Table I. Recovery of Amiben Added to Tomatoes

Amount Added, P.P.M.	Recovery, %
0.05	70, 110
0.1	85, 70, 75, 115
0.15	83
0.25	96
0.5	90
0.75	121
1.0	107
1.25	123

The ether and benzene extractions of the tomato extract (adjusted to pH 7.5) removed a considerable quantity of interfering substances which precipitated out if the extract solution was directly adjusted to pH 2 without extraction. Some of these interfering substances were acidic because the pH of the solution immediately after extraction was 8.5.

All of the solvents used in the procedure were distilled. The standard solution of amiben must be freshly prepared. Solutions of amiben in organic solvents turn yellow with time possibly because of oxidation of amine and further polymerization reactions leading to colored quinone-type compounds (*3*). Quantitative methylation of the herbicide required three treatments with BF_3-methanol because *ortho*-chloro substituted benzoic acids are less reactive due to stearic hindrance. Methanol distilled over KOH should be used for preparation of the boron trifluoride reagent.

When this method is used with a tritium electron affinity detector, the hexane should be dried over sodium sulfate before injection into the column (*7*).

A pH of 2 was optimum for extraction of amiben into benzene. In the determination of guthion residues, Adams (*1*) found an optimum pH of 4.1 for extraction of 2-aminobenzoic acid (anthranilic acid) into benzene. This pH apparently corresponded to a kind of "isoelectric point" for anthranilic acid. The presence of the two chlorines in amiben would make it a stronger acid and a weaker base. Therefore, a lower optimum pH (pH 2) is to be expected for amiben if, indeed, an isoelectric point can be said to exist for it.

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Literature Cited

(1) Adams, J. M., Chemagro Corp., Kansas City 20, Mo., personal communication.
(2) Colby, S. R., Baker, R. S., Warren, G. F., Purdue University, Lafayette, Ind., unpublished data.
(3) Fieser, L. F., Fieser, M., "Textbook of Organic Chemistry," p. 555, 1950.
(4) Goodwin, E. S., Goulden, R., Reynolds, J. G., *Analyst* **86,** 697 (1961).
(5) Goodwin, E. S., Goulden, R., Richardson, A., Reynolds, J. G., *Chem. Ind. London* **1960,** p. 1220.
(6) Metcalf, L. D., Schmitz, A. A., Chromatography Application Methods Bulletin WCA 1, Barber-Colman Co., Rockford, Ill., Sept. 22, 1960.
(7) Segal, H. S., Amchem Products Inc., Ambler, Pa., personal communication.

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DEFOLIANT RESIDUES

P^{32}- and S^{35}-Labeled *S,S,S*-Tributylphosphorotrithioate Defoliant Residue in Cottonseed

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Radioactive tributylphosphorotrithioate containing either the P^{32} or S^{35} isotopes was synthesized. The radioactive compounds were applied to intact cotton plants, and the distribution of the intact compound and its degradation products into the various sulfur and phosphorus fractions of the cottonseed kernel was traced by means of the P^{32} and S^{35} labels. Most of the radioactivity which was translocated to the seed resulted from the degradation products. Only a small amount of the total radioactivity present in the seed was represented by the unaltered thiophosphate compound.

THE FIRST organic thiophosphate compound reported to induce and accelerate abscission was *S,S,S*-tributylphosphorotrithioate (*6*, *14*). During the same period, Goyette (*5*) tested homologous trialkyl phosphorotrithioites and the corresponding trialkyl phosphates for abscission-inducing activity in the greenhouse, and noted that only the former were active. Hall *et al.* (*7*) studied a wide array of phosphorus- and sulfur-containing compounds for abscission properties and confirmed Goyette's findings. Both groups of workers concluded that the sulfur moiety was essential for effective defoliation.

The organic phosphorus insecticides, in general, are cholinesterase inhibitors, and some are extremely toxic to warm-blooded mammals. Little is known about the mammalian toxicity of organic phosphorus defoliants except that these compounds did not appear to be cholinesterase inhibitors in a preliminary study (*11*).

Because of the great potential of thiophosphate compounds in defoliation, this present study was primarily concerned with the residue of the intact P^{32}- or S^{35} - labeled *S,S,S* - tributylphosphorotrithioate and its degradation products in cottonseed.

