Metabolism of Thiofanox in Cotton Plants

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Acid-delinted cottonseeds were treated with 0.5% (w/w) [^{35}S]- or [*N-Me*-^{14}C]thiofanox (P), 3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime, before planting. This insecticide was readily taken up by the young seedlings and translocated to the cotyledons and developing leaves where it was rapidly metabolized to its sulfoxide (P_1), 3,3-dimethyl-1-(methylsulfinyl)-2-butanone *O*-[(methylamino)carbonyl]oxime. The level of P_1 declined in the cotton leaves with time resulting in the formation of the sulfone (P_2), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone *O*-[(methylamino)carbonyl]oxime, and water-soluble metabolites. Cottonseeds from laboratory and field plants grown from thiofanox-treated seeds contained less than 5 ppb of carbamate residues.

Thiofanox (P), 3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime, is a potent contact and systemic insecticide being developed by Diamond Shamrock Corporation. When applied to cottonseeds or in the seed furrow at planting, P is readily absorbed and translocated to the plant foliage where it controls many foliar feeding pests including a number of species from the following orders: Coleoptera, Homoptera, Dyptera, Thysanoptera, and Acarina. In cotton, thiofanox has demonstrated control for up to 8 weeks on thrips, *Frankliniella* spp.; cotton aphid, *Aphis gossypii* Glover; cotton fleahopper, *Pseudatomescelis beriatus* (Reuter); and spider mites, *Tetranychus* spp., when applied in the seed furrow at the time cotton is planted (Davis and Cowan, 1974) and when applied as a 0.5% by weight seed treatment (Diamond Shamrock Corporation Technical Bulletin, 1973).

Whitten and Bull (1974) studied the metabolism of ^{35}S-labeled P in cotton plants and soil. They report rapid oxidation of P to its sulfoxide (P_1), 3,3-dimethyl-1-(methylsulfinyl)-2-butanone *O*-[(methylamino)carbonyl]oxime, and sulfone (P_2), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone *O*-[(methylamino)carbonyl]oxime. Further degradation of P_1 and P_2 led to the formation of water-soluble products. Their studies with seed applied [^{35}S]P were carried to 21-day seedlings. We followed the metabolism of [^{35}S]P and [*N-Me*-^{14}C]P when applied to cottonseeds through the plant's life to determine the presence of residues in the cotton boll at maturity. We also wanted to determine the total carbamate residues of thiofanox in cottonseeds resulting from thiofanox-treated seeds or from in-furrow application of its granular formulation, Dacamox 10G (trademark of the Diamond Shamrock Corporation).

EXPERIMENTAL SECTION

Chemicals. The initial specific activities of [^{35}S]P and [*N-Me*-^{14}C]P were 8.55 mCi/mmol and 1.51 mCi/mmol, respectively. The [^{35}S]P was converted to [^{35}S]P_1 (oxidization with hydrogen peroxide in acetic acid) and [^{35}S]P_2 (oxidization with aqueous sodium periodate) to check for conversion of these metabolites during extraction. Beside these sulfur- and carbon-labeled chemicals, nonlabeled oximes such as O, 3,3-dimethyl-1-(methylthio)-2-butanone oxime, O_1, 3,3-dimethyl-1-(methylsulfinyl)-2-butanone oxime, and O_2, 3,3-dimethyl-1-(methylsulfonyl)-2-butanone oxime, were synthesized. The structure of P and its metabolites and their toxicity (rat oral LD_{50} in milligrams/kilogram) are given in Table I. The radioactive and nonradioactive compounds used in this study were greater than 99% pure as shown by thin-layer chromatography (TLC).

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Plant Culture. Cottonseeds (*Gossypium hirsutum* L. var. Stoneville 213) weighing 100 mg each were treated with 500 μg of [^{35}S]P or [*N-Me*-^{14}C]P in 10 μl of benzene. The benzene solution was evenly applied to the seed surface using a microliter syringe. This amounted to a treatment of the seeds with 0.5% P by seed weight. Four treated seeds were planted 2.54 cm deep in sterilized greenhouse potting soil contained in 22.9 cm diameter styrofoam pots. The test was replicated 25 times. After 4 weeks, the seedlings were thinned to one per pot.

