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Effect of pH on the Hydrolysis of Chlorothalonil

Aqueous solutions of chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) were prepared at 0.5 ppm, buffered at pH 5 to 9, and stored in the dark. No hydrolysis was observed at pH 7 or lower. At pH 9, chlorothalonil hydrolyzed to 4-hydroxy-2,5,6-trichloroisophthalonitrile and 3-cyano-2,4,5,6-tetrachlorobenzamide. The rate of decline of chlorothalonil followed first-order kinetics and was determined to be 1.8% per day using gas chromatographic and radiotracer techniques.

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) is the active ingredient in the fungicide Bravo commercially marketed in the United States by the Diamond Shamrock Corporation. It was first registered in 1969 for the effective control of pathogens affecting potatoes and later for use on turf, ornamentals, tree crops, and vegetables. Vincent and Sisler (1968), Turner and Battershell (1970), and Tillman et al. (1973) found the diverse fungicidal activity of chlorothalonil to be attributed to its action as an alkylating agent for cellular thiols. The action and fate of chlorothalonil in biological systems were investigated by Long and Siegel (1975).

Ballee et al. (1976) developed analytical procedures to determine residues of chlorothalonil in crops and soil. Upon analysis of field samples, it was shown that chlorothalonil does not accumulate in soil. Its rate of decline was affected by the presence of moisture. In this study the conditions under which the hydrolysis of chlorothalonil occurs are investigated as well as the nature of the products of hydrolysis.

MATERIALS AND METHODS

[^{14}C]Chlorothalonil, uniformly labeled in the ring, was synthesized with a specific activity of 0.026 mCi/mmol. Radiochemical purity, determined by thin-layer chromatography, was 99.3%. Separate benzene solutions of [^{14}C]chlorothalonil and nonradioactive chlorothalonil were prepared at concentration levels of 100 and 50 μg/ml, respectively.

Clark and Lubs pH 5.0, 7.0, and 9.0 buffer mixtures were prepared as described in Lange's Handbook of Chemistry (1949).

To each of three 500-ml glass-stoppered Erlenmeyer flasks, 3 ml of the stock nonradioactive chlorothalonil solution was added and evaporated free of benzene under a stream of dry nitrogen. To the sample to be stored at a basic pH level, the equivalent of 5.5 μg of radiolabeled chlorothalonil was added. A 6-ml volume of the appropriate buffer was added to each of the three flasks. To each flask was then added 294 ml of sterile distilled water resulting in a final volume of 300 ml. The approximate final concentrations of the samples were 0.50 ppm for the pH 5 and 7 samples and 0.52 ppm for the pH 9 sample. The flasks were sealed and covered with aluminum foil to prevent any exposure to light, shaken 2 h, and stored at room temperature.

All samples were shaken on an automatic shaker for 30 min prior to any sampling. Periodic analyses were conducted by acidifying 10-ml portions of each aqueous solution with 10 drops of 1:1 sulfuric acid and partitioning with 20 ml of isopropyl ether. A 10-ml portion of the ether extract was evaporated to dryness, diluted to a known volume with benzene, and analyzed using a gas–liquid chromatograph equipped with an electron capture detector and using a 6% DC-200 Chrom-Q column at 225 °C. Thus, the decline of chlorothalonil with time at the three pH levels was determined.

The sample stored at pH 9 was analyzed utilizing radiotracer techniques to determine the fate of chlorothalonil under the described conditions after 89-days storage. To determine any losses due to volatility, duplicate 1.0-ml portions of each aqueous sample were transferred to scintillation vials, 10 ml of Aquasol was added, and the samples were counted using a Searle Analytic ambient temperature counter.

To determine the formation of any water-soluble, nonpartitioned hydrolysis products, a known portion of the aqueous pH 9 sample was transferred to a separatory funnel, acidified with 10 drops of 1:1 sulfuric acid, and partitioned with 30 ml of isopropyl ether. Duplicate 1.0-ml portions of each phase were counted for radioactivity to determine the effect of storage time upon the partitioning of chlorothalonil residues. All data were corrected for background and counting efficiency.

Characterization of radioactivity partitioned into the organic phase was accomplished by concentrating a known

portion of the ether phase to a volume less than 0.5 ml under a stream of clean, dry air and subjecting the concentrated extract to thin-layer chromatographic analysis.

