

# Residues of 4-Amino-3,5,6-Trichloropicolinic Acid in

## Grass from Applications of Tordon Herbicides

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Residues of 4-amino-3,5,6-trichloropicolinic acid, picloram, were determined in grass from seven locations at various time intervals after application of Tordon herbicide. Four formulations of the herbicide were used, three liquid and one granular. Liquid formulations deposited residues on grass up to 200 p.p.m. for each pound per acre applied. In all experiments, the residue level decreased within one or two weeks after application, then remained

relatively constant for 8 to 16 weeks. Residues from a granular formulation generally increased through a maximum near the eighth week after application, and dropped to a lower level within the following eight weeks. Residue of the herbicide in samples of grass collected the year after application generally showed a 90 to 100% decrease over the "mid-season" level. No bound form of picloram was found.

Large land areas in the United States are capable of producing grass suitable for grazing livestock. In many of these areas, various types of brush and trees become established because of natural causes,—*i.e.* fire destroying the grass cover or overgrazing during poor growing seasons. This brush competes with the grass for moisture and nutrients resulting in greatly reduced stands of grass.

Tordon herbicides, (Dow Chemical Co.) containing picloram, can be used to remove selectively brush and other broad-leaved plants without destroying the grass. Thus, large areas of rangeland may be improved for increased livestock production.

One question which this procedure raises concerns the amount of herbicidal residue that may result in grass from this use. To be most beneficial, it is important that no limitation be on the label as to the presence of cattle on the pasture at time of treatment. Under these conditions, the grass receiving the herbicidal application could be consumed immediately afterward. The low mammalian toxicity of the compound, giving no adverse effect in calves receiving over 500 mg./kg. body weight, would permit grazing of cattle on freshly treated grass without danger of adverse effects (Jackson, 1966).

The experiments reported here were carried out to determine the residues that occur immediately after application of the herbicide and at intervals thereafter. They cover widely scattered geographic locations, therefore encompassing a great number of variables affecting residue content.

### EXPERIMENTAL PROCEDURES

**Residue Determination.** The method used is essentially that of Bjerke *et al* (1967). Because of the relatively high residues from this type of application, cleanup is not generally required, permitting the following simplified procedure.

A 10-gram sample of ground grass was extracted by blending with 50 ml. of aqueous 0.1N KOH solution and filtering on a Buchner funnel. The filter cake was washed to make a final volume of 200 ml. Then an aliquot was acidified with 6N H<sub>2</sub>SO<sub>4</sub>, saturated with NaCl, extracted with ether, and analyzed following the procedure of Bjerke *et al* (1967).

Table I. Recovery of Picloram from Fortified Controls  
P.P.M. Picloram,

Added	Found	Av. % Recovery
5.0	4.4, 4.7, 4.8, 4.2	90
10.0	9.4, 9.2	93
20.0	19.7, 17.0	92
50.0	45.0, 41.5	87
100.0	85.0, 85.0	85
200.0	184, 178, 161, 159	86
300.0	286, 270	93
		Mean 89

The aliquot size taken from the filtrate was chosen to give a picloram methyl ester concentration in the final solution within the range of 0.01 to 0.2 µg. per ml., the range of the standard curve. For example, for a residue of 200 p.p.m. a 2-ml. aliquot was diluted to 100 ml. and a 2-ml. aliquot of this diluted solution was pipetted into a 50-ml. beaker with 10-ml. of water. Recovery from fortified controls averaged 89%, Table I.

Several experiments were conducted to show that the above method was determining all of the picloram residue in the grass. Three samples of grass were extracted with 0.5N alcoholic KOH to determine if any additional chemical could be extracted with a more vigorous extraction. Five grams each of three samples previously treated with Tordon were boiled in 50 ml. of the 0.5N alcoholic KOH for 30 minutes on the steam bath. The solution was then diluted to 250 ml. with water. A 5-ml. aliquot was extracted with 10 ml. of ether. Four milliliters of the ether extract was boiled down, the methyl ester prepared, and finally diluted to 5 ml. with benzene before being chromatographed. The volatility of picloram is very low, so evaporation can be carried out without loss provided that reasonable care is used to prevent excess heating beyond dryness. Similar 5-gram portions of these same samples were shaken 15 minutes with aqueous KOH and diluted to 250 ml. Both sets of samples were treated alike after the dilution to 250 ml. With two other samples, a comparison was made between 0.5N and 0.1N aqueous KOH solutions using the normal cold extraction in both cases. Table II shows that the cold aqueous extraction actually recovered more residue than did the boiling alcoholic KOH, and the cold 0.1N KOH was as effective as cold 0.5N KOH, Table II.

