Persistence of (2,4-Dichlorophenoxy)acetic Acid and 2-(2,4-Dichlorophenoxy)propionic Acid in Agricultural and Forest Soils of Northern and Southern Ontario

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The persistence of (2,4-dichlorophenoxy)acetic acid (2,4-D) and 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP), under actual field conditions, was determined in agricultural soils of northern Ontario (New Liskeard) and southern Ontario (Elora) by utilizing gas–liquid chromatography with electron capture detection of the methyl ester derivatives. Persistence of 2,4-D in two soil types (sand and clay) typical of the forestry region of Northern Ontario (Englehart) was also determined. Herbicides were applied at typical field rates of 0.560 kg ha^{-1} (agricultural) and 2.24 kg ha^{-1} (forest). In all cases the dissipation patterns were best described by non-first-order kinetics, with the rate of dissipation declining over time. Dissipation of 50% of the herbicides (DT_{50}), in the upper 10 cm of soil, took place in less than 7 days in all except the northern sandy forest soil. The rapid dissipation of these chemicals under soil conditions typical of southern and northern Ontario appears to present no hazard in terms of crop rotation practices.

The herbicides (2,4-dichlorophenoxy)acetic acid (2,4-D) and 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP) are both recommended for use as weed control agents in cereal crops in Ontario. In addition, an increasingly important use of these herbicides has been application for site preparation and conifer release programs in silviculture. Soil persistence studies dealing with these chemicals are numerous in the literature (McCall et al., 1981; Smith, 1978, 1980; Walker and Smith, 1979; Stewart and Gaul, 1977; Smith and Hayden, 1976; Cochrane and Russell, 1975; Foster and McKercher, 1973; Altom and Strizke, 1973; Kirkland and Fryer, 1972; Alexander and Aleem, 1961). However, none of these have been conducted in Ontario, and their relevance with respect to predicting the behavior of these chemicals in Ontario soils has been questioned (National Research Council, Canada, 1978). Of particular concern is persistence in northern Ontario where soil temperature and pH may be low. Several investigators have indicated that these conditions may promote increased longevity of the phenoxy herbicides (Moreale and Van Bladel, 1980; Corbin and Upchurch, 1967; Brown and Mitchell, 1948; Derose and Newman, 1947; Kries, 1947). With these questions in mind, field studies were conducted to examine the actual persistence of 2,4-D and 2,4-DP under conditions typical of their use in agriculture and forestry in northern and southern Ontario.

MATERIALS AND METHODS

Experimental Design and Location. All studies were designed as randomized complete blocks with chemical applications to triplicate treatment areas 2 m × 30 m. Each treatment area was subdivided into subplots (2 m × 1 m) separated by buffer zones (0.5 m). Two separate studies, one involving 2,4-D and one involving 2,4-DP, were conducted in a southern agricultural soil at the Elora research station of the University of Guelph (43°4′ N; 80°26′ W) as well as in a northern agricultural soil at the New Liskeard College of Agricultural Technology, New Liskeard, Ontario (47°30′ N; 79°40′ W). Two additional studies involving 2,4-D, only in different forest soils, were located in a reforested area near Englehart, Ontario (47°49′ N; 79°52′ W). Site locations were chosen to be representative of agricultural soils in both southern and northern Ontario and of two soil types typical of the northern forest. The characteristics of the various soils are given in Table I.

Table I. Characteristics of Study Soils[a]

	Elora, loam[b]	New Liskeard, clay[b]	Englehart, fine sand[b]	Englehart, sandy clay loam[b]
pH	7.2	7.2	5.2	6.4
% clay	19	62	2	29
% silt	49	34	6	19
% sand	32	4	92	52
% organic matter	4.2	5.9	2.8	0.8

[a] Soil classification (Canada Soil Survey Committee, 1978): Elora, loam of the Gray-Brown Luvisol type; New Liskeard, clay of the Humic Gleysol type; Englehart fine sand, sand of the Gray-Wooded Luvisol type, underlain by clay; Englehart sandy clay loam, sandy clay loam found in depressions and poorly drained areas also belonging to the Gray-Wooded Luvisol type. [b] Soil texture.

