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Picloram Stability in a Sample of Forest Soil During Handling and Storage

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Picloram (4-amino-3,5,6-trichloropicolinic acid) is a broad-spectrum herbicide used to control a wide variety of broadleaf and woody vegetation. It is particularly important in forestry (Norris et al. 1983). For both research and monitoring purposes, soil samples are frequently collected and analyzed to determine the persistence and mobility of picloram in the environment. Management, regulatory, and judicial decisions depend on these data; therefore it is essential that results are reliable. Unfortunately, although quality-control and quality-assurance programs are important, sample integrity during handling and storage has received little attention. Most managers of monitoring and research programs expect to complete chemical analyses soon after sample collection. In practice, long delays often occur, yet investigators assume that residue levels found at the time of analysis are the same as those present at the time of sample collection.

Little data have been published on the stability of herbicides in samples. Investigators have warned that crop and soil samples must be stored at a temperature at which the residues and the crop do not decompose further while awaiting extraction. This may seem an obvious precaution but information on maintaining stability is seldom given in the literature. Chau and Thomson (1978) have reported that several phenoxy herbicides were stable in water samples treated with sulfuric acid, and Knyr and Sokolov (1974) have evaluated methods for stabilizing halophenoxy herbicides in soil. We have not found similar types of information on picloram in soil. Current handling and storage techniques appear to rely on studies of 2- to 3-day cold storage and to be based more on conjecture than data. The purpose of this study was, therefore, to determine the effect of handling and storage conditions on the integrity of picloram in a forest soil.

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MATERIALS AND METHODS

The study was conducted in two parts: a field phase to determine the effects on forest-soil samples of handling after collection and during transport, and a laboratory phase to determine the effects of cold storage on picloram recovery.

In the field phase, 5 kg of Calvin silt loam was collected from the 0- to 10-cm depth at a forest site near Alsea, Oregon. The soil (37% moisture) was divided into fourteen 40-g samples that were placed in individual polyethylene bags. Each sample was fortified to 100 ppb picloram by adding 4.0 ug picloram (acid equivalent, a.e.) as the potassium salt in 1 ml water. The fortification mixture was prepared from Environmental Protection Agency picloram standard (99.0% pure) dissolved in deionized water containing potassium hydroxide. After samples were mixed, half were immediately frozen with dry ice in an insulated container (cold-temperature treatment), and half were placed in a cardboard box (ambient-temperature treatment). Unfortified control samples were also collected and handled in bulk in both treatments. All samples were transported to the laboratory, where cold-temperature samples were immediately placed in a freezer at -15°C. Ambient-temperature samples were left in the vehicle for 24 hours to simulate actual sampling procedures, which often take 2 days. Extraction and analysis were begun 43 hours after fortification, at which time a set of control samples was fortified to allow us to quantify any losses during analysis and thus to provide a means of distinguishing losses due to storage alone. The two treatments (cold and ambient temperatures) were completely randomized in seven replications.

In the laboratory phase of the study, a 15-kg bulk sample of the Calvin silt loam forest soil was subdivided, and 96 samples were fortified to 100 ppb picloram, as before, or to 300 ppb (a.e.). All samples were stored at -15°C, except 0 storage-time samples, which were extracted less than 2 hours after fortification. At 0, 29, 98, 196, 280, and 330 days, eight samples of each fortification level were taken for chemical analysis. Again, a set of control samples was fortified at the beginning of the extraction phase to allow us to distinguish losses due to analytical procedures from those due to storage. This was a completely randomized factorial experiment with eight replications; fortification level (100 and 300 ppb) was one factor and length of storage (0, 29, 98, 196, 280, and 330 days) the other.

The basic analytical method, developed by Bjerke (1973), was modified by C. E. Evans to deal with the high level of organic matter common to forest soils. (Details of the modifications for forest soil, forest floor, and vegetation are available from L. A. Norris.) The modifications include larger Woelm basic alumina columns at two steps in the analysis and a more vigorous potassium permanganate oxidation. All solvents and reagents were reagent grade except the diethyl ether, which was distilled

twice. Picloram standards were from the Environmental Protection Agency (Research Triangle Park, NC). We used an HP 5880 A gas chromatograph with ^{63}Ni EC detector and a 15 m OV-101 capillary column held isothermal at 80°C between 0 to 0.5 minutes, temperature-programmed at + 30°C per minute between 0.5 and 3.5 minutes to 170°C, then held isothermal to 8.0 minutes. This method yielded 79.6% mean picloram recovery (95% confidence limits ± 4.9%) from fortified controls (n = 10).