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**Table II. Comparison between Extraction Procedures**

Sample	Picloram Found, P.P.M.	
	0.5N Alcoholic KOH Refluxed	0.1N Aqueous KOH Cold
1	12.0	13.4
2	12.8	14.6
3	10.8	13.6
0.5N KOH Cold		
4	0.2	0.2
5	3.5	3.5

**Table III. Results of Experiments to Find "Bound" Picloram**

Sample	Weeks from Application to Harvest	Gross P.P.M. of Picloram Found		
		Bjerke method	After hydrolysis of grass	After hydrolysis of extract
4	18	1	<0.01	<0.01
5	18	2	<0.01	<0.01
6	2	19	<0.01	<0.01
7	2	16	<0.01	<0.01

**Table IV. Composition of Formulations and Rates Used on Grass Plots**

Characteristic	Tordon 225 Mixture	Tordon 212 Mixture	Tordon 22K Mixture	Tordon Beads
Picloram	1 lb./gal.	1 lb./gal.	2 lb./gal.	2%
2,4-D	...	2 lb./gal	...	...
2,4,5-T	1 lb./gal.	...	...	...
Cation	Triethylamine	Triisopropanol amine	Triisopropanol amine	K <sup>+</sup>
Borontrioxide equivalent	...	...	...	43.8
Physical form	Liquid	Liquid	Liquid	Pellets
Rates of Application of Formulations on Grass				
	Gal./Acre			Lb./Acre
Michigan			1	
Georgia	3	0.5	1.5	450
Montana	3	0.5	1.5	450
North Dakota	3	3		450
South Dakota		0.5	1.5	
Oklahoma	3	0.5	0.75	450
Texas	2	0.5	1.5	450
		2		

An experiment was conducted to determine if any picloram exists in grass in a nonextractable form which can be released by hydrolysis. Samples taken two and sixteen weeks after application, were analyzed. Each sample was extracted exhaustively with 0.1N KOH until the level of picloram extracted was less than 0.01 p.p.m. They were then hydrolyzed with 100 ml. of 2N NaOH for six hours on the steam-bath. The hydrolysate was analyzed for picloram by the determinative procedure above. No additional residue was found, Table III.

An experiment was conducted to determine if an extractable form of picloram was present, e.g. a glucoside, which was not determined in the usual procedure. The original 0.1N KOH extracts from the above two samples were exhaustively extracted with diethyl ether until analysis showed that less than 0.01 p.p.m. of "free" picloram remained. Then NaOH pellets were added to the aqueous phase to make it 2N, and this was hydrolyzed six hours on the steam-bath. This

solution, when analyzed for picloram, showed that no bound extractable form was present, Table III. These experiments show that the method extracts all of the picloram residue, and that none is present in a bound form.

**Sample Collection.** Grass samples were collected from several locations at intervals following application. In all cases, grass taken during the year of application of the herbicide was taken from a fresh area of the plot at each sampling. Table IV gives the concentration and salt form of the active ingredients in the various formulations used. In all experiments in which they were used, Tordon Beads were scattered over the treated area as formulated.

All samples of grass, usually 2 pounds, were placed in plastic bags which were tied shut, then frozen prior to shipping with dry ice to Michigan for analysis. A single sample was taken from each replicate at harvest time in all but the Midland, Mich., experiment, where two subsamples were taken from each plot. The samples were maintained frozen until they were analyzed. Depending on the moisture content, they were put through a meat grinder or Wiley Mill, mixed well, and aliquots removed for analysis. Locations of experiments and details furnished with the samples are given below.

