Biochemical Transformations of Anilide Herbicides in Soil

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Respirometric and gas chromatographic studies demonstrated that the herbicides *N*-(3,4-dichlorophenyl) propionamide (propanil), *N*-(3,4-dichlorophenyl) methacrylamide (Dicryl), and *N*-(3,4-dichlorophenyl)-2-methyl pentanamide (Karsil) were metabolized by the microbial population in soil. The aliphatic moieties of these molecules were oxidized and, in each case, a substantial portion of the liberated 3,4-dichloroaniline was condensed to 3,3′,4,4′-tetrachloroazobenzene. Alkyl substitution of the acetanilide nitrogen in *N*-isopropyl-2-chloroacetanilide (Ramrod) increased persistence in soil and prevented transformation of this herbicide to aniline or azobenzene residues.

Anilide herbicides are relatively new and promising agents of weed control in turf and in a variety of economically important crops, including rice, cotton, soybeans, corn, and tomatoes. Among the attractive features of these herbicides are their effectiveness, selectivity, low mammalian toxicity, and biodegradability (Weed Society of America, 1967). However, studies in this laboratory (Bartha *et al.*, 1967; Bartha and Pramer, 1967) demonstrated that the biodegradation of *N*-(3,4-dichlorophenyl)propionamide (propanil) inhibited soil respiration and produced residues identified as 3,4-dichloroaniline (DCA) and 3,3′,4,4′-tetrachloroazobenzene (TCAB). It was of interest, therefore, to perform comparative kinetic studies of herbicide degradation and identify residues formed from several anilides.

MATERIALS AND METHODS

The herbicides used (propanil, Dicryl, Karsil, Ramrod) were analytical standards or recrystallized technical material. The DCA was purified by recrystallization from ligroin. The TCAB was synthesized by $LiAlH_4$-reduction of 3,4-dichloronitrobenzene as described by Corbett and Holt (1963). All of these materials were established as pure by thin-layer and gas chromatography and by melting point determinations. For quantitative degradation experiments in soil an equivalent of 50 grams dry weight of fresh, sieved Nixon sandy loam (pH 5.5) was treated with 25 mg. (500 p.p.m.) of the respective herbicide. The high herbicide concentrations (almost 100 times the usual field application levels) increased the accuracy of the respirometric and analytical measurements. The herbicides were incorporated into soil by mixing only; no solvents or emulsifiers were used. All the soil samples were moistened to 60% of holding capacity, and incubated at 27° C. in beakers covered with thin polyethylene film or in the special respirometric vessels described below. Periodic aeration and the addition of H_2O assured a constant oxygen supply and moisture level.

Oxygen uptake was measured in a constant pressure differential soil respirometer constructed in this laboratory. As illustrated by Figure 1, the unit consisted of six 250-ml. filter flasks, 1, connected through individual manometers, 2, and a manifold, 3, to a compensating vessel, 4. All connections were made with heavy-walled Tygon tubing. The flasks were closed with rubber stoppers pierced by syringe needles, 5. Ten-milliliter glass syringes with rubber plungers, 6, were permanently sealed to the needles with epoxy resin. Inside each flask a vial, 7, containing 5 ml. of 20% KOH was suspended on a cup hook from the stopper. In each case, the syringe was adjusted initially to the uppermost mark. Oxygen uptake by microorganisms in the soil decreased pressure so that the manometer fluid moved into the arm adjacent to the flask. The manometer fluid was returned to its original position by adjustment of the syringe, and the decrease in gas volume was read directly from the calibrated syringe barrel. Syringes were returned to their original position by opening the manometer bypass valves, 8. Measurements of oxygen uptake were made at appropriate intervals for 21 days and converted to volume under standard conditions of temperature and pressure.

