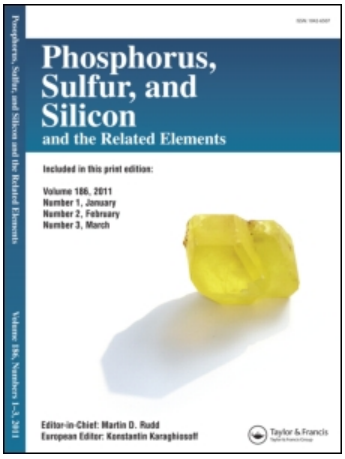

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Distribution and Degradation of ^{14}C-Ethyl Prothiofos in a Potato Plant and the Effect of Processing

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Prothiofos and some of its degradation products have been synthesized in our laboratory for investigation purposes. The residual fate of ^{14}C-ethyl prothiofos in different parts of potato plant was studied. The highest level of insecticide residues was detected in and on the leaves of potato plants. The residues of prothiofos insecticide were mainly located in the peels of potato tubers (peeling process removed 85% of the total residue after one month of the treatment), small amount penetrated into the pulp of potato tubers. The degradation products in the extracts of both peel and pulp of potato tubers were identified as prothiofos, prothiofos oxon, desethyl prothiofos, O-ethyl-S-propyl phosphorodithioate, O-ethyl phosphorothioate, O-ethyl-S-propyl phosphoric acid, O-ethyl phosphoric acid, despropylthio prothiofos, and prothiofos oxon sulfoxide. In addition 2,4-dichlorophenol was identified as such and in conjugated metabolites.

Potatoes are processed in three ways: frying, boiling, and baking, which is simulated in home preparation. The amount of prothiofos residues was found to decrease on boiling (70%) and further on baking (82%) and frying (100%). The results indicated that frying process is the most effective method for reducing the amount of pesticide residues. Detectable residues were found in boiled potatoes (2.64 ppm), in the boiling water (0.24 ppm), and in the frying oil (0.71 ppm). From these results we concluded that the processed potatoes are safely used for human consumption.

Keywords ^{14}C-Ethyl prothiofos; degradation products; potato plants; processing and residues

INTRODUCTION

Prothiofos (**I**), (Tokuthion, *O*-(2,4-dichlorophenyl) *O*-ethyl-*S*-propyl phosphorodithioate) is an organophosphorus insecticide effective for the

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We sincerely thank Prof. Dr. Issa Fakhr for his assistance in the synthesis of prothiofos insecticide.
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control of leaf—eating caterpillars, *pseudoccus* ssp., thrips, cockchafer larvae, cutworms in a range of crops including vegetables, fruit, maize, sugar cane, sugar beet, tea, tobacco, and ornamentals.[1]

It is selectively more toxic to insects than mammals due to the difference in metabolism of the thiopropyl group.[2]In spite of low toxicity (LD_{50} 950 mg/kg mouse and LD_{50} 1700 mg/kg rats), fatal and nonfatal poisoning cases have been reported in humans.[3–5] Determinations of its residues were carried out using several techniques such as GLC, GC/MS and HPLC.[6–10] Many researchers have studied how to minimize pesticide residues in food products.[11–13]

The potato is tuber crop grown in the greatest quantities.[14] Potatoes—members of the Solanaceae plant family—serve as major, expensive low-fat food sources providing energy (starch), high-quality proteins, fiber, and vitamins. Potatoes also produce biologically active secondary metabolites, which may have both adverse and beneficial effects in the diet. These include glycoalkaloids, calystegine alkaloids, protease inhibitors, lectins, phenolic compounds, and chlorophyll.[15]

Because organophosphorus insecticides are reported to affect cells, animals, and humans, a need exists to develop a clearer understanding of their roles both in the plant and in the diet. Since the typical use pattern of (**I**) is leaves treatment, its metabolic pathways in plants after foliar application have not been examined. Here we present data on (a) the distribution of the organophosphorus insecticide prothiofos in potato plant after its application in the field; (b) the identification and determination of some of its degradation products; and (c) the removal of the organophosphorus insecticide prothiofos and/or of its residues from potato by processing. Each of these steps was studied separately in order to determine which could account for residue changes.

EXPERIMENTAL

Spectral Analysis

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

Chemicals

Chemical structures and abbreviations of the insecticide prothiofos and of its supposed metabolites are shown in Figure 1.

