The Persistence of 2,4,5-T in a Pacific Northwest Forest

L. A. NORRIS, M.L. MONTGOMERY, and E.R. JOHNSON

Abstract. The concentrations of 2,4,5-T [(2,4,5-trichlorophenoxy)acetic acid] in four species of vegetation varied from 11 to 115 ppmw immediately after application at 2.24 kg/ha but were less than 0.5 ppmw after 1 yr. The 2,4,5-T level in forest floor declined 90% during the first 6 months after application and less than 0.02 kg/ha remained after 1 yr. There was little leaching of 2,4,5-T from the forest floor into soil and no residues were found deeper than 15 cm. Maximum soil residues did not exceed 0.1 ppmw. Residue levels and dissipation rates of 2,4,5-T were similar after one and two successive annual applications.

INTRODUCTION

One of the most widely used and critically needed herbicides in forestry in the Pacific Northwest is 2,4,5-T. It is also one of the most criticized and closely scrutinized. Despite intensive efforts to find substitutes, 2,4,5-T is essential for the control of woody plant species that are reducing or preventing the growth of conifers on many acres of forest land.

We believe an adequate assessment of the hazards associated with the use of 2,4,5-T in the coastal forests of Oregon and Washington must include a consideration of the toxicity of the herbicide to nontarget biota and the probability that these organisms will be exposed to biologically significant quantities. There are extensive data on the toxicity characteristics of 2,4,5-T and TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) but more information is needed on their residue levels in the forest.

Norris (13, 14, 16) reported the entry and fate of 2,4,5-T in forest streams after aerial application of the herbicide in western Oregon and described the persistence of 2,4,5-T in forest floor in laboratory experiments. Our study reports the persistence of 2,4,5-T in four kinds of vegetation and in forest floor and soil after two successive annual aerial applications of the herbicide in a western Oregon forest.

MATERIALS and METHODS

The study area was located 460 m above sea level in the Northwest Coast Ranges, near Vernonia, Oregon. The area is typical of the cool, moist, highly productive Douglas fir [Pseudotsuga menziesii (Mirb.) Franco] forests in this region. The soil is a silty clay loam in the Astoria series (pH 5.3; 14% organic matter; 13.8% sand, 58.4% silt, and 27.8% clay). The area receives more than 180 cm of rain annually, largely between October and May.

Approximately 87 ha were treated with 2.24 kg/ha 2,4,5-T as the isooctyl ester in diesel oil applied by helicopter in March, 1971. A second application of the same mixture was made in March, 1972, to 61 of the original 87 ha. At the time of the first application, a transect with nine sampling points was established in the treated area. Samples (described below) were collected at all nine sampling locations within 2 h of application and again at 1, 3, 6, and 12 months after treatment. The same sampling scheme and schedule was followed on the six plots that received a second application 1 yr later. The other three plots, which were not retreated after the first year were sampled 24 months after the first application. All samples were placed in heavy plastic bags and stored at 0°C until analyzed.

Samples of current season's growth of vine maple (Acer circinatum Pursh.), blackberry vines (Rubus spp.), composites of several grass species, and 15-cm terminal branch segments of Douglas fir were collected for analysis. A 15-cm by 15-cm square of forest floor material also was collected. To minimize contamination among samples, 15-cm increments of soil were collected from the vertical face of each of the three soil pits dug on each plot. New soil pits were made at each sampling time. All forest floor and soil sampling equipment was cleaned carefully between sampling depths and locations.

Each sample type was collected in triplicate at each of six or nine sampling points. Composite samples of vegetation or soil were prepared in the laboratory by combining equal amounts of material from each of the three subsamples from a given sampling point. The three subsamples of forest floor were combined for analysis.

Plant residues of 2,4,5-T were extracted and purified by the alkaline extraction method of Chow et al. (4). Following purification on basic alumina, the ether extracts were evaporated to dryness and methylated with BF₃-methanol (15, 16). Herbicide residues in soil were extracted with dilute alkali. A 50-g soil sample was blended with 150 ml of 0.2 M sodium hydroxide in a centrifuge bottle. After heating 1 h on the steam bath with occasional stirring, the bottle was centrifuged and the supernatant was transferred to a liter separatory funnel. The soil was re-extracted twice with 100-ml portions of 0.2 M sodium hydroxide. After acidification of the combined basic extracts with dilute sulfuric acid, the 2,4,5-T was extracted with three 200-ml portions of diethyl ether. The combined ether extracts were purified and methylated in the same manner as the plant extracts. The methylated samples were
diluted with benzene and analyzed using a Varian 2100 gas
chromatograph with Infotronics microcoulometric detector
(16). The minimum detectable residue was 0.01 ppmw. Re-
covery studies were performed on soils fortified with 2,4,5-T
at concentrations from 0.005 ppmw to 0.1 ppmw. Average
recovery ranged from 80% to 95%.

