Metabolism of the Herbicide Pronamide in Soil

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The metabolism of pronamide (3,5-dichloro-*N*-(1,1-dimethyl-2-propynyl)benzamide) in soil was studied under laboratory conditions. [*carbonyl*-^{14}C]Pronamide was applied to sterilized and nonsterilized soils and held at 25° for 33 days. Each soil was monitored for evolved $^{14}CO_2$ as well as total CO_2. Similar comparisons were also made in soil with [*carbonyl*-^{14}C]- and 3,5-[*ring*-^{14}C]dichlorobenzoic acid which is a potential metabolite of pronamide. In nonsterilized soil, approximately 13% of the [^{14}C]pronamide was mineralized to $^{14}CO_2$ after 33 days whereas *ca.* 81 and 51% of the carbonyl ^{14}C and ring ^{14}C, respectively, of 3,5-dichlorobenzoic acid were mineralized to $^{14}CO_2$ after 28 days. In sterilized soil, essentially no $^{14}CO_2$ was evolved indicating that the observed mineralization of $^{14}CO_2$ in nonsterilized soil was biologically mediated. No effects upon total CO_2 evolution were noted in any of the soil treatments. The level of pronamide plus transformation products was also determined. After 33 days, pronamide made up *ca.* 45 and 82% of the extractable ^{14}C residues in the nonsterilized and sterilized soils, respectively.

Pronamide (3,5-dichloro-*N*-(1,1-dimethyl-2-propynyl)-benzamide) is the active ingredient in Kerb herbicide, a new broad spectrum herbicide effective in the control of annual grasses and many broadleaved weeds. Kerb is very effective in the pre- and post-emergence control of weeds in lettuce (Lavalleye *et al.*, 1969) and in alfalfa and other legumes (Viste *et al.*, 1970) as well as *Poa annua* control in southern turfgrasses (Burt, 1970). Metabolism studies of [*carbonyl*-^{14}C]pronamide in soil (Yih *et al.*, 1970; Yih and Swithenbank, 1971a), alfalfa (Yih and Swithenbank, 1971a), and rat and cow excrements (Yih and Swithenbank, 1971b) have established its metabolic fate in a variety of environmental systems. In all the systems studied, extensive cyclization of pronamide occurred to give 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-methylenoxazoline followed by subsequent hydrolysis to *N*-(1,1-dimethylacetonyl)-3,5-dichlorobenzamide. Several additional minor metabolites have also been identified in these systems, all resulting from metabolism of the two terminal side-chain carbon atoms. Surprisingly, 3,5-dichlorobenzoic acid was not observed as a pronamide metabolite.

As part of an expanded study of the fate of pronamide in the environment, laboratory experiments were conducted to further delineate the metabolic fate of pronamide. In particular, it was of interest to evaluate the effects of soil microorganisms upon observed transformations of pronamide in soil by conducting incubations in sterilized *vs.* nonsterilized soil samples. Conversely, it was also of interest to learn whether total soil microbial activity was affected by low fortifications with pronamide. Included in this study was 3,5-dichlorobenzoic acid since it is a potential metabolite of pronamide and some benzoic acid herbicides (Kearney and Kaufman, 1969).

MATERIALS AND METHODS

Synthesis of Compounds. The preparations of 3,5-[*carbonyl*-^{14}C]dichloro-N-(1,1-dimethyl-2-propynyl)benzamide, 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-methylenoxazoline, and *N*-(1,1-dimethylacetonyl)-3,5-dichlorobenzamide have been previously described (Yih and Swithenbank, 1971a). 3,5-[*carbonyl*-^{14}C]Dichlorobenzoic acid was prepared by treating $^{14}CO_2$ with the Grignard reagent prepared from 1-bromo-3,5-dichlorobenzene. Purification was effected by reprecipitation with mineral acid (HCl) from an aqueous solution of the sodium salt yielding material with a specific activity of 8.78 mCi/g. Chemical and radiopurity were confirmed by tlc–autoradiography.

