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Fate of Potassium 3,4-Dichloro-5-isothiazolecarboxylate in Cotton Plants and White Rats

Ted N. Shaver,* Don L. Bull, and G. Wayne Ivie

^{14}C-labeled potassium 3,4-dichloro-5-isothiazolecarboxylate (PDIC) was applied to individual leaves (100 μg/leaf) of field-grown cotton plants for studies of absorption, photodecomposition, and metabolism and to whole plants in a small plot (1121 g of AI/ha) for studies of radioactive residues in different parts of the cotton plant. Also, ^{14}C-labeled PDIC was administered orally to white rats to determine metabolism, accumulation in tissues, and excretion. There was some photodecomposition (<1% of dose) of PDIC on leaf surfaces. The chemical was rapidly absorbed (55% after 24 h) from leaf surfaces and then readily translocated throughout the plant. Appreciable residues of radiocarbon (>200 ppm) accumulated in cottonseed; most of this was the parent compound. PDIC was rapidly excreted (ca. 95% in 24 h) in the urine of white rats; only minimum concentrations (0.01–0.17 ppm) remained in any tissues after 24 h.

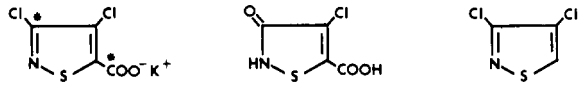
There is considerable interest at present in the use of plant growth regulating chemicals either to terminate the fruiting cycle of cotton at a certain time during the latter part of the growing season (Kittock et al., 1973; Kittock et al., 1978) or to precondition the plant so that eventual application of conventional defoliants results in a more efficient removal of foliage. Potential advantages of such procedures include (1) a reduction of latter season infestations of insect pests such as the pink bollworm (*Pectinophora gossypiella* Saunders) and the boll weevil (*Anthonomus grandis* Boheman) by eliminating squares and small bolls essential to the development of these pests, (2) improvements in overall lint quality through the removal of some bolls that might have incompletely developed lint at harvest, and (3) potential improvement in dust problems at cotton gins through a reduction of foliage contaminants of machine-harvested seed cotton.

One of the more promising of these plant growth regulators is potassium 3,4-dichloro-5-isothiazolecarboxylate (herein referred to as PDIC). This experimental chemical, under evaluation by Pennwalt Corp., is a water-soluble (48.5 g/100 mL), white crystalline powder that is essentially nontoxic to mammals (acute oral LD_{50} to rats is ca. 1.2 g/kg). The present report describes the fate of PDIC in cotton plants and in white rats.

Cotton Insects Research Laboratory, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, College Station, Texas 77840 (T.N.S., D.L.B.), and the Veterinary Toxicology and Entomology Research Laboratory, Agricultural Research Science and Education Administration, U.S. Department of Agriculture, College Station, Texas 77840 (G.W.I.).

EXPERIMENTAL SECTION

Chemicals. The Pennwalt Corp., Tacoma, WA, provided pure samples of PDIC radiolabeled with ^{14}C at the 3- and carboxyl-carbon positions of the molecule (sp act., 19.32 mCi/mmol). Also supplied were nonradioactive samples of technical PDIC and two potential metabolites: I (4-chloro-3-oxo-5-isothiazolidinecarboxylic acid) and II (3-4-dichloroisothiazole).



PDIC I II

Fate on Field-Grown Cotton. *Foliar Application to Individual Leaves.* The ^{14}C-labeled PDIC was diluted with 6.25 parts of the nonradiolabeled material and then dissolved in water to form a solution having a concentration of 1000 ppm active ingredient (AI). This solution was applied in situ to fully expanded leaves of field-grown 'SP-37' cotton by spreading a 100-μL aliquot (100 μg of AI) uniformly over the upper surface of each leaf with a micropipet.

At 0, 1, 3, 7, and 14 days posttreatment, three treated leaves were collected at random from different plants and processed immediately. Unabsorbed (external) radioactive material was recovered by rinsing the leaves thoroughly with methanol, and absorbed (internal) radioactive material was extracted by homogenizing the rinsed leaves with

a Tissuemizer in methanol. Solids separated by centrifugation were reextracted twice with methanol and then dried at 40 °C.

Combined solvent extracts of each sample were radioassayed by conventional liquid scintillation counting (LSC); then extracts were evaporated under vacuum to a convenient volume and analyzed by thin-layer chromatography (TLC) by using precoated glass plates (silica gel 60, F-254, Brinkmann Instruments, Houston, TX) with solvent mixtures: (A) benzene, methanol, and diethylamine (10:4:2, v/v) and (B) benzene, methanol, and acetic acid (79:14:7, v/v). Nonradioactive analytical standards were visualized under ultraviolet light; radioactive spots were located by autoradiography with x-ray film and then recovered and quantified by LSC.