The study was a randomized block experiment with each of
the six or nine sampling points being a block. The data were
handled in separate analyses as follows: (a) residues in vege-
tation were treated as a randomized block factorial with spe-
cies and time after application as the factors, (b) residues in
the forest floor were analyzed as a randomized complete
block, and (c) residues in soils were treated as a randomized
block, split plot experiment with time after application as a
main plot and depth in the soil profile as the subplot.

RESULTS and DISCUSSION

Herbicide residues in vegetation. Mean concentrations of
2,4,5-T in the four different kinds of vegetation after the first
application are shown in Table 1. Maximum concentrations,
ranging from 10.6 ppmw in vine maple to 114.5 ppmw in
grass, occurred immediately after application. These levels
dropped sharply during the first 3 months, after which time
the dissipation rate decreased. One year after application,
concentrations ranged from 0.48 ppmw in vine maple to 0.03
ppmw in blackberry foliage. Only vine maple contained de-
tectable 2,4,5-T residues 24 months after application.

Analysis of variance revealed mean herbicide concentrations
were markedly different among species (P < 0.001) and among
sampling times (P < 0.001). There was a highly significant

species-sampling time interaction (P < 0.001) which shows the
rate of change of concentration of 2,4,5-T in vegetation was
not the same among species. The change in herbicide concen-
tration in each species with time was best described by a cubic
function (P < 0.001). Regression equations for each species
are in Table 2.

The 2,4,5-T concentration in vegetation was also highest
immediately after the second application and again it declined.

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**Table 1.** 2,4,5-T levels in vegetation after two successive annual applications.a

<table>
<thead>
<tr>
<th>Species</th>
<th>0 (ppmw)b</th>
<th>1 (ppmw)b</th>
<th>3 (ppmw)b</th>
<th>6 (ppmw)b</th>
<th>12 (ppmw)b</th>
<th>24 (ppmw)b,c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackberries</td>
<td>45 (6.1)</td>
<td>0.59 (0.9)</td>
<td>0.05 (0.1)</td>
<td>0.02 (0.1)</td>
<td>0.03 (.01)</td>
<td>0</td>
</tr>
<tr>
<td>Douglas fir</td>
<td>52 (8.2)</td>
<td>11 (2.7)</td>
<td>0.35 (0.11)</td>
<td>0.47 (0.14)</td>
<td>0.22 (0.10)</td>
<td>0</td>
</tr>
<tr>
<td>Grass</td>
<td>120 (21)</td>
<td>3.4 (0.85)</td>
<td>0.58 (0.19)</td>
<td>0.14 (0.04)</td>
<td>0.12 (0.05)</td>
<td>0</td>
</tr>
<tr>
<td>Vine maple</td>
<td>11 (1.5)</td>
<td>0.48 (0.11)</td>
<td>0.28 (0.07)</td>
<td>0.16 (0.04)</td>
<td>0.48 (0.09)</td>
<td>0.02 (0.01)</td>
</tr>
</tbody>
</table>

First applicationd

<table>
<thead>
<tr>
<th>Species</th>
<th>0 (ppmw)</th>
<th>1 (ppmw)</th>
<th>3 (ppmw)</th>
<th>6 (ppmw)</th>
<th>12 (ppmw)</th>
<th>24 (ppmw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackberries</td>
<td>160 (35)</td>
<td>2.9 (1.8)</td>
<td>0.01</td>
<td>0</td>
<td>0</td>
<td>–</td>
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<tr>
<td>Douglas fir</td>
<td>52 (9.4)</td>
<td>14 (2.6)</td>
<td>0.10 (0.02)</td>
<td>0.04 (0.01)</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Grass</td>
<td>140 (33)</td>
<td>9.3 (1.5)</td>
<td>0.21 (0.09)</td>
<td>0.12 (0.03)</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Vine maple</td>
<td>23 (6.6)</td>
<td>10 (2.8)</td>
<td>0.10 (0.03)</td>
<td>0.10 (0.03)</td>
<td>0.02</td>
<td>–</td>
</tr>
</tbody>
</table>

