the nature and concentration of terminal daminozide residues.

LITERATURE CITED

Blanpied, G. D., Smock, R. M., Liu, F. W., in "1977 and 1978 Cornell Fruit Handling and Storage Newsletter", Pomology Department, Cornell University, Ithaca, NY, 1977.
Edgerton, L. J., Rockey, M. L., Arnold, H., Lisk, D. J., *J. Agric. Food Chem.* **15**, 812 (1967).
Publication 360, Fruit Production Recommendations 1978, Ontario Ministry of Agriculture and Food, Information Branch, Legislative Buildings, Toronto, Ontario M7A 1A5, 1978.
Sharples, R. O., in "The Biology of Apple and Pear Storage", Fidler, J. C., Wilkinson, B. G., Edney, K. L., Sharples, R. O., Ed., Commonwealth Agricultural Bureaux, Research Review No. 3, 1973, p 175.
Toth, B., Wallcave, L., Patil, K., Schmeltz, I., Hoffmann, D., *Cancer Res.* **37**, 3497 (1977).
Toth, B., Wilson, R. B., *Am. J. Pathol.* **64**, 585 (1971).

Brian D. Ripley*
Everet C. Lougheed[2]
Sherwood R. Miller[3]

[1]Provincial Pesticide Residue Testing Laboratory
Ontario Ministry of Agriculture and Food
c/o University of Guelph
Guelph, Ontario Canada N1G 2W1
[2]Department of Horticultural Science
University of Guelph
Guelph, Ontario, Canada N1G 2W1
[3]Agriculture Canada
Smithfield Experimental Farm
Trenton, Ontario, Canada K8V 5R5

Received for review January 23, 1979. Accepted March 28, 1979.

A Change in the Degradation Pathway of Parathion after Repeated Applications to Flooded Soil

The degradation pathway of parathion shifted to hydrolysis from reduction after repeated applications of parathion or its hydrolysis product, *p*-nitrophenol, to a flooded soil. This shift occurred as a result of the proliferation of parathion-hydrolyzing microorganisms that utilized *p*-nitrophenol as the energy source. This is probably the first report of the enrichment of a population capable of degrading a parent molecule upon application of the primary product of its metabolism.

The major pathways in the degradation of parathion in soil and water environments are nitro group reduction (Lichtenstein and Schulz, 1964) and hydrolysis (Sethunathan et al., 1977), both mediated essentially by microorganisms. It is well established that microbial populations with pesticide-degrading potentials readily build up in soil and water systems following repeated applications of a wide array of pesticides which serve as sources of carbon and energy for growth (Waid, 1972). But a shift from one pathway to another following repeated applications of a pesticide characterized by more than one degradation pathway as in parathion is not known. We report here a clear shift in the degradation pathway of parathion from nitro group reduction to hydrolysis after the repeated application of parathion or its hydrolysis product, *p*-nitrophenol, to a predominantly anaerobic flooded soil.

EXPERIMENTAL SECTION

At 15-day intervals, 1.72 μmol of parathion was added to 20 g of alluvial soil contained in sterile test tubes (200 × 25 mm) and the soil was flooded with 25 mL of sterile distilled water. At periodic intervals after each parathion addition, parathion and its predicted degradation products, aminoparathion and *p*-nitrophenol, were analyzed in duplicate soil samples. For quantifying nitro group reduction, parathion and aminoparathion were extracted from soil samples with methanol–acetone–benzene (1:1:1) and the residues in the benzene fraction were analyzed in a Perkin Elmer gas chromatograph Model 3920 equipped with flame photometric detector specific for phosphorus. Using this procedure, the recoveries of parathion and aminoparathion immediately after application to the soil ranged between 85 and 95%. In studies dealing with the hydrolysis of parathion, parathion and *p*-nitrophenol were extracted from the soil samples with chloroform–diethyl ether (1:1) and then analyzed after separation by thin-layer chromatography. *p*-Nitrophenol from the chromatogram was eluted directly in 0.1 N NaOH while parathion was converted first to *p*-nitrophenol by alkaline hydrolysis (Sudhakar-Barik and Sethunathan, 1978). *p*-Nitrophenol was measured at 400 nm. The recoveries of known quantities by this method ranged from 72 to 86% for parathion and from 78 to 90% for *p*-nitrophenol.

