the moisture content was approximately 35 percent by weight. Preliminary trial runs on soil samples of various moisture content showed 35 percent to be optimum for maximum microorganic activity. The flasks were closed with one hole rubber stoppers through which a short piece of glass tubing was inserted. To the outer end of this glass tubing a four inch piece of pure gum rubber

tubing was attached. Without clamping the rubber tubing the flasks were placed in a constant temperature water bath set at 30.5 degrees centigrade. This temperature was selected to be used after several trial runs proved it to be close to optimum for most active growth of the organisms for the period of incubation to be used. The open flasks were incubated for 48 hours to allow the dormant organisms to become active with a constant supply of fresh air. At the end of 48 hours of incubation a pressure clamp was placed on the rubber tubing of each flask. The flask remained closed for four hours, then the free end of the rubber tubing was attached to a suitable mercury manometer and the clamp released, (Figure 1). The pressure built up inside the flasks by evolved gases displaced the mercury in the manometer and this displacement was recorded in centimeters. The gases which built up the pressure within the flasks was assumed to be the end product of the metabolism of microorganisms present in the soil sampled. The basis for this assumption was established by running samples of soil in which microorganisms had been killed by either oven sterilizing or by treating with toluene, an accepted bactericide. After the reading was made the flask was disconnected from the manometer and the clamp opened to permit the release of accumulated gas pressure and entrance of fresh air. Flasks were then closed and incubation continued with pressure recordings repeated at the end of two additional consecutive four hour periods. By this procedure readings were made after 52, 56 and 60 hours of incubation. These periods of incubation were selected after trial runs showed they would give an adequate indication of the relative microorganic activity. Periods of 8 or 12 hours of closed incubation were tried and it was found that the pressure built up during these longer periods was sufficient to break

Figure 1. The mercury manometer used to make readings in centimeters of mercury displacement showing the flasks and the technique used in attachment. Also the constant temperature bath used for incubation of the samples is shown.

the flasks. Periods of incubation of less than 4 hours did not permit the accumulation of sufficient gas to establish relative activity.

The analysis of the data recorded was made by analyzing the readings of the samples from one run of the constant temperature bath. This was done because of the variations in pressure observed for the check samples between consecutive runs of the water bath. These variations were established as being due to slight fluctuations in temperature brought about by minor fluctuations in electrical current of the local power generators.

EXPERIMENTAL RESULTS

Untreated samples of soil were run through the procedure in quadruplicate to determine the periods of incubation to be used in this study. The constant temperature bath was adjusted for 30.5 degrees centigrade. Figure 2 presents the data of the two trials of untreated samples and a trial of 2,4-D at a rate equivalent to 4 pounds per acre. These trials were run at different times. The broken line of the graph represents the average pressure of four replicates of 2,4-D treated soil. Each solid line of the graph represents the average of the four replicate samples of untreated soil. The results show an increase in gas pressure from 52 hours of incubation to approximately 74 to 78 hours of incubation, then a decline in pressure. This curve follows the established pattern of initial bacterial activity under optimum conditions of development.

From these data it was decided that the use of readings at 52, 56 and 60 hours of incubation would be sufficient to give the desired relative data on influence that the chemicals might have on microorganic activity.

Figure 2. The pressure in centimeters of mercury displacement of two separate trials of untreated soil samples and a trial of 2,4-D using readings at 52, 56, 60, 74, 78, and 82 hours after incubation was initiated.

Sterilized Soil Samples

Oven sterilized (109° C for 48 hours) 100 gram samples of soil and 100 gram samples treated with toluene ($\frac{1}{2}$ cc. per 100 gram sample) were incubated with untreated samples to establish the efficiency of the technique described in determination of activity of microorganisms on the basis of relative volumes of gases evolved. Table 1, presents the data on relative microorganic activity in these samples based on centimeters of mercury displaced by gas pressures developed after several periods of incubation.

Table 1. Centimeters of mercury displacement for untreated, toluene treated, and oven sterilized samples of soil after 52, 56, and 60 hours of incubation.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Rep. 1	5.5	7.5	8.9
	Rep. 2	5.3	7.0	9.4
	Rep. 3	5.3	7.4	10.0
	Rep. 4	5.6	7.5	9.0
Average		5.6	7.4	9.3
Toluene	Rep. 1	0	0	0
	Rep. 2	0	0.8	0
	Rep. 3	0	0.6	0
	Rep. 4	0	0.1	0
Average		0	0.38	0
Oven Sterilized	Rep. 1	0	0	0.4
	Rep. 2	0	0	0.1
	Rep. 3	0	0	0.5
	Rep. 4	0	0.1	0
Average		0	0.025	0.25

The data show an average displacement of 5.6, 7.4, and 9.3 cm. for the untreated samples at 52, 56, and 60 hours of incubation respectively. There was no pressure in either the oven sterilized or the toluene treated samples after 52 hours of incubation. After 56 hours of incubation, the toluene treated samples averaged 0.38

centimeters of pressure; the oven sterilized 0.025 centimeters. At the end of 60 hours of incubation there was no pressure in the toluene treated samples and 0.25 centimeters in the oven sterilized samples of soil. The slight pressure found after 56 and 60 hours of incubation for the two treated series can be accounted for by the compression of air in the tube of the manometer when the flasks were attached. This fact was not discovered until the end of this trial when it was found the mercury was occasionally displaced by air compression during this attachment. This operational error was corrected for in later runs by installing a release valve in the manometer coupling so that the mercury could be brought to an established level after the flask was attached and before the gas pressure released and the reading made.

