phorimetric sensitivity of *p*-nitrophenol, it should be possible to determine low concentrations of parathion, which can be hydrolyzed to *p*-nitrophenol. Low concentrations of methyl parathion (methyl parathion showed no detectable phosphorescence) can also be determined by hydrolysis to *p*-nitrophenol. Because of the similarities in the structures and the great differences in the phosphorescence sensitivities of methyl parathion, parathion, and *p*-nitrophenol, several additional studies were carried out to prove that *p*-nitrophenol impurity was not responsible for the phosphorescence of parathion. An ether solution of the parathion sample used for these studies was shaken with aqueous base which would extract any *p*-nitrophenol. Then the aqueous base was acidified and re-extracted with ether. This solution was clear (ether solutions of *p*-nitrophenol are yellow) and was not phosphorescent, indicating no *p*-nitrophenol impurity in the parathion.

The phosphorimetric determination of the carbamates (Sevin, Zectran, Bayer 44646, Bayer 37344, NTA 10242, and U.C. 10854) and of the phosphate Imidan also appears promising. All of these, except possibly Imidan, should be relatively insensitive to the electron capture detector. At the present time, colorimetric methods are used for the determination of the carbamates. The colorimetric limits of detection are always in the microgram per milliliter range. Work in this laboratory has already been done on the determination of low concentrations of *p*-nitrophenol in urine. In addition, work is now being initiated on the phosphorimetric determination of carbamate residues on agricultural crops. The great sensitivities of many of the other pesticides and related compounds in Table I should result in many more agricultural applications. However, a poor cleanup of the sample and a poor separation of the pesticide from the sample can result in serious errors and much higher limits of detection than those given in Table I. The limits of detection in Table I are for the ultimate detection limits when the background is completely a result of the solvent.

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RESIDUE DETECTION

Tracer Study of Residues from 2-Chloro-6-(trichloromethyl)pyridine in Plants

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THE ANNOUNCEMENT of the N-Serve nitrogen-extending agent by The Dow Chemical Co. aroused interest in several problems related to 2-chloro-6-(trichloromethyl)pyridine, the active ingredient in this product. One of these topics was the detection and identification of any chemical residues which might occur in crop plants grown on soil subsequent to treatment with N-Serve. The work which resulted from this study is presented here.

Methods and Materials

2 - Chloro - 6 - (trichloromethyl)-C^{14}-pyridine. 2-Chloro-6-(trichloromethyl)-C^{14}-pyridine was prepared as described in a previous publication (*2*). The specific activity was 1 millicurie per millimole.

6-Chloropicolinic Acid. The reference sample of 6-chloropicolinic acid was prepared by the acid hydrolysis of 2-chloro-6-(trichloromethyl)pyridine, as described previously (*2*).

Treated Fertilizer. Ammonium phosphate fertilizer was treated by moistening with a 2% solution of 2-chloro-6-(trichloromethyl)-C^{14}-pyridine in ethyl ether, followed by air-drying at room temperature for 2 minutes prior to application to the soil.

Planting. Three-gallon metal cans, $9^1/_2$ inches in diameter, were filled to within 2 inches of the top with soil. Three-inch deep furrows were opened diametrically along the surface of the soil, and enough treated ammonium phosphate was added to provide 100 pounds per acre of nitrogen and 2 pounds per acre of 2-chloro-6-(trichloromethyl)-C^{14}-pyridine.

The furrows were immediately filled with soil, and the soil was seeded to a single crop. For tomatoes, transplants rather than seeds were planted in the cans of treated soil.

Subsequently, all crops were grown to maturity in the greenhouse under maintenance which avoided leaching of the soil.

Varieties. Golden cross Bantam corn was used. The carrots were Imperator. The lettuce was New York Special. The tomatoes were Pearson. The oats were unnamed.

Radioautographs. Radioautographs

To assess residue problems which might attend the use of N-Serve nitrogen-extending agent, corn, lettuce, tomatoes, oats, and carrots were planted in soil treated with 2-chloro-6-(trichloromethyl)-C^{14}-pyridine, a tagged form of the active ingredient in N-Serve. Autoradiographs showed the plants to contain radioactive residues in most of their organs. C^{14}-counting indicated that the residue level in leaves of corn and of tomatoes passed through a maximum several weeks after treatment and then decreased. Paper chromatography of extracts of oat seeds and corn, lettuce and tomato leaves demonstrated that the principal residue present was the hydrolysis product, 6-chloropicolinic acid, rather than the parent compound.

were prepared from tissue which had been dried between blotters in a botanical specimen press heated at 60° C. in a forced-circulation oven. The dried samples were mounted on cardboard with cellulose tape, covered with 1/2-mil polyethylene film, placed in intimate contact with x-ray film for 6 weeks, and processed.

