An Isotopic Study of Ethyl-*N*,*N*-di-*n*-propylthiolcarbamate (EPTC-S^{35}) Residue in Various Crops

S. C. FANG and PATRICIA THEISEN

Department of Agricultural Chemistry, Oregon State College, Corvallis, Ore.

A rapid method for the separation and subsequent determination of radioactive EPTC-S^{35} residue in crops is described. Radioactive EPTC residue as low as 0.2 γ can be detected with a windowless gas flow counter. The precision of this procedure on the recovery of EPTC is approximately 99 $\pm$ 3%. EPTC is readily absorbed by crops from soil and is rapidly metabolized. From 80 to 90% of the absorbed radioactivity is soluble in water and the rest remains in the tissues. The highest free EPTC-S^{35} residue found in plant tissues does not exceed more than 3% of the total amount absorbed.

THE HERBICIDE, ETHYL-*N*,*N*-DI-*n*-PROPYLTHIOLCARBAMATE (EPTC), when applied as a preplant, soil incorporated treatment, is of potential value in controlling many annual and perennial grasses and a number of the major annual broad-leafed weeds. A herbicide might also be taken up by the crops and remain as a residue. A colorimetric method for the analysis of EPTC residues in crops has been described (*2*). The lower limit of this method is approximately 4 γ. The following report describes a radioactive isotope technique for determining sulfur-35–labeled EPTC residues in plants with lower limits of detection of 0.2 γ. EPTC-S^{35} was applied to the soil followed by analysis for radioactive EPTC residues, in the various plants grown in the treated soil.

Experimental

Measurement of Radioactive EPTC. Radioactive EPTC is completely lost within an hour when it is directly plated on either a glass surface or on a stainless steel planchet. It is partially adsorbed on Whatman No. 1 filter paper. The amount of retention on filter paper depends largely on the drying time and the type of solvent used. Organic solvents, such as ethyl ether, acetone, hexane, iso-octane, and absolute ethyl alcohol are very satisfactory for such a purpose. Once the solvent is dried, EPTC-S^{35} is very strongly adsorbed by the filter paper without significant loss. Repeated wetting and drying either with water or with an organic solvent caused a further loss of EPTC-S^{35} from the filter paper. Coating the filter paper surface with a thin layer of activated carbon greatly increases the capacity of absorption. Furthermore, the loss of EPTC-S^{35} during solvent evaporation is greatly reduced. Because of the volatility of this compound, the conventional direct plating method cannot be used here. When a definite volume, 0.1 ml. of iso-octane or ethyl alcohol solution containing various amounts of EPTC-S^{35} is dried immediately under an infrared lamp, in a stainless steel cupped planchet containing a circle of filter paper coated with activated carbon, reproducible results can be obtained. The standard radioactivity curve of EPTC-S^{35} prepared by this method gave a linear increase.

Recovery of EPTC-S^{35} in Plant Tissues. In this study, the extraction procedure for EPTC residue in crops, as outlined by the Stauffer Chemical Co. (*2*) has been slightly modified. The plant sample is submitted directly to steam distillation without prior extraction of the crop with hexane. The steam condensate containing EPTC is extracted continuously with hexane or iso-octane by using a setup for volatile oil—lighter than water—determination (Figure 1). After the moisture trap is partially filled with water, 1 to 2 ml. of iso-octane are introduced quantitatively into the measuring column. The sample is then heated with an electric mantle and allowed to boil gently for 1 hour. At the end of this period, the volume of iso-octane is measured and then drained out through the stopcock. Aliquots of 0.1 ml. of iso-octane solution are dried, as described previously, and the radioactivity of the samples is counted either with a thin mica end window Geiger-Müller counter (1.8 mg. per sq. cm.) or with a gas-flow windowless counter in the usual manner. The amount of EPTC-S^{35} in the crops is then calculated from the standard radioactivity curve. Quantitative recoveries of added EPTC-S^{35} to various crops and soil samples are shown in Table I. After several rinsings of the distillate collector with water and iso-octane, the apparatus can be used again for the next determination.

EPTC-S^{35} Residue in Crops. The crops used in this study were kidney bean, sweet corn, garden pea, radishes, carrots, cabbage, mustard, table beets, and sugar beets. The seeds were sown separately in flats containing Newberg sandy loam soil under greenhouse conditions. One day after the seeds were planted, all the flats received a pre-emergence application of EPTC-S^{35} at a rate of either 1 or 4 pounds per acre. The appropriate amount of EPTC-S^{35} was weighed and first dissolved in 10 ml. of ethyl alcohol. The alcohol solution was then added to a volume of water equivalent to 400 gallons of water per acre. The EPTC-S^{35} solution was applied as evenly as possible to the soil surface by means of a polyethylene wash bottle. The plant samples were harvested at various times as indicated in Table II and analyzed for free EPTC-S^{35} residue.

Results and Discussion

The accuracy of this method depends largely on the initial weighing of EPTC-S^{35} and the subsequent preparation of standard solutions containing various amounts of this chemical. However, the results show that a linear increase of radioactivity can be obtained with as high a content as 200 γ of EPTC-S^{35} per planchet. This method has been used successfully, for the last two seasons, in the determination of EPTC-S^{35} residues.

