permit its use in this analytical procedure; however, it was found possible to remove the aromatics completely from the hexane extract containing lindane by this direct sulfonation technique, as interfering substances are either sulfonated and removed by the extraction procedure or are converted to products that do not interfere in subsequent analysis.

Apparatus

A specially designed all-glass digestion and nitrating apparatus (*3*). A suitable photometer.

Reagents

Fuming sulfuric acid, 30%. Reagents as described by Schechter and Hornstein (*3*).

Procedure

One hundred grams of whole mushrooms are washed under tap water, cut into small pieces, and added to 200 ml. of methylene chloride in a 500-ml. Erlenmeyer flask equipped with a reflux condenser. The mixture is refluxed gently for 3 to 4 hours, then allowed to stand overnight. The extract is decanted through a filter paper into a separatory funnel, where small amounts of water either extracted or introduced in the rinse step separate to the top. The methylene chloride phase is then drained into an Erlenmeyer flask and its volume reduced on a steam bath to approximately 50 ml. The solution is poured into a 250-ml. separatory funnel and is extracted with a 10-ml. portion of 30% fuming sulfuric acid. The methylene chloride–sulfuric acid mixture is shaken vigorously for 2 minutes with frequent venting of the separatory funnel. (Precautions should be used in handling fuming sulfuric acid.) The layers are allowed to separate, and the bottom acid layer is drained off and discarded. To ensure complete sulfonation, the methylene chloride layer is extracted in a similar fashion with two more 10-ml. portions of 30% fuming sulfuric acid.

After the third 10-ml. portion of the acid has been discarded, 25 ml. of cold water is cautiously added to the methylene chloride. The mixture is thoroughly shaken, again with frequent venting of the separatory funnel, and the two phases are allowed to separate. The water layer is discarded, and the methylene chloride fraction is drained into a second 250-ml. separatory funnel and washed with another 25 ml. of cold water. The methylene chloride phase is returned to the first separatory funnel and washed with a third 25-ml. portion of water. After phase separation the methylene chloride is emptied into a 250-ml. Erlenmeyer flask having a ⅋ 24/40 joint (traces of water will cause no harm), a boiling chip is added, and the methylene chloride is evaporated on a steam bath to a volume of 1 to 2 ml. One hundred and twenty milliliters of glacial acetic acid is added and 20 ml. of the acid is distilled out by heating in an oil bath at 130° to 140° C. to remove the last traces of volatile solvent. The glacial acetic acid solution is then analyzed (*3*).

Table I. Recoveries of Lindane Added to Untreated Mushrooms

(Weight of each sample 100 grams. Analysis run on methylene chloride extracts after sulfonation.[a])

Added, γ	Recovered, γ	Recovery, %
50	47	94
75	73	97
100	95	95

[a] Apparent lindane blank on methylene chloride extract of 100 grams of mushrooms (no lindane added) without sulfonation was 35 γ, or 0.35 p.p.m. Sulfonation reduced this to 2 γ, or 0.02 p.p.m.

Literature Cited

(1) Furman, D. P., and Hoskins, W. M., *J. Econ. Entomol.*, **41**, 106–7 (1948).
(2) Hornstein, I., *Anal. Chem.*, **24**, 1036 (1952).
(3) Schechter, M. S., and Hornstein, I., *Ibid.*, **24**, 544 (1952).
(4) Schechter, M. S., Pogorelskin, M. A., and Haller, H. L., *Ibid.*, **19**, 51–3 (1947).
(5) Shrenk, H. H., Pearce, S. J., and Yant, Y. P., U. S. Bur. Mines, Repts. Invest. **3287** (1935, revised 1937).

Received for review April 6, 1955. Accepted June 3, 1955.

HERBICIDAL ACTION

Activities and Residues of Sulfur-35–Labeled Bis(ethyl Xanthic) Disulfide

RUBLE LANGSTON

Agricultural Experiment Station, Purdue University, Lafayette, Ind.

