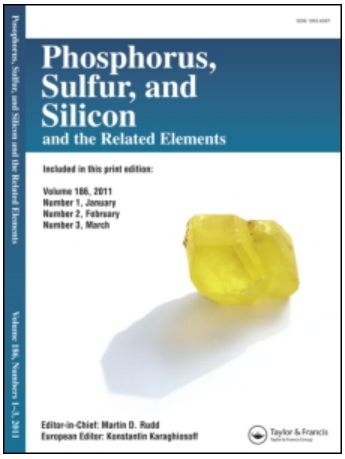

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Photolysis, Oxidation, and Hydrolysis of ^{14}C-Ethyl Prothiofos [*O*-(2, 4-Dichlorophenyl) *O*-Ethyl *S*-Propyl Phosphorodithioate]

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PHOTOLYSIS, OXIDATION, AND HYDROLYSIS OF ^{14}C-ETHYL PROTHIOFOS [*O*-(2, 4-DICHLOROPHENYL) *O*-ETHYL *S*-PROPYL PHOSPHORODITHIOATE]

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The chemical stability of the widely used organophosphorus insecticide ^{14}C-prothiofos was studied. For this study prothiofos insecticide and some of its degradation products have been prepared. The oxon of the parent compound was obtained through the oxidation of the insecticide with various oxidizing agents, where yields depended on the type and nature of oxidizing agent. The effect of ultraviolet light and direct sunlight on prothiofos were investigated. Exposure to direct sunlight caused gradual degradation of prothiofos giving the same products as UV irradiation.

The hydrolysis of ^{14}C-prothiofos at pH 5, 7, 8, and 9 in buffered aqueous media at 25°C, 40°C and 55°C was studied. The results indicated that prothiofos was stable in acid medium, but it was hydrolyzed to 2,4-dichlorophenol and O-ethyl S-propyl phosphorodithioate in alkaline medium.

The degradation products identified by TLC and GC/MS were prothiofos oxon, O-ethyl S-propyl phosphordithioate, des-propyl thioprothiofos, prothiofos oxon sulfoxide, and one unknown compound in addition to the parent compound. The phenolic compound was identified by GC/MS and by its color.

Supplemental materials are available for this article. Go to the publisher's online edition of* Phosphorus, Sulfur, and Silicon and the Related Elements *to view the free supplemental file.

Keywords ^{14}C-Prothiofos; degradation products; hydrolysis and oxidation; sunlight; UV light

INTRODUCTION

Organophosphorus pesticides are widely used in modern agriculture as an alternative to organochlorines for pest control. This common class of compounds represents more than one-third of the total insecticides used in the world[1] and acts via inhibition of the enzyme cholinesterase, which plays a key role in the insect's nervous system.[2]

Among factors affecting the persistence of pesticide in the environment (i.e., oxidation, metabolism in plants and animals, bacteriological degradation, hydrolysis), photolysis is one of the foremost.[3,4] In this regard, an increasing number of articles devoted to the photodedegradation of pesticides have appeared in the literature in recent years.[5–9]

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Previous studies have focused on the hydrolysis of organophosphorus pesticides and revealed that hydrolysis is pH- and temperature-dependent.[10,11] In the environment, hydrolysis of organic chemicals occurs in dilute solution. Under these conditions, water is present in large excess, and the concentration of water is essentially constant during hydrolysis. Hence the kinetics of hydrolysis is pseudo first-order at a fixed pH.

Phosphorothioate sulfoxides are proposed as the toxicologically relevant bioactivation products of many phosphorothioate pesticides, but they have not been characterized from biological systems.[12] The oxidative bioactivation of phosphorothioates may reflect their conversion to more potent phosphorylating agents upon treatment with peracids.[13]

The present work has been carried out to investigate the following:

(i) Effect of sunlight, ultraviolet radiation, and various pH levels on the stability of prothiofos
(ii) The effect of different oxidizing agents on the stability of prothiofos insecticide

RESULTS AND DISCUSSION

Photolysis of Prothiofos

Effect of sunlight. The percentage loss of ^{14}C-labeled prothiofos after exposure to direct sunlight for one week was 73% then increased to 100% at the end of the experiment period (Figure 1). The parent compound almost disappears after one month exposure to sunlight; when ^{14}C-labeled prothiofos was kept in darkness, the insecticide suffered no change after one month. The half-life time period $T_{1/2}$ for sunlight reaction is 2.81 days.