Chemical Application. Applications of 2,4-D (as the dimethylamine salt) and 2,4-DP (as the butoxyethanol ester) were made with a bicycle sprayer on June 3 and June 9, 1981, in the southern and northern agricultural sites, respectively. The sprayer was equipped with a five-nozzle boom (Teejet 8002LP nozzles) and delivered a spray volume of 225 L ha^{-1} under 207 kPa of pressure with a swath width of 2 m. Agricultural sites had previously been planted to barley so that applications [0.560 kg ha^{-1} acid equivalent (a.e.)] were made at the five to six leaf stage of the crop. In forest experiments, 2,4-D (as the dimethylamine salt) was applied with a (Pestex brand) backpack sprayer operated at 207 kPa with three Teejet 6503 nozzles delivering 667 L ha^{-1}. Chemical was applied at a rate of 2.24 kg ha^{-1} a.e. over a swath width of 1 m between rows of trees. Forest sites contained natural vegetation consisting predominantly of a variety of grasses and 4-year-old plantings of conifers. Stumps and brush interfering with uniform chemical application were removed prior to treatment.

Sampling Methodology. Soil samples from the northern sites were taken with a box corer measuring 10 × 10 × 10 cm and constructed of a 3.0-mm steel plate. Two cores of soil (1000-cm^3 volume each) were removed from a randomly selected 2-m^2 subplot in each treatment area at various times after treatment and pooled for analysis. Soil samples from the southern agricultural sites

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were taken in a similar way by using a soil probe (1.82-cm i.d.). In this case 10 soil cores totaling 26.0 cm^2 × 10 cm deep were taken from a randomly selected 2-m^2 subplot and pooled for analysis. Samples were immediately frozen and stored at −17 °C until analysis. Soil temperatures at 6–8-cm depth and rainfall were monitored at all experimental sites by using customized recording devices as described elsewhere (Thompson, 1983).

Sample Preparation and Extraction. A detailed discussion of the preparation and extraction methodology has been published previously (Thompson, 1983). The procedure involves acidic methanol extraction, serial liquid–liquid partitioning into ethyl ether, derivatization using BF_3 to form the methyl ester, and subsequent cleanup by a second liquid–liquid partitioning and fractionation on a Florisil column. GLC analysis was performed with separation on a column of OV-17 (4% on Gas-Chrom Q) and electron capture detection (^{63}Ni). Peak areas were integrated by a computerized data system (Varian Vista 401 data system) and quantitated by comparison with an external standard prepared from analytical-grade standards.

Results from recovery tests performed in triplicate with the appropriate fortified soils (collected as above) were used to correct the raw data for losses resulting from the chemical assay procedure. In this way losses due to extraction, derivatization, and cleanup were taken into account. All results were calculated on a soil dry mass basis.

Statistical Analysis. An analysis of variance was conducted for the observations from each experiment to determine if a statistically significant reduction in chemical residues was indicated. Means of three replicates were then subjected to multiple linear regression analysis with a variety of transformations of the dependent and independent variables. The best fit regression line was determined and slopes of the regression lines were compared by using a two-tailed test to compare pairs of regression coefficients as described at Zar (1974). Regression equations were used to calculate times for 50% dissipation (DT_{50}).

RESULTS AND DISCUSSION

Recovery from Fortified Soils. Recovery tests using the various soil substrates indicated 2,4-D recovery rates (mean ± standard error) as follows; Elora loam 67 ± 3%, New Liskeard clay 69 ± 2%, Englehart fine sand 94 ± 4%, and Englehart sandy clay loam 68 ± 3%. Similarly, recoveries for 2,4-DP were 94 ± 3% in the Elora loam and 81 ± 2% in the New Liskeard clay.

Persistence of 2,4-D and 2,4-DP in Agricultural Soils. Expected "zerotime" soil residue concentrations were calculated and compared to the actual residue concentrations as determined by the analysis of samples collected within 3 h of treatment. Recoveries approximated 60–70% of expected, indicating that 30–40% of the chemical was unaccounted for. These losses may have been due to interception of the chemical by the crop or other vegetation, drift, and volatilization. Dissipation of soil residues in the top 10 cm of soil was rapid, and in all cases the decline was highly significant ($P = 0.001$). The DT_{50} values of 4 and 6 days for 2,4-D (parts a and b of Figure 1) and 5 and 3 days for 2,4-DP (parts a and b of Figure 2), in southern and northern sites, respectively, were derived from the best fit regression equations. The values for 2,4-D are similar to those reported by other investigators (Smith, 1978, 1980; Altom and Strizke, 1973), who reported DT_{50} values between 3 and 7 days for 2,4-D. However, they differ from those of Foster and McKercher (1973), who reported DT_{50} values of 9–28 days for 2,4-D depending on the soil type and moisture content in their laboratory experiments. The kinetics of dissipation were best described by linear regression of log transforms of