The plants were grown in an environmental growth chamber (Percival PGW-108) with 14 hr, 30° day and 10 hr, 21° night temperatures under a mixture of fluorescent (24 G.E. Cool White-F96T12-1500 mA) and incandescent (12 G.E. 100 W) lamps yielding 43,200 lx at the top of the plants. The plants grown from [*N-Me*-^{14}C]P-treated seeds were contained in 30.5 cm × 35.6 cm sealed glass jars. Air was passed through the jars at a rate of 0.6 l./hr and bubbled into ethanolamine to measure $^{14}CO_2$. The jars were placed in the environmental chamber. Because of heat dissipation differences, the daytime temperature in the jars was 35°. Plants were harvested after various periods of radioisotope exposure and stages of development. After thorough washing to remove adhering soil, the plants were divided into roots, stems, and leaves and, at maturity, the seeds and boll trash. The tissue was immediately extracted or frozen and held at −20° for subsequent analysis.

Tissue Extraction and Metabolite Purification and Identification. Tissue was homogenized for 3 min in 5 vol of 80% acetone in 10^{-4} *N* HCl per gram of fresh tissue using a VirTis 45 homogenizer. Nonlabeled P, P_1, P_2, O, O_1, and O_2 (100 μg of each) were added to the homogenate to facilitate isolation and later identification of the ^{14}C- and ^{35}S-labeled metabolites. The homogenate was centrifuged at 12,000*g* for 10 min. The supernatant was decanted and the pellet resuspended in extraction medium for 1 hr at 50°. The reextracted pellet material was suction filtered, and the filtrate combined with the centrifugation supernatant. A portion of the combined extract was taken for measurement of radioactivity. The nonextractable radioactivity was determined by wet digestion of the extracted, dried plant tissue (modified after Mahin and Lofberg, 1966). Samples (200 mg) were placed in scintillation vials to which 0.2 ml of 60% perchloric acid and 0.4 ml of 30% hydrogen peroxide were added. The vials were sealed and heated for 1 hr at 80°. The vials were cooled and the digested residues diluted with 15 ml of scintillation fluid [6 g of PPO (2,5-diphenyloxazole) in 1 l. of toluene plus 500 ml of Cellosolve]. Extractable and nonextractable residues were determined for each tissue. The extraction efficiency for all experimental periods was 94.3% for cotyledons and leaves, 87.5% for stems, and 79.6% for roots regardless of the radiotracer used.

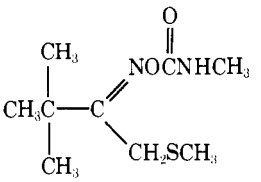
Table I. Thin-Layer Chromatographic Properties of P and Metabolites

Solvent system	R_f for metabolites[c]					
	P_1	O_1	P_2	O_2	P	O
A[a]	0.09	0.24	0.34	0.56	0.77	0.88
B[b]	0.10	0.20	0.40	0.60	0.70	0.80

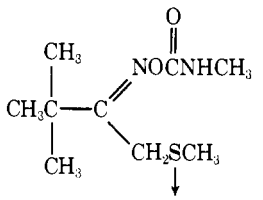
[a] Hexane–chloroform–butyl ether–benzene–ethyl acetate–dioxane, 14:8:5:4:4:4 (v/v). [b] Hexane–acetone, 7:3 (v/v). [c] See text for structures of metabolites and rat oral LD_{50} (mg/kg).

The combined extractable residues were exposed to a gentle air stream at room temperature to remove the acetone. The chlorophyll was removed by centrifugation at 5000*g* for 10 min. The discarded chlorophyll pellet did not contain any radioactivity. The resulting aqueous supernatant was extracted twice with equal volumes of chloroform. The chloroform–10^{-4} *N* HCl partition coefficients for P, P_1, and P_2 are 0.99, 0.93, and 0.98, respectively. These partition coefficients are identical in 10^{-2} *N* HCl or water.