Sulfur-35–containing bis(ethyl xanthic) disulfide, known commercially as Herbisan, was synthesized and evaluated as a pre- and postemergence herbicide. Herbicidal concentrations of bis(ethyl xanthic) disulfide as formulated were not absorbed in sufficient quantities to be detected by regular radioisotopic counting procedures in any of the vegetables studied, when it was applied as a pre-emergence spray on muck soil. Foliage applications of bis(ethyl xanthic) disulfide showed that of the 16 vegetable crops studied, only in cabbage and cauliflower was translocation ascertained by autoradiographic procedures and verified by Geiger-Müller counting. These studies indicate that bis(ethyl xanthic) disulfide is primarily a contact herbicide.

BIS(ETHYL XANTHIC) DISULFIDE, known commercially as Herbisan and Sulfasan, shows promise as both a pre- and postemergence contact herbicide (*1*, *3*, *4*). The literature contains no information on its uptake, transport, accumulation, or residual qualities in vegetable crops.

Herbisan, like most herbicides, is required in relatively minute quantities for effective weed control. Regular chemical methods for its detection are not of sufficient sensitivity to allow a critical evaluation of its action and its residues. Consequently, it appeared that labeling with sulfur-35 and studying with isotope techniques would prove of great value, as such techniques are several hundred

times more sensitive than ordinary chemical methods.

The present paper is a report of the synthesis of labeled bis(ethyl xanthic) disulfide and its action as a herbicide.

Methods and Materials

Sulfur-35–containing bis(ethyl xanthic) disulfide was synthesized from labeled carbon disulfide according to the following reactions:

$$NaOH + H_2O + C_2H_5OH + CS_2^* \longrightarrow C_2H_5OC(=S^*)-S^*-Na + H_2O$$

$$2C_2H_5OC(=S^*)-S^*-Na + Cl_2 \longrightarrow C_2H_5OC(=S^*)-S^*-C(=S^*)-OC_2H_5 + 2NaCl$$

Twenty grams of sodium hydroxide were dissolved in 20 ml. of water. The resulting solution was cooled and 31 ml. of 95% ethyl alcohol were added.

Table I. Herbicidal Activities of Sulfur-35–Labeled and Commercial Herbisan on Weeds and Grasses in Muck Soil

	No Treatment	Labeled Herbisan	Commercial Herbisan
Weeds	64.2[a]	7.0	10.7
Grasses	18.0	4.1	5.5

[a] Each figure represents an average of number of weeds or grasses contained in four 6-inch pots 7 days after spraying.

Twenty-five milliliters of CS_2^* containing 1.66 millicuries of sulfur-35 were added dropwise with continuous agitation over a 2-hour period to the solution, which was maintained at 37.5° C. Stirring was continued for an additional hour, at which time the resulting deep red solution was diluted with 100 ml. of water.

Oxidation was conducted by the addition of chlorine gas above the surface of the agitated solution for 2 hours. After oxidation, the two-phase solution possessed a pale yellow color. The lower layer, bis(ethyl xanthic) disulfide, was washed several times with water. Other impurities, primarily carbonyl sulfide, were removed by vacuum treatment at 50 to 60 mm. of mercury at 60° C. A yield of 18 grams was obtained. The final product possessed an oily yellow appearance and crystallized at 28° C. The specific activity (corrected), as determined with an end-window tube (1.8 mg. per sq. cm. of mica), was 9.02×10^5 C per minute per mg.

The labeled bis(ethyl xanthic) disulfide was formulated into commercial Herbisan, a pre-emergence herbicide, by mixing in the following ratio: bis(ethyl xanthic) disulfide 58 parts, a medium aromatic oil 48 parts, and emulsifier 2 parts. Herbisan 91, recommended as a postemergence herbicide, was formulated by mixing 9 parts of the labeled bis(ethyl xanthic) disulfide with 1 part of horticultural oil and adding 1% emulsifier.

