all these liquid phases for both sensitivity and selectivity. Interfering peaks, particularly in the 3-ethoxy region, were observed for some crops (rice, tomatoes, wheat grain) on all columns except the 5% OV-3. The chromatography of 3-ethoxycarbofuran was shown to be excellent with no crop related variation.

While the split procedure appears to be the most general, it is possible that analysis of both carbofuran and 3-hydroxycarbofuran could be done for certain crops by the 3-hydroxy procedure alone.

Practitioners of this method should be aware of possible difficulties which were discovered during the development of this method. The NPD system is very sensitive and is a selective but not a specific detector. Interfering peaks which were artifacts of the method and its reagents were observed frequently during the development of the method. Specifically, responses were found to come from the following: (1) contaminated glassware, (2) phosphorus detergents, (3) filter aids (filter paper, glasswool, cheesecloth), (4) sodium sulfate, (5) butyl rubber, (6) deterioration of the detector bead (sensitivity is achievable when selectivity is not, detectors exhibiting wide solvent regions should be considered suspect). Once these problems were identified, isolated, and dealt with, they did not recur.

The quantitative nature of all partition steps and the ethoxylation step were verified by radiotracer experiments as well as recovery of standard compounds.

Two areas where significant loss of compound occurred were noted. First, the samples must not be allowed to go to dryness during concentration. Significant loss will occur if the solvent is totally removed. Second, carbofuran was shown to be absorbed from hexane onto sodium sulfate. Radiotracer experiments showed that the loss was irreversible. This phenomenon was sodium sulfate batch related. The loss was easily avoided by adding diethyl ether (10%, v/v) to the hexane extracts before the addition of a minimum amount of sodium sulfate. Magnesium sulfate and barium oxide also resulted in significant loss of carbofuran if used as drying agents.

CONCLUSIONS

The method, as written, was designed to be as general as possible. It was applied without change to the 16 crops in Table I. Variations of the method are possible with individual crops. Some of these variations include (1) analysis of the carbofuran fraction with no cleanup column (peas, potatoes, tomatoes), (2) analysis of both carbofuran and 3-hydroxycarbofuran by the 3-hydroxycarbofuran procedure alone, (3) alteration of the Florisil column elution pattern, and (4) analysis using a different GC column. These variations may work for an individual crop and shorten the procedure. However, given a crop of unknown characteristics, the method practiced exactly as written has the best chance of success.

ACKNOWLEDGMENT

The authors wish to thank B. C. Leppert and J. C. Markle for their many helpful comments and discussions. The authors wish also to thank T. A. Bixler, J. E. Burt, L. M. Dodge, and G. E. Lover for their outstanding technical assistance.

LITERATURE CITED

Butler, L. I., McDonough, L. M., *J. Assoc. Off. Anal. Chem.* **54**, 1357 (1971).

Coburn, J. A., Ripley, B. D., Chau, A. S. Y., *J. Assoc. Off. Anal. Chem.* **59**, 188 (1976).

Cook, R. F., "Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives", Vol. 7, Academic Press, New York, 1973, p 187.

Thomson, W. T., "Agricultural Chemicals", Book 1, Thomson Publications, Indianapolis, IN, 1973, p 40.

Wong, L., Fisher, F. M., *J. Agric. Food Chem.* **23**, 315 (1975).

Received for review September 12, 1978. Accepted August 16, 1979.

Hydrolysis of Fenitrothion in Model and Natural Aquatic Systems

Roy Greenhalgh,* Kasturi L. Dhawan, and Pearl Weinberger

The hydrolysis of fenitrothion [*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate] was studied in the dark in buffered distilled water, natural lake water, and buffered lake water. Above pH 8, specific base catalysis was the predominant degradative reaction resulting in the formation of 3-methyl-4-nitrophenol. Below pH 7, a second reaction involving dealkylation also took place to form demethylfenitrothion. The extent of this reaction was temperature dependent. Amino fenitrothion was also detected as a reaction product, but only in natural lake water systems. The $t_{1/2}$ for the disappearance of fenitrothion at 23 °C and pH 7.5 in natural lake water in the dark and in the field were 49.5 and 1.5–2 days, respectively. This difference suggests that photolysis and microbiol processes are the main degradative route of fenitrothion in natural aquatic systems.