Uniformly ^{14}C-ring-labeled 3,5-dichlorobenzoic acid was prepared by converting ring-labeled benzoic acid according to conventional methods to benzoyl chloride, which was then bissulfonated to give the 3,5-disulfonic acid derivative. Chlorination then gave the 3,5-di(chlorosulfonyl)-benzoyl chloride. Thermal elimination of sulfur dioxide subsequently produced a crude ring-labeled 3,5-dichlorobenzoyl chloride which was partially purified by fractional distillation. The 3,5-[^{14}C]dichlorobenzoic acid obtained by hydrolysis was purified by sublimation. Its identity and purity were verified by its melting point and infrared spectrum. Its specific activity was 0.49 mCi/g.

Preparation and Treatment of Soil. The physical properties of the Hagerstown soil chosen for this study are presented in Table I. The moistened soil was stored at 13° until approximately 2 weeks before initiation of the present study at which time it was transferred to the laboratory, spread evenly on a bench top, and moistened daily to encourage microbial activity. After 2 weeks, the soil was screened and brought to a moisture level of *ca.* 11%. Sterilized soil for certain treatments was obtained by autoclaving soil samples in half-filled glass gallon jars at 17 psi and 120° for 3 hr and allowing them to cool before fortification.

Fortification of all soil tests was done by adding sufficient acetone solutions of each compound to a small portion (*ca.* 20 g) of each soil sample and then thoroughly mixing the treated portions with the larger portions (total soil = 200 g) after evaporation of the acetone. The final concentration for each test was 10 ppm on a wet weight basis. All tests were set up in triplicate in flasks patterned after those of Bartha and Pramer (1965) described in the next section.

Metabolism of Pronamide and 3,5-Dichlorobenzoic Acid in Soil. Experiments were conducted to determine the rates and extent of pronamide metabolism. The flasks containing the fortified soils were equipped with a side-tube charged with 10 ml of 0.5 *N* NaOH for trapping CO_2. The NaOH was replaced and CO_2-free air was passed through the flasks at periodic intervals. Half-milliliter aliquots of the NaOH solutions were removed and radioassayed by scintillation spectrometry. The remaining portions were titrated (after precipitating the carbonate with barium chloride) to determine total absorbed CO_2 as described by Bartha and Pramer (1965). Verification that the trapped ^{14}C was $^{14}CO_2$ was obtained by comparing the infrared spectrum and X-ray diffraction pattern of the [^{14}C]barium precipitate with authentic barium carbonate. The sterilized soils were sampled less frequently to minimize accidental microbial contamination.

After 33 days, the pronamide-treated soils were analyzed for degradation products as well as pronamide. Duplicate 50-g aliquots of the sterilized and nonsterilized soils were Soxhlet extracted with 250 ml of methanol for 20 hr. The extracts were concentrated to *ca.* 5 ml and ali-

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Table I. Physical Properties of Hagerstown Clay Loam Soil

Mech. anal.	Org matter	Cation exchange capacity	pH
Sand, 38% Silt, 34% Clay, 28%	3.42%	9.4 mequiv/100 g	6.4

quots were chromatographed directly on Brinkman precoated thin-layer plates of silica gel F-254, 0.5-mm thick. The thin-layer solvent systems employed were: (A) acetone-benzene (2:98, v/v); (B) acetone-benzene (25:75, v/v); and (C) ethyl acetate-isopropyl alcohol-water (65:25:10, v/v). The soil extracts were chromatographed with appropriate aliquots of a stock solution containing nonradioactive compounds known to be pronamide metabolites from prior soil and plant metabolism studies (Yih and Swithenbank, 1971a). The radioactive areas were located by autoradiography and quantitated by scraping and counting (scintillation) the appropriate silica gel spots. The nonradioactive compounds were located by using an ultraviolet light. Metabolite identifications were made based on coincidence of the nonlabeled reference compounds with the ^{14}C-labeled metabolites in two of the three tlc solvent systems.

