Isolation and Identification of Impurities in Technical Quinalphos†

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A number of impurities developed in a sample of technical quinalphos on storage were isolated by column and thin-layer chromatography, and the structures of these impurities were determined by nuclear magnetic resonance, infrared, and mass spectroscopy. Non-phosphorus-containing impurities were not active whereas phosphorus containing impurities were as active as quinalphos against *Tribolium castaneum* Herbst.

INTRODUCTION

Several organophosphorus pesticides have low mammalian toxicities and are regarded as safe for general use. However, the combination of some organophosphorus compounds (Frawley et al., 1957) or impurities present in technical materials (Casida and Sanderson, 1963), arising either from synthesis or during storage, may lead to markedly different toxicities than would be expected from the toxicities of the individual components. The potentiation of malathion and phenthoate toxicities by several trimethyl phosphorothioate and phosphorodithioate esters have been investigated (Pelligrini and Santi, 1972). Several other impurities from technical-grade malathion, acephate (Umetsu et al., 1977), and fenthion (Toia et al., 1980) and their effect on the mammalian toxicities of purified insecticide were reported.

In consideration of the widespread use of quinalphos (*O,O*-diethyl *O*-quinoxalin-2-yl phosphorothioate), a contact and stomach insecticide and acaricide with good penetrating properties, a detailed knowledge of impurities and their effect on toxicities is of importance. The present study is concerned with the identification of impurities, which are commonly present or may develop upon storage in technical quinalphos, and the effect of these impurities on insect toxicity.

MATERIALS AND METHODS

Material. All melting points were obtained on a Fisher-John's melting point apparatus and were uncorrected. The infrared (IR) spectra were recorded on a Perkin-Elmer Model 405 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were determined on a Varian EM-360L (60 MHz) spectrometer using tetramethylsilane as the internal standard.

Silica gel F-254 (E. Merck Laboratories) plates of 0.25-mm thickness were used for analytical thin-layer chromatography (TLC). Preparative TLC was conducted by using silica gel plates of 0.5-mm thickness. Several different solvent systems were used. Location of compounds on TLC plates was accomplished by 2,6-dibromoquinone 4-chlorimide (DBQ) spray reagent (Menn et al., 1963), iodine vapor, or ultraviolet detection. Silicic acid was used for column chromatography.

Mass spectra of purified compounds were recorded by direct insertion probe in a JEOL–JMSD-300 mass spectrometer; unless otherwise specified, the ionizing voltage was 70 eV. A JEOL Inc. USA Model JGC-20K gas chromatograph interfaced with a JMS-D300 mass spectrometer (equipped with computer Model JMA-D3000) was used for gas chromatography.

Gas chromatography was carried out on a Hewlett-Packard Model 5890A gas chromatograph equipped with a glass column (2 m × 2-mm i.d.) packed with 3% OV-225 on Gas Chrome Q (80–100 mesh) with a flame ionization detector. Nitrogen was used as a carrier gas with a flow rate of 40 mL/min. The operating conditions were as follows: Column temperature was kept at 130 °C for 2 min to 270 °C at 10 °C min^{-1}, with injector and detector temperature at 300 °C, respectively.

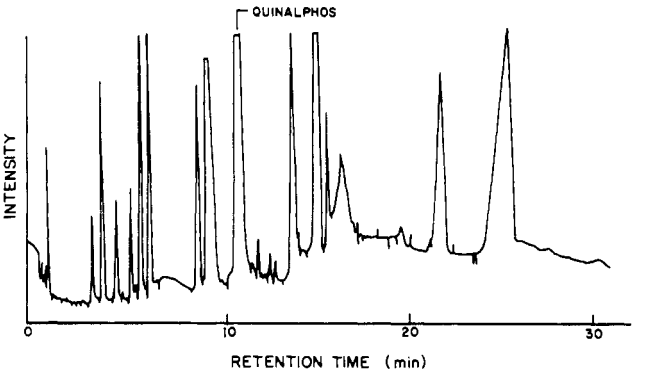

Figure 1. Gas chromatogram of degraded quinalphos.

Chemicals. Technical quinalphos (95%) was obtained from M/S Sandoz (India) Ltd., Bombay. A sample of purified quinalphos was obtained through the Environmental Protection Agency from the Battelle Memorial Institute repository, Columbus, OH.