Total Residue. Total residue-content of tissue was estimated by counting fresh, compacted 400-mg. samples of tissue in 1-inch diameter planchets with a mica end-window Geiger-Müller tube. Multiplication by an experimentally determined conversion factor transformed the net counts per minute into parts per million of 2-chloro-6-(trichloromethyl)-pyridine-equivalent residue.

Tissue Extraction. Both fresh and dried tissues were extracted by dropping thinly sliced material into boiling 85% methanol containing an excess of sodium bicarbonate beyond that required to neutralize any free acids in the sample. Subsequently, the extracts were decanted through a paper filter.

For lettuce, a single such extraction of fresh leaves was used. For fresh tomato leaves and dried oat seeds, two further such extractions ensued, and the filtrates from these extractions were combined with their respective initial extracts before further study.

Corn Leaf Extracts. Ground corn leaves, 11.9 grams, sampled at the age of 2 weeks were mixed with 2 ml. of water and 200 mg. of sodium bicarbonate and freeze-dried. The 12.8 grams of condensate were extracted with 6 grams of chloroform, and the chloroform concentrate was saved for counting. The dried ground tissue was then extracted with 85% methanol, and the 85% methanol extracts were used for further study.

Paper Strip Chromatograms. Extracts were placed on 1-inch wide strips of Whatman's No. 1 filter paper and developed with descending solvents. After air-drying at room temperature, radioactive regions were detected either by passage through a strip counter (Forro) or by cutting the chromatogram into 1/2-inch wide segments and counting these for 30 minutes each with a Geiger-Müller counter and scaler.

Nonradioactive reference compounds were detected by placing the dried strip in contact with the sensitized surface of No. 4 contrast photographic enlarging paper and exposing to the monochromatic filtered light from a mercury vapor lamp, as described by Markham and

Figure 1. Radioautograph of lettuce grown in soil treated with methyl-tagged 2-chloro-6-(trichloromethyl) pyridine

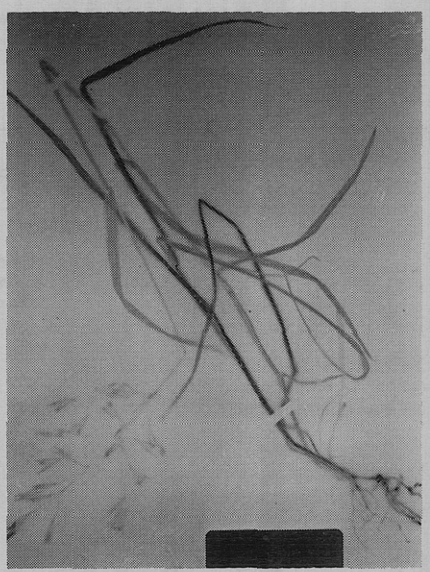

Figure 2. Radioautograph of oats grown in soil treated with methyl-tagged 2-chloro-6-(trichloromethyl)pyridine

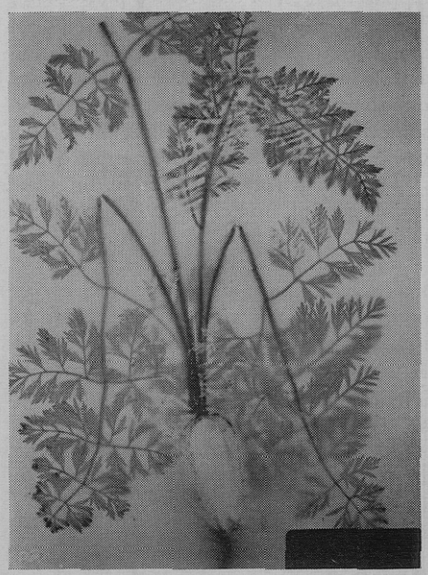

Figure 3. Radioautograph of carrot grown in soil treated with methyl-tagged 2-chloro-6-(trichloromethyl-pyridine)