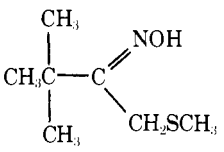
The metabolites in the chloroform extract were resolved by TLC on Brinkman precoated silica gel F-254 (fast running) plates which were developed in one of the following systems: (a) system A, hexane–chloroform–butyl ether–benzene–ethyl acetate–dioxane, 14:8:5:4:4:4 (v/v); (b) system B, hexane–acetone, 7:3 (v/v). Identification of metabolites was based on cochromatography with authentic standards visualized by spraying the plates with 2.5% potassium permanganate in 10% sodium hydroxide. The R_f values of the metabolites (see structures) in the two solvent systems are given in Table I.



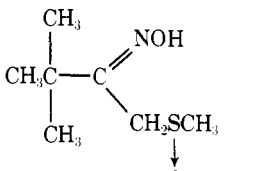
P (8.5)



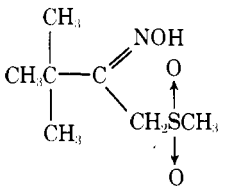
P_1 (3.8)



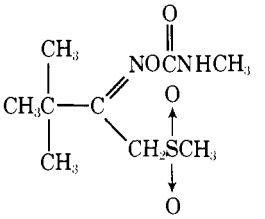
O (813)



O_1 (2000)



O_2 (734)



P_2 (1.9)

All extracts of plant tissues and radioactive areas from chromatograms were counted in Aquasol scintillation cocktail using a Packard Tri-Carb liquid scintillation spectrometer which counted ^{35}S at 84.1% efficiency and ^{14}C at 93.4% efficiency. Quenching was determined in all samples using internal standardization and all reported values are corrected for quenching, counting efficiency, and radioactive decay.

Possible degradation of P, P_1, P_2, O, O_1, and O_2 during extraction and purification was monitored by adding ^{35}S-labeled P, P_1, and P_2 and nonlabeled O, O_1, and O_2 to untreated cotton tissue. In all cases, more than 90% of the added metabolites was recovered intact.

The data reported in the tables and figures are from triplicate plant samples. In many cases, duplicate samples of each tissue extract were analyzed by TLC. Therefore, each data point represents the average of three to six individual observations.

Field Studies. In April and May of 1972 and 1973 different rates of P were applied to cotton by seed treatment (0.5–1.0 lb/100 lb) or in-furrow (0.25 to 2.0 lb/acre) applications at 18 locations in 8 states (Marwyn, Ala.; Chandler, Ariz.; Denwood, Itowah, and Keiser, Ark.; Madera, Meloland, and Wasco, Calif.; Albany, Pinehurst, Tifton, and Vienna, Ga.; Roanoke-Rapids, N.C.; Florence, S.C.; College Station, San Perlita, Thompson, and Waco, Tex.). The cottonseeds harvested at the end of the season were analyzed for total carbamate residues using a Tracor 550 gas chromatograph (Chin et al., 1975).

RESULTS

Cotton seedlings and plants grown from [^{35}S]P-treated seeds were harvested 7, 17, 27, and 42 days after planting. Plants grown from [*N-Me*-^{14}C]P-treated seeds were harvested 17 and 27 days after planting. The harvested plants were divided into cotyledons, leaves, stems, and roots (cotyledons and leaves in the ^{14}C experiments) for extraction.

Most of the radiotracer taken up by cotton plants was translocated to the cotyledons and lower leaves (Table II). The cotyledons abscised 7–8 weeks after planting and the lower leaves abscised 10–12 weeks after planting. Therefore, the majority of the radiotracer taken up by the seedlings was no longer associated with the plants as they matured. Very little of the radiotracer taken up through the roots remained there. Initially, there was some accumulation of radiotracer in the stem, but this dropped to less than 1% of the total plant residue after 27 days.

The major metabolites in the cotton seedlings and developing cotton plants from [*N-Me*-^{14}C]P- and [^{35}S]P-treated seeds were P_1, P_2, and the water-soluble metabolites. The water-soluble metabolites, P_1 and P_2, accounted for over 95% of the extractable residues from the cotyledons, leaves, stems, and roots at all stages of growth. The results from the plants harvested 17 and 27 days after planting are given in Tables III and IV, respectively.