The concentrated ether extract of the aqueous sample stored at pH 9 was applied to silica gel plates (with fluorescent indicator, Brinkman Co.) as a 1–2-cm band using disposable microliter pipets. Nonlabeled reference standards of chlorothalonil, 4-hydroxy-2,5,6-trichloroisophthalonitrile, and 3-cyano-2,4,5,6-tetrachlorobenzamide were also applied to each plate in a similar manner.

Either of three solvent systems was used to develop the TLC plate. These were as follows:

Solvent system I	(1)	Develop plate to 15 cm in 8:2 benzene–acetone
	(2)	Dry and redevelop plate to 11 cm in 2:1 benzene–methanol
Solvent system II		Develop plate to 16 cm in 1:1 hexane–acetone
Solvent system III		Develop plate to 16 cm in 9:1 benzene–acetone

The R_f values obtained in these systems for standards of chlorothalonil, 4-hydroxy-2,5,6-trichloroisophthalonitrile, and 3-cyano-2,4,5,6-tetrachlorobenzamide are presented in Table I.

After development and drying of the TLC plates, the nonlabeled standards were visualized under short-wave UV light and marked. The plate was then exposed to Kodak x-ray film Type NS54T for 10 days and the film developed according to standard procedures. On the basis of the resulting radioautogram, the TLC plate was sectioned into regions with and without radioactivity. These sections were cut with scissors, deposited into scintillation vials with 20 ml of Aquasol, and counted to determine the distribution of radioactivity on the TLC plate.

RESULTS AND DISCUSSION

Data from GLC analysis of the samples are presented in Table II. The initial concentrations were slightly less than the 0.50- and 0.52-ppm levels prepared, possibly due to incomplete solubility or some adhering of the compound to glassware. No decline of chlorothalonil was observed after 49 days storage in the absence of light at pH 5 or 7. However, a significant decline was observed at the basic pH 9 level; the concentration decreased from 0.40 ppm of chlorothalonil at $T = 0$ to 0.08 ppm after 89 days, an 80% decrease. Since this sample was amended with [^{14}C]-chlorothalonil, the partitioning of radioactivity into the organic phase was easily determined to be 98.7%; thus, the decline of chlorothalonil was not due to the formation of any nonpartitioned, polar hydrolysis products.

Upon GLC analysis of the ether extract of the pH 9 solution, two peaks were observed—one corresponding to chlorothalonil and the other to an unknown. Similar GLC analysis of a standard of 3-cyano-2,4,5,6-tetrachlorobenzamide showed this compound to have the same retention time as the unknown. Upon derivitization with 1-methyl-3-*p*-tolyltriazene, a third peak corresponding to the methyl ether of 4-hydroxy-2,5,6-trichloroisophthalonitrile was detected.

Additional quantities of the unknown were isolated and purified by TLC techniques. The unknown exhibited the same R_f values as 3-cyano-2,4,5,6-tetrachlorobenzamide in solvent systems I, II, and III (Table I).

The purified unknown was submitted for mass spectral analysis; a cluster of peaks at m/e 282, 284, and 286 was observed. These data suggested a tetrachlorinated compound having a fragmentation pattern consistent with that obtained for an authentic standard of 3-cyano-2,4,5,6-tetrachlorobenzamide.

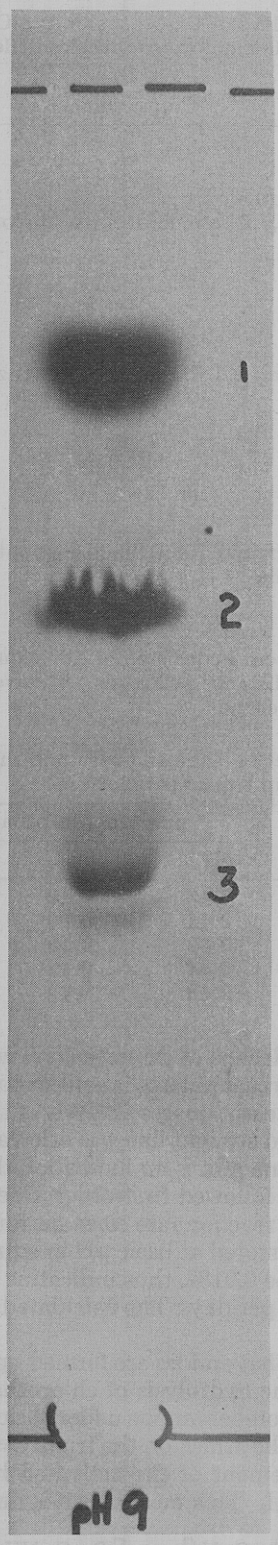
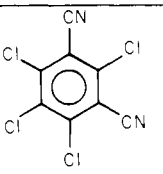
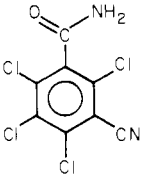
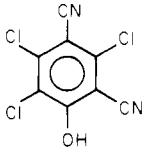