RESULTS AND DISCUSSION

The rate of degradation of parathion in a flooded soil increased progressively after each successive application (Figure 1). Interestingly, aminoparathion was recovered as the major product after the first addition of parathion, both aminoparathion and *p*-nitrophenol after the second addition and *p*-nitrophenol alone after the third addition (Table I). Thus the data clearly demonstrated that the pathway in the degradation of parathion shifted from reduction to hydrolysis after its repeated application to a flooded soil. The *p*-nitrophenol formed was metabolized subsequently as indicated by its disappearance, in agreement with an earlier report (Siddaramappa et al., 1973). The degradation of parathion in flooded soil followed first-order kinetics for all applications irrespective of the pathway involved (Figure 1), but the kinetic constant gave the indication that hydrolysis of parathion proceeded at a remarkably faster rate than reduction.

Tenfold dilutions of the soil were prepared at 5 days after each addition of parathion and 1 mL of each dilution was added to 15 mL of a sterile mineral salts medium (Sethunathan, 1972) supplemented with 0.69 μmol of parathion for most-probable-number (MPN) estimates of the population capable of hydrolyzing parathion. Uninoculated medium served as control. Direct assay of *p*-nitrophenol formed in the medium was not possible because of

0021-8561/79/1427-1391$01.00/0

Table I. Shift from Nitro Group Reduction to Hydrolysis after Repeated Additions of Parathion to Flooded Soil[a]

incubation after every parathion addition	recovered, μmol/20 g of soil					
	1st parathion addition		2nd parathion addition		3rd parathion addition	
	aminoparathion	p-nitrophenol	aminoparathion	p-nitrophenol	aminoparathion	p-nitrophenol
1 day	0	0	0.48 ± 0.03	0.30 ± 0.04	0	0.92 ± 0.02
3 days	0.11 ± 0.03	0	0.51 ± 0.03	0.27 ± 0.02	0	0.07 ± 0.01
7 days	0.30 ± 0.02	0	0.74 ± 0.02	0	0	0
15 days	0.39 ± 0.02	0	0.37 ± 0.02	0	0	0

[a] Parathion (1.72 μmol) was added to the soil at 15-day intervals. The rate of disappearance of parathion after each of the three additions is indicated in Figure 1.

Table II. Shift in the Pathway of Parathion Degradation in Flooded Soils Pretreated with p-Nitrophenol[a]

incubation after parathion addition, days	recovered, μmol/20 g of soil					
	soil not pretreated with p-nitrophenol			soil pretreated with p-nitrophenol		
	parathion	p-nitrophenol	aminoparathion	parathion	p-nitrophenol	aminoparathion
0	1.67 ± 0.02	0	0	1.63 ± 0.01	0	0
0.5	1.65 ± 0.02	0	0	1.38 ± 0.03	0.29 ± 0.03	0
1	1.65 ± 0.03	0	0	0.46 ± 0.02	1.19 ± 0.05	0
3	0.69 ± 0.01	0	0.86 ± 0.01	0.30 ± 0.01	0	0
12	0.26 ± 0.03	0	0.81 ± 0.01	0	0	0

[a] Parathion (1.72 μmol) was added to flooded soil that was never treated with p-nitrophenol or was previously treated with two applications of p-nitrophenol. Pretreatment of the soil with p-nitrophenol led to accelerated disappearance of parathion and a distinct shift to hydrolytic pathway.