Because P_1, P_2, and the water-soluble metabolites accounted for nearly all of the extracted radiotracer, the metabolism scheme of these three metabolites was followed in plants grown from [^{35}S]P-treated seeds (Figure 1). The pattern of metabolism of [^{35}S]P in cotton cotyledons (Figure 1A) and cotton leaves (Figure 1B) was similar. The major metabolite was P_1 which accounted for about 80% of the total radioactivity in the cotyledons after 7 days and in the first and second true leaves after 17 days. As the P_1 level declined in the cotyledons (Figure 1A) and leaves (Figure 1B), the levels of P_2 and water-soluble metabolites increased. The concentration of P_2 reached a higher level in the cotyledons than in the leaves suggesting slight differences in the rate at which P_1 is metabolized in these tissues. After 42 days, the level of water-soluble metabolites reached 23% in the cotyledons and 30% in the leaves.

Short-term experiments with excised cotton leaves have shown the half-life ($T_{1/2}$) of P to be less than 1 day and that it was converted almost exclusively to P_1. The $T_{1/2}$ of P_1 in the cotyledons could be calculated because after 27 days the total radioactivity had plateaued and P had reached a level of 0.1% or less. The $T_{1/2}$ for P_1 in the cotyledons was 41 days using the 27- to 42-day period for calculation. The actual level of P_1 in the cotyledons after 42 days was 51.1% of the ^{35}S-extractable radiotracer. However,

Table II. Distribution of [^{35}S]P and Metabolites in Various Cotton Tissues

Harvest time, days	Total plant uptake, % applied dose	% of total plant radioactivity					
		Cotyledons	1st leaf pair	2nd leaf pair	3rd leaf pair	Stem	Roots
7	9.0	92.3				5.5	2.2
17	10.0	94.0	2.1			1.0	3.0
27	15.2	87.5	9.2	0.7		0.6	2.0
42	16.7	78.4	10.8	6.0	3.0	0.6	1.2

Table III. Relative Concentrations of [^{35}S]P, [*N-Me*-^{14}C]P, and Metabolites in 17-Day Cotton Plants following Seed Treatment

Plant part	Label used	% of radioactivity for metabolites						
		Water soluble[a]	P_1	O_1	P_2	O_2	P	O
Cotyledons	^{35}S	8.9	79.0	1.0	10.7	0.3	0.1	<0.05
	^{14}C	10.5	68.0	1.0	19.5	0.4	0.3	0.3
Leaves and apex	^{35}S	15.2	76.8	2.2	4.7	0.6	0.5	<0.05
	^{14}C	34.1	53.4	1.9	8.4	0.5	1.2	0.5
Stems	^{35}S	41.5	43.9	5.7	2.0	0.5	5.9	0.5
Roots	^{35}S	29.7	61.1	4.6	2.2	0.6	1.8	<0.05

[a] Based on P.

Table IV. Relative Concentration of [^{35}S]P, [*N-Me*-^{14}C]P, and Metabolites in 27-Day Cotton Plants following Seed Treatment

Plant part	Label used	% of radioactivity for metabolites						
		Water soluble[a]	P_1	O_1	P_2	O_2	P	O
Cotyledons	^{35}S	14.7	61.7	1.2	21.2	0.7	0.1	<0.05
	^{14}C	26.6	30.7	1.2	40.8	0.5	0.1	0.1
1st leaf pair	^{35}S	13.4	73.7	1.1	11.0	0.7	0.1	<0.05
	^{14}C	30.2	53.6	0.8	14.3	0.8	0.1	0.2
2nd leaf pair and apical meristem	^{35}S	12.2	77.6	1.4	7.9	0.6	0.3	<0.05
	^{14}C	50.2	39.6	0.8	8.0	0.9	0	0.3
Stems	^{35}S	61.7	30.8	2.6	3.4	0.6	0.5	0.4
Roots	^{35}S	23.0	66.5	5.0	0.8	1.5	0.1	<0.05

[a] Based on P.

higher growing temperatures can shorten the $T_{1/2}$ of P_1 in cotton leaves dramatically (Tables III and IV).