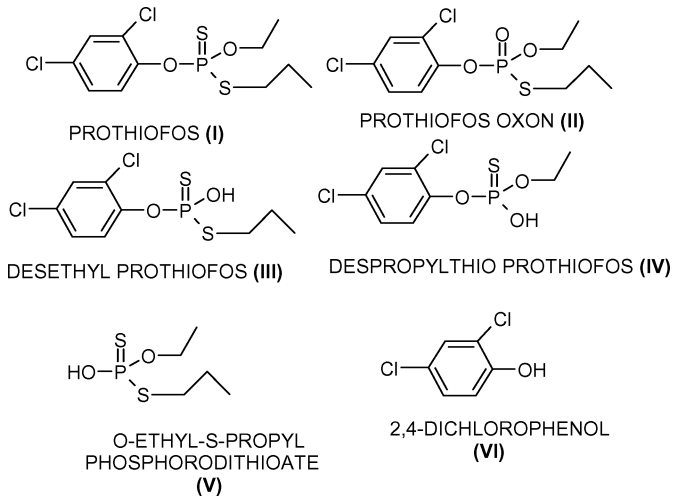
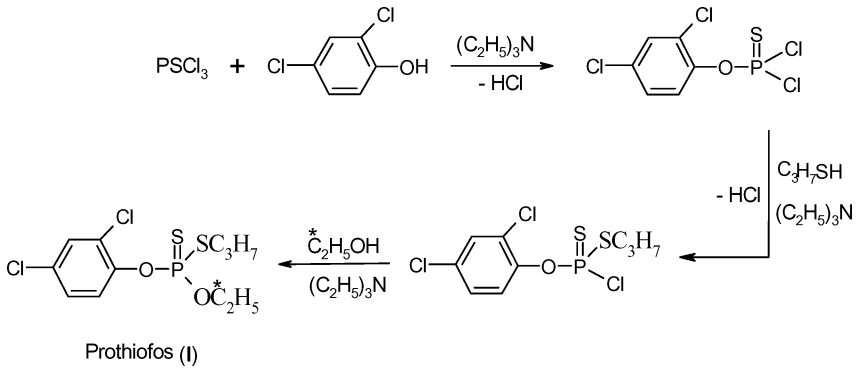


FIGURE 1 Prothiofos and its supposed degradation products.

Prothiofos, *O*-(2,4-dichlorophenyl) *O*-ethyl-*S*-propyl phosphorothioate (prothiofos oxon) (**II**), *O*-(2,4-dichlorophenyl) *S*-propyl phosphorodithioate (**III**), *O*-(2,4-dichlorophenyl) *O*-ethyl phosphorothioate (**IV**),*O*-ethyl-*S*-propyl phosphoro-dithioate (**V**) and 2,4-dichlorophenol were synthesized as follows.

Synthesis of Prothiofos (Tokuthion) O-(2,4-dichlorophenyl) O-ethyl-S-propyl Phosphorodithioate (I)

A mixture of 2,4-dichlorophenol (0.01 mol, 1.62 g) and triethylamine (0.03 mol, 4.2 mL) in dry benzene (20 mL) was added dropwise to a cooled (5–10°C) solution of thiophosphoryl chloride (0.01 mol, 1.1 mL) in dry benzene (20 mL) during 20 minutes. Stirring was continued for 4 h, followed by dropwise addition of propyl mercaptane (0.01 mol, 0.9 mL) in dry benzene (5 mL) at 5–10°C, subsequently the temperature was raised to 70–80°C for 4 h, followed by dropwise addition of (0.6 mL, 9.25 MBq, Amersham, England) ^{14}C-1-ethyl alcohol (0.01 mol) in dry benzene (5 mL). The reaction mixture was stirred at 25°C for 20 h, filtered to remove the triethylammonium hydrochloride; and from the filtrate, the solvent was evaporated under vacuum. The crude oil was purified by silica gel column chromatography using hexane : diethyl ether (9.5 : 0.5) for elution to give a colorless oil (Sp. Act. 0.02 mCi/g, 0.74 MBq/g), (yield 85%, 2.93 g). Thin layer chromatography on silica gel plate showed one spot of R_f 0.56 in *n*-hexane : ethylacetate 99.5 : 0.5. The radiochemical purity was >98% (Scheme 1). IR (KBr) ν_{max}/cm^{-1}: 720 (P═S), 1580, 1473 (C═C ring stretch), 2966 (C-H aliphatic), 1160



SCHEME 1 Route for preparation of ^{14}C-ethyl prothiofos.

(P-Oaryl), 1022 (POC-). ^{1}H NMR ($CDCl_3$/TMS): $\delta = 0.95$ (t, $J = 7.0$ Hz, 3H, CH_3CH$_2$), 1.70 (m, 2H, C-CH_2-C), 2.95 (m, 2H, S-CH_2-), 1.37 (t, $J =$ 7.2 Hz, 3H, CH_3-CH$_2$), 4.28 (m, 2H, CH_2-CH$_3$), 7.2–7.5 (m, 3H, arom-H). EI-MS (m/z): 345 (M^+), 347 (M^++2), 349 (M^++4), 183, 162, 164, 166, 155, 141, 98, 63.