Storage of Technical Quinalphos. A sample of technical quinalphos (95%), which showed only one peak in GLC, was stored in glass bottles at 30 °C in a biological oxygen demand (BOD) chamber for 6 months. The sample was monitored after every month by GLC.

Isolation and Identification of Impurities Developed in Technical Quinalphos on Storage. A sample of technical quinalphos when stored at 30 °C turned to a black viscous mass after 6 months. Gas–liquid chromatography (Figure 1) of this black mass showed a number of other products besides quinalphos. At least six impurities less polar than quinalphos and five more polar impurities were detected by TLC using chloroform and ethyl acetate (9:1, v/v) as the solvent system. Infrared spectrum of quinalphos showed a weak absorption band at 660 cm^{-1} due to P=S; 1220 cm^{-1} due to POC(aryl) and POC(alkyl) at 1030 cm^{-1}, whereas a degraded sample of quinalphos did not show many sharp bands because of the complex mixture of compounds.

In order to obtain a sufficient amount of these impurities for structure determination, 10 g of a 6-month-old sample of technical quinalphos was subjected to column chromatography on silica gel (700 g). Stepwise elution of column with hexane (50 mL), hexane–chloroform (2:1, 200 mL), hexane–chloroform (1:1, 500 mL), chloroform (1000 mL), chloroform–ethyl acetate (9:1, 1000 mL), ethyl acetate (500 mL), and ethyl acetate–methanol (9:1, 200 mL) separated the impurities. Identification of these impurities was achieved by NMR, mass spectra, and GC–MS (Table I and Figure 2).

Bioactivity. Laboratory-raised 18–20-day-old adults of red flour beetles (*Tribolium castaneum* Herbst.) were used for bioassay study. Potter's direct spray tower (Potter, 1941) was employed for spraying operation at 25 cm of mercury pressure

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Table I. NMR and Mass Spectral Data of Impurities Isolated from a Degraded Sample of Quinalphos

	structure	NMR values, δ	molecular ion (M^+)	yield, %
I	quinalphos	8.5 (1 H), 7.8–7.0 (4 H), 4.3 (4 H), and 1.8 (6 H)	298	7.5
II	isoquinalphos	8.6 (1 H), 7.8–7.2 (4 H), 3.4 (4 H), and 1.6 (6 H)	298	5.8
III	quinalphos oxon	8.5 (1 H), 7.8–7.0 (4 H), 2.8 (4 H), and 1.5 (6 H)	282	3.2
IV	ethyl quinoxalin-2-yl phosphate	8.6 (1 H), 7.8–7.0 (4 H), 4.8 (2 H), and 1.5 (3 H)	254	1.8
V	quinoxalin-2-yl phosphate	8.0 (1 H) and 7.6–7.0 (4 H)	226	1.0
VI	2-hydroxyquinoxaline	8.4 (1 H) and 7.6–7.0 (4 H)	146	12.0
VII	diquinoxalin-2-yl oxide	8.5 (2 H) and 7.8–7.0 (8 H)	274	3.0
VIII	quinoxaline-2-thiol	8.6 (1 H) and 7.8–7.0 (4 H)	162	18.0
IX	diquinoxalin-2-yl sulfide	8.4 (2 H) and 8.0–7.2 (8 H)	290	15.0
X	diquinoxalin-2-yl disulfide	8.4 (2 H) and 8.0–7.4 (4 H)	322	7.0
XI	dithienobisquinoxaline	7.8–7.0 (8 H)	320	3.0
XII	thienodiquinoxaline	8.0–7.0 (8 H)	288	2.0
XIII	triethyl phosphate[a]		192	
XIV	*O,O,O*-triethyl phosphorothioate[a]		198	
XV	diethyl phosphite[a]		138	
XVI	*O,O*-diethyl phosphorothioate[a]		170	
XVII	*O,O,O,O*-tetraethyl pyrophosphorothioate[a]		322	
XVIII	*O,O,O,O*-tetraethyl pyrophosphorodithioate[a]		338	

[a] Analyzed by GC–MS; not isolated by column chromatography.