The ^{14}C experiment was conducted in glass chambers placed in an environmental chamber maintained at the same temperature as the ^{35}S experiment (i.e., 30° day and 21° night). However, the air temperature near the plant leaf surface was 5° warmer in the glass chamber during the day cycle. The data were compared with plants grown from [^{35}S]P-treated seeds (Tables III and IV). The level of P_1 was much lower in the ^{14}C experiments as compared to the ^{35}S experiments with a corresponding increase in P_2 and the water-soluble fraction. Some radioactivity (about 2%) was detected in the O, O_1, and O_2 regions on the TLC's of tissues from the ^{14}C treatments. The oximes from the [*N-Me*-^{14}C]P would not have any radioactivity, so the radioactivity in the oxime regions of the TLC would be metabolites of other identity. They were calculated as [*N-Me*-^{14}C]P equivalents, however, for completeness of comparison.

The data in Tables III and IV show that the metabolism of [*N-Me*-^{14}C]P was increased at the higher growing temperature. The data in Table V show that [*N-Me*-^{14}C]P was absorbed and accumulated at a faster rate as compared with the [^{35}S]P experiments.

The uptake in the [*N-Me*-^{14}C]P experiment was 19% greater at 17 days and 10% greater at 27 days than in the [^{35}S]P experiment. The accumulated residue per plant was also greater at both sampling dates. These results indicate that an increase in temperature markedly increases uptake, translocation, and accumulation of P and metabolites in cotton foliage and increases the rate of metabolism.

The air passing through the chamber containing plants grown from [*N-Me*-^{14}C]P-treated seed was bubbled through ethanolamine to trap $^{14}CO_2$. Only 1.5% of the applied dose was recovered in the ethanolamine trap. However, 58% of the applied dose was recovered in the plant and from the soil and pot. Therefore, over 40% of the applied dose was lost 27 days after the treatment started.

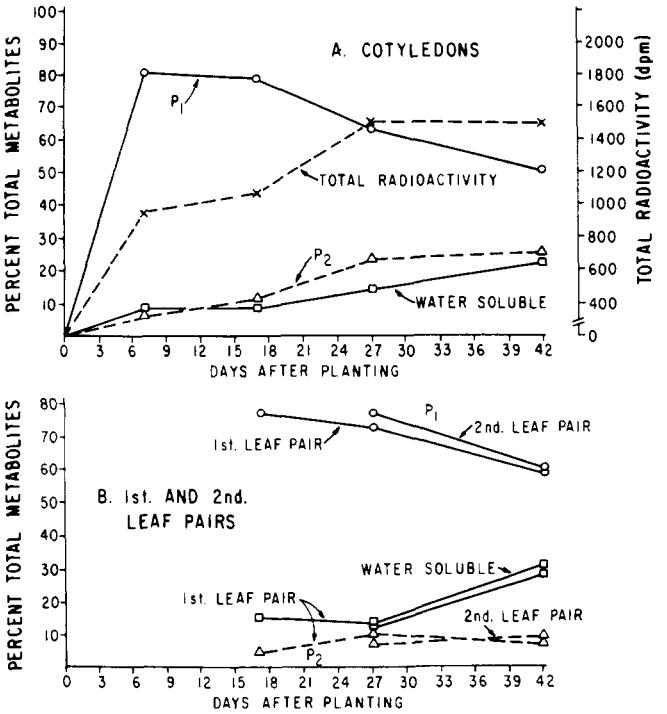


Figure 1. Distribution of [^{35}S]P metabolites in cotton. Cotyledons and leaves on cotton plants grown from seeds treated with [^{35}S]P were extracted to isolate the metabolites. The percentages refer to recovered radioactivity at each sampling date which averaged 94.3% for cotyledons and leaves during the 42-day period.

Table V. Comparison of Uptake and Accumulation of [*N*-*Me*-^{14}C]P and [^{35}S]P and Metabolites in Cotton Foliage

Label used	% uptake[a]		μg per plant residue[b]	
	17 day	27 day	17 day	27 day
^{35}S	9.6	14.7	47.7	74.2
^{14}C	11.4	16.1	62.4	88.3

[a] Percent of applied dose. [b] Sum of all metabolites in foliage.