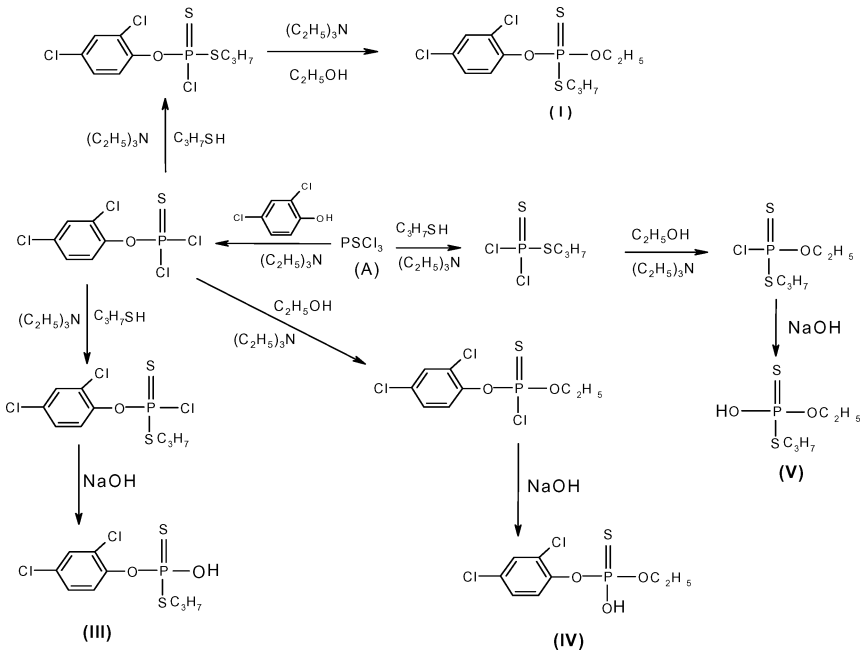
Preparation of O-(2,4-Dichlorophenyl) O-ethyl-S-propyl Phosphorothioate (Prothiofos Oxon) (II)

Nitric acid, 2.5 mL (0.059 mol, d = 1.49) was added dropwise to 252 μg of the cooled 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 (50 mL) and extracted with ether (60 mL). The ether phase was washed with 10% cold sodium bicarbonate solution and then with cold water till neutral. The ether was evaporated under vacuum. The crude oil was purified on silica gel column using hexane : diethyl ether (1:1) for elution. Yield: 64%, 162 μg. Thin layer chromatography on silica gel plate showed one spot of R_f 0.01 in *n*-hexane : ethylacetate 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$_2$), 1.76 (m, 2H, C-CH_2-C), 2.93 (m, 2H, S-CH_2-), 1.27 (t, $J = 7.1$ Hz, 3H, CH_3-CH$_2$), 4.4 (m, 2H, CH_2-CH$_3$) and 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.

Preparation of O-(2,4-Dichlorophenyl) S-propyl Phosphorodithioate (III)

A solution of 2,4-dichlorophenol (0.005 mol, 0.81 g) and triethylamine (0.01 mol, 1.4 mL) in dry benzene (20 mL) was added dropwise to a

cooled (5–10°C) solution of thiophosphoryl chloride (0.005 mol, 0.507 mL) in dry benzene (10 mL) during 20 min. Stirring was continued for 4 h, followed by dropwise addition of propyl mercaptane (0.005 mol, 0.45 mL) in dry benzene (5 mL) at 5–10°C. Subsequently the temperature was raised to 70°C for 4 h. The reaction mixture was filtered and from the filtrate most of benzene was evaporated using a rotary evaporator. The residue was dissolved in 50 mL of 0.1 N NaOH. After extraction with diethyl ether (60 mL), the aqueous phase was acidified to pH 1 with a few drops of concentrated HCl and extracted again with ether (60 mL). The ether extract was dried over anhydrous sodium sulfate and the solvent was evaporated to leave colorless oil. The crude oil was purified on silica gel column using hexane : diethyl ether (9.5 : 0.5) for elution (yield 50%, 0.8 g). Thin layer chromatography on silica gel plate showed one spot of R_f (0.83) in *n*-hexane : ethylacetate 99.5 : 0.5 (Scheme 2). IR (KBr) ν_{max}/cm^{-1}: 3411 (OH), 720 (P═S), 1580, 1475 (C═C ring stretch), 2851 (C-H aliphatic), 2921 (CH arom-H), 692 (C-S). ^{1}H NMR ($CDCl_3$/TMS): $\delta = 0.99$ (t, $J = 7.4$ Hz, 3H, CH_3CH_2), 1.76 (m, 2H, C-CH_2-C), 2.97 (m, 2H, S-CH_2-), 7.2 (s, 1H, OH), 7.3–7.6 (m, 3H, arom-H). EI-MS (m/z): 317 (M^+), 319 (M^++2), 321 (M^++4), 282, 240, 162, 164, 166, 113, 63.



SCHEME 2 Preparations of prothiofos and its degradation products.