RESULTS AND DISCUSSION

In the field phase of the study, the concentration of picloram (mean ± confidence interval) was 71 ± 6 ppb in cold-temperature samples and 69 ± 7 ppb in ambient-temperature samples. When corrected for losses during analyses, values after storage and handling were 86 ± 7% for the cold treatment, and 84 ± 8% for the ambient-temperature treament. The difference between treatments is not statistically significant (t-test, p 0.05); picloram recovery from forest soil was apparently not affected by transporting and storing samples at ambient or cold temperatures for as long as 43 hours. It would appear that special precautions to freeze picloram-containing soil samples immediately upon collection are unnecessary.

In the laboratory phase of the study, the data, both uncorrected and corrected, show no significant effect of storage at -15°C for as long as 11 months (Table 1). Regression analysis (corrected data) showed that at 100 ppb, the percentage of recovery after storage was 99.72 - 0.0313 days, and at 300 ppb was 84.69 - 0.0102 days. A comparison of regression parameters confirmed that the intercept value at storage-time 0 was less with 300-ppb than with 100-ppb fortification. The regression coefficients were the same, indicating that time in storage affected recovery the same at both fortification levels, but they were not significantly different from zero, indicating there was no change in recovery with storage time.

The reason for the significantly lower recovery of picloram at 300-ppb than at 100-ppb fortification is unknown, although we suspect some of the difference may be attributable to the larger volume of solution (3 ml vs. 1 ml) used for the 300-ppb samples. The sides of the polyethylene bags storing them seemed much wetter than those of 100-ppb samples after thawing. The bags were not rinsed when soil was removed for analysis; therefore it is possible a significant amount of picloram was left behind.

The difference in recovery between samples extracted immediately or 2 hours after fortification and those extracted after 43 hours or more in cold storage is notable. Average recovery with immediate extraction was 79%; storage of even 43 hours reduced recovery to 70%. When corrected for loss during analysis, recovery was about 13% less than expected. Since it is unlikely that this "loss" is due to degradation during 43 hours of storage at 0°C, we believe it represents a binding of the picloram to the

Table 1. Mean percentage[1] of picloram rcovery from forest soil fortified with picloram and stored at -15°C.

Fortification (ppb)	Storage Days 0	29	98	196	280	330
	Uncorrected for Loss in Analytical Recovery[2]					
100	90 ± 8	71 ± 5[3]	64 ± 9	92 ± 6	65 ± 8	83 ± 9
300	69 ± 7	64 ± 5	59 ± 5	84 ± 5	54 ± 3	78 ± 5
	Corrected for Loss in Analytical Recovery[4]					
100	103 ± 9	97 ± 7[2]	97 ± 14	94 ± 6	86 ± 11	94 ± 10
300	79 ± 8	87 ± 7	89 ± 8	86 ± 5	71 ± 4	88 ± 5[5]

[1]n = 8, ± 95% confidence interval
[2]Effects of both storage and analytical recovery
[3]Effects of storage only
[4]n = 6, two samples lost
[5]n = 7, one sample lost

soil organic matter. Adams (1973) noted bioassays for pesticide residues in soil underestimated the levels found by chemical analysis, and the discrepancy increased with time indicating increased adsorption with time (Kaufman et al. 1976, review the problem of bound residues in pesticide analysis).

The results of this study indicate that (1) cold storage of soil samples containing picloram is not necessary during collection and shipment, at least during the first 43 hours, and (2) -15°C storage for as long as 11 months has no effect on picloram recovery levels. The results also clearly show the importance of including fortified controls with stored samples in order to provide a measure of the influence on recovery of factors inherent in the analytical method or in processes such as sample drying, grinding, sieving, and storage. Failure to do so will result in reporting of residue levels that are lower than the actual levels. This study was confined to a single soil and set of storage conditions. We caution that picloram recovery may differ in other circumstances and emphasize that fortified samples should be included with unknown samples during storage and analysis as part of standard quality assurance in environmental monitoring programs.

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involving pesticides. It does not contain recommendation for their use, nor does it imply that the uses discussed have been registered. All uses of pesticides must be registered by State or Federal agencies before they can be recommended.

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