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Soil-Bound 3,4-Dichloroaniline: Source of Contamination in Rice Grain

Cecil C. Still, Tsung-Shih Hsu, and Richard Bartha
Department of Biochemistry and Microbiology, Cook College, Rutgers—The State University of New Jersey, New Brunswick, New Jersey 08903

Halogenated anilines (3,4-dichloroaniline or 3-chloroaniline) have been detected in all tested market rice samples (STILL and MANSAGER 1969). The parent compounds of these residues were assumed to be the herbicides employed on the respective fields.

The herbicide propanil (3,4-dichloropropionanilide) is a selective herbicide for weed control in rice fields (SMITH 1961). In soil and plants, especially rice plants, propanil is rapidly hydrolyzed to propionic acid and 3,4-dichloroaniline (DCA) (BARTHA and PRAMER 1970, STILL and KUZIRIAN 1967). The former is completely degraded while most of the DCA persists in form of soil-bound residues (BARTHA 1971, HSU and BARTHA 1974a). The pesticide is usually applied to rice fields when plants are very young; therefore, the aniline appearing in rice grains must have originated from soil and/or plant storage sites. We thought it important to determine the source of this contaminant of market rice and wish to report here the results of some studies on the mobility of persistent anilines using radiolabeled DCA applied to soil and to leaves of developing rice plants.

EXPERIMENTAL

Soil. The soil employed in these experiments was obtained through the courtesy of R. J. Smith, U.S.D.A., and originated from a rice field in Arkansas. To improve its texture, the soil was mixed with sand at a soil/sand ratio of 1:3 and filled to 2.5 cm from the top of nine 23 cm x 23 cm porcelain pots (side draining). The pots were watered and additional soil added so that all nine pots contained the same volume.

Rice Plants. All plants were maintained in the greenhouse for a 28-week period (December 16 to July 9). Untreated rice seed (*Oryza sativa* var. Starbonnet) was obtained from the Jacob Hartz Seed Co., Stutgart, Arkansas, and was germinated in vermiculite. Watered with a routine greenhouse nutrient solution, seedlings were allowed to develop to the 3-4 leaf stage. Plants at the 4-leaf stage were then selected and transplanted to the soil/

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sand mixture (6 plants/pot) and while watered with the same nutrient solution, were allowed to develop further to early tillering.

DCA Treatment. At the end of the pretreatment growth period (16 weeks) the pots were removed from the greenhouse and placed in a shaded area outside. For soil treatment, uniformly 14-C labeled DCA, 3.66 µCi/mg, was dissolved in acetone to give a final concentration of 16.6 mg/ml. Using syringe and needle, 1 ml was applied uniformly below the soil surface to each of these pots. For foliar treatment, 33.3 mg of the same DCA sample was dissolved in acetone to give a final concentration of 138.75 mg/ml. Each plant was then treated with 10 µl of the solution applied to the surface of the two oldest leaves only. These DCA quantities were calculated to approximate those that would result from propanil treatment at recommended field application levels. Controls were treated with the corresponding volume of acetone. The drainholes were stoppered; the pots were returned to the greenhouse. Atmospheric contact between treated and control plants was reduced by a vertical, clear polyethylene screen. After treatment, the plants were watered with distilled water and diluted (1:3) nutrient solution on alternate days.

Detection and Quantification of Radioactivity. For autoradiography, the plants were removed from the pots, dipped in water to remove soil from the roots, and blotted dry. They were then arranged for autoradiography, photographed, frozen, and lyophilized. Images were obtained on Kodak no-screen X-ray film after 21 days of exposure. Following autoradiography, radioactivities were quantitated by first removing and discarding roots as well as the originally DCA-treated leaves. The remaining leaves were cut into small pieces, 200 mg samples were subjected to "wet combustion" (ALLISON et al. 1965), and the evolved CO_2 was trapped in 250 ml of 0.1 N KOH. One ml of the trapping solution was mixed with 10 ml of "Aquasol" (New England Nuclear, Boston, Mass.) and 14-CO_2 was counted in a liquid scintillation counter (Beckman Model LS-230). Rice grains were harvested when fully ripe, combusted, and the evolved 14-CO_2 was quantitated as described for leaves.