Preparation of O-(2,4-Dichlorophenyl) O-ethyl Phosphorothioate (IV)

Triethylamine (0.01 mol, 1.4 mL) and 2,4-dichlorophenol (0.005 mol, 0.81 g) in dry benzene (10 mL) were added dropwise to a cooled (5–10°C) solution of thiophosphoryl chloride (0.005 mol, 0.507 mL) in dry benzene (10 mL) during 10 min. Stirring was continued for 4 h, followed by dropwise addition of absolute ethyl alcohol (0.005 mol, 0.29 mL) in dry benzene (5 mL). The reaction mixture was stirred at 25°C for 18 h, and filtered. From the filtrate the solvent was evaporated under reduced pressure. The residue was dissolved in 50 mL of 0.1 N NaOH. After extraction with diethyl ether (60 mL), the aqueous phase was acidified to pH 1 with a few drops of concentrated HCl and extracted again with ether (60 mL). The combined ether extract was dried over anhydrous sodium sulfate and the solvent was evaporated to leave colorless oil. The crude oil was purified on silica gel column using hexane : diethyl ether (9.5 : 0.5) for elution (yield 34%, 0.48 g). Thin layer chromatography on silica gel plate showed one spot of R_f (0.54) in *n*-hexane: ethylacetate 99.5 : 0.5 (Scheme 2). IR (KBr) ν_{max}/cm^{-1}: 3440 (OH), 722 (P═S), 1580, 1474 (C═C ring stretch), 2851 (C-H aliphatic), 2920 (CH aromatic), 670 (C─S).^{1}H NMR ($CDCl_3$/TMS): $\delta = 1.24$ (t, $J = 7.1$ Hz, 3H, CH_3CH_2), 4.4 (m, 2H, C-CH_2-C), 7.4 (s, 1H, O*H*), 7.5–7.7 (m, 3H, arom-H). EI-MS (m/z): 287 (M^+), 289 (M^++2), 291 (M^++4), 252, 224, 162, 164, 166, 126.

Preparation of O-Ethyl S-propyl Phosphorodithioate (V)

A mixture of absolute ethanol (0.005 mol, 0.29 mL) and triethylamine (0.01 mol, 1.4 mL) in dry benzene (15 mL) was added dropwise to a cooled (5–10°C) solution of thiophosphoryl chloride (0.005 mol, 0.507 mL) in dry benzene (10 mL) during 15 min. The reaction mixture was stirred at 25°C for 18 h, followed by dropwise addition of propyl mercaptane (0.005 mol, 0.45 mL) in dry benzene (5 mL) at 5–10°C, subsequently the temperature was raised to 70°C for 3 h. The reaction mixture was filtered, and from the filtrate most of the benzene was evaporated using a rotary evaporator. The residue was dissolved in 100 mL of 0.1 N NaOH. After extraction with diethyl ether (60 mL), the aqueous phase was acidified to pH 1 with a few drops of concentrated HCl and extracted again with ether (60 mL). The combined ether extract was dried over anhydrous sodium sulfate and the solvent was evaporated to leave a colorless oil. The crude oil was purified on silica gel column using hexane : diethyl ether (9.5 : 0.5) for elution to give a colorless oil (yield 68%, 0.68 g). Thin layer chromatography on silica gel plate showed one spot of R_f (0.68) in *n*-hexane : ethylacetate 99.5:0.5 (Scheme 2). IR (KBr)

ν_{max}/cm^{-1}: 2962 (OH), 707 (P=S), 2852 (C–H aliphatic), 1485 (-CH_2-), 1378 (-CH_3), 692 (C–S). ^{1}H NMR ($CDCl_3$/TMS): $\delta = 0.95$ (t, $J = 7.1$ Hz, 3H, CH_3CH_2), 1.8 (m, 2H, C-CH_2-C), 2.95 (m, 2H, S-CH_2-), 1.24 (t, $J =$ 7.3 Hz, 3H, CH_3CH_2), 4.2 (m, 2H, CH_2-CH_3), 7.3 (s, 1H, OH). EI-MS (m/z): 200 (M^+), 198, 181, 172, 158, 139, 129, 75, 43.

Preparation of 2,4-Dichlorophenol (DCP (VI)

2,4-Dichlorophenol (DCP (VI) was prepared by hydrolysis of prothiofos as follows. Prothiofos (0.01 mol, 3.45 g) was mixed with 95% ethanol containing 1% sodium hydroxide, and the reaction mixture was kept at 60°C overnight or stirred at room temperature for 5 h. The resulting precipitate was separated by filtration, dissolved in distilled water (20 mL), and acidified with a few drops of 1N HCl. Crystallization from aqueous ethanol gave (92%, 1.5 g) of 2,4-dichlorophenol, m.p. 43°C.

Field Experiment

Valor potatoes (*Solanum tuberosum L.*) were planted in an isolated area at the Experimental Station of the National Research Centre. The complete randomized block design with four replicates was used. Each plot had four 5 m rows. The complete tubers were planted in October 2005 and spaced 25 cm within the rows. Common cultural and fertilization practices were followed. The ^{14}C-ethyl prothiofos in very dilute ethanol solution (to ensure that all radioactive material is transferred) was topically applied manually with a micropipette on healthy leaves of the plant after two months of agriculture following the Egyptian Ministry of Agriculture's recommended rate (12 mg/plant each time). The pesticide was applied twice during the season with 15 days interval.