Fenitrothion [*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate] (1) has been used extensively in Canadian forests for the control of lepidopterous defoliators. Application is made by aerial spray, which can result in the inadvertent contamination of aquatic systems, either directly or indirectly from surface run-off following rainfall. Levels of fenitrothion in river water following spraying have been reported ranging from 6 ppb to 64 ppm (Symons, 1977; Flannagan, 1973; Edit and Sundaram, 1975; Moody et al., 1978). Information about the persistence and modes of degradation of this insecticide in fresh water is therefore important in assessing its impact on aquatic flora and fauna.

Like other organophosphorus insecticides, fenitrothion is known to be degraded in water by both photolysis

Agricultural Canada, Ottawa, Ontario K1A 0C6 (R.G.), and the Biology Department, Ottawa University, Ottawa, Ontario (K.L.D., P.W.).

0021-8561/80/1428-0102$01.00/0

(Ohkawa et al., 1974) and hydrolysis (Nishizawa et al., 1961; Ruzicka, 1967; Sundaram, 1973; Vitko and Cunningham, 1974). The hydrolytic data available, however, deals mainly with basic systems and adds little to our understanding of its degradation at the pHs and temperatures found in the environment. This work reports on the hydrolysis of fenitrothion at the 10 ppm level, over the pH range 3–12 and at temperatures of 23–60 °C in buffered distilled water as well as lake water. Reactions were maintained in the dark in order to diminish photolytic effects.

EXPERIMENTAL SECTION

Chemicals. Sumithion (fenitrothion) 98.7% was supplied by Sumitomo Chemical Co., Osaka, Japan, and *N*-ethyl-*N*-nitrosourea was obtained from Aldrich Chemicals, Milwaukee, OH. Lake water was collected from Lac Bourgeois, Quebec, and had the following properties: pH 7.5; organic matter, 10 ppm; Ca^{2+}, 15 ppm; Fe^{2+}, 0.18 ppm; HCO_3^-, 41.72 ppm; SO_4^{2-}, 11, 82 ppm; Cl^-, 1.55 ppm; and other ions, <1 ppm.

Gas Chromatography. A Pye Model 104 gas chromatograph (GC) was used equipped with an alkali flame ionization detector (AFID) and a RbCl annulus. Analyses were performed on a 2.8 m × 0.4 cm i.d. glass column packed with 6% SE-30/4% QF-1 on 100/120 mesh Gas-Chrom Q. A column temperature of 215 °C and a nitrogen carrier gas flow of 40 mL/min gave the following retention times: aminofenitrothion, 4.1 min; fenitrothion, 5.6 min; *O*-ethylfenitrothion, 6.3 min; *S*-methylfenitrothion, 9.4 min; *S*-ethylfenitrothion, 10.6 min.

Procedure. Standard solutions of fenitrothion (10 mg/L) were prepared by the addition of a stock solution in acetone (10 g/L) to the appropriate buffers; pH 3, 0.07 M NaH_2PO_4; pH 5–7.5, 0.07 M NaH_2PO_4/0.07 M K_2HPO_4; pH 8–9, 0.025 M borax and 0.01 M HCl; pH 10, 0.01 M borax/0.04 M NaOH. Aliquots (100 mL) were transferred to stoppered Erlenmeyer flasks in a thermostated bath (±0.1 °C), and the reaction was carried out in the dark. Duplicate samples (5 mL) were taken at appropriate intervals, acidified to pH 1 by the addition of concentrated HCl, saturated with NaCl, and extracted with ethyl acetate (3 × 2 mL). The combined extracts were evaporated to 0.5 mL by a stream of nitrogen, treated with an excess of ethereal diazoethane for 30 min at room temperature. After removal of excess reagent, the residual solution was diluted to 10 mL with ethyl acetate and analyzed by GC. The recovery of fenitrothion and demethylfenitrothion from distilled water at the 10 ppb level was >93%. 3-Methyl-4-nitrophenol was extracted with ether/acetonitrile (1:1) from the acidified solution and analyzed by GC equipped with an electron-capture detector. Lake water samples (pH 7.5) were extracted with ethyl acetate (5 × 2 mL) prior to and after acidification The first extract contained fenitrothion and aminofenitrothion and the second, demethylfenitrothion.