These finding are similar to the data obtained by El-Sayed et al.[14] in their studies about the persistence and nonbiological transformation of selecron, cyolane, and reldan insecticides as affected by sunlight. Thin layer chromatography showed the degradation products of ^{14}C-labeled prothiofos (63 μg) after exposure to direct sunlight at different time intervals and were identified as the parent compound, its oxon, and one unknown compound (Table I).

These findings are similar to the data obtained by GC/MS after one month exposure to direct sunlight [prothiofos oxon (**II**), RT 36.64 minutes and its fragments at m/z 328, 294, 266, 224, 161, 142, and 114]. Iwao et al.[15] found that exposure to sunlight caused gradual

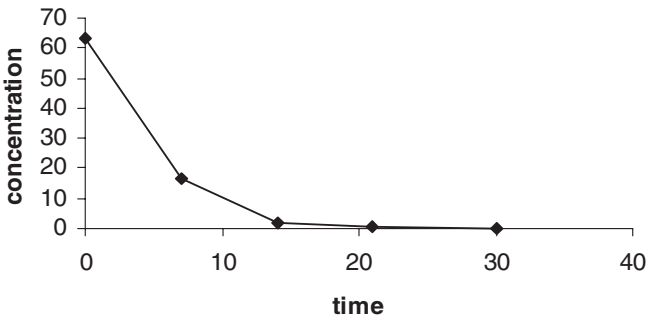


Figure 1 Effect of sunlight on prothiofos insecticide after different times.

Table I Degradation products of ^{14}C-labeled prothiofos (63 μg) after exposure to direct sunlight at different time intervals

	R_f values in various solvent system				Concentration							
					Week		2 Weeks		3 Weeks		1 Month	
Degradation products	1	2	3	4	(μg)*	%**	(μg)*	%**	(μg)*	%**	(μg)*	%**
Prothiofos (**I**)	0.56	0.47	0.21	0.80	16.7	26.5	2	3.2	0.4	0.7	0	0
Prothiofos oxon (**II**)	0.01	0.06	0.00	0.52	11.2	17.8	7.4	11.7	3.7	6	2	3.2
Unknown	0.85	0.45	0.65	0.49	1.7	2.6	4.2	6.6	3	5	0.4	0.6

System 1: *n*-hexane:ethyl acetate, 99.5:0.5 (v/v).
System 2: *n*-heptane:ethylacetate, 99:1 (v/v).
System 3: *n*-heptane pure.
System 4: *n*-heptane:chloroform:methanol, 9:4:1 (v/v).
*Data are the average of two replicates.
**Percent remaining of prothiofos and its degradation products.

degradation of prothiofos, giving the same products (oxon, despropylthio prothiofos, and 2,4-dichlorophenol).

Effect of UV light. Photodecomposition is only one of the various transformation processes determining the fate of pesticides in the environment. It may occur in water and air as well as on target organisms and on soil surface. Useful data on the photolysis of pesticides in water are available.[16] We focused our investigation on alkaline medium (pH = 8), which is similar to the pH of canal water.[17] Photodegradation of organophosphorus insecticides depended on the exposure time to ultraviolet rays. The percent loss of ^{14}C-labeled prothiofos increases as the time of exposure increases and reaches 99% after 6 h of exposure (Table II). The half-life time period was calculated from the slope of the straight line, which represented the logogrammatic relation between the concentration of prothiofos and reaction time; we found that $T_{1/2}$ of prothiofos is 1.93 h.

TLC and GC/MS analysis of extracts of photodegradation products showed seven degradation products in addition to the parent compound (Scheme 1; see also Table S1 in the Supplemental Materials, available online) and were identified as prothiofos oxon, *O*-ethyl phosphorothioate, *O*-ethyl *S*-propyl phosphorothioate, *O*-ethyl phosphoric acid, prothiofos oxon sulfoxide, *O*-ethyl *S*-propyl phosphorodithioate, and one unknown compound, which was found in the organic layer, and has R_f values (0.85, 0.45, 0.65, and 0.49) in the following solvent systems: *n*-hexane:ethyl acetate, 99.5:0.5; *n*-heptane:ethyl acetate, 99:1; *n*-heptane only, and *n*-heptane:chloroform:methanol 9:4:1, respectively.

Oxidation of Prothiofos

Table III shows the effect of different oxidizing agents on ^{14}C-labeled prothiofos. Nitric acid oxidized 64% of the insecticide after 2 h at 25°C. When the reaction was refluxed for 3 h using hydrogen peroxide, the yield of oxygen analogue was 39%. When dimethylsulfoxide and/or potassium permanganate were used as oxidizing agents, the yield of oxon was 36% and 23%, respectively.