The only reasonable conclusion that can be drawn from the lack of pressure in the toluene treated and oven sterilized samples as compared to pressures recorded from untreated check samples is that in samples of soil where microorganisms have been either destroyed or rendered inactive, there will be no gas evolved under the conditions used in this study. The evolution of gas in the closed flasks is, on a volume basis, directly proportional to the relative activity of the microorganisms.

2,4-dichlorophenoxyacetic Acid

Soil samples treated with the amine salt of 2,4-D at several rates were run with untreated checks. Gas pressure readings were made at the 52, 56, and 60 hour periods of incubation. These data were analyzed statistically. Table 2, shows the results of treatments equivalent to one and two pounds of active acid per acre in comparison with untreated soil samples.

Table 2. The readings of pressure in centimeters of mercury displacement for different periods of time with 2,4-D at one and two pounds per acre.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Rep. 1	5.1	5.3	6.3
	Rep. 2	4.9	4.9	6.4
	Rep. 3	4.8	5.0	6.5
	Rep. 4	5.0	5.1	6.5
Average		4.95	5.07	6.42
Total Average		5.48		
2,4-D 1#/A.	Rep. 1	5.0	5.6	6.8
	Rep. 2	4.6	5.2	6.4
	Rep. 3	5.2	5.6	6.9
	Rep. 4	5.0	5.5	5.9
Average		4.95	5.47	6.5
Total Average		5.64*		
2,4-D 2#/A.	Rep. 1	4.5	4.4	6.1
	Rep. 2	4.9	4.9	5.5
	Rep. 3	5.3	5.1	6.5
	Rep. 4	4.8	5.2	6.3
Average		4.88	4.90	6.1
Total Average		5.29*		

- * Least significant difference for treatments is 0.25 at the 5% level.

Analysis of Variance

Source of Variation	d.f	Ms.	F.
Total	35		
Treatments	2	.37	4.25*
Readings	2	6.955	79.94**
Treatment x Readings	4	.0825	
Within Treat. and Readings	27	.087	

- * Significant at 5% level.
 ** Significant at both 5% and 1% level.

The total average for all untreated samples including all periods of incubation was 5.48 centimeters. The one pound rate showed 5.64 centimeters. The one pound rate showed 5.64 centimeters on the same basis and two pound treatment 5.29. Using the F value to find significance, we find a significant F value for treatments, a least

significant difference analysis showed the two pound rate to be significantly lower at the 5% level in comparison to the one pound treatment but not lower than the untreated samples.

Table 3. Readings of pressure in centimeters of mercury displacement for different periods of time with three and four pound rates of 2,4-D.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Flask 1	5.4	4.8	6.0
	Flask 2	6.1	4.3	5.6
	Flask 3	5.6	5.0	5.6
	Flask 4	6.4	4.9	5.7
Average		5.87	4.87	5.72
Total Average		5.49		
2,4-D 3#/A.	Flask 1	5.3	4.9	5.7
	Flask 2	5.1	5.6	6.5
	Flask 3	6.3	4.6	5.4
	Flask 4	5.9	4.7	5.3
Average		5.65	4.92	5.72
Total Average		5.43		
2,4-D 4#/A.	Flask 1	5.3	5.0	5.6
	Flask 2	5.4	4.6	5.4
	Flask 3	6.3	5.5	6.2
	Flask 4	5.8	5.0	6.1
Average		5.7	5.02	5.82
Total Average		5.52		

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	2	.025	.1497
Readings	2	2.615	15.658**
Treatment x Readings	4	.035	
Within Treat. and Readings	27	.167	

** Significant at both 5% and 1% level.

Table 3, shows the results of treatments equivalent to 3 and 4 pound 2,4-D acid per acre in comparison to untreated samples run at the same time.

The total average centimeters of mercury displacement for untreated samples at all periods of incubation was 5.49. The 3 pound treatment showed a total average of 5.43 centimeters and the four pound treatment showed 5.52. These data were analyzed and no significant F value was found for treatments in comparison to checks. The pressures recorded in Tables 2 and 3 indicate no effect of 2,4-D on microorganic activity of soil under the conditions of this study.

2,4,5-trichlorophenoxyacetic Acid

One, two, or three pounds of 2,4,5-T per acre treated samples using three replications were compared to untreated checks in an individual trial of the constant temperature bath. Rates equivalent to four, eight, or sixteen pounds per acre were compared to untreated samples using three replications in another trial. Tables 4 and 5 show the readings in mercury displacement recorded from the above treatments of 2,4,5-T after incubation for 52, 56 and 60 hours.