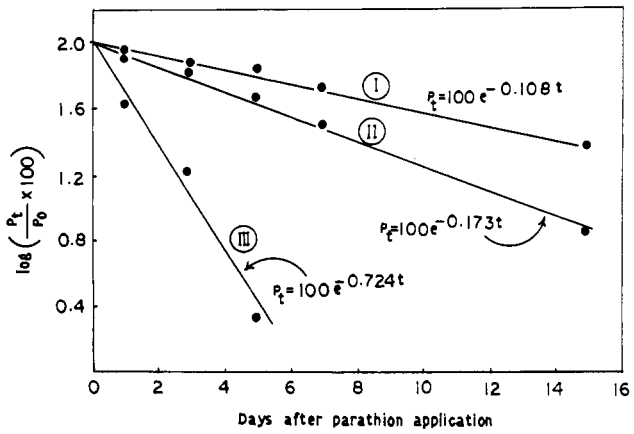

Figure 1. The rate of disappearance of parathion from a flooded soil after three (I, II, III) successive applications. The sharp slope in parathion degradation after application III was associated with the shift in its pathway to hydrolysis as indicated in Table I. P_0 and P_t indicate the concentrations of parathion at the start and at different intervals (t) after parathion addition.

its further metabolism to nitrite (Siddaramappa et al., 1973). The formation of nitrite after 14 days was therefore used as the index of parathion-hydrolyzing activity since nitrite is liberated from parathion by microorganisms only after a primary hydrolysis (Siddaramappa et al., 1973). Parathion was hydrolyzed in the medium receiving appropriate soil dilutions prepared after two or three additions of the insecticide; no hydrolysis occurred in uninoculated medium. The population of microorganisms hydrolyzing parathion increased from undetected levels after one addition to 14×10^6 and $>4300 \times 10^6$/g of soil after two and three additions, respectively. The hydrolysis of parathion by soil dilutions used in MPN estimates was certainly not chemical since even 10^{10} dilution of the soil prepared after three additions of the insecticide readily hydrolyzed parathion during 15-day incubation. Moreover, soil dilutions lost their ability to hydrolyze parathion after autoclaving. In fact, a *Pseudomonas* sp. ATCC 29353 isolated from the same soil hydrolyzed parathion with ease and then liberated nitrite (Siddaramappa et al., 1973). The buildup of a population capable of hydrolyzing parathion after repeated additions of the insecticide is worth noting since the hydrolysis of parathion is not an energy-yielding reaction.

In another experiment to explain the mechanism of the buildup of parathion-hydrolyzing microorganisms, 20-g flooded soil samples were treated with 7.19 μmol of p-nitrophenol at 15-day intervals. Parathion (1.72 μmol) was added to the soils, 12 days after the second application of p-nitrophenol. Interestingly, the degradation pathway of parathion shifted from reduction to hydrolysis in soils pretreated with p-nitrophenol (Table II). Evidently, parathion-hydrolyzing microorganisms proliferated during the metabolism of p-nitrophenol. This would explain the rapid buildup of parathion-hydrolyzing microorganisms and the resulting shift to hydrolysis in soils upon repeated applications of parathion. Enrichment of microorganisms capable of degrading a parent molecule upon the application of its breakdown product as reported in this study is perhaps new to the literature.

ACKNOWLEDGMENT

We thank H. K. Pande for facilities and C. A. Edwards, Rothamsted Experimental Station, England, for comments on the manuscript.

LITERATURE CITED

Lichtenstein, E. P., Schulz, K. R., *J. Econ. Entomol.* **57**, 618 (1964).
Sethunathan, N., *Adv. Chem. Ser.* **No. 111**, 244 (1972).
Sethunathan, N., Siddaramappa, R., Rajaram, K. P., Barik, S., Wahid, P. A., *Residue Rev.* **68**, 91 (1977).
Siddaramappa, R., Rajaram, K. P., Sethunathan, N., *Appl. Microbiol.* **26**, 846 (1973).
Sudhakar-Barik, Sethunathan, N., *J. Environ. Qual.* **7**, 345 (1978).
Waid, J. S., *Residue Rev.* **44**, 65 (1972).

Sudhakar-Barik
P. A. Wahid
C. Ramakrishna
N. Sethunathan*

Laboratory of Soil Microbiology
Central Rice Research Institute
Cuttack-753006, India

Received for review December 14, 1978. Accepted May 14, 1979. Supported by grants from the International Atomic Energy Agency, Vienna, the Department of Atomic Energy, Bombay, and the Department of Science and Technology, New Delhi.