The behavior of P = S organophosphorus compounds towards different oxidizing agents to give P = O products has been studied.[18] Prothiofos oxon (**II**), *O*-ethyl *S*-propyl phosphorodithiote (**V**), *O*-ethyl *S*-propyl phosphoric acid, and prothiofos oxon sulfoxide were detected by the thin layer chromatographic technique. In all cases, these results are

Table II Degradation products of ^{14}C-labeled prothiofos (252 μg) after exposure to UV light

					Concentration							
	R_f values in systems				1 h		2 h		4 h		6 h	
Degradation products	1	2	3	4	(μg)*	%**	(μg)*	%**	(μg)*	%**	(μg)*	%**
Prothiofos (**I**)	0.56	0.47	0.21	0.80	160	63.4	120	47.6	60	23.8	2.3	1
Prothiofos oxon (**II**)	0.01	0.06	0.00	0.52	41	16.3	11.6	4.6	2.4	1	1.6	1.3
O-Ethyl phosphorothioate (**VII**)	0.21	0.52	0.45	0.60	9.6	3.8	15	5.9	32	0	3.2	0
O-Ethyl *S*-propyl phosphorothioate (**IX**)	0.43	0.41	0.32	0.85	15.7	6.2	22	8.7	0	8	0	7.1
O-Ethyl phosphoric acid (**VIII**)	0.77	0.65	0.60	0.89	6.4	0.0	25	9.9	20	4.2	18	3.5
Prothiofos oxon sulfoxide (**X**)	0.96	0.81	0.46	0.42	0	0	0	0	10.6	0.7	8.7	0.8
O-Ethyl *S*-propyl phosphorodithioate (**V**)	0.68	0.65	0.35	0.83	0	0	0	0	1.8	0	2	0.6
Unknown	0.85	0.45	0.65	0.49	0	0	0	0	0	0	1.6	0

*Data are the average of two replicates.
System 1: *n*-hexane:ethyl acetate, 99.5:0.5 (v/v).
System 2: *n*-heptane:ethylacetate, 99:1 (v/v).
System 3: *n*-heptane pure.
System 4: *n*-heptane:chloroform:methanol, 9:4:1 (v/v).
**Percent remaining of prothiofos and its degradation product.

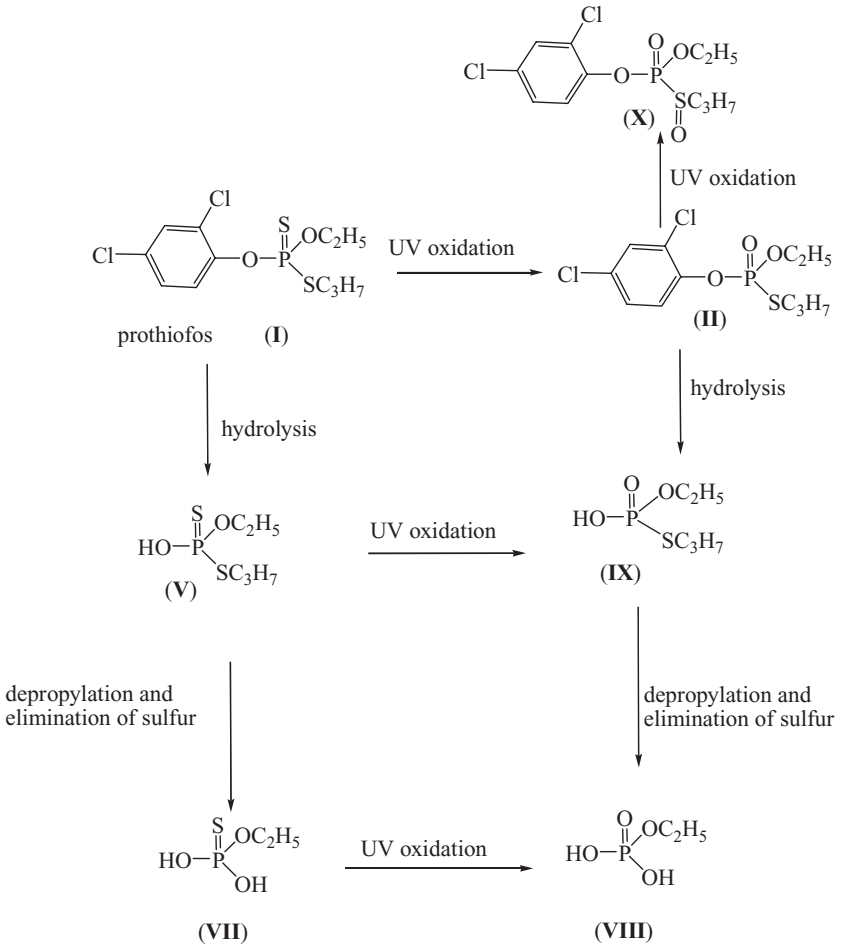
in agreement with those obtained from GC/MS as listed in Table II. These results are in accordance with those reported by Fakhr et al.[18] in their studies about the stability of organophosphorus insecticides actellic and malathion. In addition to oxidation, hydrolysis reactions occur and lead to the formation of *O*-ethyl *S*-propyl phosphorodithiote (**V**), *O*-ethyl *S*-propyl phosphoric acid, and prothiofos oxon sulfoxide. Prothiofos was oxidized by peracids, giving not only oxon but also other degradation products. Segall and Casida[19] and Miyamoto[20] found that profenofos insecticide underwent oxidation with *m*-chloroperbenzoic acid to give 86% of profenofos sulfone. Also, oxidation of diazinon by aqeuoes chlorine leads to the formation of diazoxon.[21]