The labeled Herbisan was applied as a pre-emergence spray at the rate per acre of 2 gallons of Herbisan in 50 gallons of water. Postemergence treatments with Herbisan 91 were at the rate per acre of 1 gallon in 100 gallons of water.

Results

In order to establish that the herbicidal activity of the labeled Herbisan was of the same magnitude as that of commercially produced Herbisan, comparative tests were conducted on natural weed and grass seedlings growing in muck soil. The results as shown in Table I indicate that the labeled Herbisan possessed herbicidal activity as great as or slightly greater than the commercial grade.

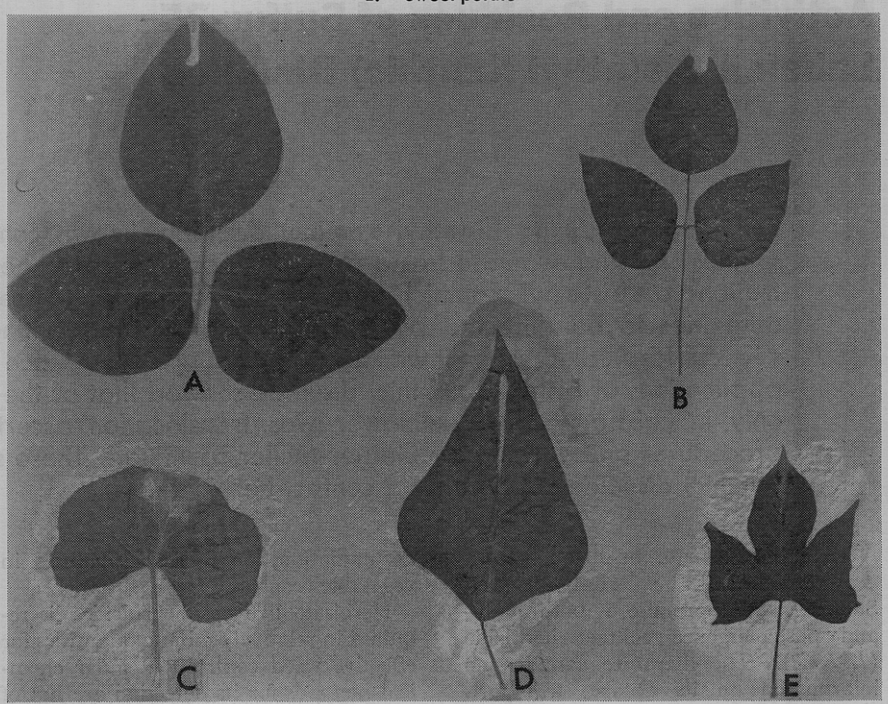
Studies were conducted to measure the uptake of Herbisan following pre-emergence applications. Spray treatments were applied in muck soil to onions, lettuce, red beets, spinach, carrots, tomatoes, lima beans, sweet corn, and red kidney beans. All pre-emergence sprays were made in the greenhouse on October 18, 1954, 3 days after planting. Leaf samples were taken November 22, dried at 65° C. for 24 hours, and ground to pass a 60-mesh screen. Radioassays were conducted on samples possessing quantities sufficient for counting of infinite thickness. All samples were compared to leaf tissue of nonradioactive ground red kidney bean of identical geometry. Five-minute counts were made upon duplicate aliquots of each vegetable. The counting data revealed no radioactivity in any of the treated vegetables based on a background count which varied between 46.1 and 50.0 counts per minute.

Studies on foliar absorption and translocation were conducted by applying one drop of labeled Herbisan 91 from a No. 27 B-D syringe to a leaf of each vegetable. This drop represented 3.8 mg. of Herbisan 91 with a total activity of 3.7×10^7 counts per minute. The vegetables treated were kale, cauliflower, watermelon, lettuce, edible soybean, lima bean, white potato, green bean, cucumber, sweet corn, sweet potato, red kidney bean, cabbage, peppermint, spinach, and onion. The treated leaves were left intact on the growing plants for 16 days, at which time they were excised from the plant, dried in a mounting press for 24 hours, and mounted on cardboard with Duco cement. The mounted leaves were then placed in direct contact with Kodak no-screen x-ray film and exposed for 30 days. The autoradiographs were developed using conventional developing procedures.