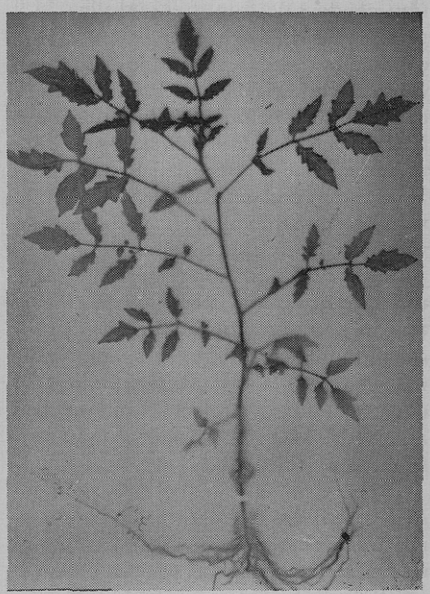

Figure 4. Radioautograph of tomato plant grown in soil treated with methyl-tagged 2-chloro-6-(trichloromethyl)pyridine

Smith (*1*) for purines. The reference compounds appeared on the developed print as light areas against a dark background.

Experimental Results

Radioautographs. Radioautographs of lettuce, oats, carrots, and tomato plants grown on soil treated with 2-chloro - 6 - (trichloromethyl) - C^{14}-pyridine are presented in Figures 1 through 4. Radioautographs of untreated plants showed no darkening. Apparently, all of the treated plants contained residues of some sort as a consequence of the soil treatment. In all cases, this residue is distributed throughout the foliar parts of the plants with a somewhat higher level occurring in the vascular tissues than in the parenchyma.

The carrot roots are unique, for residues are not apparent in their xylem tissue.

Rate Studies. Rate studies were made by sampling fully developed leaves of tomato plants and of corn plants at intervals after treatment with the tagged 2 - chloro - 6 - (trichloromethyl)pyridine. The results of counting these samples are shown in Figures 5 and 6.

For both corn and tomato plants, the total foliar residue-level lags initially then increases with time, passes through a maximum, and finally falls off.

Data published recently (*2*) for the Soil R sandy loam, in which the corn plants were grown, show that, even in the absence of volatility losses, more than 50% of the applied 2-chloro-6-(trichloromethyl)pyridine should have vanished from the soil by the time this peak leaf concentration had been reached.

Corn Leaf Extracts. The 6 grams of chloroform extract of the lyophyllization condensate from freeze-drying 11.9 grams of corn leaves counted only 8.2 counts per minute above background, whereas the original ground tissue had counted 13.9 counts per minute above background in infinite thickness. From these data, one may estimate that less than 30% of the residue in the leaf consisted of volatile compounds, such as 2-chloro-6-(trichloromethyl)pyridine.

Further fractionation of the nonvolatile radioactivity in the dried sample showed that most of the radioactivity was a substance soluble in 85% methanol. This material could not be extracted into ethyl ether or into chloroform from neutral aqueous solution, but could be extracted from an acidified one.

Paper strip chromatography demonstrated that this chloroform-soluble material was a substance having R_f values in several different solvent systems identical to those for 6-chloropicolinic acid, the simplest hydrolysis product of 2-chloro-6-(trichloromethyl)pyridine (Table I).

Lettuce Leaf Extract. The 85% methanol extract from one-half gram

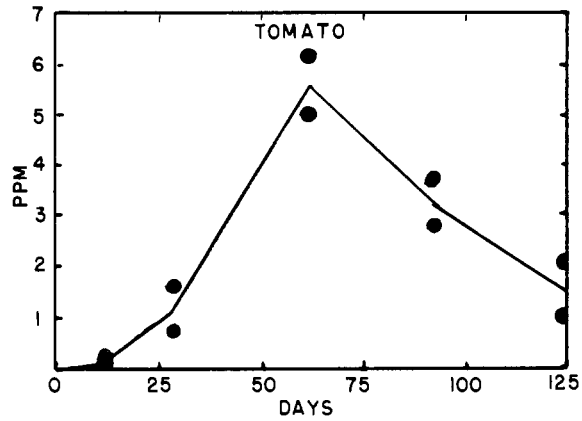

Figure 5. Rate study of residue level in leaves of tomato grown in soil treated with methyl-tagged 2-chloro-6-(trichloromethyl)pyridine