Phosphorothioate sulfoxides are proposed as the toxicologically relevant bioactivation products of many phosphorothioate pesticides, but they have not been characterized from biological systems.[12] The oxidative bioactivation of phosphorothioates may reflect their conversion to more potent phosphorylating agents upon treatment with peracids.[13]

Hydrolysis of Prothiofos

Surprisingly, there are only a few data on the reactivity of organophosphorus compounds in aquatic systems despite their prevalence in the environment. Hydrolysis pathways, for which acids, bases, or neutral species serve as catalysts, have been proposed for phosphoric and thiophosphoric trimesters.[11]

We found that prothiofos is not as resistant to hydrolysis. Under basic conditions, it hydrolyzed more rapidly than under acidic conditions. The hydrolysis reaction and pathways are given in Scheme S1 (Supplemental Materials).



Scheme 1 Some degradation products resulting from irradiation of ^{14}C-labeled prothiofos in buffered aqueous solution at pH = 8 at 254 nm.

Under acidic conditions, upon hydrolysis prothiofos undergoes dealkylation, which results in the formation of *O*-(2,4-dichlorophenyl) *O*-ethyl phosphorothioate. In an alkaline medium, hydrolysis of prothiofos results in the formation of 2,4-dichlorophenol and *O*-ethyl *S*-propyl phosphorodithioate.

All hydrolysis products were analyzed by TLC on silica gel plates using different solvent systems. The R_f values and concentration of identified ^{14}C-labeled compounds from hydrolysis are shown in Table IV. The phenolic compounds were non-labeled and could be detected qualitatively by GC/MS or spraying the plate with palladium chloride reagent and exposure to I_2 vapor.

Hydrolysis data will generally be important in assessing the fate of organic chemicals that have hydrolysable functional groups, e.g., esters, peroxides, and halogenated compounds. Besides substitution, halogen substances can also undergo elimination reactions in water to produce dechlorinated products.[22] This study indicates that hydrolysis can significantly affect the fate of prothiofos in the environment.

Table III R_f values and concentrations of ^{14}C-labeled prothiofos and its degradation products after oxidation

					Concentration							
	R_f values in systems				Nitric acid (2h) 25°C		Hydrogen peroxide (3h) reflux		Potassium permanganate (6h) reflux		Dimethyl sulfoxide (4h) reflux	
Degradation products	1	2	3	4	(μg)*	%**	(μg)*	%**	(μg)*	%**	(μg)*	%**
Prothiofos oxon (**II**)	0.01	0.06	0	0.52	162.3	64.3	97.3	38.6	90.26	35.8	56.7	22.5
O-Ethyl *S*-propyl phosphorodithiote (**V**)	0.68	0.65	0.35	0.83	67.3	26.7	95.8	38	45.4	18	49.9	19.7
O-ethyl S-pro *O*-Ethyl *S*-propyl phosphoric acph phosphorothioate (**IX**)	0.43	0.41	0.32	0.85	0	0	0	0	50.4	20	84.2	33.4
Prothiofos oxon sulfoxide (X)	0.96	0.81	0.46	0.42	22.4	8.9	58.7	23.3	66.8	26.5	61.5	24.4

*Data are the average of two replicates.
System 1: *n*-hexane:ethyl acetate, 99.5:0.5 (v/v).
System 2: *n*-heptane:ethylacetate, 99:1 (v/v).
System 3: *n*-heptane pure.
System 4: *n*-heptane:chloroform:methanol, 9:4:1 (v/v).