Isolation and identification of DCA and TCAB were performed as described previously (Bartha and Pramer, 1967). Quantitative analyses for pesticides and their metabolites were made after 0, 7, 14, and 21 days by transferring each of the 50-gram soil samples to a measuring cylinder and diluting to 250 ml. with acetone. The suspension was homogenized for 30 seconds in a Waring Blendor and filtered. Aliquots of the clear filtrate were concentrated 10-fold on a steam bath and used for the measurement of propanil, Dicryl, Karsil, TCAB, and azobenzene. Ramrod, DCA, and aniline concentrations were measured in portions of unconcentrated soil extract (injection volume = 1.0 μl.). Analyses were performed with an F & M Model 700 gas chromatograph having dual flame ionization detectors. Columns: 1.8 meters long, 3-mm. o.d. stainless steel, packed with 5% UC-W98 on

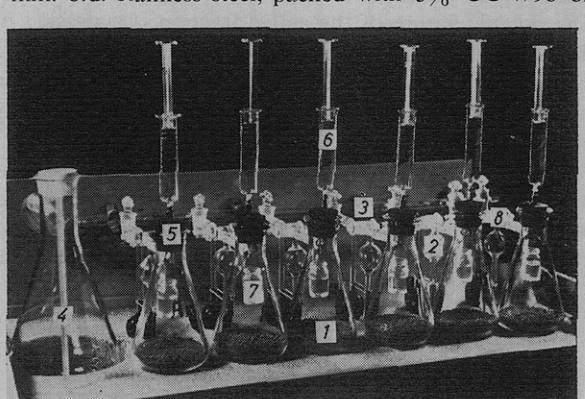

Figure 1. Soil respirometer

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Chromosorb W. Carrier: helium, 30 cc. per minute. Temperatures: injection port = 270° C.; detector = 300° C.; oven = 150° C. isothermal for aniline, 200° C. for DCA and Ramrod, 250° C. for all other compounds. Peaks were quantitated by comparing their heights to appropriate calibration curves.

Thin-layer chromatographic separations were performed on Eastman Chromagram sheets (silica gel with fluorescent indicator) developed with benzene. Spots were located under ultraviolet light. Infrared spectra were recorded in KBr pellets with a Perkin-Elmer Model 21 spectrograph.

RESULTS AND DISCUSSION

Preliminary tests demonstrated that losses of aniline, DCA, and Ramrod occurred if soil samples were dried before extraction, if extraction was performed using a Soxhlet apparatus, or if acetone extracts were concentrated by evaporation. These losses were avoided, however, because the sensitivity of the flame ionization detector to aniline, DCA, and Ramrod was great enough for analyses to be made directly on untreated extracts. There were no losses of propanil, Dicryl, Karsil, azobenzene, or TCAB during concentration of acetone extracts of soil. Since recoveries from soil of added anilide herbicides, aniline, DCA, azobenzene, and TCAB varied between 90 and 110%, the results of analyses by quantitative gas chromatography were used without correction.

Residues isolated from soil samples that were treated with propanil, Dicryl, and Karsil and incubated for 2 weeks were identified as DCA and TCAB by their retention times in the gas chromatograph, their movement on thin-layer plates, and their melting points (Table I). Furthermore, the infrared spectra of purified metabolites and authentic DCA and TCAB were compared and were identical (Figure 2).

The results of respirometric and gas chromatographic measurements on herbicide-treated soil samples are summarized in Figure 3. Soils that received propanil, Dicryl, or Karsil displayed an initial increase in oxygen consumption. The effect was interpreted as evidence for the biological oxidation of the carbon that comprised the aliphatic side chain of the added compounds. This transient increase was followed by a depression of the respiration rate, caused by inhibitory products arising from the degradation of these herbicides. On the basis of respirometric data, Ramrod was the anilide least subject to oxidation in soil. Gas chromatographic analyses supported this conclusion, since after 21 days the decrease in Ramrod concentration was only 10%, and no evidence was ob-

Table I. Melting Points, R_f Values, and Retention Times of Some Anilide Herbicides and Degradation Products

Compound	M.P., ° C.	R_f	Retention, Sec. 200° C.	Retention, Sec. 250° C.
Propanil	92	0.10	...	33
Dicryl	124	0.25	...	38
Karsil	105	0.30	...	56
Ramrod	75	0.15	70	...
DCA	71	0.65	38	...
TCAB	158	0.95	...	140