No attempt was made to control the spread of the drop, and as a result the Herbisan 91 spread over several square

Figure 1. Contact areas of labeled bis(ethyl xanthic) disulfide on vegetable leaves 16 days after application

A. Edible soybean
B. Lima bean
C. Cucumber
D. String bean
E. Sweet potato



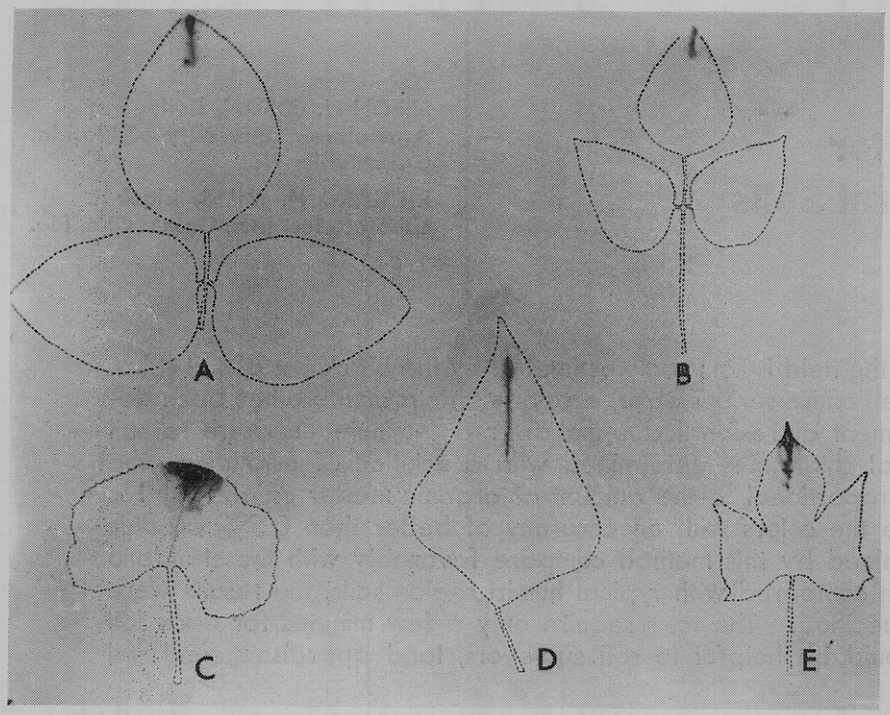


Figure 2. Autoradiograph of leaves of Figure 1, showing extent of movement of labeled bis(ethyl xanthic) disulfide

centimeters of the treated leaf surface. Observations indicated that an immediate contact-killing action occurred where the drop spread, and resulted in a discoloration within 48 hours.

Inspection of the autoradiographs showed that, with the exception of cabbage and cauliflower, radioactivity occurred only in those areas killed by contact action. Figure 1 shows the extent of damage caused by one drop of Herbisan 91 placed on the leaves of edible soybean, lima bean, cucumber, green bean, and sweet potato. All the tissues adjacent to the dead areas are apparently healthy. The autoradiograph of the leaves in Figure 1, shown in Figure 2, indicates that radioactivity is present only in tissues that had been killed. This would indicate that the herbicidal action is of a contact nature. The results shown in Figures 1 and 2 are typical of all of the vegetables studied, with the exception of cabbage and cauliflower which showed very faint quantities of radioactive sulfur distributed rather uniformly throughout the treated leaf and petiole. The autoradiographs showing this translocation were so light in color that they could not be reproduced by the photographic procedures necessary for printing and are, therefore, not shown.