The radiocarbon content of dried, extracted leaf tissues (ca. 100 mg/sample) was determined with combustion by the methods of Ivie (1978).

Foliar Treatment of Whole Plants. A plot (1.7 m^2) of cotton was enclosed with a 1.8 × 1.8 × 5.5 m long screen cage that had the top and upper one-third of the sides covered with clear plastic to protect the treated plants from rain. The plants were sprayed manually with ^{14}C-labeled PDIC (sp act., 1.93 mCi/mmol) at a rate equivalent to 1.12 kg of AI in 185 L of water/ha; there were no open bolls on the plants when treated. The treated plants were harvested 43 days posttreatment and were separated into stems, roots, leaves, and fruits; fruit was subdivided into calyx, bracts, lint, seed hulls, and seed meats. Seed and lint were not dried, but all other plant parts were dried 48 h at 40–50 °C. The leaves and woody parts of the plants were weighed and ground separately in a Wiley mill to pass a 20-mesh screen. All samples were analyzed in triplicate for radiocarbon content by oxygen combustion as described.

Some of the seed meats containing ^{14}C residues were extracted by homogenization with a mixture of methanol and hydrochloric acid (99:1, v/v), and the extract was analyzed by TLC. Quantities sufficient for GLC–mass spectral (GC-MS) identification of the major radioactive product detected in seed were obtained by preparative TLC, with further purification by liquid chromatography (LC) on a reversed-phase column (4 mm 1.0 × 30 cm; packed with μ-Bondapac/C-18) eluted with 50% methanol in 1% acetic acid at 0.8 mL/min. The GC-MS studies were done with a Varian Mat Ch-7 spectrometer coupled with a Varian 2700 gas chromatograph and a 620-L Varian computer. The isolated compound was converted to its methyl ester with diazomethane and then injected on a 0.6 m × 2 mm i.d. glass column packed with 3% SE-30 on 80–100 mesh Chromosorb W. Operating parameters for GC-MS were as follows: injector, 190 °C; column, 150 °C; detector oven, separator, and inlet, 210 °C; ion source, 225 °C; helium flow, 50 mL/min; ionizing voltage, 70 eV.

Petiole Injection of Individual Leaves. An aqueous solution of ^{14}C-labeled PDIC (sp act., 3.22 mCi/mmol) was applied by petiole injection (10 μL/leaf; 80 μg of AI) to fully expanded main-stem leaves of field-grown cotton plants. Whole plants were harvested at 3, 7, and 14 days posttreatment, separated into different parts, and analyzed for radiocarbon content as described.

Fate in Animals. Female white rats (Sprague-Dawley strain, 150–170 g) were treated orally with 0.5 mL of an aqueous solution containing 1 mg of ^{14}C-labeled PDIC (sp act., 3.86 mCi/mmol). The dose was administered with a syringe and stomach tube after light anesthesia of the rats with ether. The treated rats were held individually in metabolism cages equipped either for the entrapment of expired $^{14}CO_2$ (Bull and Ivie, 1976) or for the separate collection of urine and feces. The rats were provided only water after treatment; two rats were sacrificed at each of the specified times by vertebral fracture and processed immediately. Triplicate samples of blood and the specified tissues (muscle, brain, liver, kidney, and fat) were taken from each rat, weighed, dried for 24 h at 40–50 °C, and then analyzed for radiocarbon content by oxygen combustion. Urine was collected at the specified times and analyzed directly via LSC for radiocarbon content and by TLC to detect any metabolic conversion of the PDIC. Feces were dried, pulverized, and combusted.

Table I. Fate of Foliar Applied ^{14}C-Labeled Potassium 3,4-Dichloro-5-isothiazolecarboxylate on Individual Cotton Leaves in the Field (100 μg/leaf)

days posttreatment	% of dose in indicated fraction			
	external rinse	internal extract	unextractable	loss[a]
0	97.5	2.2	0.3	0.0
1	44.9	23.2	5.5	26.4
3	22.6	18.8	10.2	48.4
7	11.2	9.5	15.4	63.9
14	3.5	4.8	10.8	80.9

[a] Reflects volatilization, weathering, and translocation to other parts of the plant.