RESULTS AND DISCUSSION

Plants were treated when 12 weeks old (March 11) and one plant was removed from each pot five days later on March 16. Autoradiography of these plants showed a distribution of radioactivity throughout the plant body with highest activity in the treated leaves. The control plants, while showing much less activity, showed uniform distribution of radioactivity. The appearance of some radioactivity in the controls was anticipated because of atmospheric contact with volatilized DCA and 14-CO_2.

Leaf treatment and soil treatment resulted in about the same amount of 14-C activity in the plants (TABLE I). Radioactivities of the treated plants were about 10 times those of the controls.

With assurance now that the DCA has been transported throughout the plant body, we wished to determine its mobility at the time of grain development. Toward this end, plants were then maintained for an additional eight weeks, at which time flower spikes were visible. Weeds and senescent leaves were removed and discarded so as not to become sources of soil radioactivity. Five

TABLE I

Radioactivity in leaves 5 days after soil and leaf treatments with 14-C DCA

Treatment	dpm/g	µg DCA/g[a]
None	5.62×10^4	6.9
Leaf	51.00×10^4	62.8
Soil	55.60×10^4	68.4

[a]Calculated from specific activity of applied DCA

weeks after the appearance of flower spikes (July 9), about 50% of the grains were fully ripe and golden brown. The total elapsed time between DCA treatment and harvest was 120 days. Ripe grains were harvested from treated pots and controls, pulverized separately and subjected to wet combustion. The resultant data are shown in TABLE II. It has been estimated previously (STILL and MANSAGER 1969) that market rice might contain as much as 1 µg DCA/g. Our data are in remarkably close agreement with their figures.

No significant radioactivity was detected in grains of the leaf-treated rice plants, though according to TABLE I, the shoots of these contained more radioactivity than the shoots of the soil-treated plants. Therefore, the radioactivity found in the grain must originate from soil rather than plant storage sites. Persistence of propanil and of free DCA in soil is brief (BARTHA and PRAMER 1970), but DCA was demonstrated to be covalently bound to soil organic matter at a rapid rate (HSU and BARTHA 1974a, HSU and BARTHA 1976) and humus-degrading soil fungi were shown to be capable of releasing intact DCA from such complexes (HSU and BARTHA 1974b). In consideration of these findings we interpret the radioactivity in the rice grains as DCA that was temporarily immobilized in soil as humic complex and was made available for root uptake during the grain ripening period by the microbial cleavage of these humic complexes. This interpretation is supported also by the findings of STILL and MANSAGER (1969) who found DCA in the grain of rice plants that were never treated with herbicide, but grew in soil that had a history of propanil treatment. Uptake of DCA by rice roots and its translocation throughout the plant was demonstrated by STILL (1968).

TABLE II

Radioactivity in rice grains 120 days after soil and leaf treatments with 14-C DCA

Treatment	Sample weight[a] (g)	dpm/g	µg DCA/g[b]
None	1.151	0[c]	0
Leaf	1.128	0[c]	0
Soil	1.073	3327 + 400[d]	0.419

[a]Each sample was prepared from 55 rice grains

[b]Calculated from specific activity of applied DCA

[c]Statistically not different from background

[d]Standard deviation

Assuming that the radioactivity found in the rice grains (TABLE II) is due to DCA that is free or that can be liberated in the digestive process; that the toxicity of DCA is the same as that described for aniline (Merck Index, Eighth Ed.); and that the LD_{50} for dogs can be applied to humans, it can be shown by simple calculations from these data that a lethal dose of DCA might be present in 10^6 servings (assuming 30 g per serving) of rice or that serious intoxication might result from the consumption of 10^4 servings. It seems then that little or no health hazard from aniline can be associated with the consumption of market rice.

It would be remiss of us not to point out that the molecular structure associated with the 14-C activity in the rice grains has not been identified in our experiments and that the DCA found in rice grain by STILL and MANSAGER (1969) was present as a complex of a yet undetermined composition. Nevertheless, there remains little doubt that some soil-bound pesticide residues are capable of causing low-level crop contamination. Similar results were obtained recently by FUHREMAN and LICHTENSTEIN (1978) on the soil-bound residues of the insecticide O,O-diethyl-O-p-nitrophenyl phosphorothioate (parathion). Oat seedlings and earthworms became contaminated when cultivated in soil that was exhaustively extracted to contain bound residues only.

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