Figure 1. Radiochromatogram of [^{14}C]chlorothalonil stored 89 days at pH 9 in aqueous media: (1) chlorothalonil; (2) 3-cyano-2,4,5,6-tetrachlorobenzamide; (3) 4-hydroxy-2,5,6-trichloroisophthalonitrile.

By combining TLC analysis with radiotracer techniques, it was possible to quantify the pH 9 sample as containing 24% intact chlorothalonil; 22% of the chlorothalonil degraded to 4-hydroxy-2,5,6-trichloroisophthalonitrile and 54% to 3-cyano-2,4,5,6-tetrachlorobenzamide. These results are in excellent agreement with the GLC data which showed 20.0% intact chlorothalonil to remain after the 89-day storage period. A radiochromatogram of TLC analysis of the ether extract is shown in Figure 1.

Table I. R_f Values of Chlorothalonil, 3-Cyano-2,4,5,6-tetrachlorobenzamide, and 4-Hydroxy-2,5,6-trichloroisophthalonitrile in Three Thin-Layer Chromatographic Solvent Systems

Compound	Structure	R_f in system I[a]	R_f in system II[b]	R_f in system III[c]
Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile)		0.81	0.81	0.74
3-Cyano-2,4,5,6-tetrachlorobenzamide		0.67	0.70	0.43
4-Hydroxy-2,5,6-trichloroisophthalonitrile		0.41	0.21	0.04

[a] Solvent system I consisted of development in 8:2 benzene-acetone for 16 cm followed by development in 2:1 benzene-methanol to a distance of 11 cm. [b] Solvent system II consisted of 1:1 hexane-acetone. [c] Solvent system III consisted of 9:1 benzene-acetone.

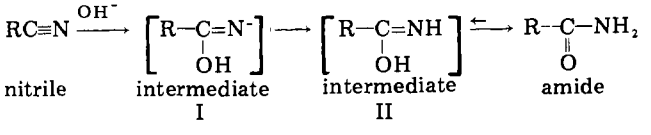
Table II. Effect of Time and pH upon the Decline of Chlorothalonil in Aqueous Solutions

	ppm of chlorothalonil		
Time, days	pH 5	pH 7	pH 9
0	0.43	0.43	0.40
14	0.44	0.40	0.36
33	0.39	0.45	0.33
43	0.44	0.42	0.22
49	0.48	0.42	0.23
89			0.08

The rate of decline of chlorothalonil was determined by plotting log percent chlorothalonil remaining vs. time; by computerized least-squares analysis of the data, the slope of the generated straight line was calculated to be −0.0079. The straight line plot is an indication that the decline of chlorothalonil followed first-order kinetics.

The specific reaction rate constant for the hydrolysis of chlorothalonil stored at basic pH in aqueous solution was calculated to be 0.0182, thus indicating a 1.8% decline of chlorothalonil per day. The calculated half-life was 38.1 days.

It is known that amides are formed upon the hydrolysis of nitriles. The hydrolysis of chlorothalonil to 3-cyano-2,4,5,6-tetrachlorobenzamide under basic conditions occurs by a nucleophilic attack on the triple C≡N bond followed by a rearrangement of the activated hydrogen atom of intermediate II. This mechanism is depicted as follows:



Hydrolysis of chlorothalonil to 4-hydroxy-2,5,6-trichloroisophthalonitrile occurred due to the labile chlorine at the four carbon position of the molecule. The cyano groups ortho and para to this position activate it, thus causing the formation of 4-hydroxy-2,5,6-trichloroisophthalonitrile.

Under the conditions of this study, these two different routes of hydrolysis of chlorothalonil were observed to account for all radiolabeled compounds used in the experiment.

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