The kinetics of the hydrolysis was followed by measuring the residual concentrations of fenitrothion (*A*) at known time intervals and plotting log *A* vs. time. The Arrhenius plots were derived from plotting the rate constants at pH 5 and 12 for four different temperatures, i.e., 23, 37, 50, and 59.5 °C. All reactions were allowed to proceed to 90% completion.

RESULTS AND DISCUSSIONS

In the dark, at room temperature, the hydrolysis of fenitrothion (10 ppm) proceeded very slowly, hence it was studied at 50 °C. All the plots of log *A* vs. time were linear for the pH range 3–11 (Figure 1), indicating the reaction to be pseudo first order. A plot of the pH vs. observed rate

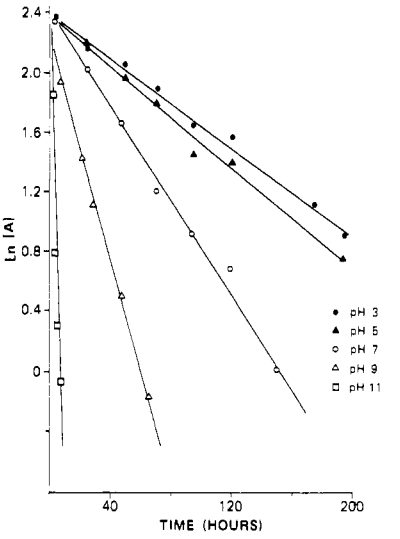
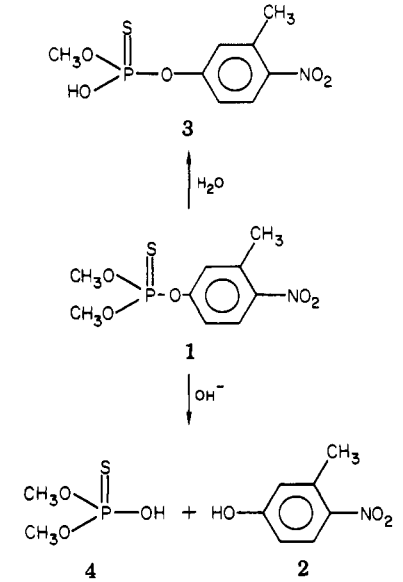


Figure 1. Hydrolysis of fenitrothion (10 ppm) in distilled water buffered at pH 3–11 at 49.5 °C and in the dark. Plot of ln residual concentration of fenitrothion [*A*] vs. time.

is shown in Figure 2, which indicates two reactions, one specific base catalyzed and the other pH independent. The overall rate expression for the disappearance of fenitrothion is $d[F]/dt = k_{obsd}[F]$ and in the pH range studied, the pseudo-first-order rate constant k_{obsd} is given by $k_{obsd} = k_{H_2O}[H_2O] + k_{OH}[OH^-]$ where $k_{H_2O} = 2.5 \times 10^{-6}$ L mol^{-1} min^{-1} and $k_{OH^-} = 1.7$ L mol^{-1} min^{-1}. A similar plot was obtained when the hydrolysis was carried out at 23 °C under similar conditions and the two rate constants, k_{H_2O} and k_{OH^-}, were 1.0×10^{-7} and 1.5×10^{-1} L mol^{-1} min^{-1}, respectively.

At 50 °C, the hydrolysis products at pH 7 and below consisted of mixtures of 3-methyl-4-nitrophenol (**2**) and demethylfenitrothion (**3**). In contrast, only **2** was formed in reactions at pH 9 and above. The two reactions occurring are shown in eq 1. Attack of the OH^- ion at the



(1)

phosphorus atom results in cleavage of the P–O aryl bond (Faust and Gomaa, 1972; Blumenthal and Herbert, 1945) to give dimethylphosphorothioic acid (**4**) and **2**. Dealkylation of dialkyl aryl phosphorothioates is associated with the reaction of "soft" nucleophiles at the methyl carbon atom, followed by cleavage of the C–OP bond (Truchlik and Kovacicova, 1977). The dealkylation of fenitrothion has previously been observed in plants (Hallet