**MIDLAND, MICHIGAN.** Three plots were sprayed with Tordon 22K Weed Killer at a rate of one gallon per acre in a 1-to-19 water solution, using a bicycle sprayer on August 10, 1965. A fourth plot, several feet from the treated plots, was left as the untreated control. Applications were made on a windless day to avoid contamination of the control plot. The grass had previously been cut and raked, and had regrown to 8 to 12 inches tall. The first samples were taken immediately after treatment using hand clippers, cutting two 2-foot X 3-foot subplots from each plot. All samples were collected in polyethylene bags and quick-frozen until analysis. Samples collected the spring following application were taken without reference to the previous subplots. After the spring sampling the year after application, all plots were mowed and raked to allow fresh growth for samples collected later. One sample per plot was taken two times during the year following treatment, and once the second year.

Plot 2 was protected from rainfall during the first month after treatment by covering it with a polyethylene sheet mounted on a gable-roof shaped frame. The bottom of the plastic was about a foot above ground level to allow air circulation. The cover was put on every evening and removed every morning during the first 30 days after application of the herbicide, unless it was raining or rain threatened.

**TIFTON, GEORGIA.** Established coastal bermuda grass at the Coastal Plains Experiment Station, University of Georgia, was treated with water sprays of 40 gallons per acre on June 27, 1966. The grass was at the hay stage at time of application. Three replicated plots were established for each treatment.

**MOCCASIN, MONTANA.** Native grass, at the boot stage of heading at the Central Montana Branch Station, Montana State University, was treated with 23.4 gallons per acre of spray solution on June 18, 1966. Plots were in triplicate. An experimental plot sprayer was used, with a 4-nozzle, 6.5-foot boom.

**FARGO, NORTH DAKOTA.** A bicycle wheel-type plot sprayer was used at North Dakota State University to apply the herbicide on native bluegrass on June 9, 1966. The grass had headed at time of application. Sampling was begun one week after the herbicide was applied. All plots were put out in triplicate. Grass was not available the spring following application.

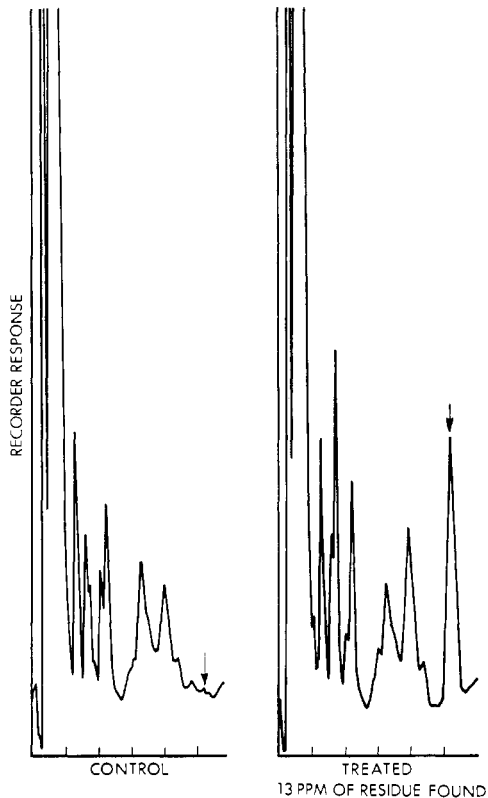


Figure 1. Typical chromatograms of grass samples from Texas

BROOKINGS, SOUTH DAKOTA. Native bluegrass, grown six inches after clipping, was sprayed on August 2, 1966, with 18.2 gallons per acre of solution with a tractor mounted plot sprayer. Three replicates of each treatment were established.

STILLWATER, OKLAHOMA. Mature established native grass was sprayed with a tractor-sprayer on June 6, 1966. Three replicates of each treatment were established.

COLLEGE STATION, TEXAS. At Texas A&M, established brown-seed paspalum, little bluestem, and other native grasses

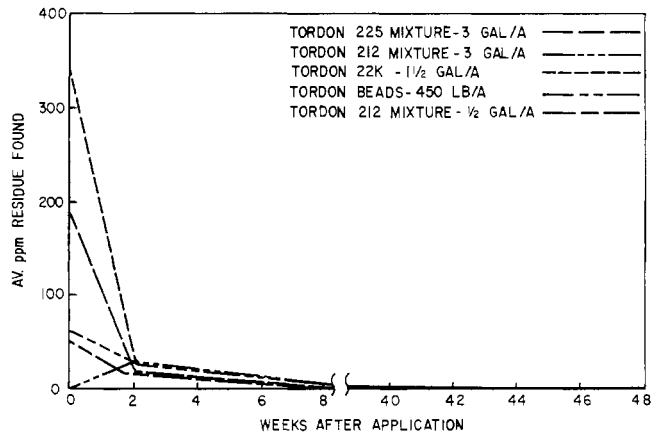


Figure 2. Residues of picloram in grass from Georgia

were treated with broadcast sprays on May 27, 1966. The plots were replicated three times.