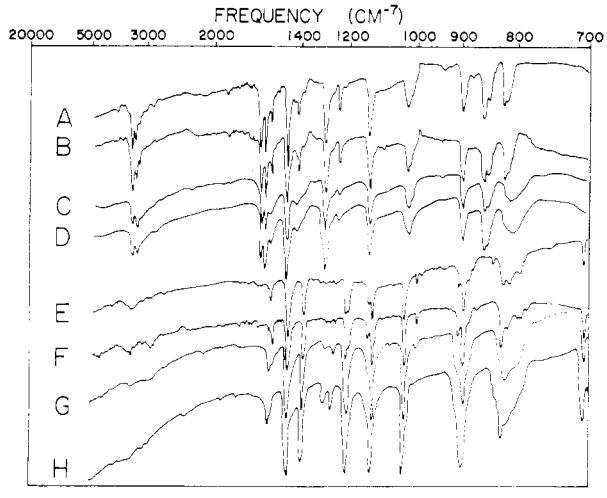

Figure 2. Infrared spectra

Authentic 3,4-dichloroaniline (*A*), 3,3′,4,4′-tetrachloroazobenzene (*E*), and substances isolated from soil treated with propanil (*B*, *F*), Dicryl (*C*, *G*), and Karsil (*D*, *H*)

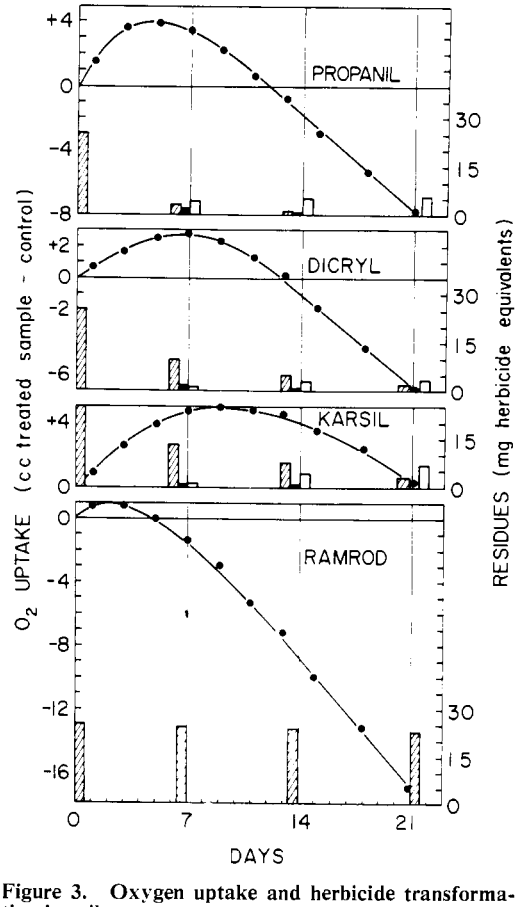

Figure 3. Oxygen uptake and herbicide transformation in soil

Oxygen uptake curves are differences between treated and control soil samples (positive values indicate increased O_2 consumption, negative values represent inhibition). Shaded bars represent unaltered herbicide, solid bars 3,4-dichloroaniline, and open bars 3,3′,4,4′-tetrachloroazobenzene. Metabolites are represented in milligram-equivalents of their parent herbicide

tained for production of an aniline or azobenzene from Ramrod in soil. The inhibition of respiration in Ramrod-treated soil was apparently not caused by metabolites but by the herbicide itself. At the same level of application (500 p.p.m.), no respiratory inhibition was caused by unchanged propanil, Dicryl, or Karsil. At normal field application levels all the described respiratory inhibitions are expected to be insignificant.