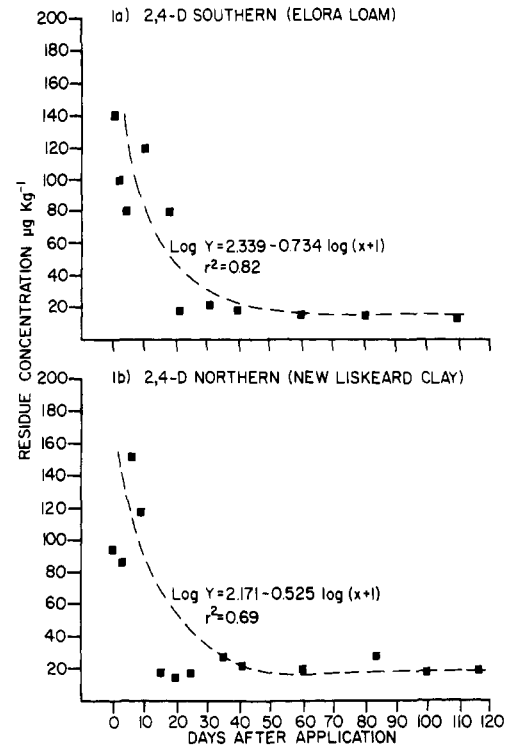

Figure 1. Dissipation of 2,4-D in agricultural soils of Ontario. Experiments were initiated on June 3, 1981, at Elora and June 9, 1981, at New Liskeard, with chemical application at a rate of 0.560 kg ha^{-1} a.e. Mean residue concentrations (three replicates) in the upper 10 cm of the soil profile are presented with curves developed from "best fit" regression equations of log transformed data.

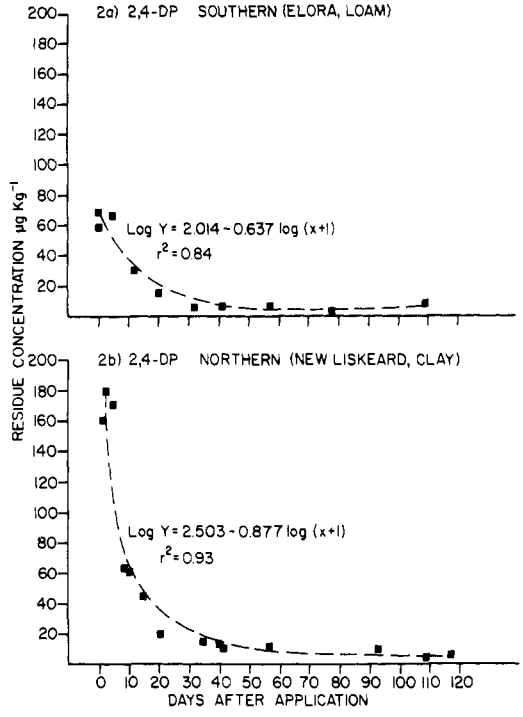

Figure 2. Dissipation of 2,4-DP in agricultural soils of Ontario. Experiments were initiated on June 3, 1981, at Elora and June 9, 1981, at New Liskeard, with chemical application at a rate of 0.560 kg ha^{-1} a.e. Mean residue concentrations (three replicates) in the upper 10 cm of the soil profile are presented with curves developed from "best fit" regression equations of log transformed data.

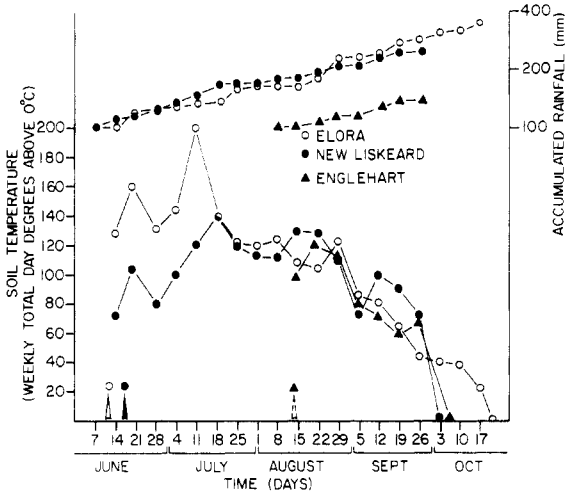

Figure 3. Soil temperatures and rainfall observed at Elora, New Liskeard, and Englehart, Ontario, in 1981. Data were obtained by monitoring soil temperatures in the 6–8-cm zone of the soil profile in each of the experimental sites. Arrowheads indicate the time of initiation of the persistence studies.