Effect of pH on the Fate of Prothiofos Insecticide

Chemical hydrolysis of the organophosphorus insecticide prothiofos at 25°C, 40°C, and 55°C was studied using buffered solution at pH values of 5, 7, 8, and 9, which are usual conditions in natural water. The data presented in Figure 2 show that in acidic medium (pH 5, 25°C) 52% of prothiofos was degraded after 24 h, while at pH 7 about 73% of prothiofos was decomposed after the same time. At pH 9, the loss of prothiofos was 82%. From this

Table IV Concentration and R_f values of ^{14}C-labeled prothiofos and some of its hydrolysis products

					Hydrolysis reagents							
					Hydrochloric acid				Sodium hydroxide			
	R_f values in various solvent system				Aqueous		Chloroform		Aqueous		Chloroform	
Degradation products	1	2	3	4	μg*	%**	μg*	%**	μg*	%**	μg*	%**
Prothiofos (**I**)	0.56	0.47	0.21	0.80	0	0	167.2	66.0	0	0	63.8	25
O-Ethyl *S*-propyl phosphorodithiote(**V**)	0.68	0.65	0.35	0.83	0	0	0	0	133.3	53	0	0
Des-propylthio prothiofos (**IV**)	0.54	0.49	0.30	0.78	32.2	13	0	0	0	0	0	0

System 1: *n*-hexane:ethyl acetate, 99.5:0.5 (v/v).
System 2: *n*-heptane:ethylacetate, 99:1 (v/v).
System 3: *n*-heptane pure.
System 4: *n*-heptane:chloroform:methanol, 9:4:1 (v/v).
*Data are the average of two replicates.
**Percent remaining of prothiofos and its degradation products.

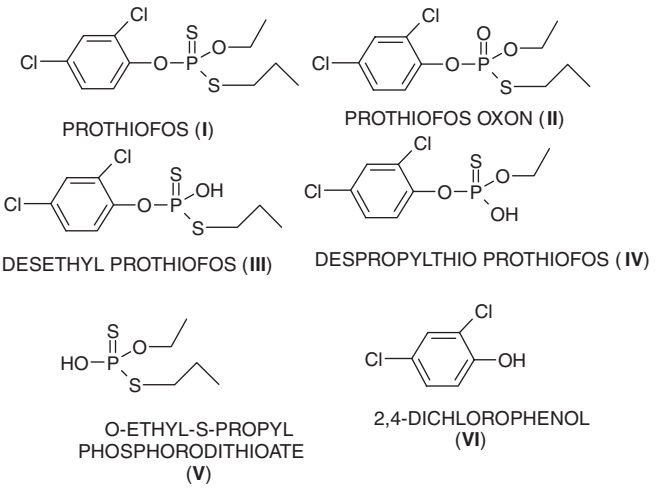


Figure 2 Prothiofos and its main degradation products.

data it becomes evident that the stability of the compound decreases as the pH increases in the following order: pH 5 < pH 7 < pH 8 < pH 9. The same trend was also observed at 40°C and 55°C. These data are in agreement with those reported by Aly and Badawy[2] in their studies on the hydrolysis of organophosphorus insecticides (profenofos, bromophos, and fenitrothion) in aqueous medium.

The effect of pH on the hydrolysis of prothiofos is shown in several figures and tables available in the Supplemental Materials. Throughout the hydrolysis of prothiofos at 25°C, there was an excellent linear relationship between log(a/(a–x)) (concentration of prothiofos) and t (time). This indicated that the hydrolysis of prothiofos is of the first order. The linear regression equations are as follows:

$$-\frac{d[\mathrm{C}]}{dt} = k_{\mathrm{app}}[\mathrm{C}]$$

$$\mathrm{k_{app}} = (2.303/\mathrm{t}) \log (\mathrm{a}/\mathrm{a} - \mathrm{x})$$

The half times of degradation ($t_{1/2}$) at 25°C were 22.9, 12.6, 10.5, and 9.6 h at pH 5, 7, 8, and 9, respectively (Figure S1, Supplemental Materials); the half times of degradation ($t_{1/2}$) at 40°C were 18, 10.28, 10, and 7.8 h, respectively (Figure S2). At the same pH values, the half times of degradation ($t_{1/2}$) at 55°C were 14.5, 9, 7.8, and 6.5 h, respectively (Figure S3).