Sampling

Three potato plants were collected after 7, 14, 21, and 30 days from the last application of the labeled prothiofos. Each plant was differentiated into leaves, stems, roots, and tubers and they were weighed and stored at −20°C till analysis.

Three tubers were peeled with a knife in the way the peeling is usually carried out by consumers. The weights of the flesh (pulp) and of the peel of each sample were recorded. The analysis was carried out separately. The concentration in whole unwashed tubers was estimated taking into account the concentration in the flesh and the peel, and the respective weights.

Processing

Potatoes are processed in three phases: frying, boiling, and backing, which is simulated in home preparation.

1. Three tubers were peeled, and cut into pieces for french fries. The weights of the peel, and the flesh were recorded. Each subsample was fried in soybean oil for 15 min. The weight of fries after cooking was taken. The fries, as well as the oil, were analyzed.
2. Three tubers were boiled for 15 min in a pressure kettle, containing 1 l of hot water. The weight of each subsample was recorded before and after boiling. The above methods of cooking were chosen since they are among the most common cooking methods used around the world. Also, the products are highly consumed by children.
3. Three tubers wrapped in aluminum foil were baked in an oven at 220°C for 90 min.

Extraction and Isolation of Degradation Products

The potato samples (whole, peels, pulps, french fries, baked, and boiled) were separately blended three times with methanol (100 mL each) for three minutes and the mixture of each sample was filtered. The filtrate was extracted three times each with 100 mL of chloroform and sodium chloride solution (salting out, make separation easily) and was shaken for 2 min. The combined chloroform extracts were dried over anhydrous sodium sulfate, filtered, and from the filtrate the solvent was evaporated under reduced pressure. Triplicates of organic and water layer samples were radioassayed. The extract was analyzed by TLC for identification of the metabolites.

The aqueous layer was divided into two parts: the first part was evaporated to dryness in special dessicator over P_2O_5, extracted with methanol (5 mL) and analyzed by thin-layer chromatography. The second part was acidified by 50 mL 2N HCl, and heated for 12 h at 100°C to decompose conjugated phenolic compounds. After cooling the acidified water phase was extracted with 50 mL chloroform three times and analyzed by TLC. The ^{14}C distribution among the aqueous, organic soluble fraction and the non-extractable residues was then calculated.

Chromatographic Analysis

Extracts of organic and aqueous layer of potato samples were achieved by thin-layer chromatography (TLC) using silica gel 60 F_{254} thin-layer

chromatoplates (20–20 cm, 0.25 mm thickness, E. 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; and
- 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[16] or by subjecting to I_2 vapor after preliminary spray with $PdCl_2$ solution.[17,18] To detected the phenolic compounds, the plate were developed in the above systems and sprayed with ferric chloride/potassium ferricyanide solution, where blue spots on yellow background appeared.[19]

Radioassay

Radioactivity in the liquid extracts was determined by mixing each aliquot with 10 mL of Packard Emulsifier Scintillator cocktail and analyzed by liquid scintillation counting (LSC) with Packard Model TRICARB 2300 TR. The background level of radioactivity in LSC averaged 30 dpm and was subtracted from the values for measured samples. Radioactivity in the air dried unextractable residues from the treated plants was combusted by using a Harvey Biological Oxidizer (model OX-600). The librated $^{14}CO_2$ was trapped in carbsorb scintillation cocktail and assayed for radioactivity. The internal standard technique was used for quench correction. The efficiency of combustion was determined to be greater than 90%.

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

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 GC/MS Finnigan Mat SSQ 7000, EI 70 eV. Operating conditions: 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 0 min, then

increasing at rate of 5°C /min, until it reaches 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, and 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.

RESULTS AND DISCUSSION

Distribution of Radioactivity in Potato Tubers

The distribution of radioactivity of ^{14}C-prothiofos was examined in potato tubers samples. The use of a radiolabeled compound in this investigation was based on the assumption that the radiolabeled molecule would be metabolized in the same manner as the unlabelled molecule.[20]

The topically applied radioactive material was subsequently translocated through the plant. Some of this translocated radioactive material was detected in the whole parts of the potato plants. Fourteen days after the last treatment, the highest level of insecticide residues (49.5 ppm) was detected in and on the leaves of the potato plants. This high level might be expected since the leaves are the most exposed parts of the plant to the insecticide application. The accumulation of insecticide detected in the roots after 14 days from the last treatment of insecticide was 20 ppm, while the stem of the potato plant had 28.5 ppm. The level of the residues was low on tubers and amounted to 4.8 ppm.

Table I shows the prothiofos residues in peels and pulps of potato tubers collected at different time intervals from the last treatment with

TABLE I ^{14}C-Residues in Potato Tubers (Peels and Pulps) After the Last Application of ^{14}C-Prothiofos

	^{14}C-residues (ppm)					
	Peels		Pulps			
Time (Days)*	Extract Mean ± SD***	Bound	Extract Mean ± SD***	Bound	Wt. of tubers (g)	%**
7	0	0	0.22 ± 0.04	0	118	0.00011
14	3.76 ± 1.96	0	0.54 ± 0.04	0	175	0.00314
21	11.7 ± 0.35	0	8.2 ± 2.02	0	161	0.01339
30	3.6 ± 0.23	0	0.64 ± 0.19	0	196	0.00350

*Samples taken after the last application; **percentage of the applied dose; and ***results are expressed as mean ±SD for three determinations of prothiofos level for each sample.