Table II. Distribution of Radioactivity in Cotton Plants Sprayed with ^{14}C-Labeled Potassium 3,4-Dichloro-5-isothiazolecarboxylate and Harvested 43 Days Posttreatment

plant sample	ppm (dry wt) ^{14}C-labeled PDIC equiv (±SE)	dry wt, g
stems	16.9 ± 0.9	113.2
roots	3.3 ± 0.2	15.5
leaves (on ground)	83.0 ± 4.7	115.5
leaves (on plant)	145.7 ± 5.4	70.8
bolls		
calyx	45.0 ± 2.6	43.8
bracts	429.7 ± 19.1	3.0
lint	4.9 ± 1.8	44.7
seed	233.7 ± 23.8	86.3
hulls	24.5 ± 4.8	

RESULTS

Persistence on Individual Leaves of Field-Grown Cotton. Following a single application, residues of ^{14}C-labeled PDIC disappeared rapidly from the leaves. Only 44.9 and 3.5% of the treatment dose remained on leaf surfaces after 1 and 14 days, respectively (Table I). Absorbed radioactive materials reached a peak at 1 day posttreatment and then declined at subsequent sampling intervals due probably to volatilization and translocation from the treated leaves. On two occasions in preliminary tests, rainfall of less than 1 cm washed essentially all of the unabsorbed ^{14}C-labeled PDIC from treated leaves.

Analyses of washes and extracts with TLC revealed the presence of only the parent compound after 1 day; however, traces of a second radioactive compound were detected on the third and subsequent sampling dates. This compound did not correspond to either of the two potential metabolites (I, II) that were available and amounted to <5% of the recovered radioactivity.

Foliar Treatment of Whole Plants. It was evident from combustion analyses that there was considerable accumulation (>200 ppm) of radiocarbon in seed collected from cotton plants 43 days after the single spray application of

Table III. Recovery of Radioactivity from Cotton Plants Treated with ^{14}C-Labeled Potassium 3,4-Dichloro-5-isothiazolecarboxylate by Leaf Petiole Injection or Topical Application to Leaves

plant part	ppm (dry wt) ^{14}C-labeled PDIC equivalents at indicated day posttreatments			
	3	7	14	14 (topical)
root	1.5 ± 0.1	0.8 ± 0.1	0.1 ± 0.0	0.4 ± 0.1
stems	1.4 ± 0.2	0.8 ± 0.1	0.5 ± 0.1	1.1 ± 0.3
terminal	6.9 ± 0.6	17.3 ± 0.6	2.7 ± 0.1	35.8 ± 6.4
leaves	4.0 ± 0.8	6.6 ± 1.5	1.6 ± 0.4	5.3 ± 1.8
treated leaves	25.2 ± 4.9	16.6 ± 1.5	19.7 ± 5.3	
bracts	16.0 ± 1.6	20.1 ± 3.6	13.7 ± 4.2	76.7 ± 15.6
whole fruit (less bracts)	22.6 ± 3.7	7.7 ± 1.4	6.9 ± 1.7	8.3 ± 1.7

Table IV. Radiocarbon Residues in Tissues of Rats following Oral Administration of ^{14}C-Labeled Potassium 3,4-Dichloro-5-isothiazolecarboxylate (1 mg/rat)[a]

hours after treatment	ppm of ^{14}C-labeled PDIC equivalents in[b]					
	muscle	brain	liver	kidney	blood	fat
1/4	3.2 ± 0.8	1.6 ± 0.3	7.9 ± 0.8	198.0 ± 9.8	16.08 ± 5.23	0.9 ± 0.3
1/2	0.6 ± 0.2	0.5 ± 0.1	1.9 ± 0.2	32.6 ± 2.7	5.85 ± 1.05	0.3 ± 0.1
1	0.4 < 0.1	0.2 < 0.1	0.9 ± 0.1	27.8 ± 3.8	2.57 ± 0.47	0.3 ± 0.1
2	0.3 ± 0.1	0.2 < 0.1	0.7 ± 0.1	16.3 ± 1.7	2.41 ± 0.31	0.1 ± 0.0
4	0.1 < 0.1	0.1 < 0.1	0.4 ± 0.1	7.1 ± 0.8	0.89 ± 0.16	tr[c]
8	tr	tr	0.1 ± 0.0	1.8 ± 0.1	0.19 ± 0.04	tr
24	tr	tr	tr	0.2 < 0.1	tr	tr

[a] At 24 h posttreatment, ca. 95% of the administered dose was recovered in the urine and ca. 0.9% in the feces; <0.01% was expired as $^{14}CO_2$. [b] Data represent means (±SE) of triplicate samples of each tissue from each of two rats per time. [c] Trace represents less than 0.1 ppm in the sample.