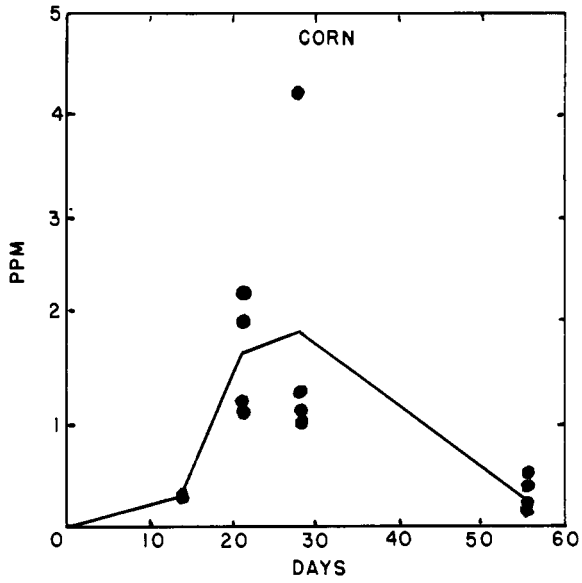

Figure 6. Rate study of residue level in leaves of corn grown in soil treated with methyl-tagged 2-chloro-6-(trichloromethyl)pyridine

Table I. R_f Value Comparisons

Compound	Solvent System: 1-Butanol saturated with 1.5N NH_4OH	1-Butanol, triethylamine, water, 5:1:2 v./v.	tert-Amyl alcohol, water, concd. NH_4OH 10:5:1 v./v.	Benzene, propionic acid, water 2:2:1 v./v.
2-Chloro-6-(trichloromethyl)pyridine	0.94	0.88	0.95	0.99
6-Hydroxypicolinic acid	0.16	0.28	0.26	0.76
6-Chloropicolinic acid	0.33	0.59	0.23	0.74
	0.37	0.60	0.26	0.70
	0.35	0.50	...	0.80
Metabolite of 2-chloro-6-(trichloromethyl)pyridine in corn leaves	0.33	0.56	0.23	...
	0.37	0.60	0.28	...
Metabolite of 2-chloro-6-(trichloromethyl)pyridine in lettuce leaves	0.36	0.50	...	0.73
Metabolite of 2-chloro-6-(trichloromethyl)pyridine in oat seeds	0.33	0.46	...	0.66
	0.37	0.50	...	0.67
Metabolite of 2-chloro-6-(trichloromethyl)pyridine in tomato leaves	0.35	0.52	...	0.80
	0.35	0.51	...	0.80

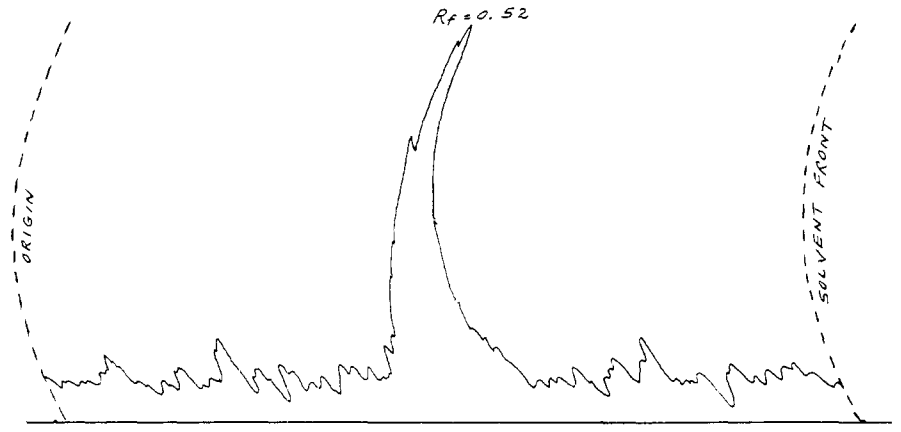

Figure 7. Radioactivity scan of paper strip chromatogram prepared from leaf of tomato grown in soil treated with methyl-tagged 2-chloro-6-(trichloromethyl)pyridine

Developer: 1-butanol, triethylamine, water, 5:1:2v./v.

of treated lettuce leaves was vacuum-evaporated to dryness, taken up in 4 ml. of water, adjusted to a pH of 2 with 6*N* hydrochloric acid, and shaken out with 4 ml. of ethyl acetate. Paper chromatography of the ethyl acetate extract showed the presence of a radioactive material having R_f values in agreement with those for 6-chloropicolinic acid. This comparison is shown in Table I.