^{14}C-compound. The prothiofos residues in whole potato tubers increased with time from 0.22 ppm to 19.9 ppm during the first three weeks, then decreased rapidly at the end of 30 days of the last application and amounted to 4.24 ppm. These results indicate that the highest level of the insecticide residues was detected in and on the peels of potato tubers and amounted to 3.6 ppm after 30 days of the last application. Peeling process removed 85% of the total residues. Small amount of insecticide residues (0.64 ppm) penetrated into the pulps. These results are in agreement with those reported by many authors.[21–23] In their studies on distribution and degradation of some organophosphorus insecticides on potato plants, Lentza-Rizos and Balokas[24] found that the amounts of chloropropham herbicide removed by peeling were 98% and 91% from the first and second potato sample treated with this pesticide at 10 and 28 days, respectively. Mohamed[25] found, that the loss was 49.6 and 52% for thin peeled, 57 and 68% for thick peeled, and 54 and 70% for carbandum peeled in case of DDT and Lindan residues, respectively.

By extracting the potato tubers (peels and pulps) with methanol and partitioning between chloroform and water, the activity in extractable ^{14}C-residues in peels increased from 3.76 ppm to 11.7 ppm during the first three weeks of the second application, then it decreased rapidly and reached 3.6 ppm at the end of experimental period. The ^{14}C-residues in extractable layer of pulps also showed the same trends. There were no bound residues detected in both peels and pulps of potato tuber and the percent of recovery ranged from 90 to 97% as shown in Table II. Our observations support the findings of other researchers,[26,27] who found that percentage of bound residues in the total ^{14}C-labeled residues of crop samples differs with the variety of pesticides and crops.

Identification and Characterization of Radioactive Degradation Products in Potato Tubers

Because metabolic factors may be of importance in the risk assessment of prothiofos and because there are very little data available on prothiofos metabolism, we performed rather extensive series of experiments to elucidate interactions and metabolic routes.[28] Tables III and IV represent the R_f values and the amounts (ppm) of the ^{14}C insecticide and some of its degradation products after three weeks and after one month of the second application. In addition to the parent compound (prothiofos) (**I**), prothiofos oxon (**II**), despropylthio prothiofos (**IV**), *O*-ethyl-*S*-propyl phosphorodithioate (**V**), *O*-ethyl phosphorothioate, *O*-ethyl-*S*-propyl phosphorothioic acid, *O*-ethyl phosphoric acid and prothiofos oxon sulfoxide were detected. The phenolic compound

TABLE II Extractable and Nonextractable ^{14}C-Activities in Different Parts of Potato Tubers After the Last Application of ^{14}C-Prothiofos

	^{14}C-residue (ppm)*							
	7 days		14 days		21 days		30 days	
Fraction	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Total ^{14}C activity	0.23		4.8		20.5		4.5	
Extractable								
Organic layer	0.00	0.22 ± 0.04	3.76 ± 1.69	0.54 ± 0.04	3.6 ± 0.2	0.54 ± 0.04	0.33 ± 0.2	0.00
Water layer	0.00	0.00	0.00	0.00	8.1 ± 0.35	7.66 ± 2.03	3.27 ± 0.2	0.64 ± 0.19
Nonextractable	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total residues	0.00	0.22 ± 0.04	3.76 ± 1.69	0.54 ± 0.04	11.7 ± 0.3	8.2 ± 2.02	3.6 ± 0.2	0.64 ± 0.19
Recovery (%)**	95.6		90.0		97.0		94.2	

*Results are expressed as mean ±SD for three determinations of prothiofos level for each sample; and ** related to the original residues.

TABLE III The Amount and R_f Values of ^{14}C Prothiofos and Its Active Degradation Products in Extracts of Different Parts of Potato Tubers After 21 Days of Treatment

	R_f values in various solvent system				After three weeks (ppm)*			
					Pulp		Peel	
Metabolites	1	2	3	4	Chloroform	Aqueous	Chloroform	Aqueous
Prothiofos (**I**)	0.56	0.47	0.21	0.80	0.49	0.00	1.40	0.00
Prothiofos oxon (**II**)	0.01	0.06	0.00	0.52	0.00	0.00	2.00	0.00
Despropylthio prothiofos (**IV**)	0.54	0.49	0.30	0.78	0.00	0.00	0.00	0.00
O-ethyl-*S*-propyl phosphorodithiote (**V**)	0.68	0.65	0.35	0.83	0.00	1.61	0.00	2.80
O-ethyl phosphorothioate (**VII**)	0.21	0.52	0.45	0.60	0.00	3.52	0.00	3.32
O-ethyl-*S*-propyl phosphorothioate (**IX**)	0.43	0.41	0.32	0.85	0.00	0.00	0.00	0.00
O-ethyl phosphoric acid (**VIII**)	0.77	0.65	0.60	0.89	0.00	0.00	0.00	1.03
Prothiofos oxon sulfoxide (**X**)	0.96	0.81	0.46	0.42	0.00	1.80	0.00	0.87

