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Effects of Pesticides on Soil Microflora Using Dalapon as an Example

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Abstract. Effects of the herbicide dalapon on the soil microflora are evaluated. Measurements were made on populations and pure cultures of micro-organisms, dehydrogenase and phosphatase, soil respiration, and nitrogen transformations. At normal concentrations of 2.6 and 26 ppm, dalapon had little effect and is unlikely to harm the soil microflora or soil fertility. At abnormal concentrations of 266 and 2,660 ppm, marked effects occurred on the microflora and its activities. In one soil, 2,660 ppm dalapon significantly increased production of ammonium-nitrogen; nitrification was almost totally inhibited in this soil. The results are discussed in terms of the validity of the tests for detecting effects of pesticides on the soil microflora. Some problems, particularly of data interpretation and evaluation, are highlighted.

Agriculture relies on chemicals to control weeds, pests and diseases. This reliance is unlikely to diminish and the risk that uncontrolled pesticide use may impair soil fertility has caused concern. Thus, registration authorities now impose more stringent controls. The United States Environmental Protection Agency (EPA) requirements for testing effects of pesticides on soil micro-organisms have been strengthened recently (Anon 1978). Discussions in the Council of Europe and elsewhere may result in similar guidelines. It is widely held that laboratory tests are inadequate for assessing and predicting the likely impact of pesticides on microbial functions. Nonetheless, some form of testing is necessary, if only to allay public concern. Thus, Anderson (1973) suggested a program of tests, later modified by Johnen and Drew (1977). Similar programs are described by Atlas *et al.* (1978) and Greaves *et al.* (1978a). Recently, an international group of microbiologists has recommended a simple test program (Greaves *et al.* 1980). These programs were not intended to be rigid, but to be flexible to enable factors such as the intended use of a pesticide and its mode of action to be considered. All the suggested programs have disadvantages which are difficult or impossible to overcome. However, they are among the most suitable available at present. The purpose of this paper is to examine some of these tests for detecting the effects of pesticides on micro-organisms. The

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Table 1. Some characteristics of the soils used

Soil	Begbroke North (BN)	Boddington Barn (BB)
Particle size class[a]	Loamy sand	Sandy loam
pH (in water)	6.35	7.6
Available P, μgP g^{-1} soil	10.6	12.6
Organic C %	2.4	1.7
Total N %	0.24	0.17
Ammonium-N, μgN g^{-1} dry soil	0.5	0.8
Nitrate-N, μgN g^{-1} dry soil	11.5	4.7
CEC mEq 100 g^{-1}	23.9	21.7
Clay %	9.5	14.0
Silt %	15.5	16.5
Coarse sand %	48.0	32.0
Fine sand %	28.0	37.5

[a] Hodgson *et al.* (1976)

herbicide dalapon (2,2-dichloropropionic acid, sodium salt) was selected for this assessment, because it has a high water solubility and low adsorption in soil. In addition, dalapon causes some repeatable effects, especially on nitrification (Worsham and Giddens 1957, Davies and Marsh 1977). It is of short persistence and is degraded microbiologically (Kaufman 1964) and its environmental safety has been reviewed by Kenaga (1974). Some concentrations used in this work were many times in excess of those which might arise in field conditions, and were used solely as a means of provoking effects on the soil microflora.

Methods

Soils: Two soils, sampled from fields at the Weed Research Organization, were taken in April after a winter fallow following spring barley (Begbroke North, BN) or winter barley (Boddington Barn, BB). Soil characteristics are given in Table 1. Sampling methods are described by Greaves *et al.* (1978a).

Herbicide: Dalapon (2,2-dichloropropionic acid, sodium salt; Boots Dalapon Weedkiller, Boots Farm Sales Ltd., Nottingham, UK), containing 72% (w/w) acid equivalent, was used at concentrations of 2.6, 26.6, 266 and 2,660 ppm of acid equivalent in dry soil. The first two concentrations are equivalent to approximately 3 and 30 kg ha^{-1} (recommended rates), respectively, assuming the herbicide is uniformly distributed in the top 10 cm of the soil profile. Localized accumulations of herbicide which can occur in soil were simulated by 266 ppm and the highest concentration, 2,660 ppm, was selected as it is known to cause repeatable effects on certain microbial populations and processes.

Soil treatment and incubation: Soils were sprayed with herbicide and mixed as described by Greaves *et al.* (1978a) and incubated at 23°C in the dark for 32 weeks. Control samples were sprayed with deionized water instead of herbicide and all samples were adjusted to and maintained at 80% of moisture holding capacity. BN soil received all concentrations of the herbicide but BB soil only the highest.