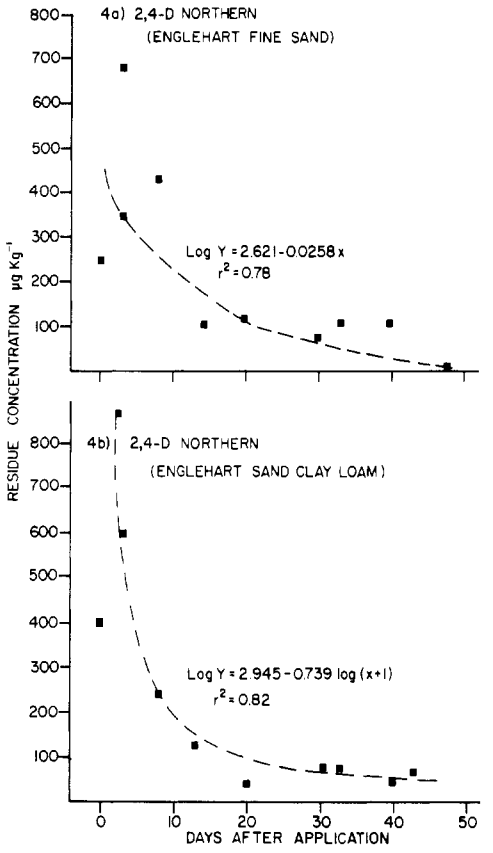

Figure 4. Dissipation of 2,4-D in forest soils of northern Ontario. Experiments were initiated on August 13, 1981, with the chemical applied at a rate of 2.24 kg ha^{-1} a.e. Mean residue concentrations (three replicates) in the upper 10 cm of the soil profile are presented with curves developed from "best fit" regression equations of log transformed data.

both time and concentration. This showed that, unlike the findings of Smith (1980), dissipation did not approximate to first-order kinetics. The implications of this are not clear except at they suggest more than one mechanism of dissipation in the soil.

Comparison of the slopes of the regression lines showed statistically significant differences between the rates of dissipation of 2,4-D and 2,4-DP ($P = 0.001$). The rate of dissipation was greatest for 2,4-D in Elora loam, but 2,4-DP dissipated more rapidly than 2,4-D in New Liskeard clay. Statistical differences in dissipation rates ($P = 0.001$) were also noted between the northern and southern agricultural sites, with rates being greater at the Elora site compared to those at New Liskeard for 2,4-D. In contrast, 2,4-DP residues declined more rapidly in New Liskeard clay. Although soil temperatures in the early part of the season (Figure 3) were higher at Elora than at New Liskeard, dissipation of the two chemicals was not consistently faster at Elora, suggesting that soil temperatures did not have an important effect on dissipation rates. In this study 2,4-DP was slightly less persistent than has been previously reported in the literature (Smith, 1978; Kirkland and Fryer, 1972; Altom and Strizke, 1973). The difference between the previously reported DT_{50} values for 2,4-DP (about 10 days) and our results of 3–5 days is unimportant from a practical standpoint. Although some investigators have suggested that the persistence of 2,4-DP is greater than that of 2,4-D (National Research Council, Canada, 1978), a better generalization would be that the persistence of these two chemicals, in the soils studied, is very similar.

In some of the above, as well as in the forest soil studies, the initial residues were slightly lower than those observed 1 day after application. Although this is a common phenomenon observed in various types of residue analysis (Ripley, 1983), the causal factors are not well understood. In this case interception of the applied chemical by grass or crop cover and subsequent removal to the soil through dripping or washing off the foilage by dew best explains the higher residues observed after zero time. Rainfall did not occur the day after treatment and was not the cause of the phenomenon.

In our studies, chemical residues were close to detection limits (approximately 5 $\mu g\ kg^{-1}$, depending on the soil type) by the end of the growing season. Residues of this magnitude are not likely to be phytotoxic to subsequent sensitive crops, especially if, as is most likely, they are strongly adsorbed to organic constituents in the soil. Other investigators have also suggested that such low levels of chemical residue in the soil are unlikely to have any biological significance (Stewart and Gaul, 1977).