Second applicatione

<table>
<thead>
<tr>
<th>Species</th>
<th>0, 1, 3, 6, 12 and 18 months</th>
<th>0, 1, 3, 6, 12 and 18 months</th>
<th>0, 1, 3, 6, 12 and 18 months</th>
<th>0, 1, 3, 6, 12 and 18 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackberries</td>
<td>0.59 (.09)</td>
<td>0.05 (.01)</td>
<td>0.02 (.01)</td>
<td>0.03 (.01)</td>
</tr>
<tr>
<td>Douglas fir</td>
<td>8.2 (1.7)</td>
<td>0.35 (0.11)</td>
<td>0.47 (0.14)</td>
<td>0.22 (0.10)</td>
</tr>
<tr>
<td>Grass</td>
<td>3.4 (0.85)</td>
<td>0.58 (0.19)</td>
<td>0.14 (0.04)</td>
<td>0.12 (0.05)</td>
</tr>
<tr>
<td>Vine maple</td>
<td>1.5 (0.11)</td>
<td>0.28 (0.07)</td>
<td>0.16 (0.04)</td>
<td>0.48 (0.09)</td>
</tr>
</tbody>
</table>

Table 2. Results of regression analysis of herbicide residue data in vegetation.

<table>
<thead>
<tr>
<th>Species</th>
<th>After first applicationa,b</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackberry</td>
<td>y = 3.38 - 4.03x + 0.65x² - 0.03x³</td>
<td>0.94</td>
</tr>
<tr>
<td>Douglas fir</td>
<td>y = 4.13 - 3.13x + 0.53x² - 0.03x³</td>
<td>0.82</td>
</tr>
<tr>
<td>Grass</td>
<td>y = 4.21 - 3.14x + 0.48x² - 0.02x³</td>
<td>0.88</td>
</tr>
<tr>
<td>Vine maple</td>
<td>y = 1.85 - 2.34x + 0.40x² - 0.02x³</td>
<td>0.78</td>
</tr>
</tbody>
</table>

After second applicationb

<table>
<thead>
<tr>
<th>Species</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackberryc</td>
<td>0.92</td>
</tr>
<tr>
<td>Douglas fir</td>
<td>0.97</td>
</tr>
<tr>
<td>Grassd</td>
<td>0.92</td>
</tr>
<tr>
<td>Vine maplee</td>
<td>0.91</td>
</tr>
</tbody>
</table>

a Covers 12 months data.

b y = ln ppmw herbicide, x = months after application.

c0, 1, and 3 months data.
d0, 1, 3, and 6 months data.
e0, 1, 3, 6, and 12 months data.

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sharply with time (Table 1). The initial concentrations ranged from 164.7 ppmw in blackberries to 23.2 ppmw in vine maple. Mean residue levels were < 0.2 ppmw in all species after 3 months, and were nondetectable 1 yr after the second application except in vine maple, which contained 0.02 ppmw.

The large number of nondetectable values in the second year's data prevented the full range of balanced statistical tests performed on the first year's data. Analysis of variance of various groupings of the second year residue data for vegetation consistently showed a significant difference among species in average herbicide concentration (P < 0.001). The forms of the regression equations that best describe the data are linear for blackberry, quadratic for grass, and cubic for Douglas fir and vine maple (Table 2). The regression equations fit the data well for both years with 82 to 97% of the variation explained by the single independent variable, time after application.

Marked differences were found in the initial residue levels in vegetation, both after the first and second application. The grass and Douglas fir show highest initial residue levels probably because they either have relatively large surface to mass ratios or occupy a prominent position in the canopy. Vine maple residues were lowest because the buds were not open at the time of spraying. Thus, the surface to mass ratios were probably low. Residues declined most rapidly and completely in blackberry shoots. This is a little surprising since both grass and Douglas fir are more resistant and, assuming detoxification has an important role in determining selectivity, these species might be expected to metabolize the herbicide more rapidly than blackberries. Residue levels were higher in blackberries, grass, and vine maple which probably reflects some decrease in the density of overstory vegetation by the first application. Figure 1 shows the average residue levels for all vegetation after the first and second applications. The concentration scale is logarithmic which tends to de-emphasize high values and accentuate low values. While we cannot test the difference between the two applications statistically, it seems clear that the rate of residue loss is as fast or faster and more complete after the second application.