Experimental

Preliminary results indicated that in vivo degradation of the trithioite and trithioate defoliants did not differ from each other because the relatively unstable trithioite is converted to the trithioate in the presence of oxygen (*9*). Therefore, *S,S,S*-tributylphosphorotrithioate (DEF) was used throughout the study and was synthesized with either the P^{32} or the S^{35} label as described previously (*13*).

P^{32} and S^{35} activity in the kernels, as well as in subsequent liquid and solid fractions, was assayed by means of a gas

flow proportional counter with 2-pi geometry. Corrections for isotopic decay and self-absorption were made by standard methods (*1*).

P^{32} and S^{35} Residues in Cottonseed. Three field plots, each containing four rows of eight to 10 plants of Deltapine 15 cotton per row, were used for the experiment. When nearly 30% of the bolls were open, one plot was sprayed with the P^{32}-labeled DEF (0.37 mc. per mmole); a second plot received the S^{35}-labeled compound (0.22 mc. per mmole); and the last plot served as a control. Each treated plot received the equivalent of 1.5 pounds of DEF per acre emulsified in 35 gallons of water. Defoliation was well initiated within 48 hours.

All of the open bolls were harvested 5 hours after spraying. The bolls that subsequently opened were harvested at 7 days, and again at 14 days, after treatment. Thus, the three samplings represented bolls of different ages at the time of DEF application. Before decortication and grinding of the kernels to 60-mesh fineness, each age group of ginned seeds from the S^{35} and P^{32} treatments was divided into two lots. One lot in each group was eluted with 95% ethanol until no radioactivity was present in the effluent. The difference in radioactivity between the ground kernels of the eluted and noneluted lots of seed is assumed to represent the amount of contaminant raioactivity worked into the ground kernels by the mechanical action of ginning, decorticating, and grinding.

Determination of Undegraded DEF in Seed Kernels. Five-gram samples of P^{32}- as well as S^{35}-labeled ground kernels were extracted with petroleum ether (b.p. 30° to 60° C., ACS reagent) with the Soxhlet apparatus for 18 hours. This solvent was used since it would not extract any of the phospholipids (*12*)—the only group of naturally occurring compounds soluble in nonpolar solvents which could acquire an isotopic label as a result of DEF degradation. To determine how much of the radioactivity in the petroleum ether extract from the seed meal was undegraded DEF, paper chromatography employing the three solvent systems described by Fukuto *et al.* (*4*) was used. The petroleum ether extract of the kernels was cleared of oil and pigments (*10*) before chromatography in order to obtain clear resolution of the radioactive spot or spots on the chromatogram. After chromatography, the resulting R_f values of the radioactive spots in each of the three solvent systems were compared, by means of radioautography, to those of P^{32}- and S^{35}-labeled DEF standards. The latter had been quantitatively added to and extracted from unlabeled ground kernels and treated the same as the unknown.

Phosphorus and Sulfur Determinations. The methods of Pons *et al.* (*12*) and Ergle and Eaton (*3*) were employed for the fractionation and analysis of phosphorus and sulfur, respectively, in the seed kernels of the control plants. The same methods were used to fractionate the radioactive forms of these elements in the kernels from DEF-treated plants. The analysis, however, of both P^{32} and S^{35} in the resulting liquid and solid fractions was carried out as mentioned above.

Results and Discussion

DEF Residue in Kernels. Chromatography of the P^{32} and S^{35} present in the petroleum ether extracts in all of the age groups indicated that DEF was the only radioactive component present; consequently, the amount of DEF in the kernels was calculated as follows. After correction for sample self-absorption and isotopic decay, the micrograms of DEF in a given sample = (c.p.m. of ether extract) × (k), where k is composed of the extraction factor of DEF from seed kernels times the ratio of micrograms to c.p.m. of standard P^{32}- or S^{35}-labeled DEF. ($k = 3.1 \times 10^{-3}$ for P^{32} and 3.8×10^{-4} for the S^{35} form.) When the total radioactivity of the kernels is compared with that of their petroleum ether extracts, all of the radioactivity shown in Table I (eluted and noneluted) for the 5-hour, 89% (±2%) for the 7-day, and 5.4% (±2%) for the 14-day harvests of seed kernels represented undegraded DEF.

Table I shows that nearly all of the P^{32} activity was removed from the 5-hour harvest of kernels by ethanol washing of the intact seed. Thus, a substantial amount of DEF (Table II) was incorporated into the kernels after normal processing. Such was not the problem, however, with kernels from bolls that opened after DEF application since little or no radioactivity was removed by the ethanol washing of the intact seeds (Table I).