Table VI. Residues of Metabolites of [^{35}S]P in Cotton Trash and Seeds

Parameter measured	Harvest date: 101 days		145 days	
	Trash	Seed	Trash	Seed
Residue[a] (ppb)	13.0	8.0	82.0	17.2
% water soluble[b]	100	87	88	86
Seed dpm/boll		449		422
Weight/seed (mg)		249		136
Trash dpm/boll	1126		1265	
Trash weight/boll (g)	8.76		2.26	
% extracted[c]	100	77	90	92

[a] Based on P. [b] Percent of extracted radiotracer remaining in aqueous phase after two chloroform extractions. [c] Percent of total seed radioactivity removed by homogenization.

Whitten and Bull (1974) reported a 50% loss of radiotracer from cotton petioles injected with [^{35}S]P 32 days after treatment and were unable to trap the volatile radioactivity.

The bolls were harvested 101 and 145 days after the experiment was initiated. The early harvest was from the lower portion of the plants where flowering and boll set occurred first. After 145 days, the plants were mature and bolls were opened in contrast to their closed state at 101 days. There was an average of 15 bolls per plant and 30 seeds per boll. The total residue in the seeds and boll trash as measured by radiotracer did not increase between the two harvest dates (Table VI). However, residues expressed on a parts per billion basis increased due to the loss in fresh weight of the seeds and boll trash as they matured. The seeds lost 45.4% fresh weight between the two harvest dates, while the boll trash lost 74.2% fresh weight while there was little or no change in radiotracer residues on a per organ basis. Almost 90% of the residues were found in the water-soluble fraction. The level of chloroform-soluble metabolites in the seeds was so low (between 1 and 2 ppb) that their exact identity could not be determined.

There were no detectable carbamate residues (at the 5-ppb level) in the seed or gin trash from either seed or in-furrow applications of thiofanox at any of the 18 locations.

DISCUSSION

The results presented in this paper demonstrate that the major metabolic pathway for P was its oxidation to P_1 and subsequent oxidation of P_1 to P_2. The conversion of P to P_1 was much more rapid ($T_{1/2}$ less than 1 day) than the conversion of P_1 to P. Degradation of P_1 and P_2 appeared to occur mainly by conversion to water-soluble metabolites. The level of radioactivity in the water-soluble metabolite pool was much higher in the [*N*-*Me*-^{14}C]P experiments where higher temperatures enhanced the rate of P_1 metabolism. The oximes resulting from [*N*-*Me*-^{14}C]P, -P_1, and -P_2 would be nonradioactive. However, the methyl group was not released as $^{14}CO_2$ indicating possible incorporation of the methyl group into the C_1 pool. The oximes of P_1 and probably P_2 and P may have been intermediates in the formation of the water-soluble metabolites because their concentrations were never higher than 3% of the total residue. Therefore, it is unlikely that the water-soluble fraction contained conjugates of the intact carbamates P, P_1, or P_2, although our data do not conclusively prove this point.

Our results are in good agreement with those of Whitten and Bull (1974) who used petiole injection and seed treatments with [^{35}S]P and [^{35}S]P_2 to study metabolism of these compounds in cotton plants. Both studies are in general agreement with those for temik, 2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime, which has been shown to be metabolized to its sulfinyl and sulfonyl derivatives and further degraded to water-soluble metabolites in cotton plants (Bartley et al., 1970; Bull, 1968; Coppedge et al., 1967; Metcalf et al., 1966) and in potatoes (Andrawes et al., 1971).

In addition, our studies showed that temperature had a marked influence on metabolism of P in cotton tissue. An increase in temperature of 5° reduced the $T_{1/2}$ of P_1 from 42 days to 23 days and increased the uptake and accumulation of radiotracer in cotton foliage. Coleby et al. (1972) reported that an increase in temperature increased the uptake, translocation, and metabolism of a systemic phosphonate insecticide in cotton plants.