These data suggest previous exposure to 2,4,5-T may enhance the rate of degradation of subsequent applications to vegetation. While this has been noted often in soil, we are not aware of similar reports in treated plants. Possibly, the first application removed the less resistant individuals of a species leaving the more resistant individuals alive at the time of the second application. The more resistant individuals may be better able to metabolize, excrete, or otherwise detoxify 2,4,5-T residues. A second possibility is that the number or activity of microorganisms responsible for some of the herbicide degradation on the surfaces of the vegetation may increase as has been noted for soil microorganisms. In any case, our data show two successive annual applications did not result in an accumulation of herbicides in the sampled tissues of any of the four species.

Morton et al. (12) and Bovey and Baur (2) also reported rapid decreases in 2,4,5-T residue levels on grass. Erné and Haartman (7) showed a decrease in 2,4,5-T residues on foliage of several species of woodland berries, but the rate of loss was slower than the rates we report. Brady (3) reported 2,4,5-T half-lives ranging from 5.5 to 12.4 weeks in foliage of one conifer and three hardwoods in the south. Initial half-lives for 2,4,5-T in our study were much shorter, ranging from 2 weeks in blackberry shoots and grass to 2.5 weeks in Douglas fir after the first application. Various processes undoubtedly operated to reduce the residue levels of 2,4,5-T in vegetation, but volatility, rain washing, growth dilution, and metabolism are probably of principal importance.

Herbicide residues in forest floor. Forest floor residues of 2,4,5-T increased from 35.7 to 40.6 mg/m² during the first month after the first application and then declined sharply (Figure 2). Eliasson (6) and Bovey and Baur (2) reported that rain washing was an important factor in moving residues from the canopy to the ground. About 0.7 mg 2,4,5-T/m² (0.3% of original application) remained after 24 months. Residue levels
transformed to natural log, \ln) declined linearly with time after application (P < 0.001) suggesting the disappearance rate approximates first order kinetics.

Regression analysis of the residue data for forest floor for 12 months on all nine points showed \( y_1 = 3.3055 - 0.269x \) with \( r^2 = 0.70 \) where \( y_1 \) is ln mg 2,4,5-T/m² in forest floor and \( x \) is months after application. Regression analysis for the three points that did not receive a second application (thus permitting a 24-month post application sample collection) showed \( y_1 = 3.0095 + 0.1920x \) with \( r^2 = 0.73 \).

Residue levels present immediately after the second application were markedly higher than after the first application (137.4 vs 35.7 mg/m²) (Figure 2). There was no lag period before rapid decomposition of the herbicide began after the second application, but there was a slight increase in herbicide levels 3 months after application. This could be due to chance variation although it did occur on three of the six plots. The 2,4,5-T level was 1.5 mg/m² 1 yr after the second application which is nearly identical to the level attained 1 yr after the first application. The percent lost was appreciably greater because the initial levels were much higher after the second application. Regression analysis showed \( y_2 = 3.9572 - 0.6909x + 0.0317x^2 \) where \( y_2 = \ln \) mg 2,4,5-T/m² and \( x \) = months after second application. The independent variable accounts for 71% of the variation of the dependent variable in this equation.

The higher initial levels of 2,4,5-T in forest floor after the second application are also reflected in the understory vegetation and probably resulted from a reduction in vegetation density due to the first application. After 3 months, the rate of 2,4,5-T degradation was virtually the same for both the first and second applications (Figure 2). Although the initial rate of degradation was much different, a change in half-lives with time is consistent with the mixed order kinetics of 2,4,5-T degradation in forest floor reported by Norris (16). After peak concentrations were observed in forest floor, the time required for 50% dissipation was approximately 6 and 2 weeks for the first and second application respectively (Figure 2). Residue levels after 1 yr were about 0.017 kg/ha or 0.76% of the originally applied 2,4,5-T.

**Herbicide residues in soil.** Residue levels in soil at all sample times were low when compared with forest floor levels of 2,4,5-T (Table 3). The residues found immediately after the first application probably represent contamination of the surface soil with fine particles of organic material from the overlying forest floor. Residue levels increased in the 0- to 15-cm layer to a maximum of 0.08 ppmw in 3 months and then declined to nondetectable levels 1 yr after the first application. There was no significant movement of herbicide into the 15- to 30-cm zone of soil despite rain of 24 cm the first month, and 70 cm the first 3 months after application.