RESULTS AND DISCUSSION

Carbon Dioxide Determinations. The quantity of $^{14}CO_2$ evolved from the pronamide fortified soils is shown in Figure 1 and that evolved from 3,5-dichlorobenzoic acid soils is shown in Figure 2. All data are expressed as the per cent of total ^{14}C theoretically available from the initial 200 g of 10 ppm of fortified soils. The soils were radioassayed at termination and found to contain the exact complement of the trapped $^{14}CO_2$ so that all initial ^{14}C was accountable.

It is apparent from the data that pronamide is degraded to the point of releasing the carbonyl carbon as $^{14}CO_2$ at a substantial rate in nonsterilized soil. In sterilized soil, however, the rate of $^{14}CO_2$ generated was barely detectable and could have been due to spontaneous decomposition of pronamide or to the eventual presence of a low level of microbial contamination.

In Figure 2, it is seen that the rates of $^{14}CO_2$ evolution from 3,5-dichlorobenzoic acid were very high, exceeding 80% of initial ^{14}C for carbonyl-labeled and 50% for ring-labeled 3,5-dichlorobenzoic acid after 28 days incubation in nonsterilized soils. Again, negligible $^{14}CO_2$ was detectable from sterilized soils. The rapid rate of decarboxylation of 3,5-dichlorobenzoic acid is consistent with published reports of similar substituted aromatic decarboxylations in soil (Horvath, 1971; Martin and Ervin, 1970). However, the relatively rapid rate of $^{14}CO_2$ released from 3,5-[*ring*-^{14}C]dichlorobenzoic acid is in contrast with data reported on other dichlorinated benzoates. Although complete degradation of benzoate in 3 days and monochlorinated benzoates in 21 days has been reported for soil suspensions by MacRae and Alexander (1965), they also reported that 2,4-, 3,4- and 2,5-dichlorobenzoic acids were not significantly degraded by soil microflora in 60 days as measured by a decrease in ultraviolet absorbancy. Perhaps the 3,5-dichloro substitution of benzoic acid renders the aromatic ring more susceptible to microbial attack than does 2,4-, 3,4-, or 2,5-dichloro substitution or perhaps rates of decomposition cannot be compared between soil and a soil suspension.

To monitor the relative microbial activities of the sterilized and nonsterilized soils and to determine if 10 ppm of pronamide or 3,5-dichlorobenzoic acid affected overall soil microbial activity, total CO_2 production by the various soils was assayed by titration of the NaOH trapping solutions. These data are plotted in Figures 3 and 4 for pronamide and 3,5-dichlorobenzoic acid, respectively. No sig-

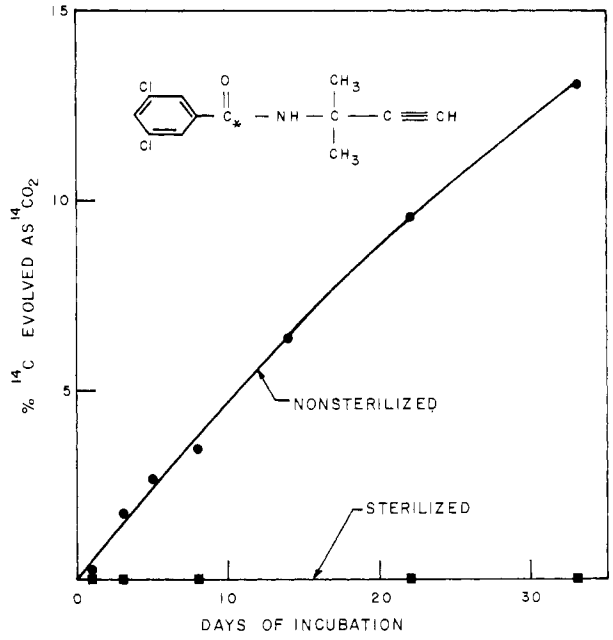


Figure 1. $^{14}CO_2$ evolution from 3,5-[*carbonyl*-^{14}C]dichloro-*N*-(1,1-dimethyl-2-propynyl)benzamide in soil.

