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Mass Spectrometric Identification of Methyl-isofenphos

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Isofenphos (Amaze®), 1-methylethyl 2-[[ethoxy [(1-methylethyl) amino] phosphinothioyl]-oxy] benzoate, is a soil insecticide that had been used commercially in the midwestern corn belt since 1981. Previous studies of this compound tentatively identified a stable oxygen analog metabolite in soil by gas chromatography. An unknown component was also detected in soil by GC and eluted between the parent isofenphos and its oxon metabolite. This unidentified compound was found in the Amaze 20G formulation. It degraded in soil within three weeks after application of the formulated insecticide (Felsot 1984).

An in vivo metabolite, N-desisopropylisofenphos oxon, has been shown recently to be the ultimate inhibitor of acetylcholinesterase which produces the characteristic symptoms of acute organophosphate toxicity (Gorder et al. 1986). A recent report indicated that technical isofenphos (92% purity) may also cause delayed neurotoxicity (Wilson et al. 1984). Francis et al. (1985) showed that technical isofenphos extracted from a commercial sample of Amaze granular insecticide could also induce delayed neurotoxicity. Ryan and Fukuto (1984) have shown that trialkyl phosphate impurities in malathion formulations can be potent delayed neurotoxicants. We do not know if a relationship exists between the unknown impurity in the Amaze 20G formulation and its reported neurotoxicity. Using GC/MS methods, we now report the identification of this unknown, which we are calling methyl-isofenphos. We have also determined the GC/MS spectra of parent isofenphos and its oxon metabolite because these are important environmental residues.

MATERIALS AND METHODS

GC/MS spectra were measured on a 100 μg/mL solution of Amaze 20G in acetone. Analytical standards of isofenphos, isofenphos oxon, and methyl-isofenfos were also studied. The methyl-isofenfos was supplied gratis by the Mobay Chemical Corporation. The GC method was similar to one previously reported (Felsot 1984). A glass

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column (183 cm x 2 mm i.d.) packed with 2% Apiezon plus 1% Carbowax 20M-terephthalic acid on 100/120 mesh Chromosorb support was used in a Varian Model 1700 GC interfaced to a Varian MAT CH-7 mass spectrometer (Wei and Felsot 1982). Column and injector temperatures were 205° and 240°C, respectively. Helium flow rate was 15 mL/min. MS operating conditions were as follows: electron energy, 70 eV; ion source temperature, 250°C; separator temperature, 250°C; emission current, 300 uA; ion accelerating voltage, 3 kV. The mass spectra were recorded on a Spectrosystem 100. The chemical ionization spectra were obtained on a Hewlett Packard Model 5988 mass spectrometer equipped with a RTE Data System. The mass spectrometric parameters were as follows: electron energy, 200 eV; emission current, 300 uA; reagent gas, methane.

RESULTS AND DISCUSSION

The identification of isofenphos and its metabolites was carried out by GC/MS. At 70 eV ionization potential, numerous fragmentations of the molecules occurred. Figures 1-6 show the 70 eV mass spectra and major fragmentation pathways of isofenphos, isofenphos oxon, and the unknown. The characteristic fragment ions of these compounds are shown in Table 1.

Isofenphos, isofenphos oxon, and the unknown gave parent ions of m/z 345, 329, and 317, respectively. All three compounds showed common fragments of m/z 43, 58, 93 and 120 corresponding to either cleavage and fragmentation of the benzoate or the N-isopropyl group (Figures 1, 3, and 5). These results suggested that the unknown may be 1-methyl 2-[[ethoxy[(1-methylethyl) amino] phosphinothioyl]-oxy] benzoate, and we have called it methyl-isofenphos.

Isofenphos oxon showed a unique ion in relatively large abundance at m/z 271. The ion at m/z 271 corresponded to a loss of N-isopropyl amino group (i.e., M-58). The isofenphos and methyl-isofenphos also had ions corresponding to M-58, but their relative abundance was less than 10%. The correlation between the M-58 ion abundance and the presence or absence of oxygen on the phosphorus atom results from the relative electronegativities of oxygen and sulfur atoms. The M-58 ion is produced by cleavage of the N-P bond adjacent to the oxygen atom (Figure 4) or sulfur atom (Figures 2 and 6) of the parent molecules. The charge remains with the oxygenated fragment or sulfurated fragment. Since oxygen is more electronegative than sulfur, the M-58 ion of isofenphos oxon is more abundant than those of isofenphos or methyl-isofenphos. Similarly, the intense M-15 ion (i.e., M-CH_3) of isofenphos oxon also reflects the oxygen effect that stabilizes a positively charged ion.