Effect of Temperature on the Fate of ^{14}C-Labeled Prothiofos Insecticide

Four groups of experiments were carried out to study the effect of temperature on the hydrolysis of prothiofos under different buffer solutions (pH = 5, 7, 8, and 9). In all cases, the hydrolysis rate of prothiofos increased with temperature (see Figures S4–S7, Supplemental Materials). The linear regression equations are as follows:

$$-\frac{d[\mathrm{C}]}{dt} = k_{\mathrm{app}}[\mathrm{C}]$$

$$\mathrm{k_{app}} = (2.303/\mathrm{t}) \log (\mathrm{a}/\mathrm{a} - \mathrm{x})$$

Where: [C] = concentration.
(a) = the original concentration of prothiofos.
(x) = the amount of hydrolytic products.
(t) = time of hydrolysis.

The apparent rate constant k_{app} is usually assumed to be the sum of three terms: k_a $[H^+]$, k_n, and k_b $[HO^-]$, respectively, corresponding to acid, neutral, and basic pathways.[23] Hence, the plot of the logarithm of the normalized concentration as a function of the reaction time gives straight lines, the slope of which identifies $-k_{app}$.

The temperature has a great effect on the hydrolysis of prothiofos. At pH 5, 7, 8, and 9, the rate of hydrolysis at 55°C is higher than at other temperatures. From the experimental results, we can see that prothiofos hydrolyzes in water, but the hydrolysis rate constant is different at different pH values and at different temperatures. At pH 5 and 25°C, the half time of prothiofos is 22.5 h, i.e., prothiofos is relatively stable in water under these conditions. At pH 9 and 55°C, the half time of prothiofos is 6.57 h, i.e., the compound is relatively unstable in water under these conditions.

CONCLUSION

In conclusion, this paper provides useful knowledge on the degradation of the organophosphorus insecticide prothiofos in the environment; the results of the investigations comprise the reactions of prothiofos with various oxidizing reagents; the hydrolysis as well as the photolysis is described. The yield of oxon depends on the type of oxidizing agent. The effect of ultraviolet light and direct sunlight on prothiofos was investigated. Exposure to direct sunlight caused gradual degradation of prothiofos, giving the same products as UV irradiation. Prothiofos was relatively stable in acidic but less stable in alkaline medium; stability of prothiofos in alkaline medium (pH 8–9), similar to canal water, decreases with increasing temperature.

MATERIALS AND METHODS

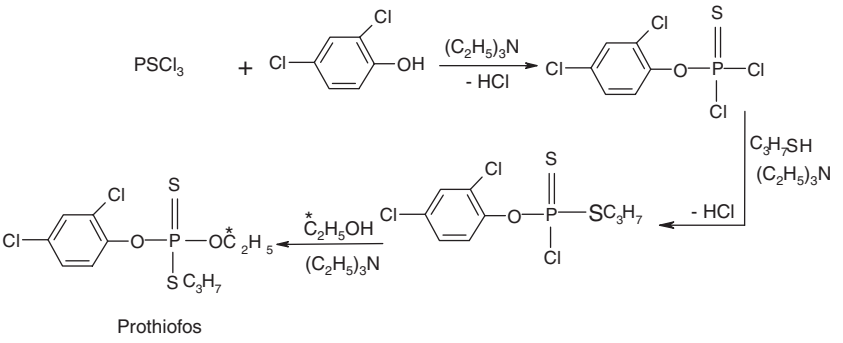
Chemicals

^{14}C-prothiofos labeled at the carbon atom of the ethyl group was prepared in one-pot reaction according to Abdel-Gawad et al.[24] The synthesized ^{14}C-prothiofos had specific activity (0.02 mCi/g) (0.74 MBq/g), and the radiometric purity was greater than 98% (Scheme 2).

Non-labeled prothiofos and some of its degradation products were synthesized for comparison purposes.[24] Chemical structures and abbreviations of the insecticide prothiofos and some of its degradation products are shown in Figure 1.

Spectral Analysis

The ^{1}H NMR spectra were obtained with a Jeol-EX (270 MHz) spectrometer with tetramethylsilane (TMS) as internal standard for samples dissolved in $CDCl_3$. The EI–MS (70 eV) spectra were obtained with a Jeol JMS-AX500 mass spectrometer. The IR spectra were recorded in potassium bromide rolled (KBr) on Nexus 670 FTIR spectrometer (Nicolet) at the National Research Centre, Cairo, Egypt.



Scheme 2 Pathway of ^{14}C-prothiofos synthesis.