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Table 4. Centimeters of mercury displacement by gas pressure for 1, 2, or 3 pounds per acre of 2,4,5-T treated 100 gram soil samples after incubation for 52, 56 and 60 hours.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Flask 1	3.1	5.3	4.8
	Flask 2	4.1	6.5	7.9
	Flask 3	3.1	4.5	6.1
	Average	3.43	5.43	6.27
Total Average		5.04		
2,4,5-T 1#/A.	Flask 1	3.2	5.4	7.5
	Flask 2	3.1	6.1	8.7
	Flask 3	3.3	4.7	6.3
	Average	3.2	5.4	7.5
Total Average		5.36		
2,4,5-T 2#/A.	Flask 1	4.5	7.0	8.1
	Flask 2	3.1	5.3	7.0
	Flask 3	4.0	5.6	8.1
	Average	3.87	5.97	7.73
Total Average		5.85		
2,4,5-T 3#/A.	Flask 1	2.8	4.6	5.5
	Flask 2	3.9	5.6	7.1
	Flask 3	3.4	5.1	6.3
	Average	3.37	5.1	6.3
Total Average		4.91		

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	3	1.56	2.17
Readings	2	36.86	50.92**
Treatment x Readings	6	.44	
Within Treat. and Readings	24	.72	

** Significant at both 5% and 1% level.

Table 5. Centimeters of mercury displacement by gas pressure for 4, 8, or 16 pounds of 2,4,5-T per acre treated 100 gram soil samples after incubation for 52, 56 and 60 hours.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Flask 1	2.7	5.5	6.3
	Flask 2	2.0	4.1	5.6
	Flask 3	2.0	4.1	5.2
Average		2.23	4.56	5.7
Total Average		4.28		
2,4,5-T 4#/A.	Flask 1	1.3	3.6	4.1
	Flask 2	2.3	4.8	5.3
	Flask 3	2.4	4.9	6.4
Average		2.0	4.43	5.43
Total Average		3.95		
2,4,5-T 8#/A.	Flask 1	2.8	5.4	7.6
	Flask 2	1.6	4.0	4.5
	Flask 3	3.4	6.2	6.3
Average		2.6	5.2	6.3
Total Average		4.70		
2,4,5-T 16#/A.	Flask 1	2.9	6.0	7.7
	Flask 2	2.5	4.1	6.4
	Flask 3	2.4	4.5	6.5
Average		2.6	4.87	6.87
Total Average		4.77		

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	3	1.45	1.79
Readings	2	42.65	52.65**
Treatment x Readings	6	.19	
Within Treat. and Readings	24	.81	

** Significant at both 5% and 1% level.

No apparent difference was indicated by the pressures recorded for any rate of 2,4,5-T in comparison to the untreated checks. Analysis of these data did not show a significant F value for treatments in either trial.

Recommended field rates of 2,4,5-T are approximately one pound active acid per acre. From data of this study rates up to sixteen pounds active acid per acre did not affect microorganic activity, therefore, it is concluded that field application of 2,4,5-T for its specific uses are not detrimental to soil microorganisms.

Pentachlorophenol

Samples of soil were treated with rates of PCP equivalent to 2 or 16 pounds per acre and compared to untreated check samples. Four and eight pound rates of PCP were compared to untreated samples in a separate trial. Gas pressure readings were made after 52, 56, and 60 hours of incubation. Table 6 compares the activity of microorganisms in terms of centimeters of mercury displacement for quadruplicate samples of untreated 2 or 16 pound PCP for the establishment periods of incubation.

Untreated checks showed a total average displacement of 7.03 centimeters while the 2 pound treated samples showed 7.55 centimeters and the 16 pound samples 3.06 centimeters. These data were analyzed and a highly significant F value was established for treatment. A least significant difference application showed the 16 pound rate to be significantly lower at both the 5% level and the 1% level in comparison to either untreated checks or the 2 pound rate samples. The 2 pound samples did not show a significant difference when compared to untreated check samples.

Table 6. Centimeters of mercury displacement recorded for untreated checks, 2 or 16 pounds of PCP after 52, 56, and 60 hours of incubation.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Flask 1	7.4	8.6	13.2
	Flask 2	4.0	4.8	7.1
	Flask 3	3.0	3.7	7.0
	Flask 4	6.3	8.1	11.2
	Average	5.17	6.3	9.62
Total Average		7.03		
PCP 2#/A.	Flask 1	6.6	7.9	11.2
	Flask 2	3.6	5.0	7.9
	Flask 3	5.9	7.0	9.8
	Flask 4	6.5	8.0	11.2
	Average	5.65	6.97	10.02
Total Average		7.55		
PCP 16#/A.	Flask 1	2.3	3.8	5.6
	Flask 2	1.1	1.9	2.8
	Flask 3	2.1	3.1	4.2
	Flask 4	1.9	2.9	4.3
	Average	1.87	2.92	4.27
Total Average		3.06**		

** Least significant difference was 1.48 centimeters at 5% level and 2.003 at 1% level.

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	2	72.49	22.94**
Readings	2	43.34	13.72**
Treatment x Readings	4	1.78	
Within Treat. and Readings	27	3.16	

** Significant at 5% and 1% level.

Table 7 presents the pressure readings of samples treated with 4 or 8 pounds of PCP per acre and compared to untreated samples.