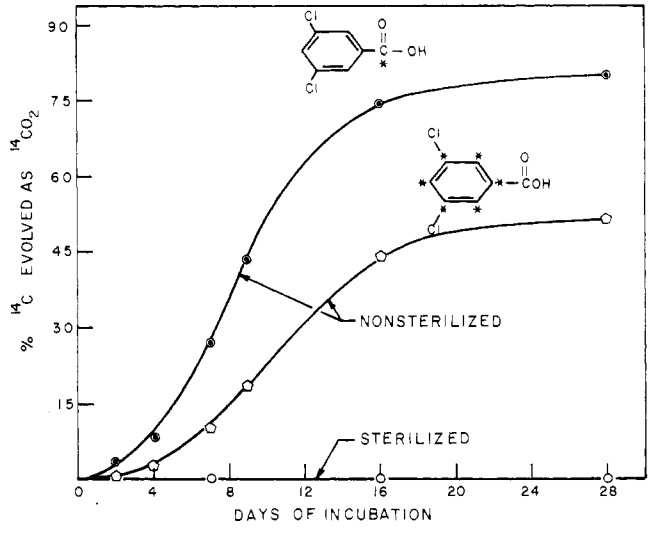


Figure 2. $^{14}CO_2$ evolution from carbonyl-^{14}C- and ring-^{14}C-labeled 3,5-dichlorobenzoic acid.

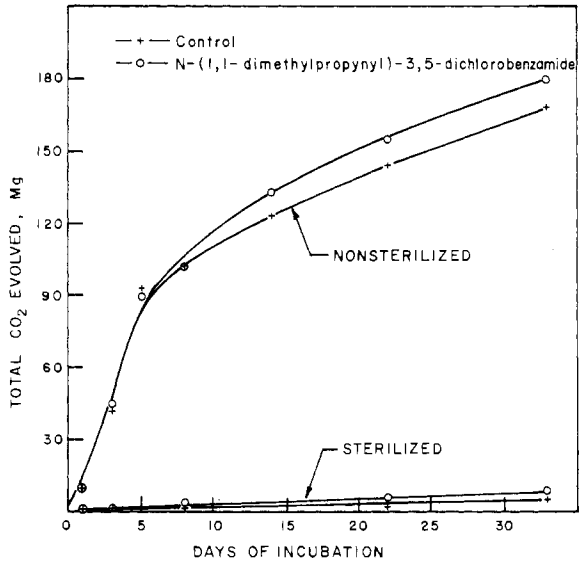
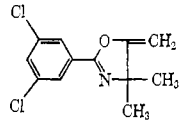
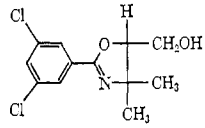


Figure 3. Total CO_2 evolution from control and 3,5-dichloro-*N*-(1,1-dimethyl-2-propynyl)benzamide fortified soils.

Table II. Metabolites of 3,5-Dichloro-*N*-(1,1-dimethyl-2-propynyl)benzamide in Sterilized and Nonsterilized Soils

Metabolite no.	Structure	% of extractable ^{14}C[a]	
		Sterilized soil	Nonsterilized soil
I	Unknown	3.46	1.93
II		2.41	33.33
Pronamide	$Cl_2C_6H_3CONHC(CH_3)_2C{\equiv}CH$	82.22	44.66
III	$Cl_2C_6H_3CONHC(CH_3)_2COCH_3$	9.37	14.63
IV		0.30	1.03
V	$Cl_2C_6H_3CONHC(CH_3)_2CH_2COOH$	0.50	1.01
VI	$Cl_2C_6H_3CONHC(CH_3)_2COOH$	0.60	1.11
VII	Unknown	1.14	2.30
	Total	100.00	100.00

[a] Extraction recoveries were 97 and 94%, respectively, for sterilized and nonsterilized soils.