Sampling: Treatment of soils and pot filling took a full day and the first samples were taken one day after treatment (referred to as Week 0). At each sample date, four replicate pots were taken from each treatment, soil in each pot thoroughly mixed and subsampled for the different measurements. Residual soil from each pot was stored at −15°C, until it was analyzed for dalapon residues.

Microbial populations: Populations of total viable bacteria, cellulolytic bacteria, sporeforming bacteria, actinomycetes, fungi, cellulolytic fungi, and algae were counted by the spread-plate technique with the appropriate medium (Greaves *et al.* 1978a). Limitations of time and labor made it possible to count populations only in bulked replicates of BN soil. No counts were done for BB soil.

Pure cultures: Bacteria (98), actinomycetes (40), fungi (54) and algae (22) were isolated from BN soil by taking colonies from spread-plates. These isolates were purified and maintained at 4°C. Herbicide solutions, sterilized by membrane filtration (0.22 μm pore diam.), were added to molten agar at 50°C to give a final concentration of 2,660 ppm dalapon. The agar for each microbial group was that used for counting. Fungi were inoculated on to the centers of herbicide-agar plates as 5 mm discs, cut from young colonies with a sterile cork-borer. Other organisms were inoculated at the periphery of plates (5 cultures/plate). Algae were incubated under illumination with a day length of 16 hr at 25 ± 1°C for 14 days. Other organisms were incubated in the dark at 19 ± 1°C for 7 days. Growth, pigmentation, and sporulation of the cultures were examined visually and recorded. A similar experiment used liquid media containing 13,000 ppm of the herbicide. Dalapon at 2,660 ppm in soil at 20% moisture content (80% moisture holding capacity) is equal to 13,000 ppm in the soil solution. Two replicate 10 ml aliquots of medium were inoculated for each organism.

Microbial activities: Dehydrogenase and phosphatase activities, carbon dioxide evolution from soils, and the production of nitrate-, nitrite- and ammonium-nitrogen were measured as described by Greaves *et al.* (1978a).

Herbicide residues: Dalapon residues were extracted from soil and analyzed as described by Cotterill (1975).

Statistical analyses: Analyses of variance were carried out on the data at each sampling time and treatment means were compared with control by t-tests.

Results

Microbial populations and activities in soils treated with low concentrations of dalapon were both increased and decreased. The changes occurred infrequently and randomly during the experiment. Often control data fluctuated widely with time. The difference between treatment and control often is significant only because the control changed whereas the treatment did not change. These apparently significant effects, often appearing after the chemical disappeared, probably indicate a lack of synchrony between fluctuations in the samples, and not effects of the chemical. Data will be presented only for concentrations showing marked effects.

Herbicide residues: The three lower concentrations of dalapon degraded rapidly in BN soil. No residues of the 2.6 and 26 ppm concentrations were detected after 2 and 4 weeks, respectively. Even when the initial concentration was 266 ppm, residues could not be detected after 12 weeks. At 2,660 ppm, degradation was slower with 1,215 ppm still present after 32 weeks. In contrast, in BB soil, 2,660 ppm dalapon declined to 190 ppm after 32 weeks and extrapolation suggests that it would have disappeared after 40 weeks.

Microbial populations: Even with dalapon at 2,660 ppm, bacterial spores showed little response (Table 2). The changes recorded are probably random fluctuations rather than effects of the herbicide. Dalapon at 2,660 ppm caused an initial statistically significant reduction in bacterial numbers followed by a significant increase at weeks 3 and 4 (Table 2) and significant decreases at weeks 8, 10, and 16. No effects were found at 266 ppm, but at 2.6 and 26 ppm significant decreases occurred in five samples. The lack of effect at 266 ppm make it unlikely that these are due to the chemical. The effects of 2.6 ppm at weeks 8 and 10 are probably random fluctuations, since no herbicide was

Table 2. The effect of dalapon on numbers of total bacteria, bacterial spores and actinomycetes ($\times 10^6$ g^{-1} dry soil) and fungi ($\times 10^5$ g^{-1} dry soil) Begbroke North soil