The total average displacement for untreated samples in this trial was 6.2 centimeters to 2.9 centimeters for the 4 pound rates and 3.38 centimeters for the 8 pound rates. An analysis of variance of these data established a highly significant F value for treatments. A least

significant difference analysis showed the 4 or 8 pound PCP treatments to have significantly lowered the gas pressure or micro-activity, when compared to untreated check samples. This was established at both the 5% level and the 1% level.

Table 7. Pressure readings for untreated samples, 4 or 8 pounds of PCP after 52, 56, and 60 hours of incubation.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Flask 1	8.6	7.4	9.6
	Flask 2	8.8	8.8	9.7
	Flask 3	1.6	2.8	2.4
	Flask 4	5.5	7.1	7.1
	Average	4.92	6.52	7.2
Total Average		6.2		
PCP 4#/A.	Flask 1	1.0	1.6	2.9
	Flask 2	3.0	4.5	4.6
	Flask 3	2.0	3.0	3.5
	Flask 4	2.0	3.0	3.7
	Average	2.0	3.02	3.67
Total Average		2.9**		
PCP 8#/A.	Flask 1	2.0	3.3	3.8
	Flask 2	2.5	4.0	4.5
	Flask 3	2.6	3.3	4.4
	Flask 4	2.4	3.5	4.2
	Average	2.36	3.52	4.22
Total Average		3.38**		

** Least significant difference was 1.432 at 5% level and 1.934 at 1% level.

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	2	38.195	13.04**
Readings	2	11.77	4.02*
Treatment x Readings	4	.1475	
Within Treat. and Readings	27	2.929	

** Significant at 5% and 1% level.

* Significant at 5% level.

Recommended rates of application of PCP in field use range from 4 to 8 pounds per acre. This investigation indicates that applications in excess of 2 pounds per acre have a depressing effect on microorganic activity. This depressing effect is reasonable considering the fact that PCP is being used generally as a wood preservative.

Trichloroacetic Acid

Soil samples treated with the sodium salt of TCA at rates equivalent to 10 or 50 pounds per acre were compared to untreated samples. Four replications were used. Rates equivalent to 100 or 150 pounds of TCA per acre were likewise compared to untreated samples. Table 8 compares the effect of 10 or 50 pounds per acre application of TCA on microorganic activity to that of untreated soil samples in terms of evolved gas pressure following incubation periods of 5E, 56, or 60 hours.

Total average pressure readings for TCA at all treatment rates were lower than for untreated check samples. Data showed that samples treated with 10 pounds per acre evolved gas which displaced 5.69 centimeters of mercury. The 50 pound per acre samples displaced 4.08 centimeters. Comparing these averages to 9.84 centimeters recorded for the untreated samples by an analysis of variance established a highly significant F value for treatments. A least significant difference analysis showed both treatments to have significantly lowered the gas pressure at the 5% level and at the 1% level.

Table 8. Centimeters of mercury displacement after 52, 56, and 60 hours of incubation for soil samples untreated in comparison to rates of TCA equivalent to 10 or 50 pounds per acre.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Flask 1	5.3	7.9	10.3
	Flask 2	6.5	9.7	12.0
	Flask 3	6.6	10.3	12.4
	Flask 4	5.1	7.9	9.7
	Average	5.87	8.95	11.1
Total Average		5.84		
TCA 10#/A.	Flask 1	3.0	6.4	7.5
	Flask 2	3.8	5.5	8.3
	Flask 3	2.8	5.1	5.3
	Flask 4	4.5	7.2	8.9
	Average	3.52	6.05	7.5
Total Average		5.59**		
TCA 50#/A.	Flask 1	2.8	4.5	5.0
	Flask 2	2.5	3.9	5.0
	Flask 3	3.1	4.7	5.1
	Flask 4	3.0	4.1	5.2
	Average	2.85	4.3	5.07
Total Average		4.08**		

** Least significant difference was .789 centimeters at the 5% level and 1.07 centimeters at the 1% level.

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	2	64.34	72.29**
Readings	2	44.31	49.79**
Treatment x Readings	4	2.29	
Within Treat. and Readings	27	.89	

** Significant at the 5% and 1% level.

Table 9 shows the mercury displacement readings for untreated samples and for samples treated with 100 or 150 pounds per acre of TCA.