### RESULTS AND CONCLUSIONS

Typical chromatograms of control and treated grass samples from Texas are given in Figure 1. Chromatograms of grass from other experiments were similar. Figures 2 through 8 show the average residues of picloram found in grass from each location over the entire sampling period. The residue of the covered plot in Michigan (Figure 3) decreased less rapidly than from the uncovered plots. This indicates slower loss of herbicide from the covered grass because of protection from rain. The pattern of residues from applications of liquid formulations in the other experiments generally follows the pattern observed in the uncovered plots in Michigan. In the North Dakota experiment, the first samples were collected one week after application so it is not possible to determine the shape of the early part of the curve.

With the solid bead formulation, the residue pattern is different. Because the beads do not readily adhere to grass foliage, the initial deposits are small. The trend is for the residue to be higher 8 and 16 weeks after application, than

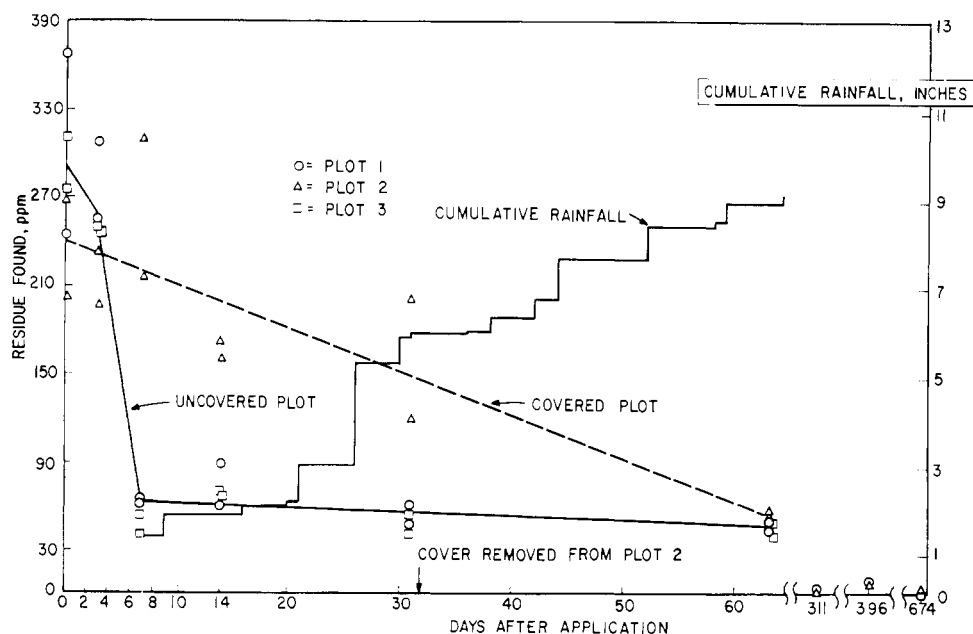


Figure 3. Residues of picloram in grass from Michigan

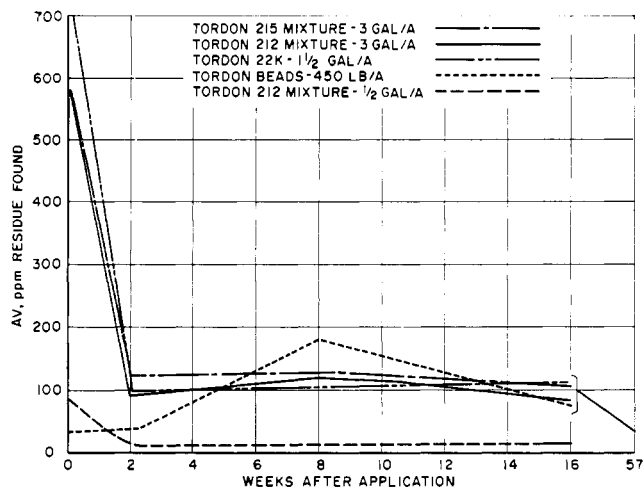


Figure 4. Residues of picloram in grass from Montana

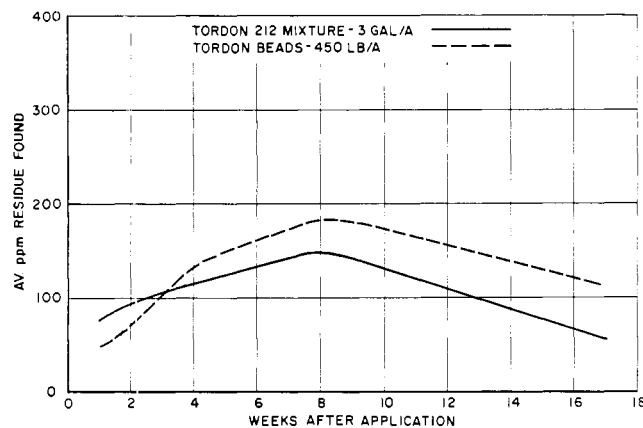


Figure 5. Residues of picloram in grass from North Dakota

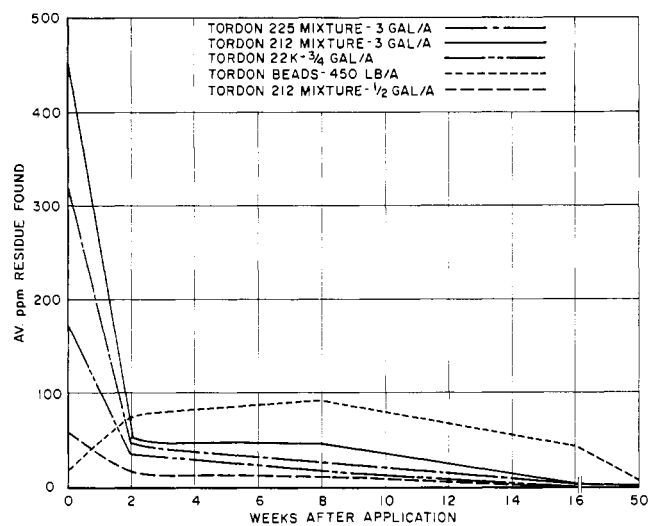


Figure 6. Residues of picloram in grass from Oklahoma

initially, although in the Texas experiment, the residue in grass immediately after application was never exceeded by later samples. This pattern seems to reflect the systemic manner in which the herbicide acts, by being translocated from the soil through the roots to the foliar portions of the grass. The rate at which this translocation occurs is undoubtedly related to the rate of leaching into the soil, which is dependent on rainfall.