Herbicide dose (ppm)	Time (weeks) 0	1	2	3	4	6	8	10	12	16	21	24	28	32
Bacteria														
0	50.0	69.5	31.8	36.0	16.5	18.7	44.8	32.6	17.2	17.8	13.2	12.3	11.2	16.2
2,660	31.1[c]	14.7[a]	34.0	81.1[a]	39.2[c]	22.6	31.5[c]	13.7[b]	14.2	7.0[c]	10.9	8.9	7.8	24.8
± S.E.	4.9	6.3	2.7	6.1	6.9	3.2	4.7	3.6	2.1	3.4	2.5	1.3	1.6	3.4
Bacterial spores														
0	2.9	4.2	4.7	4.2	4.4	3.7	6.2	3.4	3.2	1.1	2.9	1.1	3.2	2.5
2,660	2.6	4.9	4.0	3.6	5.1	4.5	4.1[b]	4.4	4.1	3.8[a]	4.2[c]	1.1	1.8[b]	2.5
± S.E.	0.3	0.3	0.3	0.3	0.3	0.4	0.5	0.4	0.6	0.2	0.3	0.2	0.3	0.3
Actinomycetes														
0	2.5	4.8	3.3	2.4	7.0	5.7	8.7	4.0	2.2	3.4	3.5	2.5	3.4	4.2
2,660	4.7	3.0	2.3	4.0	7.2	1.4[c]	4.4[c]	0.9[c]	0.3	0.2[b]	1.2	1.1	0.6[c]	ND
± S.E.	0.9	0.8	0.9	0.8	1.5	1.2	1.1	0.9	0.7	0.5	0.8	0.8	0.7	0.8
Fungi														
0	1.7	2.2	1.4	3.4	1.5	1.3	1.9	1.8	1.7	1.7	2.4	1.5	1.2	1.3
2,660	1.8	2.3	3.3[a]	0.8[a]	4.6[a]	3.7[a]	3.1	2.2	6.4[a]	6.9[a]	4.9[a]	3.0[b]	7.9[a]	5.1[a]
± S.E.	0.2	0.2	0.2	0.2	0.2	0.3	0.5	0.2	0.2	0.2	0.3	0.3	0.4	0.2

[a,b,c] Significantly different from control at P = 0.001, 0.01, 0.05, respectively. ND not determined

detected after week 2. Dalapon at 2,660 ppm caused statistically significant reductions in actinomycete populations (Table 2) only after 6 weeks exposure. Increased fungal populations in soil treated with 2,660 ppm dalapon persisted throughout most of the experiment (Table 2), after a decrease at week 3. At the lower doses, there were random small inhibitory effects, many occurring after disappearance of the chemical. *Acremonium, Gliocladium, Trichoderma, Fusarium* and *Penicillium* were dominant, only *Penicillium* showing marked response to the herbicide. Thus, in the fourth and subsequent weeks, a 15-fold increase in *Penicillium* colonies occurred, this genus forming about 95% of the total population from treated soil during this time. The population of cellulolytic fungi responded to dalapon (Table 3) in exactly the same way. After 3 weeks exposure to 2,660 ppm dalapon, numbers of cellulolytic bacteria fell to about 50% of those in the control and stayed low to the end of the experiment. At lower concentrations, both reductions and increases were noted, mainly after the herbicide had disappeared. All concentrations of dalapon caused an initial reduction in the numbers of algae other than diatoms (Table 4). At the three lower concentrations the effect was transient lasting a maximum of 2 weeks at 266 ppm. Subsequently, random significant increases in population were noted, but only up to week 24. At the lowest concentration two significant decreases occurred, 8 and 28 weeks after application of the herbicide. Dalapon at 2,660 ppm was inhibitory to the algae in most samples during the 32 weeks incubation. Effects on diatoms (Table 4) initially were similar to those on other algae, but the decreases immediately after treatment were only statistically significant at the two highest concentrations. At all concentrations, a significant increase was found one week after treatment and further small increases occurred at the 2.6 and 26 ppm levels. Two transient small decreases were detected at weeks 2 and 28 in the 2.6 ppm treatment. At the two highest concentrations, the increase at week 1 was immediately followed by marked persistent decreases and, at 2,660 ppm, diatoms could be detected in only one subsequent sample (12 wks). It must be stressed that these results for algae were obtained from soils incubated in the dark and may be atypical. The algae may be sensitized to dalapon by their lack of photosynthesis.

Pure cultures: In both agar and liquid media, 2,660 ppm dalapon had little effect on three microbial groups tested. Only the algae were markedly affected, 32% inhibition. Herbicide at 13,000 ppm produced severe effects, 87% of actinomycetes and all the bacteria and algae were killed. However, 70% of the fungi were unaffected, 12% showing increased growth and only 18% inhibited. Visual examination of these fungi indicated that 10%, mainly *Penicillium* spp., showed increased spore production, which may account for the increases in the soil (Table 2). Many were cellulolytic, this possibly accounted for their increase in the soil (Table 3).