*Data are mean 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).

was found as the free phenol in the organic extract and as conjugate in the water extract, which was liberated by acid hydrolysis. The conjugated metabolites are DCP–Glu [*O*-ß–D-glucopyranosyl-2,4-dichlorophenol] and DCP–Glu–Mal [(6-*O*-malonyl)-*O*-ß-D-glucopyranosyl-2,4-dichlorophenol]. These findings are similar to those obtained by GC/ MS for the extract of potato pulp after three weeks of the last treatment as shown in Table V.

It is important to distinguish between the primary metabolic process (triester to diester) and the secondary process involving the further metabolism of the 'leaving group'. In plants, pesticides generally conjugate with sugars to form sugar conjugates as secondary metabolites. The alcohol groups of primary metabolites are usually readily conjugated.[29] Laurent et al.[30] found that 2,4-dichlorophenol was conjugated with sugar in edible plants (potato, sugar beet and tomatoes) when treated with 2,4-dichlorophenoxy acetic acid (2,4-D).

TABLE IV The Amount and R_f Values of ^{14}C Prothiofos and Its Active Degradation Products in Extracts of Different Parts of Potato Tubers After 30 Days of Treatment

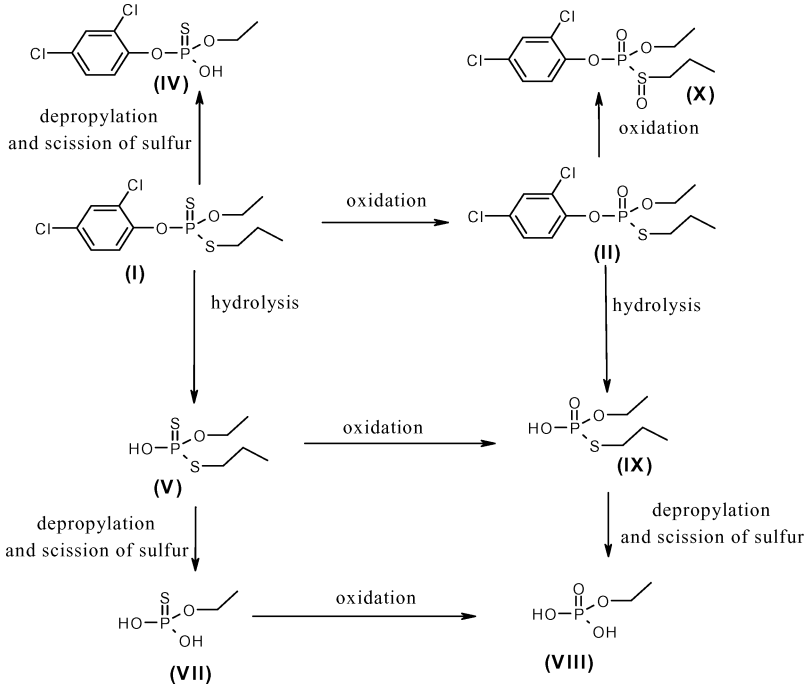
	R_f values in various solvent system				After three weeks (ppm)*			
					Pulp		Peel	
Metabolites	1	2	3	4	chloroform	aqueous	chloroform	aqueous
Prothiofos (**I**)	0.56	0.47	0.21	0.80	0.00	0.00	0.167	0.00
Prothiofos oxon (**II**)	0.01	0.06	0.00	0.52	0.00	0.00	0.162	0.00
Despropylthio prothiofos (**IV**)	0.54	0.49	0.30	0.78	0.00	0.00	0.00	0.87
O-ethyl-S-propyl phosphorodithiote (**V**)	0.68	0.65	0.35	0.83	0.00	0.00	0.00	0.00
O-ethyl phosphorothioate (**VII**)	0.21	0.52	0.45	0.60	0.00	0.15	0.00	1.10
O-ethyl-S-propyl phosphorothioate (**IX**)	0.43	0.41	0.32	0.85	0.00	0.23	0.00	0.00
O-ethyl phosphoric acid (**VIII**)	0.77	0.65	0.60	0.89	0.00	0.25	0.00	0.52
Prothiofos oxon sulfoxide (**X**)	0.96	0.81	0.46	0.42	0.00	0.00	0.00	0.60

*Data are mean 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).

