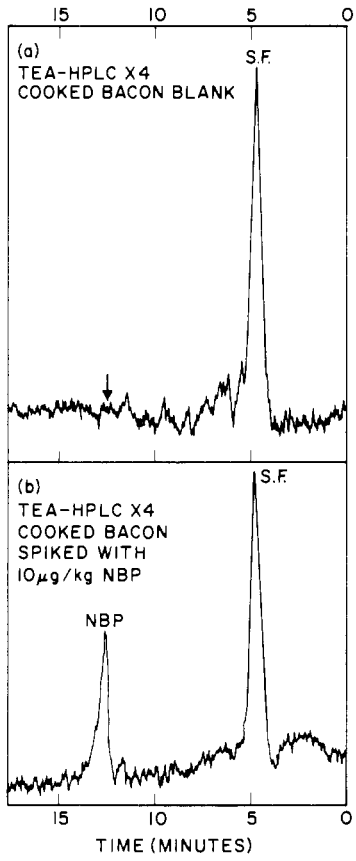

Figure 2. TEA-HPLC chromatograms of cooked bacon extracts using 2 ml/min of 1% acetone in 2,4,6-trimethylpentane on a μm Porasil column, with the TEA on attenuation ×4: (a) the cooked bacon blank; (b) a similar following recovery of 10 μg/kg of *N*-nitrosobenzylphenylamine.

N-nitroso compounds in complex foodstuffs and other environmental samples. Application of these procedures will provide previously unobtainable data on environmental levels of nonionic nonvolatile *N*-nitroso compounds. Several as yet unknown TEA responsive compounds are found to be present in all liquor, fish, and meat samples analyzed so far. The identity of the unknown compounds needs to be determined, because, given the specificity of the TEA, there is a high probability that some of the unknowns may correspond to *N*-nitroso compounds. Although the ability to evaluate environmental levels of *N*-nitroso compounds is now greatly extended, determination of total *N*-nitroso levels must still await adequate methodologies for highly ionic *N*-nitroso compounds.

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Mechanism of Degradation of Thiofanox in Aqueous Solutions

Thiofanox (P), 3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime, is stable in acidic and neutral solutions. At pH 10, it undergoes a two-step oxidation to its sulfone derivative with subsequent hydrolysis of the sulfone. The rate of hydrolysis of the sulfone derivative at pH 10 was determined at 4, 25, and 45 °C, but no direct hydrolysis of P at pH 10 was detected.

Thiofanox (P), 3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime, is an effective systemic and contact insecticide. Metabolic studies in plants (Whitten and Bull, 1974; Chin et al., 1976), animals (Tallant and Sullivan, 1974), and soils (Duane, 1974) indicated that the two-step oxidation of P to its sulfoxide derivative (P_1), 3,3-dimethyl-1-(methylsulfinyl)-2-butanone *O*-[(methylamino)carbonyl]oxime, and sulfone derivative (P_2), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone *O*-[(methylamino)carbonyl]oxime, is the primary mode of degradation. In most cases, only P_2 was found to undergo hydrolysis to its oxime (O_2), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone oxime. Although hydrolysis of P_1 to its oxime (O_1), 3,3-dimethyl-1-(methylsulfinyl)-2-butanone oxime, was occasionally detected, hydrolysis of P to its oxime (O), 3,3-dimethyl-1-(methylthio)-2-butanone oxime, was seldom detected in meaningful quantities in the environmental studies of P (Holm et al., 1975). The purpose of this investigation was to study the mechanism of the degradation of P in aqueous solutions by comparing the

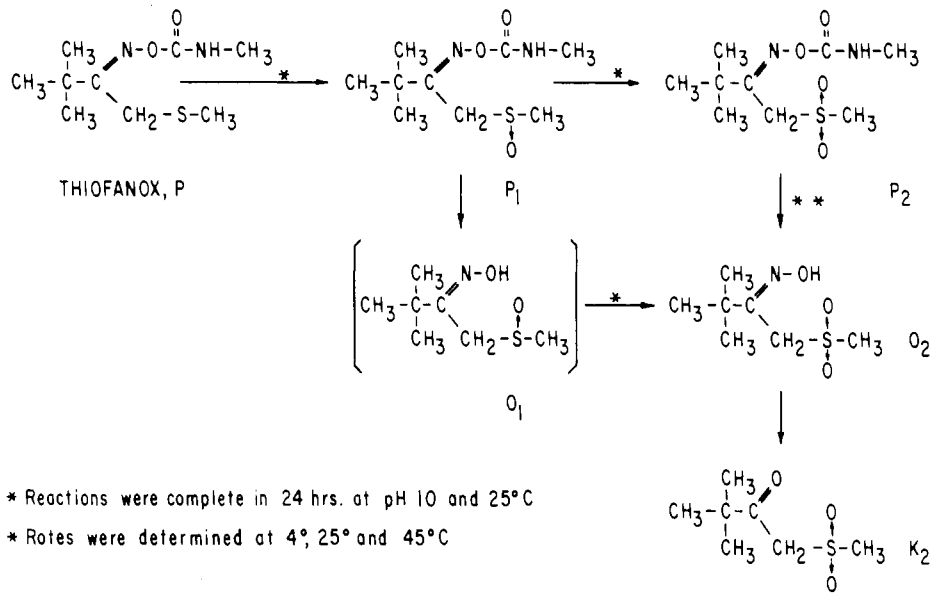

Figure 1. Mechanism of degradation of thiofanox in pH 10 solutions.

rates of oxidation and hydrolysis of P, P_1, and P_2 at pH 10 and various temperatures.