Oat Seed Extract. A radioactivity study indicated that the 85% methanol extraction had removed 85% of the radioactivity from the oat seeds. Evaporation of the extracts from 5 grams of seeds to dryness, followed by reconstitution in 2 ml. of water, acidification to a pH of 2 with dilute hydrochloric acid, and extraction into two successive 3-ml. portions of ethyl acetate yielded a concentrate which contained all but 5% of the radioactivity initially in the aqueous phase.

Paper chromatography of this ethyl acetate concentrate showed the presence of a single compound having R_f values in good agreement with those observed for 6-chloropicolinic acid.

This comparison is presented in Table I.

Tomato Leaf Extract. Radioactivity measurements showed that the methanol extraction had removed 91% of the radioactivity from the tomato leaves. This crude extract from 5½-gram leaves from 30-day old plants was vacuum-evaporated, adjusted to pH 3 with dilute hydrochloric acid, mixed with nonradioactive 6-chloropicolinic acid, and chromatographed on paper strips.

The position of the added 6-chloropicolinic acid coincided exactly with that observed for the chief radioactive spot in every instance. Observed R_f values for these chromatograms are presented in Table I.

One of the radioactivity scans prepared from this tomato leaf extract is reproduced in Figure 7 as a typical example of the results which were obtained. The single peak is well above random fluctuations in background count and is much larger than any other peak which might have gone undetected.

Discussion

The data show that crops planted on soil treated with 2-chloro-6-(trichloromethyl)pyridine may be expected to contain residues. These residues appear generally to be distributed throughout the plant with the highest concentrations appearing in vascular tissue.

The fact that the most important of the residues detected can be extracted from acidified aqueous solutions by such immiscible solvents as chloroform, ethyl ether, or ethyl acetate—but not from neutral solutions—suggests that this chief residue is an acidic material. Inasmuch as the R_f values observed on paper chromatography of this substance agree well with those found for 6-chloropicolinic acid, one can be almost certain that 6-chloropicolinic acid is the residue which was repeatedly observed. The fact that 6-chloropicolinic acid is readily prepared by the hydrolysis of 2-chloro-6-(trichloromethyl)pyridine (*2*) adds credence to this identification.

Experimental studies not described here in detail have established that the extraction methods employed will not hydrolyze 2-chloro-6-(trichloromethyl)-pyridine to 6-chloropicolinic acid. Hence, the 6-chloropicolinic acid detected is not an artifact formed during extraction of the tissues.

If 2-chloro-6-(t ichloromethyl)pyridine itself formed a large part of the residues encountered, a portion of this material would probably have been lost through volatilization during evaporation of extracts and application to the filter paper strips prior to chromatography. Unchanged 2-chloro-6-(trichloromethyl)pyridine should have condensed with the water during freeze-drying. For young corn leaves, at least, less than 30% of the total radioactivity appeared in the condensate. Furthermore, no sign of a peak corresponding to 2-chloro-6-(trichloromethyl)pyridine appeared in any of the chromatograms described here. Therefore the authors feel that 6-chloropicolinic acid, rather than the parent compound, forms the major residue in crops grown on soil treated with 2-chloro-6-(trichloromethyl)pyridine.

The fact that residues appear to be missing from the xylem tissue of carrot roots was unexpected. Because of the position of the labeling in the 2-chloro-6-(trichloromethyl) - C^{14} - pyridine, decarboxylation of the resulting 6-chloropicolinic acid would lead to loss of tagging. The difficulties which were encountered in attempting to obtain consistent background count-rates after counting fresh carrot tissue slices led to the belief that carrot roots can decarboxylate 6-chloropicolinic acid. This is perhaps the reason for the absence of radioactivity in the carrot root sections from which the radioautographs were made.

The fact that the residue level in corn leaf tissue passed through a maximum at a time when most of the added 2-chloro-6-(trichloromethyl)pyridine should either have hydrolyzed to 6-chloropicolinic acid or been lost through volatilization led to speculation that much of the residue in the plant consisted of 6-chloropicolinic acid, which was formed in the soil by hydrolysis of the 2-chloro-6-(trichloromethyl)pyridine. Indeed, work currently in progress supports this hypothesis.

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