The degradation rates of the three 3,4-dichloroacylanilides correlated with the number of carbon atoms in their aliphatic moiety. Propanil (C_3) was subject to the fastest degradation; Dicryl (C_4) decomposed more slowly; Karsil (C_6) was the most persistent. This decrease in degradation rate of chloroanilides with increased length of the aliphatic moiety may be explained either by the hydrophobic properties of longer aliphatic chains rendering compounds less soluble in water, or by the possible interference of such chains with the formation of an enzyme-substrate complex during biochemical degradation.

The early production and subsequent disappearance of DCA suggested that this compound was an intermediate in the formation of TCAB from propanil, Dicryl, and Karsil. The percentage of TCAB produced in 21 days from the decomposed portion of propanil, Dicryl, and Karsil was 22, 17, and 29%, respectively. DCA and TCAB combined did not balance the disappearance of the parent herbicide. Separate studies demonstrated that added DCA rapidly disappeared from soil, but not all of the transformed aniline was recovered as TCAB. After incubation in soil for 21 days, added TCAB was recovered quantitatively and could be considered persistent at least for the duration of the experiment. Consequently, the lack of balance between decomposed herbicide and recovered metabolites indicated that DCA liberated from the 3,4-dichloroacylanilides was converted not only to TCAB but also to other, as yet unidentified, products. Respirometric evidence did not support the possibility of extensive oxidation of DCA (Bartha *et al.*, 1967). However, red-brown residues present in extracts of treated soils may represent complex polyaromatic products of condensation of DCA with itself and/or with its derivatives. In several separate experiments the proportion of the DCA that could be recovered as TCAB varied from 22 to 46%, but factors controlling the rate and extent of the transformation and the identity and number of unknown reaction products remain to be investigated. The respiratory inhibition of soil caused by the transformation products of propanil, Dicryl, and Karsil did not correlate well with the measured concentrations of either DCA or TCAB. Apparently, the as yet unidentified metabolites are primarily responsible for this effect.

Gas chromatographic analyses failed to detect aniline or azo compounds in extracts of control soils that were sterilized with steam before treatment with filter-sterilized herbicide solutions, or in extracts of unsterile, herbicide-treated soils that were poisoned with the metabolic inhibitors NaN_3 and $HgCl_2$. Moreover, no TCAB was formed from DCA by these control soils. It was concluded, therefore, that the production of aniline and azo compounds from propanil, Dicryl, and Karsil in unsterilized soil was the result of a series of biochemical transformations mediated by soil microorganisms.

One objective of the present comparative study of anilide herbicides was to correlate molecular configuration with stability and with residue formation in soil. Although tests were performed with only four compounds, some relationships were apparent, and it will be of future interest to use different and new anilides to test the validity of the following generalizations: 3,4-Dichloroacylanilides are degraded microbiologically in soil and, within the tested range, the rate of transformation is an inverse function of length of the aliphatic side chain. The degradation liberates 3,4-dichloroaniline and in soil this compound undergoes biochemical condensation to 3,3′,4,4′-tetrachloroazobenzene. Other unidentified products are produced simultaneously. Alkyl substitution of the anilide-nitrogen of an acyl-anilide greatly increases stability in soil.

Since substituted aniline moieties are present in phenylcarbamate and phenylurea herbicides, and since they have been described as residues of the degradation of these compounds in soil (Dalton *et al.*, 1966; Geissbühler *et al.*, 1963; Kaufman and Kearney, 1965; Kearney, 1965; Kearney and Kaufman, 1965), the significance of condensation reactions and the azo compounds they produce extends beyond the present interest in anilide herbicides. Resistance of some plant species to propanil was attributed to their ability to degrade the herbicide with the release of DCA (Still and Kuzirian, 1967). Therefore, plant tissue may be an additional source of aniline residues that may enter soil and undergo transformation in part to azo compounds.

A literature search failed to reveal any data on the biological activity of chlorinated azobenzenes. Since some azo compounds are known to be carcinogenic (Weisburger and Weisburger, 1966), TCAB was synthetized in bulk and a sample was sent to the National Cancer Institute for evaluation of the safety of this relatively persistent residue. The outcome of this study should indicate whether or not any public health significance is attached to the described biochemical herbicide transformations.

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