Preparation of O-(2, 4-Dichlorophenyl) O-Ethyl S-Propyl Phosphorothioate (Prothiofos Oxon) (II)

This compound was prepared by adding equimolar amounts of 2,4-dichlorophenol (0.01 mol, 1.62 g) and triethylamine (0.03 mol, 4.2 mL) in dry benzene (20 mL) to a cooled (5–10°C) solution of phosphorus oxychloride (0.01 mol, 0.9 mL) in dry benzene (20 mL) for 15 min. The reaction mixture was stirred for 4 h, followed by dropwise addition of propyl mercaptane (0.01 mol, 0.9 mL) in dry benzene (5 mL) in an ice bath at 5–10°C. Then the temperature was raised to 70°C for 4 h, followed by dropwise addition of absolute ethyl alcohol (0.01 mol, 0.6 mL) in dry benzene (5 mL). The reaction mixture was stirred at 25°C for 20 h and filtered. The crude oil was purified on silica gel column using hexane:diethyl ether (1:1) for elution and isolated in 45% yield. Thin layer chromatography on silica gel plate showed one spot of R_f 0.01 in solvent system *n*-hexane:ethyl acetate 99.5:0.5. IR (KBr) ν_{max}/cm^{-1}: 1220 (P=O), 1559, 1475 (C=C ring stretch), 2922 (C−H aliphatic), 1037 (POC-).^{1}H NMR ($CDCl_3$/TMS): $\delta = 0.99$ (t, $J = 7.0$ Hz, 3H, CH_3CH), 1.76 (m, 2H, C−CH_2−C), 2.93 (m, 2H, S−CH_2−), 1.27 (t, $J = 7.11$ Hz, 3H, CH_3−CH_2), 4.4 (m, 2H, CH_2−CH_3), 7.2–7.5 (m, 3H, arom-H). EI-MS (m/z): 329 (M^+), 331 ($M^+ +2$), 333 ($M^+ +4$), 294, 252, 162, 164, 166, 98, 63.

Photolysis of Prothiofos

Effect of direct sunlight. In sealed tubes, 63 μg of pure ^{14}C-prothiofos was exposed to direct sunlight during May 2006 for 1, 2, 3, and 4 weeks. The samples were qualitatively and quantitatively analyzed.

Effect of ultraviolet rays. Prothiofos (252 μg, 0.0007 mmol) in 5 mL of acetone and 20 mL of buffer solution at pH 8 was applied in a Pyrex Petri dish and was exposed to short wave length ultraviolet rays ($\lambda = 254$ nm) at a distance of 10 cm for period of 1, 2, 4, and 6 h. Experiments were carried out at 28°C. The insecticide products were extracted three times with chloroform; the aqueous and organic layers were analyzed by TLC and GC-MS.

Oxidation of Prothiofos

Solutions of definite concentrations of prothiofos were subjected to different oxidizing agents such as concentrated nitric acid, hydrogen peroxide, dimethyl sulfoxide, and potassium permanganate.

With conc. nitric acid. Nitric acid [2.5 mL (0.059 mol, d = 1.49)] was added dropwise to 252 μg (0.0007 mmol) of the cooled ^{14}C- labeled insecticide at 5°C, and the mixture was stirred at room temperature for 2 h. After removal of nitrogen dioxide gas in vacuum, the mixture was poured into ice water and extracted with diethyl ether. The ether extract was washed with 10% cold sodium bicarbonate solution and then with cold water until neutral. The ether layer was evaporated under vacuum, and oxidation products were determined by thin layer chromatography.

With hydrogen peroxide. To a solution of 252 μg (0.0007 mmol) of the ^{14}C-labeled insecticide in 2 mL of glacial acetic acid, 4 mL of 30% hydrogen peroxide were added, and the mixture was refluxed for 3 h. The reaction mixture was poured on ice. After extraction with diethyl ether and washing of the extract with 10% sodium bicarbonate solution and water, the products were qualitatively and quantitatively analyzed.

With dimethyl sulfoxide. A mixture of 252 μg (0.0007 mmol) of ^{14}C-labeled prothiofos in excess of dimethyl sulfoxide and 0.1 mL of concentrated sulfuric acid was refluxed for 6 h. After removal of the separated sulfur and concentration in vacuum, the oxidation products were determined by thin-layer chromatography (TLC) and liquid scintillation counting (LSC).

With potassium permanganate. A solution of 252 μg (0.0007 mmole) ^{14}C-prothiofos in 20 mL of acetone was mixed with 15 mL solution of sodium carbonate in the least amount of water, and powdered potassium permanganate (1.25 g, 0.008 mol) was added. The mixture was refluxed for 2 h, filtered from precipitated manganese dioxide, and evaporated to give the oxidation products, which were determined by TLC and LSC.