Table I. Rate Constant (k_{obsd}) for the Hydrolysis of Fenitrothion in Buffered Distilled Water at Various Temperatures with Light Excluded

	k_{obsd}, min^{-1}								
pH	23.0 °C	37.5 °C	49.7 °C	59.5 °C	pH	23.0 °C	37.5 °C	49.7 °C	59.5 °C
3			1.26×10^{-4}		9.0	1.91×10^{-5}		5.99×10^{-4}	
5	5.75×10^{-6}	3.28×10^{-5}	1.39×10^{-4}	3.86×10^{-4}	10.0	2.13×10^{-5}		7.08×10^{-4}	
7	6.63×10^{-6}		1.55×10^{-4}		11.00			4.89×10^{-3}	
7.5	1.05×10^{-5}				12.00	1.54×10^{-3}	4.67×10^{-3}	1.72×10^{-2}	2.05×10^{-2}
8.0	9.33×10^{-6}		2.39×10^{-4}						

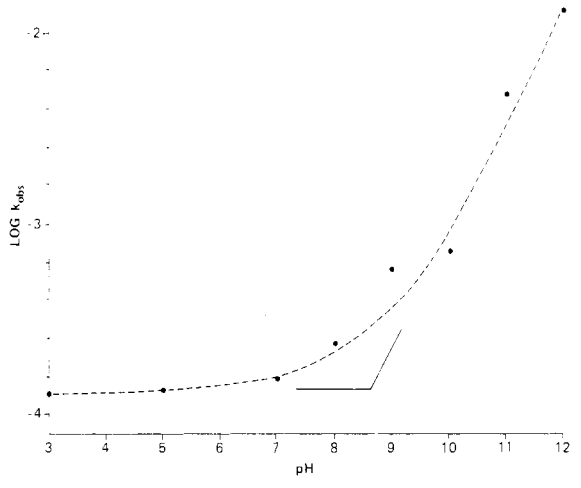


Figure 2. Plot of pH vs. observed rate for the hydrolysis of fenitrothion (10 ppm) at 49.5 °C and in the dark.

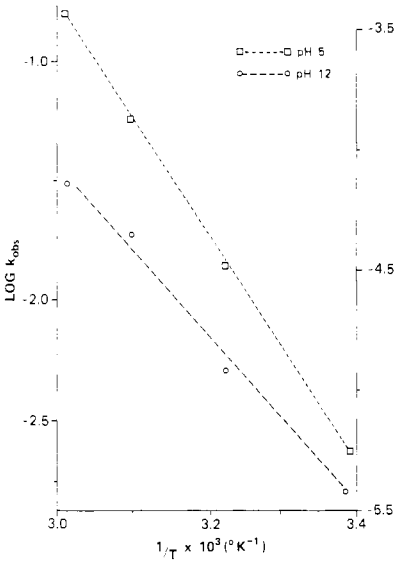


Figure 3. Arrhenius plot for the hydrolysis of fenitrothion in buffered distilled water (pH 5 and 12).

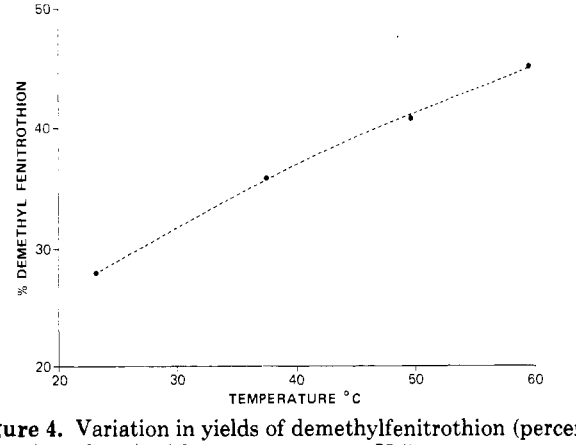


Figure 4. Variation in yields of demethylfenitrothion (percent of total products) with temperature at pH 5.

