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Detection and Analysis of Aminoparathion in Human Postmortem Specimens

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ABSTRACT: Postmortem samples from two fatalities involving parathion ingestion were examined. Parathion could not be detected in the liver tissue but a significant quantity of a related compound was detected. This was shown to be aminoparathion, a biotransformation product of parathion. The substances were extracted with hexane and analyzed by gas-liquid chromatography on a 3% OV-210 column. Thin-layer chromatography and mass spectrometery were also performed. The transformation of parathion to aminoparathion in human tissue has not been previously reported.

KEYWORDS: toxicology, chemical analysis, parathion, aminoparathion, poisoning, gas-liquid chromatography, thin-layer chromatography, mass spectrometry

Parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate) is a widely used organophosphorus pesticide in the state of New South Wales. The estimated lethal dose of parathion in man is 20 to 100 mg [*1,2*]. Parathion is oxidized to paraoxon (*O,O*-diethyl *O*-*p*-nitrophenyl phosphate) by the liver microsomal enzymes [*3*], and the two compounds are hydrolyzed by plasma and tissue esterases to produce diethylphosphorothionic acid, diethylphosphoric acid, and *p*-nitrophenol [*4*]. The last three substances represent the major urinary excretory products following a dose of parathion.

Parathion is reduced to aminoparathion (*O,O*-diethyl *O*-*p*-aminophenyl phosphorothioate) by microorganisms and mammals. The reduction process is a major degradation route for parathion in microorganisms [*5*]. Lichtenstein and Schulz [*6*] showed that yeast was primarily responsible for this reduction step in soil. Furthermore, aminoparathion was found to be a substantial reduction product when cows were fed with parathion [*7*]. Rat liver fractions have also been shown to convert parathion to aminoparathion [*8*].

In two recent suicide cases lethal quantities of an insecticide spray containing parathion were ingested. In both cases an autopsy was performed within 18 h of the reported time of death. In this paper an unidentified compound extracted from postmortem specimens, is shown to be aminoparathion. The finding is confirmed by thin-layer chromatography, gas-liquid chromatography, and mass spectrometry. In previous parathion poisoning cases received in our laboratory, an unidentified compound having chromatographic properties

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similar to those of aminoparathion has been detected. These findings suggest that the presence of aminoparathion in human postmortem specimens may be of a wider occurrence. A literature search has failed to locate any report of the transformation of parathion to aminoparathion in human tissue.

Experimental Procedure

Materials

Parathion (ethyl): a 98% technical grade sample was obtained through the courtesy of Bayer, Australia. Paraoxon: a 99% pure sample was obtained through the courtesy of Bayer, West Germany. Aminoparathion: this compound was synthesized (93% purity) in our laboratory, as it was not available commercially. Working standard solutions of the above three compounds were prepared by diluting standard stock solutions (1 mg/mL in hexane) to give concentrations of 0.002 mg/mL. pH 7 buffer solution: a mixture of a saturated solution of disodium hydrogen phosphate and a 1*M* solution of sodium dihydrogenphosphate (70:30) was prepared. All solvents and chemicals used were of analytical grade.

Preparation of Aminoparathion

The method of synthesis was adapted from previously published methods [*9,10*]. A 1-g parathion specimen was dissolved in 50 mL of ethanol and water (1:1). Twenty-five milliliters of 5*M* hydrochloric acid was added, and the solution was refluxed with 2 g of zinc dust for 1 h. The mixture was made alkaline, diluted to 500 mL with distilled water, and extracted three times with 200 mL of hexane. The concentrated extract was then purified by column chromatography using silica gel GF_{254} and a mixture of hexane/ethanol/acetone (3:1:1, v/v) [*11*]. The appropriate fraction containing the amine compound was further purified by preparative thin-layer chromatography using the above solvent mixture. A dark amber-colored liquid with an amine-like odor was obtained. Purity was determined to be 93% by nonaqueous titration [*12*]. Infrared analysis confirmed the presence of an amine group with characteristic absorption bands at 3460, 3380, and 1630 cm^{-1}. Mass spectrometric analysis confirmed that the chemical structure of the prepared compound was that of aminoparathion having a molecular weight of 261 (see Discussion).

Extraction Procedures

Tissues

A direct hexane extraction procedure was used [*1*]. To a 10-g homogenized tissue specimen in a 250-mL glass stoppered conical flask, 5 g of anhydrous sodium sulphate plus 50 mL of hexane was added. The mixture was shaken vigorously for 2 min. The hexane layer was decanted into a 25-mL screw cap centrifuge tube and centrifuged for 3 min at 2000 rpm. An aliquot of the hexane was then directly injected into the gas chromatograph.

Blood, Urine, and Bile

To a 4-mL specimen in a centrifuge tube, 1 mL of pH 7 buffer solution was added. The mixture was shaken on a vortex mixer for 10 s. Five millilitres of hexane was then added, the tube capped, and the contents gently shaken by inversion for 2 min. The mixture was then centrifuged for 3 min at 2000 rpm. A 4-mL aliquot of the hexane layer was transferred to a 5-mL glass tube and gently evaporated to dryness in a 40°C water bath using nitrogen. The extract was reconstituted with hexane and an aliquot injected into the gas chromatograph.