Table 9. Centimeters of mercury displacement after 52, 56, or 60 hours of incubation for soil samples treated with rates of TCA equivalent to 100 or 150 pounds per acre in comparison to untreated samples.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Flask 1	6.6	8.8	11.7
	Flask 2	6.4	8.8	11.4
	Flask 3	5.0	8.1	10.0
	Flask 4	7.4	11.5	13.5
	Average	6.2	9.3	11.6
Total Average		9.09		
TCA 100#/A.	Flask 1	3.7	3.3	4.9
	Flask 2	2.0	3.2	4.2
	Flask 3	1.8	3.2	3.2
	Flask 4	2.4	3.9	4.4
	Average	2.3	3.4	4.2
Total Average		8.27**		
TCA 150#/A.	Flask 1	1.5	2.3	3.3
	Flask 2	1.4	1.7	2.5
	Flask 3	1.2	2.2	2.9
	Flask 4	1.6	2.1	3.0
	Average	1.4	2.1	2.9
Total Average		2.14**		

** Least significant difference was 0.719 centimeters at the 5% level and 0.672 centimeters at the 1% level.

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	2	167.00	226.59**
Readings	2	25.745	34.93**
Treatment x Readings	4	4.43	
Within Treat. and Readings	27	.737	

** Significant at the 5% level and 1% level.

Total average centimeters of displacement for untreated checks is 9.09. The 100 or 150 pound treated samples showed a total average of 8.27 centimeters and 2.14 centimeters respectively. Analysis of these data establish a highly significant F value and show the treatments to have caused reduction in gas pressure at both the 5% level and 1% level.

All rates of TCA used in this investigation resulted in reduced gas pressure. This reduction in pressure was found to increase with increase in rates of chemical application. TCA at 150 pounds per acre reduced microorganic activity more than treatments of 15 pounds per acre with PCP. Rates of application of TCA recommended for control of perennial grasses range from 40 to 100 pounds per acre. This investigation indicates that these rates greatly depress activity of soil microorganisms.

Isopropylphenyl Carbamate

Soil samples treated with four or eight pounds of IPC per acre, using four replications, were directly compared to untreated checks. Rates of IPC equivalent to 15 or 32 pounds per acre were compared to untreated samples in a second trial. Table 10 compares the readings in mercury displacement recorded after 52, 56 and 60 hours of incubation for the four and eight pound rates of IPC in comparison to untreated checks.

The untreated samples showed an average of 8.41 centimeters of mercury displacement for all periods of incubation. IPC treated samples showed an average of 8.57 centimeters, and 6.63 centimeters, for the four and eight pound per acre rates respectively. Analysis of these data established a significant *F* value for treatments. The least significant difference analysis showed the eight pound per acre treatments to have significantly lowered microorganic activity at both the 5% level and 1% level in comparison to untreated checks. The four pound treatment was not found to be significantly different from the untreated checks.

Table 10. Centimeters of mercury displacement after 52, 56 and 60 hours of incubation for soil samples treated with rates of IPC equivalent to 4 or 8 pounds per acre in comparison to untreated samples.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Flask 1	4.5	6.4	10.5
	Flask 2	7.1	8.0	11.7
	Flask 3	6.9	8.3	11.9
	Flask 4	6.4	7.9	11.3
	Average	6.2	7.7	11.4
Total Average		8.41		
IPC 4#/A.	Flask 1	7.3	8.9	12.1
	Flask 2	4.8	7.2	10.0
	Flask 3	7.1	8.4	12.0
	Flask 4	8.5	7.1	10.0
	Average	6.2	7.9	11.0
Total Average		8.37		
IPC 8#/A.	Flask 1	4.9	6.1	9.0
	Flask 2	7.1	8.5	12.3
	Flask 3	3.1	4.2	6.5
	Flask 4	4.2	5.5	8.2
	Average	4.8	6.1	9.0
Total Average		6.63**		

** Least significant difference was 0.355 centimeters at the 5% level and 0.479 centimeters at the 1% level.

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	2	12.32	68.44**
Readings	2	69.92	388.44**
Treatment x Readings	4	.31	
Within Treat. and Readings	27	.18	

** Significant at the 5% and 1% level.

Table 11. Centimeters of mercury displacement after 52, 56, and 60 hours of incubation for soil samples treated with rates of IPC equivalent to 16 or 32 pounds per acre in comparison to untreated check samples.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Flask 1	7.0	8.1	9.1
	Flask 2	4.0	4.9	6.0
	Flask 3	6.8	7.9	7.7
	Flask 4	6.5	7.1	7.0
	Average	6.1	7.0	7.5
Total Average		6.84		
IPC 16#/A.	Flask 1	5.5	6.5	6.8
	Flask 2	4.3	5.1	6.6
	Flask 3	4.1	5.4	6.8
	Flask 4	2.8	3.6	4.5
	Average	4.2	5.2	6.2
Total Average		5.17**		
IPC 32#/A.	Flask 1	4.8	5.8	6.6
	Flask 2	4.0	4.8	5.2
	Flask 3	4.4	5.4	5.8
	Flask 4	5.2	5.2	5.9
	Average	4.6	5.3	5.9
Total Average		5.26**		