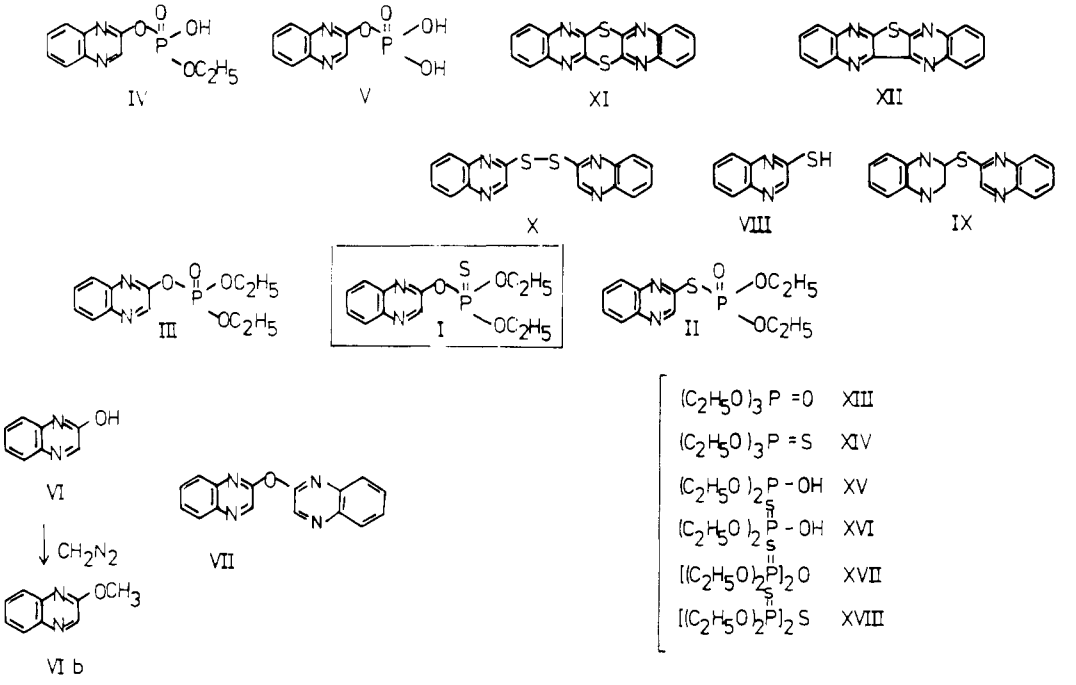

Figure 2. Degradation products of quinalphos on storage.

(0.35 kg cm^{-1}) at room temperature (25 °C). The sprayings were done in Petri dishes of 9-cm diameter, and the gap between the lower end of the tower and spray stage was 3 cm. Toluene and the surfactant Triton X-100 (0.5%) were maintained in spray solutions. Treated Petri dishes were incubated in a BOD incubator maintained at 28 ± 1 °C for posttreatment observations recorded 48 h after treatments. The average percent mortality of three replicates was calculated for each concentration and was corrected by Abbott's formula (Abbott, 1925). The regression equation and LC_{50} value for each sample were calculated statistically by probit analysis (Finney, 1971).

DISCUSSION

A sample of technical quinalphos when stored at 30 °C in a BOD for 6 months underwent degradation to give a black viscous mass, which when subjected to column chromatography gave a number of different products besides quinalphos. These products were isolated by column chromatography, purified, and identified by spectroscopic methods (Table I, Figure 2).

These products in technical quinalphos may arise due to the following reactions: (i) oxidation, (ii) thiono–thiolo rearrangement, (iii) hydrolysis, and (iv) dimerization. The proposed mechanism for the formation of these degradation products in technical quinalphos is shown in Figure 3.

The diethyl phosphorothioate, used for the synthesis of quinalphos, may be present in trace amounts in the sample of technical quinalphos, which may increase the acidity of stored sample of technical quinalphos. Due to protonation at the nitrogen atom of the quinoxaline ring, the carbon atom in the 2-position of the quinalphos may become activated. Protonated quinalphos then may undergo the Pitschimuka rearrangement to give a protonated thiolate isomer (Figure 3). This protonated thiolate isomer is further stabilized to give isoquinalphos (II).