Recovery Experiment

Recovery experiments were performed on duplicate blank postmortem specimens and also water specimens by spiking one with parathion and the duplicate with aminoparathion. The results are shown in Table 1.

Thin-Layer Chromatography

Thin-layer chromatography (TLC) was performed on precoated plates of silica gel 60 F_{254}, 0.25 mm thick and 10 by 20 cm in area. (E. Merck, Darmstadt, West Germany). Two solvent systems were used: (1) hexane/ethanol/acetone (3:1:1, v/v) [*11*] and (2) diethyl ether.[3]

The plates were developed, air dried at 25°C, and visualized by the following methods:

1. Viewing under 254-nm ultraviolet light. Parathion, aminoparathion, and paraoxon appeared as purple spots on a fluorescent green backround.
2. Spraying with a 0.25% solution of sodium fluorescein in ethanol, then exposing the plate to bromine vapour for 30 s using a dilute aqueous solution of bromine [*13*]. Parathion and aminoparathion gave yellow spots on a pink background. Paraoxon gave no reaction.
3. Spraying with a 1% solution of sodium nitrite in 1*M* hydrochloric acid, leaving the plate for 1 min and then spraying the plate with a 0.4% solution of *N*-(1-naphthyl)-ethylene diammonium dichloride in methanol [*14*]. Aminoparathion gave a purple-red spot on a white background. Parathion and paraoxon gave no reactions. The R_f values of the above mentioned compounds are shown in Table 2.

It was necessary to purify the liver extracts because of the significant quantity of extraneous material that was co-extracted. Preparative TLC plates (1 mm thick and 20 by 20 cm in area) were used. The above solvent systems and sprays were used.

Gas-Liquid Chromatography

Gas-liquid chromatography (GLC) analysis was carried out on a Hewlett Packard model 5730A gas chromatograph equipped with an auto sampler and coupled to a nitrogen phosphorus detector, model 18789A. Four different column packings were examined: (1) 3% OV-210 liquid phase on 100-120 mesh Chromosorb W, acid washed (AW), dimethyldichlorosilane (DMCS); (2) 3% OV-101 on 100-120 mesh Chromosorb W, AW, DMCS; (3) 3% OV-17 on 100-120 mesh Chromosorb W, AW, DMCS; and (4) GP 3% SP2250DB on 100-120 Supelcoport, AW, DMCS. All glass columns were 1.8 m in length and 1 mm in inner

TABLE 1—*Recoveries of parathion and aminoparathion from postmortem specimens and water specimens.*

Specimen	Recovery, %	
	Parathion	Aminoparathion
Stomach and contents	96	90
Liver	102	86
Blood	88	80
Urine	97	101
Bile	92	48
Water	100	95

[3]I. G. Ferris, personal communication, Agricultural Research Centre, Tamworth, New South Wales, Australia, 1982.

TABLE 2—*Chromatographic behavior of parathion, aminoparathion, and paraoxon.*[a]

Compound	Relative Retention on GLC Phase				Mobility on TLC Solvent System, R_f value	
	OV-210	OV-101	OV-17	SP 2250 DB	1	2
Parathion	1.00	1.00	1.00	1.00	0.62	0.65
Aminoparathion	0.61	0.81	1.03	1.04	0.48	0.41
Paraoxon	1.31	0.92	0.84	0.87	0.51	0.29

[a]GLC relative retention times measured at 190°C and TLC mobility measured at 25°C.

diameter. The flow rate of the carrier gas (nitrogen) was 25 mL/min. Chromatographic retention times were determined by isothermal analysis at 190°C for all four column packings.

Relative retention times for parathion, aminoparathion, and paraoxon on these four columns are shown in Table 2. Quantitative analysis was performed using the 3% OV-210 column kept at 220°C. The results are shown in Table 3. The practical limit of detection of the above three compounds was 0.005 mg/L.

Mass Spectrometry

Methane chemical ionization (CI) mass spectra were obtained using a quadrupole Finnigan 4021 GLC/MS system. The gas chromatograph was equipped with a 1.2-m-long, 3-mm-inner diameter glass column packed with 3% OV-17 on 80-100 mesh Chromosorb W, AW, DMCS. The carrier flow rate was 25 mL/min. The temperature was programmed to increase from 165 to 250°C at a rate of 10°C/min.

Electron impact (EI) mass spectra at 70 eV were obtained using a Hewlett-Packard 5985 GLC/MS system. The gas chromatograph was equipped with a 1.8-m-long, 1-mm inner diameter glass column packed with 3% OV-210 on 100-120 mesh Chromosorb W, AW, DMCS. The flow rate of the carrier gas (nitrogen) was 30 mL/min. The temperature was set at 210°C.