** Least significant difference was 0.904 centimeters at the 5% level and 1.22 centimeters at the 1% level.

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	2	10.645	9.145**
Readings	2	7.265	6.241**
Treatment x Readings	4	.165	
Within Treat. and Readings	27	1.164	

** Significant at the 5% level and the 1% level.

Table 11 presents the data and analysis of these data for samples treated with 16 or 32 pounds IPC per acre and compared to untreated check samples. These rates of treatment reduced gas pressure in comparison to untreated samples. The untreated check samples showed an average of 6.84

centimeters of mercury displacement for all periods of incubation while the 16 pound rate showed 5.17 centimeters and the 32 pound rate 5.26 centimeters. Analysis of these data established a highly significant F value for treatments. A least significant difference application showed the IPC treatments to have significantly depressed micro-activity in comparison to checks.

Results of this investigation using IPC at rates equivalent to 4, 8, 16, or 32 pounds per acre indicate there is a range of application of this herbicide at which microorganic activity is inhibited. This range was found to be above 4 pounds per acre.

Endothal

Soil samples treated with Endothal at several rates equivalent to experimental field rates of application compared to untreated check samples in two separate trials. Gas pressure readings were recorded after 52, 56, and 60 hours of incubation. Table 12 shows the pressure readings for rates of 1 or 8 pounds per acre treatments as compared to untreated check samples. Table 13 shows similar time period readings for rates of application of 2 or 4 pounds per acre compared to check samples.

Gas pressure readings of Tables 12 or 13, indicate that micro-activity was not depressed by Endothal at any rate of application tested. Untreated samples of Table 12 showed a total average reading for all periods of incubation of 7.23 centimeters. The 1 pound per acre treatments with Endothal show a total average of 8.73 centimeters. An analysis of variance showed pressure recorded for samples treated with this rate to be significantly higher than for the untreated checks at both the 1% level and the 5% level of significance. Pressures recorded for

Table 12. Centimeters of mercury displacement after 52, 56 and 60 hours of incubation for soil samples treated with rates of Endothal equivalent to 1 or 8 pounds per acre in comparison to untreated samples.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Flask 1	6.1	7.8	10.3
	Flask 2	5.4	6.8	9.1
	Flask 3	4.5	6.1	7.6
	Flask 4	6.1	7.4	9.5
	Average	5.5	7.0	9.1
Total Average		7.23		
Endothal 1 $\frac{1}{2}$ /A.	Flask 1	5.8	7.7	9.6
	Flask 2	6.8	8.1	11.3
	Flask 3	7.4	9.1	12.3
	Flask 4	7.3	8.7	10.7
	Average	6.8	8.4	11.0
Total Average		8.73**		
Endothal 8 $\frac{1}{2}$ /A.	Flask 1	7.0	8.5	10.7
	Flask 2	5.6	6.8	8.7
	Flask 3	4.4	5.2	7.2
	Flask 4	6.0	7.6	9.8
	Average	5.8	7.0	9.1
Total Average		7.29		

** Least significant difference was 0.88 centimeters at the 5% level and 1.19 centimeters at the 1% level.

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	2	8.72	7.86**
Readings	2	41.71	37.58**
Treatment x Readings	4	.18	
Within Treat. and Readings	27	1.11	

** Significant at the 5% and 1% level.

Table 13. Centimeters of mercury displacement after 52, 56 and 60 hours of incubation for soil samples treated with rates of Endothal equivalent to 2 or 4 pounds per acre in comparison to untreated samples.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Flask 1	6.1	8.5	11.1
	Flask 2	5.9	8.0	11.6
	Flask 3	5.6	8.0	8.8
	Flask 4	6.3	9.1	11.5
	Average	6.0	8.4	10.8
Total Average		6.38		
Endothal 2#/A.	Flask 1	5.5	8.6	11.0
	Flask 2	6.5	8.0	10.6
	Flask 3	6.0	8.0	11.2
	Flask 4	6.6	10.0	12.9
	Average	6.2	8.7	11.4
Total Average		6.74		
Endothal 4#/A.	Flask 1	5.4	8.1	8.9
	Flask 2	4.9	7.6	10.4
	Flask 3	5.8	8.6	14.3
	Flask 4	4.8	7.4	9.5
	Average	5.2	7.9	10.8
Total Average		7.98		

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	2	1.77	1.49
Readings	2	81.13	68.18**
Treatment x Readings	4	.17	
Within Treat. and Readings	27	1.19	

** Significant at the 5% level and the 1% level.

other rates of application were not shown to be significantly different from those of untreated check samples.

Endothal is a herbicide that is considered to be still in the experimental stage. Recommendations at the present time indicate rates of application of approximately 2 to 4 pounds active ingredient per acre for control of annual grass weeds. This investigation indicates a

stimulatory affect on soil microorganisms resulting from the use of 1 pound of the active acid per acre, and no affect or microorganic activity was established in using rates equivalent to 8 pounds per acre.