The level of residue in the mature seed and boll trash was low. This was not surprising since most of the uptake of radiotracer into the plant from seed treatment was completed 27 days after planting. Also, almost 90% of the plant residue was contained in the cotyledons and first leaf pair which abscised 7 to 12 weeks after planting. The major portion (86%) of the seed residue was in the water-soluble fraction. Whitten and Bull (1974) resolved six metabolites from the water-soluble fraction from cotton leaves and found

that aliquots containing as much as 3 μg equiv of P exhibited no detectable cholinesterase inhibiting activity. This indicated that the water-soluble fractions contained little or no intact carbamates. Thus, intact carbamates could not be expected to exceed 2.4 ppb in the seed. This proposal was documented by the field studies which showed no detectable carbamate residues at the 5-ppb sensitivity level from cottonseeds grown from seed-treated and in-furrow-treated cotton. These low carbamate levels in cotton (<5 ppb) have been confirmed using two additional gas–liquid chromatography column detection conditions (unpublished results).

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Fate of Microbicidal 3-Isothiazolone Compounds in the Environment: Modes and Rates of Dissipation

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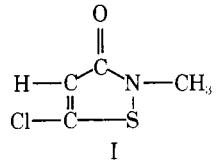
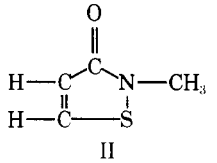
Modes and rates of dissipation of two microbicidal 3-isothiazolone compounds—5-chloro-2-methyl-4-isothiazolin-3-one calcium chloride and 2-methyl-4-isothiazolin-3-one calcium chloride—were determined over a range of conditions likely to occur in the environment. Degradation of both compounds at levels near 1 ppm was observed to occur readily by hydrolytic, photochemical, and biological action in the aquatic and terrestrial environments. Hydrolysis increased with increasing pH and increasing temperature. Adsorption by river silt or soil was not rapid; accumulation and subsequent metabolism by certain aquatic ferns were rapid. Metabolism by rats was extensive. Although moderately toxic to fish, at sub-lethal levels fish did not concentrate the compounds within themselves to any appreciable extent upon continuous exposure and rapidly excreted the residues upon withdrawal from the microbiocidal source. The decomposition of both 3-isothiazolones by several chemical and biological mechanisms ensures that the compounds will not persist in the environment.

Recently, certain 3-isothiazolone compounds have been found to exhibit a high degree of biological activity toward a number of microorganisms. Because of this activity, such compounds are effective as slimicides in industrial waters of cooling towers and paper mills and as preservatives for cosmetics, cutting oils, and jet and heating fuels (Lewis et al., 1973). Two such compounds are 5-chloro-2-methyl-4-isothiazolin-3-one (I) and, to a lesser extent, its unchlorinated counterpart (II). While the free bases I and II are inherently unstable, their shelf lives are markedly extended by the formation of adducts with $CaCl_2$, presumably occurring through the oxygen of the carbonyl group, to give III and IV (Lindquist, 1963).


I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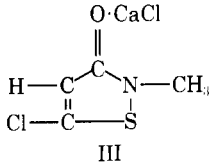
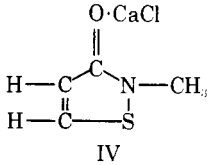

III


IV

Although both III and IV show biological activity against microorganisms, III is significantly more active than IV. Compound III alone or as a 3:1 mixture with IV, a normal by-product of the synthesis of III, shows promise as a potent, broad spectrum industrial microbicide. Because of high biological activity, these compounds are effective for most uses at low concentrations (several parts per million). Even so, safety considerations dictate that the fate of these compounds be defined, and their impact on the environment be evaluated. We have evaluated the environmental fate of both III and IV in a series of studies. These include measuring accumulation and elimination in rats and fish, defining the modes and rates of dissipation (degradation, translocation, and adsorption) in the environment, and isolating and identifying the transformation products. This paper concerns itself with the rat and fish studies and the modes and rates of dissipation of III and IV; a second paper details the isolation and identification of transformation products of III and IV (Krzeminski et al., 1975).

EXPERIMENTAL SECTION

All work reported below entailed the use of either compounds III or IV or a 3:1 mixture of the two. Each compound was available with and without a carbon-14 label. Synthesis of the free base I, whether labeled or unlabeled, was according to the method of Miller et al. (1971) for the chlorine-induced cyclization of 3,3′-dithiodipropionamides. For the carbon-14 compounds the appropriate 3,3′-dithiodipropionamide was formed from the reaction of ammo-

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