^{14}C-labeled PDIC (Table II); small amounts (ca. 5 ppm) were also found in the lint. The highest concentration (>400 ppm) of radiocarbon was found in bracts; lower levels were detected in other parts of the plant, particularly in leaves remaining on the plant at harvest (ca. 145 ppm).

Extraction and subsequent TLC analyses of the radiocarbon material in seed revealed the presence of two ^{14}C-labeled compounds; one of these cochromotographed on TLC with PDIC and accounted for 95.2% of the radioactivity in the extract. GC-MS analysis of the methyl ester of this compound showed that it was the same as the methyl ester from authentic PDIC, thus indicating that the major radioactive component in the seed was the unaltered parent compound. The mass spectral data were as follows: m/e 211 (molecular ion, Cl = 35), 180 (base peak, $M^+ - OCH_3$), 176 (base peak, $M^+ - Cl$), 152 ($M^+ - CO_2CH_3$), 117 ($M^+ - CO_2CH_3Cl$).

The unknown radioactive compound comprising 4.8% of the total radioactivity recovered from seed was apparently identical with the radioactive compound recovered in trace amounts from washes and extracts of individual leaves. The extraction technique recovered 86.2% of the radiocarbon in the seed. No attempt was made to determine the nature of the remaining 13.8%.

Petiole Injection of Individual Leaves. There was a rapid translocation of ^{14}C-labeled PDIC in cotton plants after petiole injection (or topical treatments) of individual leaves (Table III). At 3 days postinjection 22.6 ppm of ^{14}C-labeled PDIC equivalents were detected in debracted fruit (flowerbuds, flowers, and bolls) and 16.0 ppm in bracts. Small concentrations were also found in roots and stems at all sampling times. Although not shown in Table III, the percentages of applied dose recovered in the fruit were 33.4, 15.3, and 33.5% for plants harvested 3, 7, and 14 days after treatment by petiole injection; 12.8% was found in the fruit at 14 days after topical treatment.

Extraction and TLC analysis of the radioactive material in fruit from plants treated by petiole injection revealed the presence of PDIC (95.8% of total radioactive material in the extract) and 4.2% of the same compound detected in trace amounts in leaf washes and extracts of individual leaves and in methanol extracts of seed from plants treated by foliar application. Approximately 90% of the radioactivity in the seed was recovered by the methanol extraction technique.

Fate in White Rats. After oral administration, ^{14}C-labeled PDIC was absorbed from the gut into the circulatory system at a very rapid rate (Table IV). The greatest concentrations of radioactivity in blood and all tissues were measured at 15 min posttreatment. Levels of radiocarbon were consistently higher in the blood and kidneys than in other organs at all sample times. Radioactivity was rapidly eliminated from the body via the urine (ca. 75% in 4 h, 95% in 24 h). Minimal concentrations (0.01–0.17 ppm) remained in any tissues after 24 h. Only traces (<0.01 μg equivalents of ^{14}C-labeled PDIC) of radiocarbon were found in the trapping solutions used for tests of $^{14}CO_2$ expiration by treated rats, thus indicating essentially no metabolism of PDIC to CO_2 by the rats. Less than 1% of the dose was excreted in the feces through 24 h. Direct TLC analysis of urine samples at each collection time indicated the presence of a single radioactive spot that was identical in TLC behavior to authentic PDIC. Subsequent GC-MS analysis as described above confirmed that the product from rat urine was in fact unmetabolized PDIC.

DISCUSSION

The experiments reported herein have demonstrated that PDIC, whether absorbed into the plant system following foliar application or injected into the petioles of leaves, was readily translocated throughout the plant. The accumulation of rather large concentrations of the chemical in the fruit, especially the seeds, indicated that it entered the phloem tissue and was transported with the assimilate stream. This pattern of source to sink movement is characteristic of a symplastic systemic molecule (Crisp, 1972).

The assumed photodecomposition of PDIC on leaf surfaces led to the formation of trace amounts of a single unidentified product. This compound was apparently identical with the one detected in extracts of individual leaves and seeds of whole plants treated with PDIC. Since

the relative proportions of PDIC and the metabolite were approximately the same in seed extracts and external washes of leaves, it is possible that this product was formed on the leaf by photodecomposition and then absorbed and translocated to the seed. However, the metabolite was also detected in seed of plants that were treated by petiole injection and had no surface exposure of the PDIC to sunlight, suggesting that it may be a metabolic product as well.