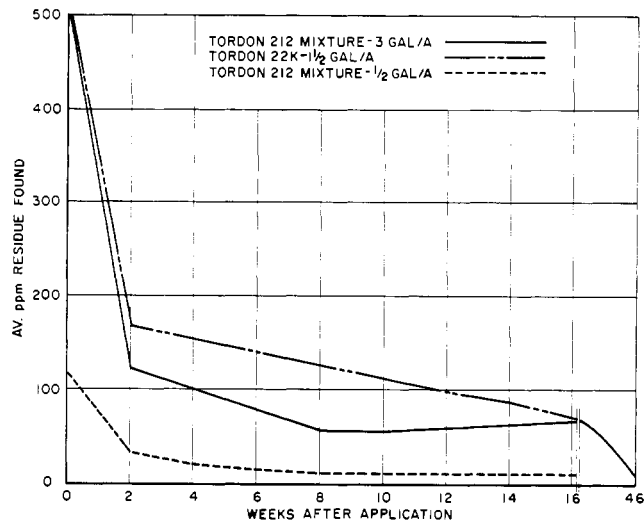


Figure 7. Residues of picloram in grass from South Dakota

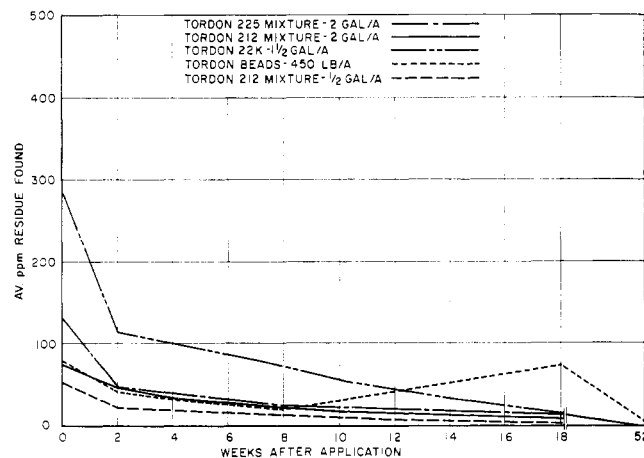


Figure 8. Residues of picloram in grass from Texas

Reasonably, in a situation such as this, where the herbicide affects the grass very little, if any, the residue content will be proportional to the amount applied. This would hold only where the other conditions affecting the grass are the same for all levels of application, as would be essentially true in all the plots in one location. Based on these assumptions, a "specific residue" figure has been calculated for the samples from each location. By doing this, residues from different rates of application of the same or similar formulations can be averaged for each location and a direct comparison can be made between different locations. By definition:

$$\text{Specific Residue} = \frac{\text{p.p.m. of residue}}{\text{pounds per acre applied}} = \text{Sp.R.}$$

where the parts per million and pounds per acre are both expressed in terms of 4-amino-3,5,6-trichloropicolinic acid.

The average specific residue figures for all samples analyzed are given in Table V. Since the route of residue accumulation with a sprayed liquid is different from that of the granules, data from the latter have been tabulated separately from the liquid formulations. The greatest coefficient of variation occurs with the initial application. This is probably a function of the uniformity of application and stand of grass. Figure 9 shows a plot of the average specific residue data for the entire series of experiments, with boundaries for 95% confidence limits. Since these data are from widely scattered

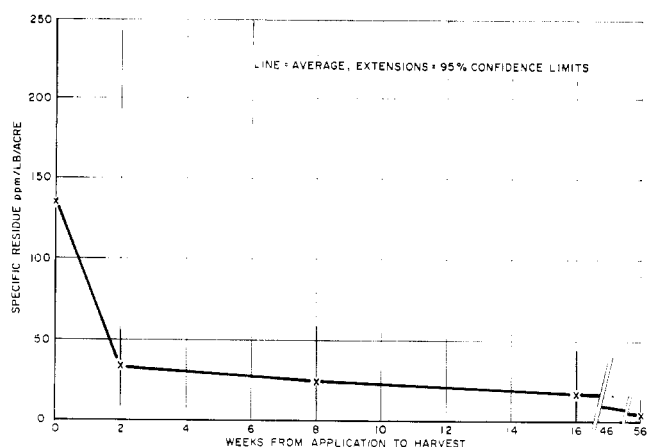
**Table V. Specific Residues in Grass from Application of Tordon Herbicides**  
Specific Residues, P.P.M. of Picloram per Lb./Acre Applied<sup>a</sup>

	Weeks from Application to Harvest							
	0	3/7	1	2	4	8	16-18	52
<b>Liquid Formulations</b>								
Georgia	74 ± 82	...	...	10 ± 7	...	1 ± 2	...	0
Michigan	141 ± 54	125 ± 32	28 ± 11	34 ± 11	26 ± 7	24 ± 2	...	3 ± 1
Michigan <sup>b</sup>	...	125 ± 32	132 ± 58	83 ± 9	80 ± 45	29 ± 3	...	...
Montana	200 ± 76	...	...	33 ± 17	...	38 ± 16	33 ± 22	12 ± 2
North Dakota	...	...	26 ± 2	33 ± 15	44 ± 28	51 ± 10	19 ± 13	...
Oklahoma	121 ± 51	...	...	32 ± 13	...	16 ± 22	1 ± 2	0
South Dakota	200 ± 111	...	...	50 ± 23	...	20 ± 11	22 ± 9	3 ± 1
Texas	78 ± 62	...	...	30 ± 19	...	15 ± 11	4 ± 5	0
Av.	135 ± 112	...	...	32 ± 24 <sup>c</sup>	...	24 ± 34 <sup>c</sup>	16 ± 26	3 ± 9
<b>Granular Formulation</b>								
Georgia	0	...	...	3 ± 1	...	1 ± 1	...	...
Montana	4 ± 5	...	5 ± 2	4 ± 2	...	20 ± 9	8 ± 6	...
North Dakota	...	...	5 ± 2	11 ± 5	15 ± 7	21 ± 4	13 ± 5	...
Oklahoma	2 ± 1	...	...	8 ± 1	...	10 ± 2	3 ± 6	0.3
Texas	9 ± 9	...	...	5 ± 2	...	2 ± 1	8 ± 6	0.4