The proposed pathway for the degradation of ^{14}C-prothiofos in potato plant is shown in Scheme 3. Metabolic routes include oxidation to prothiofos oxon, hydrolysis to 2,4-dichlorophenol, which is further conjugated with sugar in the plant, and cleavage of the propyl group leading to the formation of despropylthio prothiofos as well as hydrolysis of the

TABLE V Structures and GC-MS Fragments of Extractable Potato Pulp After Three Weeks from the Last Treatment

	RT (min)	GC-MS (m/z)
Prothiofos (**I**)	44.08	345, 347, 349, 183, 162, 164, 166, 155, 141
O-ethyl-S-propyl phosphorodithioate (**V**)	6.54	200, 198, 181, 172, 158, 139, 129
Prothiofos oxon sulfoxide (**X**)	40.47	344, 308, 266, 207, 182, 161, 112
O-ethyl phosphorothioate (**VII**)	24.6	143, 115
2,4-dichlorophenol (**VI**)	3.79	163, 105



SCHEME 3 Proposed pathways for degradation of ^{14}C-prothiofos in potato tubers.

ethoxy group leading to the formation of desethyl prothiofos. Katagi and Mikami[31] noted that the metabolism of organophosphorus pesticides in plants have revealed cleavage of the P-O-aryl linkage and *O*-dealkylation to be among the most predominant metabolic pathways. Enzymatic and acid hydrolysis released an aglycon and direct spectroscopic analyses of the metabolites implied that **I** primarily underwent cleavage of the P-O-aryl linkage or hydroxylation of the aryl methyl group similarly to other organophosphorus pesticides in plants. These metabolites were also detected in rats[5] and in human urine.[2]

Effect of Processing

Peeling process was the most effective way to remove the pesticide residues from the crops.[23,32–34] After three weeks of the last application, peeling removed 59% of the ^{14}C-residues, while 30 days after the experiment it removed 85% of the total ^{14}C-residues. The processing methods lead to a high decrease in the total amount of ^{14}C-residues

TABLE VI Effect of Processing Methods on the Residues of ^{14}C Prothiofos in Potato Tubers After Three Weeks of the Last Application

	Total ^{14}C prothiofos (ppm) Mean ± SD*	Reduction (%)
Fresh whole potatoes (peeled)	8.20 ± 2.02	0.00
Boiled whole potatoes (peeled)	2.48 ± 0.17	69.80
Baking whole potatoes (peeled)	1.49 ± 0.10	81.80
french fries from peeled potatoes	0.00	100.00

*Results are expressed as mean ±SD for three determinations of prothiofos level for each sample.

in peeled potato. Obvious reduction was detected after boiling (70%) and was increased after baking (82%) and frying (100%) as shown in Table VI. This decrease could be attributed to the time of exposure, the effect of temperature and/or evolution of $^{14}CO_2$ gas. Soliman[35] found that peeling, blanching, and frying are necessary to remove pesticide residues from contaminated potato tubers. The residues detected in boiling water were 0.24 ppm and in frying oil 0.71 ppm. Boiling is effective in reducing the level of water soluble pesticide residues and the use of oil in cooking processes decreases the level of fat soluble ones.

The obtained data indicated that frying was more effective than the other investigated potato processing methods in reducing the levels of prothiofos residues. This finding is in accord with the results obtained by many authors.[12,21,25] Also these findings are in agreement with those obtained by Lewis et al.[36], who found that the residues of the pesticides were significantly reduced to less than 2% and 10% of the maximum theoretical residues level for potato crisp and jacket crisps, respectively. The results demonstrate that the level of pesticide residues in potato tubers is lowered, sometimes far below the recommended levels by washing or cooking.[33]

TLC and GC-MS revealed the presence of four degradation products in addition to the parent compound in the extracts of both boiled and baked potatoes, which were identified as prothiofos, prothiofos oxon, *O*-ethyl phosphorothioate, prothiofos oxon sulfoxide and despropylthio prothiofos, as shown in Table VII. The prothiofos residues level in the different processing methods was found in the range of 0 to 0.04 ppm, which was below the maximum residue limits (MRLs) of prothiofos in potatoes (0.05 ppm).[37] It can be concluded that the processed potatoes can be safely used for human consumption.

TABLE VII The Amount, R_f Values and GC-MS Fragments of ^{14}C-Prothiofos and Its Active Degradation Products in Extracts of Pulp of Potato Tubers After Processing

	RT (min)	GC/MS (m/z)	R_f values in systems			Boiling (ppm)*	Baked (ppm)*
			Sys 1	Sys 2	Sys 3		
Prothiofos (**I**)	44.08	345, 347, 349, 183, 162, 164, 166, 155, 141	0.56	0.47	0.21	0.04	0.00
Prothiofos oxon (**II**)	36.64	328, 294, 266, 224, 161, 142, 114	0.01	0.06	0.00	0.03	0.00
O-ethyl phosphorothioate (**VII**)	24.6	143, 115	0.21	0.52	0.45	0.14	1.12
Prothiofos oxon sulfoxide (**X**)	40.47	344, 308, 66, 207, 182, 161, 112	0.96	0.81	0.46	0.26	0.00
Despropylthio prothiofos (**IV**)	21.2	287, 289, 291, 252, 162, 164, 166 , 126	0.54	0.49	0.30	0.00	0.37

*Data are mean 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.

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