Table II. Rate Constants (k_{obsd}) and $t_{1/2}$ Values for the Hydrolysis of Fenitrothion in Lake Water

medium	temp, °C	k_{obsd}, min^{-1}	$t_{1/2}$, days
lake water	23	9.72×10^{-6}	49.5
	49.7	1.03×10^{-4}	4.7
buffered lake water	23	2.22×10^{-5}	21.6
	49.7	1.93×10^{-4}	2.5

et al., 1977) and animals (Hollingworth et al., 1967) where it serves as a deactivation process; in these instances, reaction is attributed to the SH moiety of glutathion alkyl transferase. In the case of the hydrolysis reactions, the nucleophile is presumably water. The dealkylation of other organophosphorus insecticides has also been reported in both sea and distilled water for parathion (Weber, 1976) and for methyl parathion in buffered and natural water systems (Smith et al., 1976).

The rate constants for the hydrolysis of fenitrothion (10 ppm) in buffered distilled water, pH 3–12, and at temperatures 23.0, 37.5, 49.7, and 59.5 °C are given in Table I. Activation parameters were calculated from the rate constants at pH 5 and 12 at different temperatures (Figure 3) and gave E_0 values of 22.6 and 18.0 kcal/mol, respectively, for the two reactions. The difference in activation energies for the two reactions could account for the fact that the amount of dealkylation taking place at any one pH is temperature dependent. This phenomenon is illustrated in Figure 4, which shows the amount of demethylfenitrothion as a percent of the reaction products formed at pH 5 for various temperatures; **2** was the other product in these reactions.

The hydrolysis of fenitrothion was also examined in natural lake water (pH 7.5) and buffered lake water at the same pH. These reactions followed first-order kinetics and the rate constants are given in Table II. Comparison of the rate constants at 23 °C indicates only a slight enhancement of k_{obsd} (2×) in the buffered system. This suggests that natural water systems have a considerable buffering capacity, especially for reactions involving contaminants at the ppm level.

In addition to the reaction products **2** and **3**, aminofenitrothion was formed in all the reactions involving lake water although it was never detected in the experiments with distilled water. This is in accord with field experiments where amino fenitrothion has been reported in river water (Vitko and Cunningham, 1974) and in the sediments of lakes and was attributed to bacterial action. In buffered lake water (23 °C), aminofenitrothion comprised 16% of the reaction products compared with less than 5% in natural lake water. It is possible that the buffers support the growth and activity of the microfauna present in the lake water.

In a field experiment recently reported (Greenhalgh et al., 1979), eight model systems, fabricated from PVC (1-m cubes), and open at the top were immersed in Lac Bour-

geous, Quebec. They were filled with lake water, algae, plants and sediment in various combinations. Each system was sprayed by hand with aqueous 10% fenitrothion EC formulation (1% Atlox 3409 and 1% Aerotex 3470) to simulate an aerial spray treatment equivalent to 4 oz/acre. The lake water temperature varied from 19–23 °C and the pH from 7.0–7.5 during the 14-day study. The lake water was analyzed periodically and a half-life of 1.5–2 days determined for fenitrothion in the systems. Despite absorption by the PVC, the observed half-life is consistent with the values, 0.73–5 days reported for shallow ponds (1 m deep) and small lakes (Symons, 1977). Thus, the half-life of fenitrothion in the field experiments is much less than the values determined in the laboratory study at the same pH and temperature but in the absence of light. Although surface evaporation (Marshall and Roberts, 1977), absorption by aquatic plants, algae and sediment will contribute to the disappearance of fenitrothion, the hydrolysis study and the formation of oxidation products in the field study strongly suggest that photolytic or microbial processes are the primary means of its removal from natural water systems.

ACKNOWLEDGMENT

The authors wish to acknowledge the technical help of M. Wilson.