Enzymes: Phosphatase in BN soil (Table 5) was inhibited by dalapon at all concentrations. At the two lower concentrations, the effect had disappeared by week 2 and at 266 ppm by week 3. Subsequently, small, statistically significant increases occurred, especially at 266 ppm. The herbicide was inhibitory throughout the experiment at the highest concentration in BB soil although in BN soil the effect was less consistent. Dehydrogenase activity (Table 6) was inhibited in a few samples by 2.6 and 26 ppm herbicide. At 266 and 2,660 ppm, inhibition in BN soil was marked throughout the experiment, especially at the

Table 3. The effect of dalapon on numbers ($\times 10^4$ g^{-1} dry soil) of cellulolytic fungi in Begbroke North soil

Herbicide dose (ppm)	Time (weeks) 0	1	2	3	4	6	8	10	12	16	21	24	28	32
0	7.4	6.1	6.0	14.1	5.2	3.0	2.4	3.1	2.6	2.2	1.9	2.3	2.4	2.3
2,660	8.0	8.1^c	11.6^b	4.8^a	14.9^a	4.9^a	3.5^b	4.5^c	4.0^b	3.5^a	2.6	4.1^a	4.9^a	4.5
± S.E.	0.8	0.6	1.0	0.6	1.3	0.3	0.2	0.4	0.3	0.2	0.3	0.2	0.2	0.2

[a,b,c] Significantly different from control at P = 0.001, 0.01, 0.05, respectively

Table 4. The effect of dalapon on numbers ($\times 10^4$ g^{-1} dry soil) of algae in Begbroke North soil

Herbicide dose (ppm)	Time (weeks) 0	1	2	3	4	6	8	10	12	21	24	28	32
Algae other than diatoms													
0	1.3	ND	42.2	31.9	34.2	17.8	52.0	9.6	30.7	2.2	12.7	29.2	27.7
2,660	0.2^c	ND	24.9^a	13.5^a	14.6^b	19.8	41.2^c	6.5	27.7	0.6	4.2^b	4.9^a	7.1^a
± S.E.	0.3	ND	2.9	5.1	4.2	5.8	3.1	1.7	1.4	0.6	1.6	2.2	3.0
Diatoms													
0	3.0	26.8	14.3	5.8	11.3	5.7	7.7	2.8	4.4	<0.1	0.2	1.1	1.1
266	0.8^c	36.6^c	2.6^a	4.3^c	6.7^c	5.0	4.4^b	1.3^a	3.1	<0.1	0.3	$<0.1^b$	0.3
± S.E.	0.7	2.4	0.9	0.5	1.2	0.7	0.7	0.2	0.6		0.2	0.2	0.3

[a,b,c] Significantly different from control at P = 0.001, 0.01, 0.05, respectively. ND not determined

Table 5. The effect of dalapon on phosphatase activity of soil as mg phenol released g^{-1} dry soil

Soil	Begbroke North				Boddington Barn		
Herbicide dose (ppm)	0	266	2,660	± SE	0	2,660	± SE
Time (wks)							
0	0.43	0.43	0.42	0.008	0.22	0.21	0.005
1	0.45	0.42[a]	0.38[a]	0.004	0.21	0.18[a]	0.002
2	0.43	0.38[c]	0.36[a]	0.010	0.19	0.16[c]	0.005
3	0.39	0.39	0.35	0.020	0.19	0.15[a]	0.003
4	0.44	0.44	0.41[b]	0.006	0.21	0.18[a]	0.002
6	0.52	0.53	0.49[c]	0.008	0.26	0.20[a]	0.004
10	0.43	0.45[c]	0.42	0.006	0.25	0.17[a]	0.004
12	0.47	0.47	0.44[c]	0.006	0.25	0.19[a]	0.003
16	0.41	0.45[a]	0.40[c]	0.004	0.23	0.15[a]	0.004
20	0.38	0.39	0.35[c]	0.006	0.19	0.14[a]	0.003
24	0.44	0.50[a]	0.44	0.007	0.24	0.19[a]	0.010
28	0.44	0.47[b]	0.41[b]	0.006	0.24	0.20[a]	0.005
32	0.40	0.42	0.42	0.043	0.20	0.16[a]	0.002