EXPERIMENTAL SECTION

Reagents and Apparatus. Analytical standards of P and its metabolites P_1, P_2, O, O_1, and O_2 and the ketone (K_2), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone, were synthesized. Carbon-14 tracer analogues of P, P_1, and P_2 were prepared by labeling the methyl carbon attached to the sulfur atom. The specific activities were 17.9 to 27.5 mCi/g. All the standards were greater than 99% pure. Test mixtures were made to pH 10 with a pH 10 buffer solution (Fisher Scientific Co.). Analysis was conducted using thin-layer chromatography and autoradiography. The TLC plates were precoated silica gel on aluminum (Brinkman Instruments, Inc.). The chromatography solvent system was a mixture of *n*-hexane and acetone (7:3, v/v). The developed plates were exposed to x-ray films (SB-54, Kodak) for 2–4 weeks. The spots on the plates were cut for the determination of radioactive distribution by liquid scintillation counting. All test data represent averages of five replicates and each set of replicates included untreated controls as standards. According to both TLC radioautography and subsequent counts, all standards proved to be stable throughout the analytical procedures. At room temperature, losses of the standards through volatilization were found to be insignificant.

TEST PROCEDURES

Stability of P, P_1, and P_2 in Neutral and Acidic Solutions. The water solubility of P at 22 °C was measured as 5200 ppm, and P_1 and P_2 are readily soluble in water. To study the stability of these three carbamates, both ^{14}C and nonradioactive standards were dissolved in deionized water and 10^{-3} N HCl and stored in the dark at 4 and 25 °C. TLC tests and countings proved that no changes of these carbamates took place even after 10 weeks. This indicates that these three carbamates are stable in neutral and acidic solutions at 25 °C or lower temperatures. In order to study the mechanism of the degradation of these carbamates, the rest of the experiments were conducted at pH 10.

Oxidation of P and P_1 to P_2. Approximately 15 000 dpm of [^{14}C]P (0.4 μg of P) in 3.5 μl of 10^{-3} N HCl was mixed with 2.0 ml of pH 10 buffer solution in each of eight 60-ml separatory funnels. At room temperature and after different time intervals, 20 ml of 0.2 N HCl was added and the radioactivity was extracted with 2 × 10 ml of $CHCl_3$. The $CHCl_3$ extract was dried over Na_2SO_4 and evaporated to dryness at room temperature with a gentle current of dry N_2 gas. The residues were dissolved in benzene for TLC analysis. Similar tests were conducted with [^{14}C]P_1 and [^{14}C]P_2.

Table I. Degradation of Thiofanox in pH 10 Solutions

Compds	R_f	Hourly test (25 °C), %			Daily test (4 °C), %	
		<5 s	1 h	3 h	1 day	5 days
P	0.48	60.8	50.5	43.8	0.0	0.0
P_1	0.06	27.9	42.0	44.9	0.0	0.0
P_2	0.25	1.5	1.8	4.3	93.5	90.0
O	0.57	0.2	0.2	0.2	0.0	0.0
O_1	0.15	1.8	4.0	2.9	0.0	0.0
O_2	0.36	0.1	0.1	0.1	4.5	5.4
K_2	0.42	0.0	0.0	0.0	0.0	0.6
Recovery[a]		92.3	98.6	96.2	98.0	96.0

[a] For each test, 15 000 dpm of [^{14}C]P was used and was considered as 100% in spot countings.

Kinetic Hydrolysis of P_2 to O_2. Approximately 4 × 10^5 dpm of [^{14}C]P (1.0 μg of P) was mixed with 25 ml of pH 10 buffer solution in a 50-ml flask. Three flasks were prepared and tightly sealed during their incubation at 4, 25, and 45 °C in the dark. At different time intervals, 1.0 ml of each of the radioactive solutions was taken and mixed with 20 ml of 0.2 N HCl in a 60-ml separatory funnel. The radioactivity was extracted with 2 × 10 ml of $CHCl_3$ which was dried over Na_2SO_4 and evaporated to dryness at room temperature for TLC analysis.