LITERATURE CITED

Blumenthal, E., Herbert, J. B., *Trans. Faraday Soc.* **41**, 611 (1945).
Edit, D. C., Sundaram, K. M. S., *Can. Entomol.* **107**, 735 (1975).
Faust, S. D., Gomaa, H. M., *Environ. Lett.* **3**, 171 (1972).
Flannagan, J. F., *Manitoba Entomol.* **7**, 1 (1973).
Greenhalgh, R., Weinberger, P., Moody, R. P., presented at the Spray Drift Symposium, Fredericton, New Brunswick, Feb 18–21, 1979.
Hallet, D., Greenhalgh, R., Weinberger, P., Prasad, R. *J. Environ. Sci. Health B* **12**, 53 (1977).
Hollingworth, R. W., Metcalfe, R. L., Fukuto, T. R., *J. Agric. Food Chem.* **15**, 242 (1967).
Marshall, W. K., Roberts, J. R., Fenitrothion Symposium, Ottawa, Canada, April, 1977, N.R.C. Publication No. NRCC 16073, pp 253.
Moody, R. P., Greenhalgh, R., Lockhart, L., Weinberger, P., *Bull. Environ. Contam. Toxicol.* **78**, 8 (1978).
Nishizawa, Y., Fujii, K., Kadota, T., Miyamoto, J., Sakamoto, H., *Agric. Biol. Chem.* **25**, 605 (1961).
Ohkawa, H., Mikami, N., Miyamoto, J., *J. Agric. Biol. Chem.* **38**, 2247 (1974).
Ruzicka, J. H., *J. Chromatogr.* **31**, 37 (1967).
Smith, J. H., Mabey, W. R., Bohonos, N., Holt, B. R., Lee, S. S., Mill, T, Bomberger, D. C., presented at the 172nd National Meeting of the American Chemical Society, San Francisco, Aug 1976.
Sundaram, K. M. S., *Chem. Contr. Res. Inst., Ottawa, Inf. Rep. CC–X* **44** (1973).
Symons, P.E.K., *Res. Rev.* **68**, 1 (1977).
Truchlik, S., Kovacicova, J., Fenitrothion Symposium, Ottawa, Canada, April, 1977, NRC Publication No. NRCC 16073, pp 17.
Weber, K., *Water Res.* **10**, 237 (1976).
Vitko, V., Cunningham, T. D., Fish & Marine Serv., Environ. Can., Report 458, 1974.

Received for review June 29, 1979. Accepted September 4, 1979. C.B.R.I. Contribution No. 1107.

Enzymatic Detoxification of Waste Organophosphate Pesticides

Douglas M. Munnecke[1]

A bacterial enzyme preparation which could hydrolyze eight organophosphate pesticides was examined for its ability to detoxify pesticides derived from pesticide containers, spray tanks, and spray solutions and from industrial pesticide production facilities. The commercial pesticide formulations, parathion emulsifiable concentrate (EC) 48%, Dursban EC 50%, Diazinon EC 25%, cyanophos EC 50%, and methyl parathion wettable powder 25%, were examined at 0.02–0.04% (spray solution) and at 1% (container residue) concentrations. The enzyme preparation could hydrolyze these pesticides significantly faster than chemical hydrolysis procedures currently recommended, had a half-life in diluted formulated pesticide solutions ranging from 27 to 80 h, and was not strongly inhibited by the detergent and solvent ingredients in the commercial formulations. Residual amounts of formulated ethyl parathion in industrial pesticide containers could be enzymatically hydrolyzed (>95%) in 16 h using 13–16 mg of enzyme preparation/L of treated pesticide-containing waste water.

The use of pesticides throughout the world has been expanding quite rapidly since the first introduction of synthetic chemicals for use in crop protection, and growth rates of 6–8% per year (Doyle, 1975) are expected for the next several years. In 1980, it is estimated that 2.27 billion kg of pesticides will be produced (Munnecke, 1978). Hazardous wastes are generated in this section of the chemical industry at both the consumer and producer levels and in order to insure safe production, handling, and utilization of these agricultural chemicals, environmental guidelines and regulations governing these procedures have been established. Pesticide producers and formulators have a seemingly wide range of pesticide waste disposal technology available for meeting these air and water point source discharge requirements; however, once the pesticides have been distributed to the individual consumer, the methods available for the disposal and/or detoxification of excess pesticides, pesticide containers, and spray

Institut für Bodenbiologie, Forschungsanstalt für Landwirtschaft, 3300 Braunschweig, Germany.
[1] Present address: Department of Botany–Microbiology, University of Oklahoma, Norman, OK 73019.

0021-8561/80/1428-0105$01.00/0