[a,b,c] Significantly different from control at P = 0.001, 0.01, 0.05, respectively

Table 6. The effect of dalapon on the dehydrogenase activity of soil as μl H g^{-1} dry soil

Soil	Begbroke North				Boddington Barn		
Herbicide dose (ppm)	0	266	2,660	± SE	0	2,660	± SE
Time (wks)							
0	18.5	18.8	15.9[c]	0.8	6.2	7.1[c]	0.4
2	17.8	11.8[a]	4.8[a]	0.8	3.9	1.8[b]	0.3
3	17.4	11.4[b]	3.7[a]	1.1	5.9	1.5[a]	0.4
4	18.8	12.4[a]	4.6[a]	0.8	5.9	2.9[a]	0.4
6	16.0	10.8[a]	2.7[a]	0.8	5.4	3.4	0.7
8	15.1	10.7[a]	3.1[a]	0.7	5.5	2.4[a]	0.2
10	15.4	11.7[a]	1.5[a]	0.6	5.1	1.1[a]	0.4
12	15.7	9.8[a]	1.9[a]	0.5	4.0	2.2[a]	0.2
16	12.2	9.9[b]	1.8[a]	0.5	3.7	1.3[b]	0.3
20	11.2	7.4[a]	1.6[a]	0.4	3.0	0.8[a]	0.1
24	8.0	5.8[b]	1.3[a]	0.5	2.7	0.9[b]	0.2
28	7.8	4.6[a]	1.4[a]	0.4	2.6	1.1[b]	0.2
32	6.9	3.9[a]	1.2[a]	0.3	2.5	1.0[b]	0.3

[a,b,c] Significantly different from control at P = 0.001, 0.01, 0.05, respectively

highest concentration where it was reduced ultimately by more than 80% compared to the control. In BB soil a similar prolonged inhibition followed a small, significant increase immediately after treatment.

Soil respiration: Carbon dioxide evolution from BN soil was not immediately affected by dalapon (Table 7). At the two lowest concentrations, evolution was reduced for a short time four weeks after treatment and, at 2.6 ppm, followed by small intermittent stimulations. Dalapon at 266 ppm stimulated evolution at week 3 but subsequently inhibited it for most of the remaining period. Respiration was inhibited by 2,660 ppm from week 2 to week 30. Respi-

Table 7. The effect of dalapon on CO_2 evolution from soil as μg C g^{-1} dry soil $week^{-1}$

Soil	Begbroke North				Boddington Barn		
Herbicide dose (ppm)	0	266	2,660	± SE	0	2,660	± SE
Time (wks)							
1	115.8	116.7	114.2	1.1	74.6	101.1[a]	0.6
2	90.8	88.9	69.3[a]	1.8	36.1	57.7[a]	1.2
3	67.7	79.7[b]	52.7[a]	2.1	33.5	63.3[a]	1.3
4	78.8	73.8[c]	62.8[a]	1.3	33.2	74.1[a]	1.8
6	66.8	53.9[a]	54.8[a]	1.3	33.0	60.4[a]	1.2
8	56.8	42.1[a]	43.7[a]	1.8	26.4	106.0[a]	4.2
10	46.4	36.8[a]	37.4[a]	0.6	24.7	79.8[a]	1.8
12	42.7	32.4[a]	32.0[a]	1.6	24.9	52.7[a]	0.8
14	45.6	35.2[a]	34.0[a]	1.0	26.4	88.6[a]	6.0
16	36.7	30.0[b]	25.1[a]	0.6	24.4	26.6	1.2
18	38.6	31.7[c]	25.8[a]	1.8	31.6	24.8[b]	1.1
20	37.4	33.7[c]	23.6[a]	1.2	29.6	20.0[b]	1.5
22	48.8	42.6[b]	30.2[a]	1.5	38.4	27.9[a]	0.5
24	32.0	25.4[a]	19.3[a]	0.8	24.4	15.0[a]	0.6
26	31.9	23.8[a]	19.0[a]	0.8	23.1	16.9[b]	0.8
28	24.8	24.1	18.4[b]	1.0	24.2	19.5[b]	0.9
30	23.8	20.4[b]	16.7[a]	0.7	20.1	16.0[b]	0.8
32	19.8	17.4	17.4	2.5	16.8	10.8[a]	0.4

[a,b,c] Significantly different from control at P = 0.001, 0.01, 0.05, respectively

ration of BB soil was higher in treated samples than in the controls during the first 14 weeks. During this period, the rate of evolution from treated samples did not decline as in the control. From 18 weeks, dalapon significantly reduced carbon dioxide evolution compared to the control soil.