E.H. No. 2

Micro-activity of untreated 100 gram soil samples was compared to similar samples, treated with E.H. No. 2 at rates equivalent to 2, 4, or 8 pounds per acre. Gas pressure readings were made after 52, 56, and 60 hours of incubation using three replications.

Table 14 indicates the mercury displacement readings recorded for samples in this trial. Samples treated with all rates of the herbicide tested showed a total average reading for all periods of incubation lower than directly comparable untreated check samples. The averages were 8.19 centimeters for untreated samples and 7.19, 5.74, and 6.53 centimeters for the 2, 4, and 8 pound per acre rates of E.H. 2 respectively. An analysis of these data shows a significant F value for treatments. Using the least significant difference determination it was established that all rates of application of E.H. 2 had significantly lowered the gas pressure readings at both the 5% level and the 1% level in comparison to untreated samples.

Table 14. Centimeters of mercury displacement after 52, 56 and 60 hours of incubation for soil samples treated with rates of E.H. No. 2 equivalent to 2, 4, or 8 pounds per acre in comparison to untreated samples.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Flask 1	6.1	8.2	8.8
	Flask 2	6.4	8.8	9.5
	Flask 3	6.6	8.7	10.6
	Average	6.4	8.6	9.6
Total Average		8.19		
E.H. No. 2 2#/A.	Flask 1	5.9	8.0	10.3
	Flask 2	5.4	7.4	7.8
	Flask 3	4.8	6.9	8.2
	Average	5.4	7.4	8.8
Total Average		7.19**		
E.H. No. 2 4#/A.	Flask 1	4.7	5.8	7.0
	Flask 2	4.5	5.7	7.2
	Flask 3	3.8	5.7	7.5
	Average	4.3	5.7	7.2
Total Average		5.74**		
E.H. No. 2 8#/A.	Flask 1	4.6	6.8	7.4
	Flask 2	5.3	7.2	8.5
	Flask 3	4.8	6.5	8.7
	Average	4.9	6.8	8.2
Total Average		6.83**		

** Least significant difference was 0.615 centimeters at the 5% level and 0.854 centimeters at the 1% level.

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	3	9.647	6.89**
Readings	2	31.185	22.28**
Treatment x Readings	6	.14	
Within Treat. and Readings	24	.40	

** Significant at the 5% level and 1% level.

Results indicate that E.H. No. 2 at all rates of application tested here markedly depress microorganic activity in treated soil. This depressing effect does not appear to be as severe as that induced by TGA or PCP at comparable field levels of application.

E.H. No. 2 is an experimental herbicide being investigated at the present time for control of grass weeds. Recommended field application is approximately 2 to 4 pounds per acre.

E.H. No. 1

Micro-activity in soil samples treated with E.H. 1 at rates equivalent to 1 or 8 pounds per acre were compared to that in untreated check samples in a single run of the constant temperature bath. Rates of 2 or 4 pounds per acre were compared to untreated checks in a similar trial. Periods of incubation were the same as those used for the other herbicides. Tables 15 and 16 show the gas pressure readings of soil samples treated with the several rates of E.H. No. 1 in comparison to untreated samples.

All rates of E.H. No. 1 increased evolution of gas as indicated by total average pressure reading data. In Table 15 the untreated samples are shown to have averaged 7.54 centimeters for all time periods; the 1 pound per acre treatments average 7.98 centimeters, and the 8 pound per acre treatment 8.56 centimeters. Analysis of these data establish a significant F value. A least significant difference analysis showed the 8 pound treatment rate had increased gas pressure significantly at the 5% level in comparison to untreated soil samples.

Table 15. Centimeters of mercury displacement after 52, 56 and 60 hours of incubation for soil samples treated with rates of E.H. No. 1 equivalent to 1 or 8 pounds per acre in comparison to untreated samples.

Treatment	Pressure Readings in Centimeters		
	52 hours	56 hours	60 hours
Untreated			
Flask 1	5.4	7.7	10.0
Flask 2	4.7	6.8	7.7
Flask 3	6.1	8.7	11.8
Flask 4	5.4	7.4	8.8
Average	5.4	7.7	9.6
Total Average	7.54		
E.H. No. 1 1 $\frac{1}{2}$ /A.			
Flask 1	6.1	8.6	11.3
Flask 2	4.7	7.8	10.4
Flask 3	5.7	8.3	10.5
Flask 4	5.4	7.9	9.1
Average	5.5	8.2	10.3
Total Average	7.98		
E.H. No. 1 8 $\frac{1}{2}$ /A.			
Flask 1	6.3	9.5	11.6
Flask 2	5.4	8.0	9.6
Flask 3	5.6	8.1	11.0
Flask 4	6.9	8.9	11.8
Average	6.1	8.6	11.0
Total Average	8.56*		

* Least significant difference was 0.753 centimeters at the 5% level.