Discussion

The EI mass spectrum of our synthesized product is shown in Fig. 1. The spectrum is characterized by the following major *m/e* ions: 261, 233, 205, 125, 109, 108, 97, and 80. The

TABLE 3—*Levels of parathion and aminoparathion found in cases 1 and 2.*

Specimen	Case 1		Case 2	
	Parathion	Aminoparathion	Parathion	Aminoparathion
Stomach and contents	360 mg[b,c]	ND[a]	940 mg[b,c]	ND[a]
Liver	ND[a]	0.47 mg/kg[b,c,d]	ND[a]	32 mg/kg[b,c,d]
Blood	0.01 mg/L[b]	0.27 mg/L[b,c,d]	0.15 mg/L[b,c,d]	6.7 mg/L[b,c,d]
Urine	ND[a]	0.02 mg/L[b]	NA[e]	NA[e]
Bile	NA[e]	NA[e]	0.63 mg/L[b,c]	0.31 mg/L[b,c]

[a]ND—not detected.
[b]GLC confirmed.
[c]TLC confirmed, solvent Systems 1 and 2.
[d]GLC/MS confirmed.
[e]NA = not available.

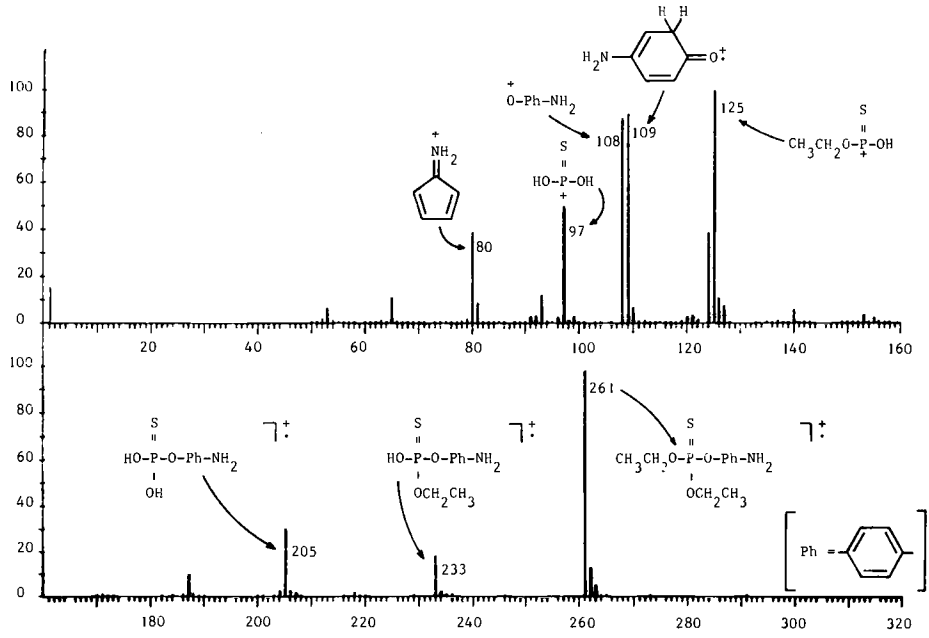

FIG. 1—*Electron impact mass spectrum of aminoparathion.*

compound gives an intense ion peak at *m/e* 261 (confirmed by methane chemical ionization mass spectrometry). Sequential loss of the ethyl groups gives rise to two fragment ions at *m/e* 233 and 205. These two ions undergo typical ether cleavage to produce the remaining major fragment ions in the spectrum. The ions at *m/e* 125 and 97 are a result of the loss of the radical $\dot{O}C_6H_4NH_2$. The *m/e* 233 and 205 ions also lose the neutral fragments, $CH_3CH_2OP(S)(O)$ and $HOP(S)(O)$, respectively, to yield the odd electron ion at *m/e* 109. This ion then loses a hydrogen radical to form the resonance stabilized ion at *m/e* 108. A loss of CO from this ion results in the formation of the *m/e* 80 ion.

The chromatographic data shown in Table 2 demonstrate that the more polar OV-210 column gave better separation (baseline) of parathion, aminoparathion, and paraoxon. Thus, it was chosen to quantitate the extracts. None of the extracts, including postmortem blanks, produced any unusual peaks on the GLC chromatograms. The only peaks detected were those of parathion and aminoparathion or both. Both TLC solvent systems used were found to give adequate separation of the above three compounds. The sprays used in Procedure 3 of the TLC section were particularly useful in that they confirmed the presence of a primary aromatic amino group in the extracts. Paraoxon, a metabolite of parathion, although not detected in the postmortem specimens examined, has been included in Table 2 so that its chromatographic behavior can be compared with those of parathion and aminoparathion.

The amounts of parathion found in the stomach and contents of both cases (Table 3) represent the ingestion of lethal doses of the pesticide [*1,2*]. The results also show that no aminoparathion was found in the stomach, whereas the corresponding liver extracts contained aminoparathion but no parathion.

Recovery results (Table 1) show that greater than 80% of the free parathion and aminoparathion present in the postmortem specimens was extracted. The exception was the low recovery of aminoparathion from bile.

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