Nitrogen mineralization: No ammonium-nitrogen accumulated in BN soil treated with the three lower concentrations of dalapon, or with 2,660 ppm in BB soil. The highest concentration in BN, however, caused an increase from 2 to 94 μg N g^{-1} dry soil during the experiment. This indicated almost complete inhibition of nitrification since little increase in nitrate occurred (Table 8). In contrast, at the three lower concentrations, nitrate production was stimulated one week after exposure, the effect lasting longer with increased concentration. Some decrease of nitrate was found at the end of the incubation period at these lower concentrations. Also, results for BB soil show that 2,660 ppm dalapon stimulated nitrate-nitrogen production. No nitrite was detected in either soil. All concentrations of dalapon increased mineralization of nitrogen (ammonium-plus nitrate-nitrogen) (Table 9). The size and persistence of this increase was dependent on the herbicide concentration in BN soil, lasting longest at the highest concentration and followed by a minor decrease during the last few weeks of the experiment. This decrease was not seen in BB soil.

Discussion

The objective of this work was to investigate tests likely to detect effects on soil micro-organisms that may harm soil fertility. No attempt was made to investigate changes in 'environmental quality'. The soil microflora and its activities

Table 8. The effect of dalapon on nitrate levels in soil as $\mu g\ NO_3^- - N\ g^{-1}$ dry soil

Soil	Begbroke North						Boddington Barn		
Herbicide dose (ppm)	0	2.6	26	266	2,660	± SE	0	2,660	± SE
Time (wks)									
0	11.6	12.2	12.3	10.9[c]	9.7[a]	0.2	4.7	4.7	0.2
1	14.5	16.1[b]	18.5[a]	19.9[a]	9.7[a]	0.3	6.7	11.2[a]	0.2
2	19.5	20.8[b]	24.2[a]	26.8[a]	10.1[a]	0.3	7.6	15.4[a]	0.3
3	21.5	21.1	25.2[a]	30.7[a]	9.8[a]	0.4	8.2	17.4[a]	0.4
4	28.7	27.3	33.2[b]	38.5[a]	13.0[a]	0.9	11.7	22.5[a]	0.7
6	36.8	37.8	38.9	47.7[a]	12.6[a]	0.8	14.4	32.1[a]	1.3
8	46.8	45.0	50.2	56.7[a]	13.1[a]	1.3	18.2	32.1[a]	0.9
10	46.1	45.2	45.2	52.5[a]	12.3[a]	0.8	18.0	31.9[a]	1.4
12	65.6	61.9	60.7	67.8	14.9[a]	0.9	25.1	29.1[c]	0.8
16	63.0	62.8	61.3	63.2	12.9[a]	1.3	27.7	44.2[a]	2.0
20	75.4	81.0	79.1	81.0	13.9[a]	3.4	39.3	45.6	2.3
24	109.4	110.4	109.7	101.5[b]	15.2[a]	1.5	52.6	60.7	3.5
28	120.7	110.8[c]	110.9[c]	102.5[a]	15.0[a]	2.9	56.2	57.5	3.2
32	109.8	108.6	104.7[c]	102.1[a]	16.1[a]	1.2	54.0	55.9	1.4

[a,b,c] Significantly different from control at P = 0.001, 0.01, 0.05, respectively

Table 9. The effect of dalapon on total mineral nitrogen levels in soil as $\mu g\ NH_4^+ + NO_3^- - N\ g^{-1}$ dry soil