Persistence of 2,4-D in Forest Soils of Northern Ontario. Degradation patterns of 2,4-D in forest soils (figure 4) were similar to those observed in agricultural soils. Initial residues were 3–4 times greater than initial residues in the agricultural sites, reflecting the approximate 4-fold increase in the application rate. Residue levels toward the end of the growing season were also higher than those in the agricultural soils. This may be due to lower temperatures in the forest soils at this time (Figure 3) or to differences in the pH of these soils. Dissipation of 2,4-D in the Englehart sandy clay loam was significantly faster than in the Englehart fine sand ($P = 0.001$), with DT_{50} values of 7 and 23 days, respectively. The latter soil is characterized by a lower pH (5.2) and a higher organic matter content (2.8%), both of which would be expected to promote greater adsorption and longer persistence of 2,4-D in comparison to those of the sandy clay loam. The results of this study support the findings of Torstensson (1975), who observed greater persistence of 2,4-D in low-pH forestry soils compared to that of agricultural soils. Altom and Strizke (1973) have also studied 2,4-D degradation in sandy forest soils in the laboratory and reported DT_{50} values of 4–5 days, which are in reasonable agreement with the DT_{50} value of 7 days reported in this study for clay soil but out of line with the 23-day value observed in our sandy soil. This difference may be related to differences in soil characteristics, particularly pH, which were not reported

by Altom and Strizke (1973). This study also supports the data of Smith (1980), who showed a slightly faster rate of 2,4-D dissipation in clay loam as opposed to that in sandy loam soil. The rapidity of degradation in the clay soil seems consistent with the hypothesis that 2,4-D is rapidly dissociated to its anion in most soils (National Research Council, Canada, 1978) and is repulsed by negative sites on soil colloids, making it more readily available to soil organisms and leaching (Bailey and White, 1970; Weber et al., 1965; Grover and Smith, 1974).

Leaching of 2,4-D to depths lower than the 10-cm sampling horizon may also account for dissipation in our studies. Studies by Smith and Hayden (1976) and Stewart and Gaul (1977) have shown that 2,4-D does not leach to a great extent; however, Wilson and Cheng (1976) reported leaching in soils of low organic matter (<3%). On this basis, the soils from the Englehart location would be most likely to allow leaching. The somewhat greater persistence in these soils and the small amount of rainfall (91-mm total, Figure 3) at the Englehart site suggest that this was not a significant mechanism of dissipation.

In our opinion, the low levels of residue remaining in the soil at the end of the growing season [12–82 μg (kg of dry mass)$^{-1}$] would not pose any environmental hazard. This is especially true if the residues are strongly bound and therefore less available to soil organisms and for transport with runoff water.

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Registry No. 2,4-D, 94-75-7; 2,4-D·dimethylamine, 2008-39-1; 2,4-DP, 120-36-5; 2,4-DP butoxyethanol ester, 53404-31-2.

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Deethylsimazine: Bacterial Dechlorination, Deamination, and Complete Degradation

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Enrichment cultures utilizing deethylsimazine (6-chloro-*N*-ethyl-1,3,5-triazine-2,4-diamine) as a sole nitrogen source were obtained from soils that had had long exposure to *s*-triazine herbicides. A bacterium was isolated that utilized deethylsimazine quantitatively as a nitrogen source for growth, but only 1 mol of nitrogen was obtained/mol of deethylsimazine, whereas all six atoms in melamine were utilized. The bacterium, which was identified as a strain of *Rhodococcus corallinus*, converted deethylsimazine to 1 mol of 6-(ethylamino)-1,3,5-triazine-2,4(1*H*,3*H*)-dione, 1 mol of chloride ion, and 1 mol of ammonium ion. 4-Amino-6-(ethylamino)-1,3,5-triazin-2(1*H*)-one was tentatively identified as a transient intermediate in the degradation, which was presumed to be due to a dechlorination followed by a deamination. Growth of *R. corallinus* together with *Pseudomonas* sp. strain NRRL B-12228, which utilized 6-(ethylamino)-1,3,5-triazine-2,4(1*H*,3*H*)-dione, resulted in complete conversion of deethylsimazine nitrogen to cell material.

Deethylsimazine (6-chloro-*N*-ethyl-1,3,5-triazine-2,4-diamine; CEAT, see Table I and Figure 2) is the first compound to be conclusively identified as a microbial (fungal) product from a chloro-*s*-triazine herbicide [Kaufman et al., 1965; Kearney et al., 1965; see also Kaufman and Blake (1970)]. The product is formed in about 70% yield, the other 30% being an unidentified product; traces of ammelide (OOAT) have been tentatively

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