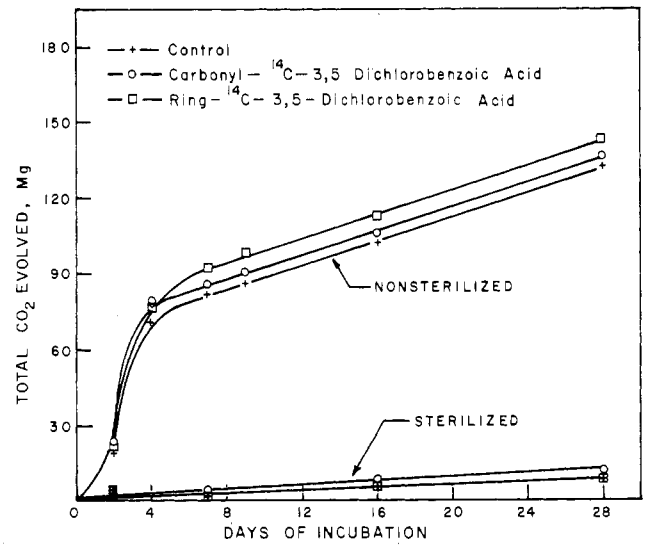


Figure 4. Total CO_2 evolution from control, carbonyl-^{14}C- and ring-^{14}C-labeled 3,5-dichlorobenzoic acid fortified soils.

nificant effect of the two compounds on total microbial activity was noted as indicated by the similarity of total CO_2 produced by control and fortified soils. This indicates that while certain organism types may or may not have been affected, the overall microbial population was not altered by a compound level at least five times the level that would result from a normal 2 lb/acre (2.25 kg/ha) application of pronamide distributed in the top 3 in. of soil.

Metabolism of Pronamide. After 33 days incubation, the pronamide-fortified soils were extracted and the extracts chromatographed to determine the extent of pronamide metabolism. Methanol extraction of the sterilized and nonsterilized soils resulted in 97 and 94% recovery, respectively, of total ^{14}C residues as determined by combustion and radioanalysis of the extracted soils. The distribution of pronamide and identified metabolites observed in the sterilized and nonsterilized soil extracts is presented in Table II in the order of increasing chromatographic polarity. In nonsterilized soil, pronamide makes up slightly less than half of the total extractable ^{14}C with metabolites III and especially II making up most of the remaining ^{14}C. In the sterilized soil, pronamide transformation has occurred but to a lesser extent than in the nonsterilized soil. Transformation of pronamide in sterilized soil is to be expected since cyclization of pronamide to metabolite II and thence to III occurs readily *in vitro* (Yih and Swithenbank, 1970). However, further transformations are obviously biologically mediated as indicated by the $^{14}CO_2$ data and metabolite data of the sterilized *vs.* nonsterilized pronamide-fortified soils of this study.

It is noteworthy that no 3,5-dichlorobenzoic acid was detected in the pronamide-fortified soils. This suggests that either 3,5-dichlorobenzoic acid is not an intermediate in the transformations of pronamide leading to release of $^{14}CO_2$ observed in this study, or that any 3,5-dichlorobenzoic acid formed as an intermediate is immediately decarboxylated. The rapid rate of $^{14}CO_2$ released by the 3,5-[*carbonyl*-^{14}C]dichlorobenzoic acid in this study supports the latter possibility and likely explains why 3,5-dichlorobenzoic acid was not observed as a metabolite in this and prior studies (Yih and Swithenbank, 1971b).

In summary, carbonyl-^{14}C-labeled pronamide undergoes appreciable metabolic transformation in nonsterilized soil under laboratory conditions with concomitant release of significant levels of $^{14}CO_2$. Likewise, carbonyl-^{14}C- and ring-labeled 3,5-dichlorobenzoic acid undergo metabolic transformations leading to substantial rates of $^{14}CO_2$ release in nonsterilized soil. Barely detectable levels of $^{14}CO_2$ were evolved from these same compounds in sterilized soil. The pronamide metabolites observed in this study are qualitatively similar to those observed in prior plant and animal studies (Yih and Swithenbank, 1971b) in which proposed metabolic pathways were presented.

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