Soil	Begbroke North						Boddington Barn		
Herbicide dose (ppm)	0	2.6	26	266	2,660	± SE	0	2,660	± SE
Time (wks)									
0	11.6	12.7	12.6	11.9	11.7	0.4	5.3	5.7	0.9
1	14.7	16.1[c]	18.7[a]	20.2[a]	22.6[a]	0.4	6.7	11.2[a]	0.2
2	19.8	21.0	24.3[a]	27.2[a]	32.3[a]	0.4	7.9	15.9[a]	0.3
3	22.3	21.6	26.1[a]	31.4[a]	37.0[a]	0.5	8.7	17.8[a]	0.3
4	30.4	28.5	34.7[c]	40.0[a]	43.7[a]	1.3	12.8	23.6[a]	1.0
6	37.2	37.8	39.7	48.5[a]	56.2[a]	0.9	15.6	34.0[a]	1.4
8	47.5	45.4	51.4	57.2[a]	64.5[a]	1.6	19.5	33.5[a]	1.2
10	46.5	46.6	47.6	53.7[a]	70.4[a]	0.9	19.9	33.1[a]	1.5
12	65.8	62.1	61.3	68.5	74.8[a]	1.0	25.5	29.6[a]	0.7
16	63.7	63.7	62.1	64.1	86.4[a]	1.3	29.5	45.0[b]	2.1
20	75.4	81.0	79.5	81.0	98.5[a]	3.5	39.6	45.6	2.3
24	109.5	111.0	109.8	101.6[b]	106.8	1.6	53.1	61.0	3.3
28	120.7	110.8[c]	111.0[c]	102.5[a]	108.6[c]	2.9	56.3	57.5	2.2
32	109.9	109.1	104.8[b]	102.1[a]	109.9	1.1	54.1	55.9	1.4

[a,b,c] Significantly different from control at P = 0.001, 0.01, 0.05, respectively

change seasonally and with climate over periods of hours, topography over distances of meters, or soil chemistry over distances of centimeters and, thus, it is difficult to identify 'environmental quality' in microbiological terms. Nor is it possible to define soil fertility in microbiological terms as all of the organisms which make beneficial or harmful contributions cannot be identified. However, there are some important processes, such as nitrogen transformations, and most test programs are based on their measurements.

Laboratory tests "do not eliminate the need for long-term monitoring of changes in soil fertility during actual use of pesticides in the field" (Atlas *et al.* 1978). While laboratory tests could allow better design of a field test program, this has not happened in practice, because the data in the literature have not been reproducible (Greaves *et al.* 1976, Johnen 1978, Anderson 1978b), owing to the variety of experimental conditions and methods used (Anderson 1978a).

Most investigations use pesticide concentrations derived by assuming uniform distribution through a defined depth (usually 10 cm) of soil. This ignores the concentration gradients formed by pesticides. Our preliminary experiments showed that dalapon applied at 40 kg ha^{-1} to soil cores produced concentrations in successive one cm soil layers of 180, 60, 50, 25, 8 and 0.8 ppm. Between 6 and 10 cm the concentration was less than 0.5 ppm. The average for 10 cm was about 30 ppm, but 70% of the profile (below 3 cm) contained less than this concentration. Insoluble or poorly soluble pesticides applied to the soil surface, or those adsorbed in soils, could produce steeper concentration gradients and more of the profile would have less than the calculated average concentration. Incorporation of powder formulations would reduce the range of concentrations produced in the soil profile whereas granules produce many localized high concentrations. Concentrations calculated from assumptions of uniform distribution through a 10 cm depth may, therefore, be misleading.

For safety, effects of concentrations higher than are likely to result from normal applications must be tested; most investigations have used a rate ten times higher than normal. This is an unrealistically high level for herbicides, since it would almost certainly kill most crops. Recent guidelines on measurement of hazards to nontarget plants and micro-organisms suggest concentrations derived from five times the maximum label rate (Anon. 1978).

Soil type affects microbial populations and may affect interactions between pesticides and micro-organisms (Johnen 1978). Considerably different effects of dalapon were found in soils from fields about 500 meters apart, sampled at different times in the crop rotation. In particular, effects on carbon dioxide evolution differed (Table 7). At the highest concentration, dalapon was inhibitory at all times in BN soil but stimulatory for 14 weeks in BB soil. Also nitrification was inhibited in BN soil but not in BB soil. These effects may be associated with the greater persistence of the herbicide in BN soil and this in turn may be due to the lower pH in this soil (Kaufman 1964; Davies and Marsh 1977).

Other variables may interact with soil type to modify the effects of pesticides (Stotzky 1975; Greaves *et al.* 1976; Gray 1978). Wingfield *et al.* (1977) showed that responses of the microflora to dalapon were less in 'undisturbed' cores than in 'disturbed' soil and were further modified by temperature. Similarly, Marsh and Greaves (1979) showed that effects of dalapon on soil nitrogen transformation changed with temperature, moisture, and soil characteristics. Many interactions between herbicide treatment, soil, temperature, and moisture were significant to varying extents at different sampling times. The effects were generally greatest at high temperature and low moisture. Most of the reported data on effects of pesticides on the soil microflora, reviewed by Anderson (1978b), Greaves *et al.* (1976), and Grossbard (1976), are on short incubations, and these may miss the effects of pesticide metabolites. Experiments