^{14}C-labeled PDIC was eliminated very rapidly from rats after oral administration and no significant concentrations of radiocarbon were retained by any of the tissues after 24 h. The low mammalian toxicity of PDIC and its rapid elimination by treated animals, coupled with the fact that only minor amounts of a single photochemical and/or metabolic degradation product are formed in or on the cotton plant, may tend to minimize the impact of the relatively large accumulations of PDIC in the seed. If the chemical is eventually used only as a conditioner for defoliation at the somewhat lower recommended rates of application and at a later stage of plant maturity, residues in seed would likely occur at reduced levels.

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Uptake, Metabolism, and Elimination of the Lampricide 3-Trifluoromethyl-4-nitrophenol by Largemouth Bass (*Micropterus salmoides*)

Donald P. Schultz,* Paul D. Harman, and Charles W. Luhning

Largemouth bass (*Micropterus salmoides*) exposed to a 1.0-μg/mL solution of the lampricide 3-trifluoromethyl-4-nitro[^{14}C]phenol (TFM) for up to 24 h accumulated radioactive residues in all tissues analyzed at each of five successive sampling periods. Maximum concentrations occurred after 8 h in brain and muscle and after 12 h in blood, liver, kidney, and head plus viscera. Concentrations of radioactivity in the bile increased throughout the experiment. In a second group of fish exposed to 1.0 μg/mL of [^{14}C]TFM for 12 h and then transferred to lampricide-free flowing water, the concentration of radioactive materials in tissues generally decreased with time throughout a 72-h elimination period. No TFM was detected in muscle tissue 12 h after the fish were transferred to lampricide-free water. The presence of conjugated TFM in the bile was confirmed. Hexane/ether extracts contained [^{14}C]TFM and other unidentified ^{14}C materials from muscle and head plus viscera, whereas methanol extracts taken after the hexane/ether extraction contained only a negligible amount of [^{14}C]TFM but large quantities of unidentified, polar ^{14}C compounds.

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is currently applied to tributaries of the Great Lakes to control populations of the sea lamprey (*Petromyzon marinus*). Registration for the use of TFM as a lampricide in the United States requires the collection of data concerning its fate in fish and water. Sills and Allen (1975) measured residues in muscle tissues of eight species of fish exposed to TFM. Hunn and Allen (1975) investigated the effects of exposure to TFM on the renal excretion of coho salmon (*Oncorhynchus kisutch*), and Allen and Hunn (1977) assessed the effect of an intraperitoneal injection of TFM on the renal function of channel catfish (*Ictalurus punctatus*). Lech (1973) and Lech and Costrini (1972) investigated the metabolism of TFM in rainbow trout (*Salmo gairdneri*). In this study we attempted to determine the uptake, distribution, and elimination of [^{14}C]TFM by various tissues in largemouth bass (*Micropterus salmoides*) and, using [^{14}C]TFM, to corroborate the method of Sills and Allen (1975) for TFM residues in fish muscle.

U.S. Department of the Interior, Fish and Wildlife Service, Southeastern Fish Control Laboratory, Warm Springs, Georgia 31830.

MATERIALS AND METHODS

Stock solutions of [^{14}C]TFM (uniformly ring labeled, sp act. 3.66 mCi/mM, Mallinckrodt Chemical Works, St. Louis, MO) and technical grade TFM (82.4%, Hoechst Ag, Frankfurt, Germany) in methanol were used to prepare the 1.0-μg/mL treatment solutions.

Largemouth bass were exposed to a mixture of 1:74 (w/w) of [^{14}C]TFM and technical grade TFM (82.4%) in polyethylene tanks containing 75 L of water (pH 6.8, temperature 12.0 ± 1.0 °C). Constant water bath temperature was maintained with a chilling unit, and oxygen content of the treatment solution was sustained by aeration.

Samples of three fish each were removed from the treatment solution at 2, 4, 8, 12, and 24 h in the uptake experiment. For the elimination experiment, fish were exposed in the treatment solution for 12 h and then placed in lampricide-free flowing water (pH 6.8, temperature 14.0 ± 1.0 °C). Samples of three fish each were taken immediately after treatment and at 4, 8, 12, 24, 48 and 72 h posttreatment. Fish in the elimination experiment were fed ad libitum after they had been placed in the lampricide-free water. Fish used in the uptake experiments averaged 22.6 cm in length and 171 g in weight and those in the elimination experiment, 21.5 cm and 146 g.