2,4,5-T levels were barely above detectable limits in soil after the second application. In contrast with the first year’s data, there was no indication of accumulation of herbicide in the 0- to 15-cm zone, despite markedly higher levels of herbicide in the overlying forest floor and rainfall of 59 cm during the first 3 months after the second application. No further analysis of data was attempted because of the low residue levels.

The extremely low levels of 2,4,5-T in soil demonstrated the strong filtering action of the forest floor, which is consistent with laboratory studies showing extensive and rapid adsorption of 2,4,5-T by forest floor material (17). The rapid disappearance of herbicide from the forest floor suggests abundant microbial activity, which was reported to be important in disappearance of 2,4,5-T from forest floor material in the laboratory (13). Although the study site received abundant rainfall each year, there probably was no appreciable loss of herbicide in overland flow because the forest floor had an infiltration capacity which far exceeded rates of precipitation (19). There was no significant leaching of the herbicide in soil and detectable residues were gone in less than 1 yr.

The short persistence and limited mobility of 2,4,5-T in the soil is consistent with previous reports of the importance of organic matter in influencing herbicide behavior. Organic matter is principally responsible for limited leaching. Strong positive correlations have been reported between organic matter content and rate of degradation (20). Forest soils in the study area typically are rich in organic matter (14%) and the abundant moisture and warm weather of spring and early summer offer ideal conditions for the limited movement and rapid dissipation of 2,4,5-T residues.
Toxicological implications. Douglas fir and vine maple were selected for sampling in this study because they are a source of food (and, therefore, 2,4,5-T residues) for deer and elk. Grass and blackberry shoots are consumed by a variety of small forest animals.

Norris (18) surveyed the literature to identify no-effect exposure levels for several herbicides including 2,4,5-T. He reported no acute toxic effects ranging from 200 to 1,200 ppmw in the diet for various types of higher animals. Chronic feeding studies showed no observable effect levels for 2,4,5-T at dosages equivalent to 100 ppmw for 90 days in dogs, 250 ppmw for 15 days in cattle, and 1,000 ppmw for 481 days in sheep. The 2,4,5-T residue data we have reported show that neither the magnitude nor the duration of exposure thought to be required for an acute or a chronic toxic response to 2,4,5-T occurred as a result of the use of 2,4,5-T in this study. Way (21) concluded the principal problem from the use of auxin herbicides in relation to wildlife is not toxicological, but ecological in terms of habitat modification. Levels of 2,4,5-T considerably greater than those occurring in this study are required to adversely affect soil microorganism functions important in the maintenance of soil fertility (1).

The presence of TCDD as a contaminant of 2,4,5-T is a central issue in the controversy about the hazards that may result from the use of 2,4,5-T. We did not measure TCDD residues in this study. Based on initial herbicide levels and an assumed herbicide:TCDD ratio of 1:1 x 10^{-7} in the formulation, we have calculated initial TCDD levels in vegetation, forest floor, and soil (Table 4). We do not know the persistence characteristics of TCDD in forest vegetation or forest floor so we have not attempted to calculate changes in TCDD levels with time. Getzendaner (1975), however, found TCDD residues declined faster than 2,4,5-T in forage grass and Crosby and Wong (5) reported TCDD half-life on vegetation of only a few hours. TCDD has a half-life of about 1 yr in soil in the laboratory (9, 10) but Crosby and Wong (5) found a much more rapid disappearance from soil exposed to sunlight.

There have been few dose response experiments with TCDD at the low levels expected in forest vegetation. Kociba et al. (11) reported a feeding study in which male and female rats were intubated with 1.0, 0.1, 0.01, and 0.001 \( \mu \)g TCDD/kg/day five times a week for 13 weeks. This study showed essen-
tially no difference in response between controls (animals receiving no TCDD) and those receiving 0.01 µg/kg/day. If we assume an animal consumes 10% of its body weight in food per day, the “no effect” dose reported by Kociba et al. (11) is equivalent to 100 x 10^-6 ppmw TCDD in the diet. The TCDD levels we calculated for forest vegetation in this study are below this value (Table 3). Therefore, we conclude the probability of significant toxicological impact from either TCDD or the 2,4,5-T applied to this forest land is small.

ACKNOWLEDGMENT

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LITERATURE CITED