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	2	3.12	5.85*
Readings	2	65.22	80.52**
Treatment x Readings	4	.19	
Within Treat. and Readings	27	.81	

* Significant at the 5% level.

** Significant at the 1% level.

Table 16 shows the untreated samples averaged 8.96 centimeters for all periods of incubation while the treatments averaged 9.18 for the 2 pound rate and 9.66 for the 4 pound rate respectively. No significance was established for treatments at these rates.

Table 16. Centimeters of mercury displacement after 52, 56 and 60 hours of incubation for soil samples treated with rates of E.H. No. 1 equivalent to 2 or 4 pounds per acre in comparison to untreated samples.

Treatment		Pressure Readings in Centimeters		
		52 hours	56 hours	60 hours
Untreated	Flask 1	6.2	9.9	10.8
	Flask 2	6.3	10.1	9.3
	Flask 3	6.7	11.0	11.2
	Flask 4	6.4	10.5	9.3
Average		6.4	10.4	10.2
Total Average		8.96		
E.H. No. 1 2#/A.	Flask 1	7.8	11.7	12.3
	Flask 2	5.8	9.5	9.8
	Flask 3	6.1	9.5	8.7
	Flask 4	7.4	11.3	10.5
Average		6.8	10.5	10.3
Total Average		9.18		
E.H. No. 1 4#/A.	Flask 1	7.3	11.4	11.8
	Flask 2	7.3	12.2	12.2
	Flask 3	6.1	9.8	9.7
	Flask 4	6.2	10.7	11.2
Average		6.7	11.0	11.2
Total Average		9.66		

Analysis of Variance

Source of Variation	d.f.	Ms.	F.
Total	35		
Treatments	2	1.48	1.54
Readings	2	62.70	65.31**
Treatment x Readings	4	.27	
Within Treat. and Readings	27	.96	

** Significant at the 5% level and 1% level.

Results of these trials indicate that E.H. 1 at field rates of application exerts a stimulatory influence on microorganic activity in soil. This stimulation appeared to increase with the increase in rate of herbicide within levels of applications included in these tests.

SUMMARY AND CONCLUSIONS

One hundred gram samples of Barnes silt loam soil were treated at several field rates of eight herbicidal chemicals. Soil samples containing 35% moisture were placed in 250 cc. Erlenmeyer flasks and incubated in a constant temperature water bath at 30.5 degrees centigrade. Following a short period of incubation, to activate microorganisms, the flasks were closed and incubation continued for a total period of 52, 56 or 60 hours. Determinations of gas pressure evolved through the activity of the soil borne bacteria and fungi were made at these intervals. The determinations were made by using a suitable mercury manometer. The validity of using evolved gas pressure as an indication of microorganic activity was established by comparing the relative micro-activity and gas pressures evolved in oven sterilized or toluene treated samples with untreated check samples of soil.

Relative effect of the several chemicals at the various rates of application investigated here was established by analyzing data comparing microorganic activity expressed as gas pressure evolved as the result of micro-activity in treated soils, after consecutive and fixed periods of incubation, in terms of mercury displacement; with similar determinations recorded for untreated, but otherwise comparable, check samples of soil.

The recapitulation of the analyzed data is presented in graphic form by Figures 3 and 4.

Figure 3. Microorganic activity in soil treated with several herbicides at various rates and measured in centimeters of mercury pressure after uniform periods of incubation. A. One, 2, 3, or 4 pounds per acre 2,4-D. B. One, 2, 3, 4, 8, or 16 pounds per acre 2,4,5-T. C. Ten, 50, 100, or 150 pounds per acre TCA. D. Two, 4, 8, or 16 pounds per acre PCP.

Figure 4. Microorganic activity of soil treated with several herbicides at various rates and measured in centimeters of mercury pressure after uniform periods of incubation. E. Four, 8, 16, or 32 pounds per acre IPC. F. One, 2, 4, or 8 pounds per acre Endothal. G. Two, 4, or 8 pounds per acre E.H. No. 2. H. One, 2, 4, or 8 pounds per acre E.H. No. 1.

The graphs represent the gas pressure readings for each treatment rate of each chemical included in this study. The untreated check line of each graph was established as an average of the pressure readings recorded for untreated check samples included in all trials with a single herbicide. The graphs indicate that TCA, PCP, IPC and E.H. No. 2 depressed microorganic activity to a greater degree than comparable field rates of other herbicides included in this investigation. Trichloroacetic acid and PCP indicate greater depression than does IPC or E.H. No. 2. The depressing effect of the high rates of TCA and PCP on microorganic activity is indicated to be less severe at later sampling dates following longer incubation periods.

Three herbicides; 2,4-D, 2,4,5-T and Endothal indicate no significant influence on relative microorganic activity at rates of treatment included in this study.

E.H. No. 1 was found to have a stimulatory effect on micro-activity. The apparent stimulatory effect increased as the rate of treatment increased.

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