Since it is unlikely that translocation from the leaves to the fully mature seeds of the 5-hour harvest would have taken place, the residue of intact DEF in the kernels after ethanol washing of the whole seed (Table II) would have been that absorbed by the seed coat into the lipid fraction of the kernel. For the developing kernels, on the other hand, despite a significant influx of P^{32} between 7 and 14 days, the level of DEF remains fairly constant and does not exceed the value of the mature kernels. This suggests that DEF was supplied to the developing seed by the adjacent carpels and not by translocation from nonproximal portions of the plant.

The residue of intact DEF and the increase of S^{35} in the seed kernels of the treated plants followed essentially the same trend during the 14-day period as that outlined for P^{32} in Tables I and II.

Table I. Relative P^{32} Activity in Seed Kernels (60 Mesh) from Ethanol-Eluted and -Noneluted Seed Lots

Samples	P^{32} Activity, C.P.M. per Gram[a] 5 Hours	7 Days	14 Days
Eluted	53	33	608
Noneluted	288	40	599

[a] Uncorrected for self-absorption. Standard error of radioactive count between duplicates of samples not over 5%.

Table II. DEF Concentration in Seed Kernels (60 Mesh) from Ethanol-Eluted and -Noneluted Seed Lots

Samples	DEF Concentration, μg. per Gram[a] 5 Hours	7 Days	14 Days
Eluted	0.39	0.21	0.25
Noneluted	2.13	0.27	0.24

[a] Consult text for method of calculation.

Distribution of P^{32} and S^{35} in Seed Kernels. The final phase of this experiment was the determination and comparison of the per cent distribution of the remaining P^{32} and S^{35} in the seed kernels with that of phosphorus and sulfur in the untreated kernels which had been extracted with petroleum ether. Since the general distribution patterns of P^{32} as well as of S^{35} were quite similar for the 7- and 14-day harvests, only the 14-day seed kernels of both P^{32} and S^{35} are represented in Tables III and IV, respectively.

Nearly all of the P^{32} was found in the inorganic phosphorus and phytin—i.e., inositol hexaphosphate—fractions (Table III). This agrees, in general, with the per cent distribution of normal phosphorus, but unlike the latter, most of the P^{32} was in the inorganic rather than the phytin form. Possibly inorganic P^{32} arising from DEF degradation in leaves and carpel regions of unopened bolls had accumulated in the developing kernels during a period of near maturity, and was not completely incorporated into the principal storage form of phytin.

S^{35} distribution in the seed, on the other hand, agrees quite closely with the sulfur found in the seeds of control plants. Table IV shows that the majority of the total sulfur, both the isotopic sulfur from DEF and the nonradioactive form in the untreated controls, is present in the insoluble organic or protein fraction of the seed. This would be expected if normal sulfur metabolism is being followed (*3*). The significant translocation and incorporation of S^{35} into the seed from DEF breakdown products in the leaves (*8*) agrees with the work of Biddulph *et al.* (*2*), who found that sulfur was just as effectively utilized from foliar application

Table III. Distribution of P^{32} in Seed Kernels from P^{32}-Labeled DEF-Treated Plants as Compared to Unlabeled Phosphorus Distribution in Control Plants

	Treated Plants		Control Plants	
Phosphorus Fraction	P^{32}, c.p.m. per gram[a]	Total P^{32} in fractions, %	P, mg. per gram	Total P in fractions, %
Inorganic	1125	61.2	0.34	3.2
Phytin	540	29.4	8.80	82.6
Carbohydrate ester	0	0.0	0.07	0.7
Phosphatide	44	2.4	0.62	5.8
Nucleic acid	130	7.1	0.82	7.7
Total P[b]	1839	100.1	10.65	100.0
Separate determination	1835		11.15	

[a] Corrected for self-absorption. Standard error of radioactive count between duplicates of samples not over 5%.
[b] Sum of individual fractions.

Table IV. Distribution of S^{35} in Seed Kernels from S^{35}-Labeled DEF-Treated Plants as Compared to Unlabeled Sulfur Distribution in Control Plants

	Treated Plants		Control Plants	
Sulfur Fraction	S^{35}, c.p.m. per gram[a]	Total S^{35} in fractions, %	S, mg. per gram	Total S in fractions, %
Soluble organic (nonprotein)	265	8.3	0.146	9.6
Soluble sulfate	5	0.2	0.063	4.2
Insoluble sulfate	266	8.4	0.165	10.9
Insoluble organic (protein)	2649	83.2	1.140	75.3
Total sulfur[b]	3185	100.1	1.514	100.0
Separate determination	3120		...	