should account for production of major metabolites. Incubation should also allow recovery from effects. Ideally, the incubations in the present study should have been prolonged until, for example, soil dehydrogenase and nitrification had fully recovered. Sampling frequency and timing must be considered. It is misleading to quote data from the end of an incubation, unless time course data have also been considered in interpretation of effects. Widely spaced sampling intervals may miss peaks of effects and do not allow measurement of duration. Detection of effects in the laboratory means decisions must be made (Atlas *et al.* 1978). For example, should further laboratory or field tests be done? As these authors said "The interpretation of the laboratory experiments for this decision making is by necessity a complex task. There is obviously a need for an objective statistical evaluation of all data. . . .". The statistical evaluation of our data only compares treatments and controls at individual sampling times and makes no allowance for changes with time in the control. As many of these changes are as large as, or larger than, those in treated soil, allowance for them in the evaluation would probably eliminate many statistically significant effects. Obviously, care must be taken in selecting statistical evaluation procedures able to identify correctly effects due to treatment. Statistical evaluation of effects is only part of data interpretation. Thus, it is difficult to argue that the inhibitions at 4 and 8 weeks of carbon dioxide evolution from BN soil treated with 2.6 ppm dalapon are due to the herbicide, which was not detectable after two weeks. Stimulation at weeks 16 and 26 and inhibition at week 30 are even more unlikely to be due to dalapon. Dalapon metabolites are simple (Kenaga 1974), their concentration would be extremely low, and are unlikely to cause effects.

How does one decide the biological (and agronomic) significance of an effect of one microbial function? Dalapon at 2,660 ppm generally produced long-duration inhibitory effects on most of the microbial activities measured. Had these effects resulted from a normal herbicide application their interpretation would be critical. Thus, what is the significance of the long-term, though small, reduction in phosphatase activity in both soils? Similarly, how should the considerable reduction in dehydrogenase activity in each soil be interpreted? This effect on respiratory cycle enzymes in BN soil occurs at the same time as reduced carbon dioxide evolution. In BB soil, however, carbon dioxide evolution initially increased while dehydrogenase declined. The different responses of respiration to dalapon in two similar soils further complicates data interpretation. Effects on nitrogen transformations are also difficult to interpret. Would the inhibition of nitrification in BN soil be detrimental to nutrition and growth of any crop? As ammonium-nitrogen is not readily leached from soils its presence could be beneficial.

The importance of the sum of detected effects on all functions and populations to soil fertility is more uncertain. Knowledge of the soil microflora and its relationship with soil fertility is insufficient to answer these questions. One simple approach is to compare magnitude and duration of effects in laboratory experiments with those of responses to natural phenomena and agricultural practices. Marsh (1978) has suggested that the importance of lowered soil nitrate level following herbicide treatment may be assessed in terms of the natural variation in soil nitrate levels. Professor K. H. Domsch has proposed a similar approach (Greaves *et al.* 1980) as has Laskowski (1979). Our work shows no

effects at herbicide concentrations likely to occur in the field. This agrees with the work reviewed by Kenaga (1974). The conclusion must be that dalapon will not impair soil fertility, if used in the recommended way. Despite the problems, all practicable efforts should be made to ensure that soil fertility is not endangered by the use of agricultural chemicals. Testing of side-effects on soil micro-organisms may be important, although it is debatable whether tests with root-free soil will ever be effective. Most pesticides are used where the soil microflora and its activities are dominated by roots and root exudates. Clearly, valid investigations of pesticide effects on micro-organisms will be made only in the context of root region microflora, which has many effects on crop growth (Rovira and McDougall 1967; Russell 1977). Unfortunately, knowledge of the root-region microflora is too limited to allow suitable tests to detect effects on rhizosphere microflora. One exception is the examination of effects on *Rhizobium* spp. but even here, there are problems of assessing effects on nodulation (Johnen *et al.* 1979; Greaves *et al.* 1978b). Until rhizosphere research gives the necessary fundamental information, testing must be limited to root-free soils. A more radical approach would be possible if effects on soil fertility were the sole concern. The ultimate expression of soil fertility is growth vigor and yield of crop-plants. Laskowski (1979) suggested that "the best course of action, the one most straightforward and meaningful, would be to do the yield experiments to begin with and dispense with all the population counts, the microbial function studies, and the plant/microbe interaction studies". Certainly we agree that, if pesticides significantly disrupt soil fertility, this will be shown by the crop in yield experiments early in pre-registration testing.

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