The results of other workers (*1*, *2*) indicate that bis(ethyl xanthic) disulfide is effective as a pre- and postemergence herbicide in onions. In an effort to evaluate its residual aspects further, applications were made pre- and postemergently. The pre-emergence spray was made one day prior to emergence of the onions, while the postemergence sprays were made about one week following the flag stage. The postemergence sprays were applied both as an over-the-top spray and as a directed spray, which resulted in the herbicide's striking the lower half inch of the onions. The onions were seeded October 20, 1954, and final radioassays were conducted January 28, 1955, after the onions were well into the bulbing stage. The final assay is shown in Table II and is representative of all the periodic radioassays conducted. None of the labeled bis(ethyl xanthic) disulfide treatments showed any radioactivity beyond that of normal background radiation.

Discussion

The studies reported here present a simple, convenient procedure for the synthesis of sulfur-35–labeled bis(ethyl xanthic) disulfide. Comparative herbicidal tests showed that the labeled, laboratory-synthesized, and formulated Herbisan compared favorably with that produced commercially. Each formulation produced an approximately 85% kill of broad-leaved plants and 75% kill of the grasses.

The results of these studies indicate that in the case of the vegetables tested, bis(ethyl xanthic) disulfide, as formulated, was not absorbed by the plants when applied pre-emergently at the rate of 2 gallons in 100 gallons of water per acre, as none of the plants showed a measurable quantity of radioactivity. Under the conditions of these studies the possibility exists that the herbicide could have been so tightly absorbed by the soil surface that none of it actually reached the root zone. However, water was always applied to the soil surface and as a result the soil received several inches.

Foliar applications of bis(ethyl xanthic) disulfide as formulated and applied as a drop to the leaves showed some degree of translocation in cabbage and cauliflower as determined by autoradiographic procedures. Similar treatments showed no detectable translocation beyond the areas of original contact in any of the other 14 vegetables tested. The radioactivity translocated in cabbage and cauliflower may or may not be in the molecular form of bis(ethyl xanthic) disulfide. As the tissues adjoining the original contact area appeared normal, this radioactivity may be the result of metabolized breakdown of sulfur-35 products. Further studies utilizing chromatographic and autoradiographic procedures are necessary before this point can be clarified.

Bis(ethyl xanthic) disulfide in herbicidal concentrations and in the formulations tested possesses two primary advantages as a herbicide. It is not absorbed by the roots of the crop plants tested when applied to the surface of muck soil, and it is not translocated by a majority of the common vegetable plants when applied as foliar sprays. The data indicate that bis (ethyl xanthic) disulfide may be used safely on the crops tested in regard to residue hazards.

Table II. Radioactivity in Onions Sprayed with Sulfur-35–Labeled Formulations of Bis(ethyl Xanthic) Disulfide

Treatment	Plant Part	Counts per Minute[a]
Herbisan pre-emergence	Leaves	54.7
	Bulbs	53.6
Herbisan 91 post-emergence[b]	Leaves	50.5
	Bulbs	55.0
Herbisan 91 post-emergence[c]	Leaves	54.1
	Bulbs	53.3
Background		53.4

[a] Counting time 10 minutes.
[b] Directed at 45° angle.
[c] Over-all spray application.

Literature Cited

(1) Alban, E. K., and McCombs, L., Proc. 6th Annual North Central Weed Control Conference, p. 81, 1949.
(2) Lee, O. C., and Warren, G. F., Purdue University, Agr. Ext. Ser., Lafayette, Ind., Mimeo. **ID-1** (**BP + HO**) (1955).
(3) Wilson, J. D., *Farm and Home Research*, **35**, No. 263, 21 (1950).
(4) Young, H. G., and Wilson, J. D., Ohio Agr. Expt. Sta., Botany Mimeo. **11** (1951).

Received for review April 12, 1955. Accepted July 26, 1955. Journal paper 854, Purdue University, Agricultural Experiment Station.