RESULTS AND DISCUSSION

Degradation of P at pH 10 and 25 °C. Knowing that the degradation of P at pH 10 and 25 °C is rapid, this experiment was conducted on an hourly time basis. Results obtained are summarized in Table I which indicate that in less than 5 s, one-third of the applied [^{14}C]P is degraded mainly to P_1 with minor quantities of O_1 and P_2. The decrease in P and the corresponding increase in P_1 during the first 3 h indicate that the first-step oxidation of P to P_1 proceeds rapidly under the conditions studied. However, the second-step oxidation of P_1 to P_2 is relatively

slower since only 4.3% of P_2 had accumulated by the end of the 3-h test. The insignificant amount of O detected in this test indicates that hydrolysis of P to O is negligible under these conditions. This may explain the infrequent detection of O in the environmental studies of P. On the other hand, the detection of O_1 indicates partial hydrolysis of P_1 to O_1. Since the quantities of both P_1 and O_1 do not increase with time, further oxidation of P_1 to P_2 and O_1 to O_2 may be proceeding subsequently. Of all the degradation products identified in Table I, only P_2 shows a slow, but consistent increase in quantity with time. This suggests that P_2 is the major, stable product derived from P under the conditions studied.

Degradation of P at pH 10 and 4 °C. The mechanism of the degradation of P at pH 10 was further elaborated by a 5-day time course study as shown in Table I. The hourly results showed the approximate difference in rates of the oxidation of P to P_1 and P_1 to P_2. The daily results show that the two-step oxidation of P to P_2 is complete after the first 24 h, even at 4 °C. This rapid oxidation was confirmed by testing [^{14}C]P_1 under the same conditions. The daily results demonstrated that after 24 h, both P and P_1 had disappeared and that P_2 was the major product. The secondary residue, O_2, may be derived in two ways. It may come from the direct hydrolysis of P_2 or by hydrolysis of P_1 to O_1 and subsequent oxidation of O_1 to O_2. The hydrolysis of P_2 as the principal mechanism for producing O_2 was proved by testing [^{14}C]P_2 under the same conditions. On the other hand, the O_1 present in the hourly study was absent in the daily study, indicating rapid oxidation of O_1 to O_2 at least within 24 h, under the conditions studied. The infrequent detection of O_1 in environmental studies involving thiofanox may then be explained by the observation that O_1 was produced in limited quantities by partial hydrolysis of P_1, followed by the rapid oxidation to O_2 as shown here. In addition, a small quantity of K_2 was detected during the fifth day of the daily test. This indicated subsequent deoximation of O_2 under the conditions studied.

Kinetic Hydrolysis of P_2 at pH 10. Based on the information obtained in the above experiments, it is seen that the oxidation of P to P_2 and the partial hydrolysis of P_1 to O_1 and subsequent oxidation of O_1 to O_2 are all complete in 24 h at pH 10 and 4 to 25 °C. The remaining reaction rate of interest that can be determined from these data is the hydrolysis of P_2 to O_2. The pseudo-first-order rate constants for hydrolysis of P_2 to O_2 at pH 10 and 4, 25, and 45 °C were determined to be 0.0036, 0.0653, and 0.2427 per day with half-lives of 192.5, 10.6, and 2.9 days, respectively. An overall summary of the degradation of P at pH 10 is given in Figure 1. Because P_2 is the most significant degradation product of P and has the highest toxicity of the three carbamates, the hydrolysis of P_2 to O_2, which toxicity is 386 times less than that of P_2 (Chin et al., 1975), may be regarded as a detoxifying process.

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Selenium Content of Bangladeshi Rice by Chemical and Biological Assay

Rice samples from three districts of Bangladesh were analyzed for selenium by bioassay (prevention of liver necrosis in rats) and by chemical analysis. The latter gave a range of 0.06–0.17 ppm of selenium, whereas a U.S. sample had 0.46 ppm. Bioassay of samples from both countries indicated that not all the selenium was biologically active.

Selenium is now recognized as an essential trace element for many species, including man, and its biochemical role as a cofactor for the enzyme glutathione peroxidase has been established (Rotruck et al., 1973). The selenium content of foodstuffs has thus taken on new significance, and is particularly important in populations in which usual diets rely heavily on a few commodities. Such a situation exists in Bangladesh, where rice provides 85% of the calories and 68% of the protein in the rural population (Nutrition Survey of East Pakistan, 1966).

METHODS

Rice samples were purchased in the market in three different districts of Bangladesh: Barisal, Mymensingh, and Comilla. In Barisal, samples included white rice, brown rice, and bran; the samples from the other districts were white or brown rice only. For comparison, a sample of U.S. Louisiana white rice was included.

The bioassay for selenium was based on the prevention of liver necrosis in rats by dietary selenium. Female Fisher strain rats with litters 1 week old were kept in plastic cages with wood chips. Over a 3–4 day period, a selenium and vitamin E deficient torula yeast diet (Schwarz, 1951; Bieri and Poukka Evarts, 1974) was mixed in increasing proportions into the ground stock ration until the mothers were consuming only the deficient diet. The young rats were weaned when 21 days old and were caged individually in suspended cages and fed the experimental diets ad libitum. Addition of finely ground rice was made at the expense of the sucrose component. To estimate the selenium content of rice samples, the incidence of death due to liver necrosis or hemorrhage and the average survival