<sup>a</sup> ± figures are for 95% confidence limits.

<sup>b</sup> Plot protected from rain for 1 month after application.

<sup>c</sup> Not counting the protected Michigan plot.



**Figure 9. Average specific residues of picloram in grass from seven locations following applications of liquid formulations**

areas representing typical conditions, it is reasonable to conclude that residues from the use of Tordon herbicides on mature grass will fall within these limits. This graph can be used to predict levels of residue in grass at any time after application of any liquid Tordon formulation at a known rate, by multiplication of the specific residue value interpolated from the graph by the pounds per acre of 4-amino-3,5,6-trichloropicolinic acid applied.

Small residues of 1% to 6% of the initial deposit, were found about a year after application of the liquid formulations to grass in Michigan, South Dakota, and Montana. Grass from Michigan sampled approximately two years after application contained less than 1 p.p.m. of residue, if any. In none of the other experiments was a residue found in grass a year after treatment with liquid formulations. In samples from plots treated with the bead formulation in Oklahoma and Texas, only small residues were present a year later. Corresponding samples from the other locations treated with the bead formulation were not available. This low level or complete absence of residue in grass collected a year after application of Tordon herbicides, leads to the reassuring conclusion that within a year, loss of chemical from the root zone

**Table VI. Decrease of Residues of Picloram in Grass for One Year after Application of Tordon Herbicide**

Location	Average Specific Residue, P.P.M.			% Decrease	
	Initial	Mid-season	Ca. 1 year	Mid-season/Initial	1-year/Mid-season
<b>Liquid Formulation</b>					
Georgia	74	10	0	86	100
Michigan	141	30	3	79	90
Montana	200	38	12	81	68
South Dakota	200	22	3	89	86
Oklahoma	121	16	0	92	100
Texas	78	15	0	81	100
Av.				85	91
<b>Bead Formulation</b>					
Oklahoma	2	10	0.3	Increase	97
Texas	9	8	0.4	11	95

of the soil was complete in most experiments. The loss was nearly complete in all of the other experiments, with rates of application as high as 9 pounds of picloram per acre.

The "midseason" residue, the nearly constant level following the initial rapid decrease, appears to represent the equilibrium level of chemical between soil and grass. Average Sp.R. values are tabulated (Table VI) and percentage decrease between the initial deposit and midseason level, and between the midseason residue and that found a year after application. An average of 85% of the initial deposit disappears at midseason, while an average of 91% of that amount is gone by the next growing season. Only in Montana and South Dakota was less than 90% of midseason residue gone the year after application.

#### CONCLUSIONS

It is concluded that no bound form of picloram exists in grass. The residue level in or on grass decreases very rapidly from that resulting from the initial deposit of liquid sprays. An equilibrium level between soil and grass is established in a

few weeks after application. The average specific residue is below 150 p.p.m. per pound per acre at the time of application, decreasing to less than 50 p.p.m. in two weeks. Grass taken the following spring shows no residue in most cases, with a maximum specific residue of 12 p.p.m. per pound per acre. The specific residue from the bead formulation was generally below that from the liquid formulation.

#### ACKNOWLEDGMENT

Grass was treated and samples were collected and shipped as follows: H. R. Guenther, Central Montana Branch Ex-

periment Station, Moccasin, Mont.; G. W. Burton, University of Georgia Coastal Plain Experiment Station, Tifton, Ga.; W. G. Wright, The Dow Chemical Company; John Nalewaja, Fargo, N. D.; S. K. Lehman, College of Agriculture, Texas A&M University, College Station, Tex.

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*Received for review February 10, 1969. Accepted July 7, 1969.*