Isoquinalphos (II) is very labile and undergoes rapid hydrolysis to give quinoxaline 2-mercaptide anion, which may either abstract a proton to give quinoxaline-2-thiol (VIII) or disproportionate and undergo dimerization by radical–radical combination to give products such as X, XI and XII.

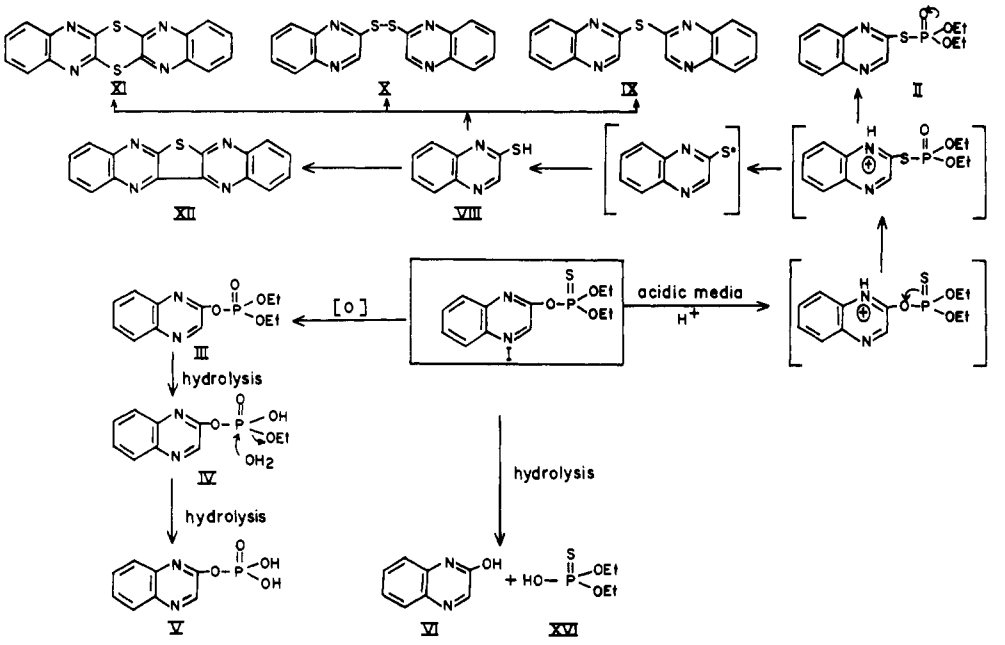

Figure 3. Proposed mechanism for degradation of technical quinalphos on storage.

Quinalphos is susceptible to oxidation at the P=S to form quinalphos oxon (III). Similar to isoquinalphos, product III may undergo hydrolysis by nucleophilic attack at the phosphorus atom followed by displacement of ethoxy group to give ethyl quinoxalin-2-yl phosphate (IV), which may further undergo hydrolysis to give quinoxalin-2-yl phosphate. Similarly nucleophilic attack at phosphorus atom of quinalphos followed by cleavage of POC (aromatic) bond may occur giving 2-hydroxyquinoxaline (VI) and diethyl phosphorothioate (XVI). The products such as X, XIII, XIV, XV, XVI, and XVII identified by GC–MS (Desmarchelier et al., 1976) may be formed from *O,O*-diethyl phosphorothioate by further oxidation and dimerization.

Effect of Impurities on Insect Toxicity. The impurities isolated from technical quinalphos were evaluated for their insecticidal activity against laboratory-reared red flour beetles (*Tribolium castaneum* Herbst.). The calculated LC_{50} values of quinalphos (I), isoquinalphos (II), and quinalphos oxon (III) are 11.34, 19.08, and 20.67 μg/plate, respectively where as products VI, VIII, IX, X, and XI are found to be nontoxic up to a level of 1% ai. It can be concluded that non-phosphorus-containing impurities were nonactive whereas phosphorus-containing impurities were as toxic as quinalphos.

From this study we can conclude that when a technical sample of quinalphos is stored at ambient temperature (30 °C) in glass bottles for more than 6 months, it undergoes degradation and loses its bioefficacy. Therefore, technical quinalphos should be stored in the refrigerator with some stabilizer. Further work on the toxicological evaluation of these impurities and stabilization of quinalphos is under progress.

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