Hydrolysis of Prothiofos

For determining the hydrolysis products of the insecticide prothiofos, a solution of 252 μg ^{14}C-labeled prothiofos in 10 mL acetone was heated to reflux for 1/2 h with 20 mL of 1N HCl or 1N NaOH solution. The aqueous and organic layers were subjected to LSC. The hydrolyzed products were identified by thin layer chromatography.

Preparation of the Buffer Solutions

pH 5.00: Solutions of 238 mL of 0.1 M NaOH and 500 mL of 0.1 M monobasic potassium phthalate ($C_6H_4C_2O_4HK$) were mixed and then diluted to 1000 mL.

pH 7.00: Solutions of 297 mL of 0.1 M NaOH and 500 mL of 0.1 M potassium dihydrogen phosphate (KH_2PO_4) were mixed and then diluted to 1000 mL.

pH 8.00: Solutions of 125 mL of 0.1 M potassium dihydrogen phosphate (KH_2PO_4) and 116.5 mL of 0.1 M NaOH were mixed and then diluted to 250 mL.

pH 9.00: Solutions of 125 mL of 0.025 M sodium borate ($Na_2B_4O_7$) and 11.5 mL of 0.1 M HCl were mixed and then diluted to 250 mL.

All of the pH values of the buffer solutions were measured by a pH meter.

Effect of pH on the Hydrolysis of Prothiofos

Four samples of 20 mL of various buffer solutions (pH 5, 7, 8, and 9) in 30 mL screw-top test tubes were rapped with foil to preclude photolysis and were fortified with 252 μg of ^{14}C-labeled prothiofos in 10 mL of acetone by vigorous shaking. These samples

were then kept at fixed temperatures (25°C, 40°C, and 55°C). At different time intervals, 3 mL duplicate samples were taken and extracted three times with chloroform. The organic and aqueous layer extracts were radioassayed.

Effect of Temperature on the Hydrolysis of Prothiofos

The effect of temperature on the hydrolysis of prothiofos at fixed pHs (pH 5, 7, 8, and 9) was studied at 25°C, 40°C, and 55°C. The container containing 20 mL of various buffer solutions in 30 mL screw-top test tubes was fortified with 252 μg of ^{14}C-labeled prothiofos in acetone by vigorous shaking and kept at certain temperatures in the thermostat. At different time intervals (6, 18, and 24 h), 3 mL duplicate samples were taken and extracted three times with chloroform. The organic and aqueous layer extracts were radioassayed.

Chromatography

Extracts of organic and aqueous layer were investigated by thin-layer chromatography (TLC) using silica gel 60 F254 thin-layer chromatoplates (20–20 cm, 0.25 mm thickness, Merck). The following solvent systems were used for development:

System 1: *n*-hexane:ethyl acetate 99.5:0.5 (v/v)
System 2: *n*-heptane:ethylacetate 99:1 (v/v)
System 3: *n*-heptane pure
System 4: *n*-heptane:chloroform:methanol 9:4:1 (v/v)

Prepared degradation products were run alongside as references, and spots were detected under ultraviolet light at 254 nm and made visible by spraying the plates with a freshly prepared Hanes–Isherwood reagent[25] or by subjecting to I_2 vapor, after preliminary spray with $PdCl_2$ solution.[26,27] To detect the phenolic compounds, the plates were developed in the above systems and sprayed with ferric chloride–potassium ferricyanide solution, where blue spots on yellow background appeared.[28]

Radioassay

Thin layer chromatographic technique was used to identify the degradation products. After development, each plate was divided into 1 cm zones which were separately scrapped, extracted with 2 mL of methanol, and then counted for radioactivity in a liquid scintillation counter.

Radioactivity was determined by mixing each zone with 2 mL ethanol and 10 ml of Packard Emulsifier Scintillator cocktail and analyzed by LSC with Packard Model TRI-CARB 2300 TR. The background level of radioactivity in LSC averaged 30 dpm and was subtracted from the values of the measured samples.

GC/MS Analysis

GC/MS analysis was used for the identification of the parent compound and its degradation products in this study. It was carried out with a GC/MS Finnigan Mat SSQ 7000, EI 70 eV. Operating conditions included the following: a capillary column DB-5, 30 m $\times$ 0.25 mm I.D. [(5%-phenyl)methylpolysiloxane]. The analysis was carried out at

a programmed temperature: initial temperature 50°C for zero min, then increasing at rate of 5°C /min until 300°C (kept for 5 min). The injector temperature was set at 250°C, and the detector temperature at 280°C. Helium was used as a carrier gas at 1 mL/min, injected volume was 2 μL, injection mode was splitless. The compounds were identified by matching their MS with those recorded in the MS library (Wiley) and comparison with those of reference compounds.

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