Recovery studies (Table I) reveal this

Table I. Recovery of EPTC-S^{35} by the Steam Distillation Method

Crop	Sample Size, Grams	Added, γ	Found, γ	Recovery, %
None	..	400	384	96
	..	400	408	102
	..	200	200	100
	..	80	78	97
	..	20	19.8	99
	..	10	10.1	101
Bean seedling	10	400	387	96
	10	400	376	94
Pea seedling	10	400	412	103
	10	400	415	104
Sugar beet leaves	10	400	405	101
	10	400	399	100

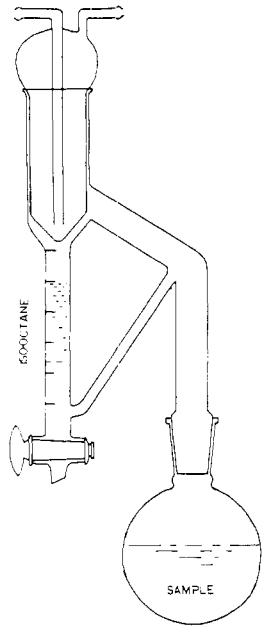

Figure 1. Apparatus for recovery of EPTC-S^{35} residue in crops

procedure to be satisfactory in amounts ranging from 10 to 400 γ. The precision of the method on the recovery of EPTC is approximately 99 $\pm$ 3%. The lower limit of detection is 0.5 γ when a thin mica end window Geiger-Müller counter is employed. When a gas flow windowless counter is used for the detection of radioactivity, the sensitivity is increased to 0.2 γ in the sample tested. The recommended 1-hour boiling time is generally sufficient to remove all EPTC-S^{35} residue from crops, as indicated by a study of continuous extraction of the distillate with an hourly change of iso-octane. No radioactivity is found in the iso-octane solution after the first hour.

Twenty-three different samples of various crops grown in EPTC-S^{35} treated soil were analyzed for free EPTC (Table II). Examination of the radioactivities of the water extract and of the plant tissue after complete removal of the volatile EPTC-S^{35} by boiling, revealed that approximately 80 to 90% of the absorbed radioactivity remains in the water solution. The largest amount of free EPTC-S^{35} in the tissues was less than 3% of the total amount absorbed. This suggests that EPTC is rapidly metabolized by plants and therefore should present little residue problem for the crops tested.

Table II. EPTC-S^{35} Residue in Plant Tissues from Crops Which Received Various Rates of Pre-emergence Treatment

Crops	Plant Part	Fresh Weight, Grams	EPTC-S^{35} Application, Lb./Acre	Treatment to Harvest, Days	Free EPTC-S^{35} Residue, γ	Concentration of EPTC-S^{35} in Plants, P.P.M.
Kidney bean	Whole plant	7.5	4	14	2.40	0.32
	Whole plant	30	4	38	1.97	0.066
	Flowers, buds	5	4	38	0	0
	Root	5	4	38	1.98	0.396
	Leaves, stem	23	4	38	0	0
	Pods	5[a]	1	66	0	0
Corn	Whole plant	7.5	4	14	1.20	0.16
	Leaves	30	4	38	0	0
	Ear	35	1	114	0	0
	Roots	1.2[a]	4	38	0.34	...
Pea	Whole plant	5.0	4	14	0.92	0.184
	Leaves, stem	0.68[a]	4	38	0.57	...
	Whole plant	10	4	38	2.47	0.247
Radish	Whole plant	5.9	4	54	0.45	0.076
	Leaves	21.6	4	55	0.51	0.024
	Roots	5.5	4	55	0	0
Carrot	Whole plant	1.5[a]	4	67	0.94	...
	Root	3.5	1	83	0	0
Beet, table	Leaves	3.3	4	38	0.26	0.079
	Whole plant	10	4	64	0.42	0.042
Sugar beets	Whole plant	5	4	64	0.66	0.132
Cabbage	Leaves	10	1	83	0	0
Mustard	Leaves	10	1	83	0	0

[a] Dry weight.

References

(1) Antognini, J., Division of Agricultural and Food Chemistry, 133rd Meeting, ACS, San Francisco, Calif., April 1958.
(2) Batchelder, G. H., Patchette, G. C., Stauffer Chemical Co., Richmond, Calif., personal communication, 1958.
(3) Lee, W. O., *Proc. 16th Western Weed Control Conf.*, p. 84–7, 1958.

Received for review April 24, 1959. Accepted July 6, 1959. Division of Agricultural and Food Chemistry, 133rd Meeting, ACS, San Francisco, Calif., April 1958. Work supported by Stauffer Chemical Co. and the Atomic Energy Commission. Approved by the director of the Oregon Agricultural Experiment Station as Technical Paper No. 1219

VITAMIN B_{12} IN FOODSTUFFS

Comparative Vitamin B_{12} Assay of Foods of Animal Origin by *Lactobacillus leichmannii* and *Ochromonas malhamensis*

HAROLD LICHTENSTEIN, ARAM BELOIAN, and HOWARD REYNOLDS[1]

Human Nutrition Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.

OF THE MICROBIOLOGICAL METHODS available for the determination of vitamin B_{12}, two appear to have special merit, the *Lactobacillus leichmannii* (*9*) and the *Ochromonas malhamensis* (*1, 5*) methods. The *L. leichmannii* assay has, in several modifications, been more widely accepted because of its rapidity, uniformity of response, and precision of results. Comparative data on the vitamin B_{12} content of foods of animal origin as assayed by the *L. leichmannii* and *O. malhamensis* methods are presented.

[1] Present address, Cellular Metabolism Laboratory, Human Nutrition Research Division, Agricultural Research Service, Washington 25, D. C.

Experimental Procedure

To provide comparative data, a number of food products were selected so as to comprise a cross-sectional survey of the various classes of foods of animal origin in customary use. These were assayed by both the *L. leichmannii* and *O. malhamensis* methods. Food products with a fermentation history and organ