[a] Corrected for self-absorption. Standard error of radioactive count between duplicate samples not over 5%.
[b] Sum of individual fractions.

as was phosphorus. Apparently the increase of radioactivity in the cottonseed kernel after treatment with labeled-DEF is due mainly to breakdown products of the DEF and not from the unaltered defoliant itself. The concentration of the latter in the developing kernel remains at a constant level (0.2 to 0.3 p.p.m.).

Entrance of DEF into the kernels from surface contamination is somewhat higher in this case than in actual practice since the open bolls were harvested 5 hours after spraying instead of 7 to 10 days after defoliation, as is customary. Contamination could also be minimized by using a suitable method of kernel processing. More investigations should be carried out since it is not known conclusively at present whether DEF or its breakdown products (*8*) are toxic with respect to cholinesterase in vitro.

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Literature Cited

(1) Aronoff, S., "Techniques of Radiobiochemistry," pp. 35–46, Iowa State College Press, Ames, Iowa, 1955.
(2) Biddulph, O., Cory, R., Biddulph, S., *Plant Physiol.* **31,** 28 (1956).
(3) Ergle, D. R., Eaton, F. M., *Ibid.*, **26,** 639 (1951).
(4) Fukuto, T. R., Metcalf, R. B., March, R. B., Maxon, M. G., *J. Econ. Entomol.* **48,** 347 (1955).
(5) Goyette, L. E., *Proc. 11th Ann. Beltwide Cotton Defol. and Physiol. Conf.*, Birmingham, Ala., p. 9 (1956).
(6) Hall, W. C., Texas Agr. Expt. Sta. Progr. Rept. **1935** (1957).
(7) Hall, W. C., Katterman, F. R. H., Miller, C. S., Herrero, F. A., Abstracts, 134th Meeting, ACS, Chicago, September 1958.
(8) Katterman, F. R. H., Hall, W. C., *Plant Physiol.* **36,** 816 (1961).
(9) Kosolapoff, G. M., "Organophosphorus Compounds," p. 198, Wiley, London, 1950.
(10) Meagher, W. R., "Colorimetric Determination of Guthion Residues in Plant Material," Chemagro Corp. Research Department, Neville Island, Pittsburgh, Pa., 1956.
(11) Murphy, S. D., Dubois, K. P., *A.M.A. Arch. Ind. Health* **20,** 161 (1959).
(12) Pons, W. A., Jr., Stansbury, F., Hoffpauir, C. S., *J. Assoc. Offic. Agr. Chemist* **36,** 492 (1953).
(13) Sprayberry, B. A., Liverman, J. L., Hall, W. C., *Proc. 12th Ann. Beltwide Cotton Defol. and Physiol. Conf.* Birmingham, Ala., p. 22 (1957).
(14) Woofter, D. H., *Proc. 11th Ann. Beltwide Cotton Defol. and Physiol. Conf. Birmingham, Ala.*, p. 27 (1956).

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FUNGICIDE EFFECTS ON FLAVOR

The Effect of Some Fungicides on the Flavor of Canned Strawberries

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OFF-FLAVOR in canned strawberries has been attributed to captan and thylate. Tapio (*9*) mentioned captan induces in strawberries a "bitter flavor" which persists in canned fruit and jam. Marsh *et al.* (*6*) were unable to detect captan in canned fruit. Crang and Clarke (*2*) found 11 to 19 p.p.m. captan and an undescribed off-flavor in canned fruit in one year.

Strawberries sprayed with thiram were found by Marsh *et al.* (*6*) to have a marked off-flavor when canned, while Tapio (*9*) found no off-flavor. Crang and Clarke (*2*) report definite off-flavor in canned fruit in two years out of five, although certain individuals were able to detect differences in all trials.

The objective of the present study was to determine whether off-flavors were detectable in strawberries that were canned subsequent to field treatment with Phaltan, captan, or Thylate. The ability of individuals to detect off-flavor due to added fungicide was also determined.

Materials and Methods

In 1960 and 1961, Sparkle strawberries were sprayed with Phaltan (50W, 50% *N*-trichloromethylthiophthalimide), captan (50W, 50% *N*-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide), or Thylate (65% bisdimethylthiocarbamoyl disulfide). The rate of application