Table 1 and Figures 2 and 4 show the most abundant ions for isofenphos and isofenphos oxon at m/z 213 and m/z 229, respectively. These ions are resulted from the rearrangement of one hydrogen atom with the elimination of a neutral olefin. A

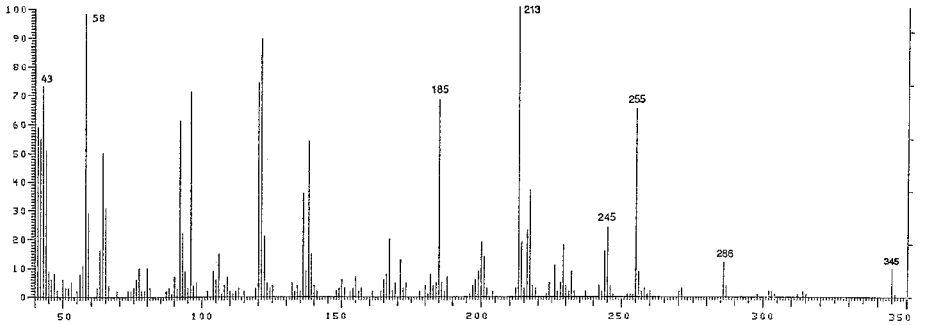

Figure 1. Mass spectrum of isofenphos

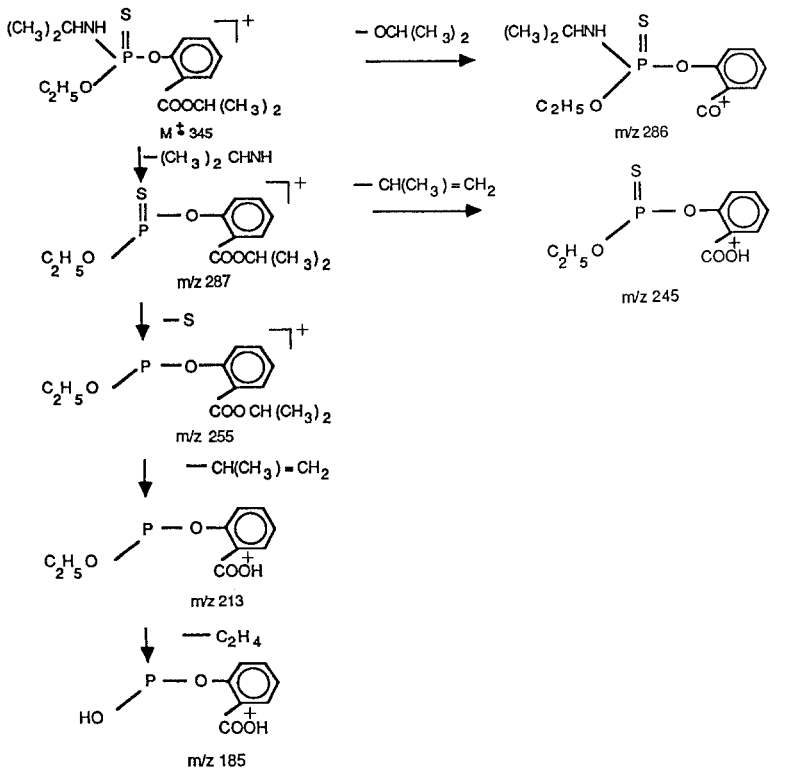

Figure 2. Major fragmentation pathways of isofenphos

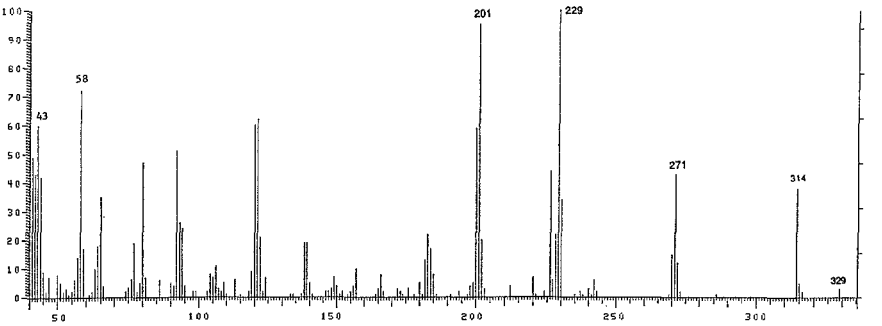

Figure 3. Mass spectrum of isofenphos oxon

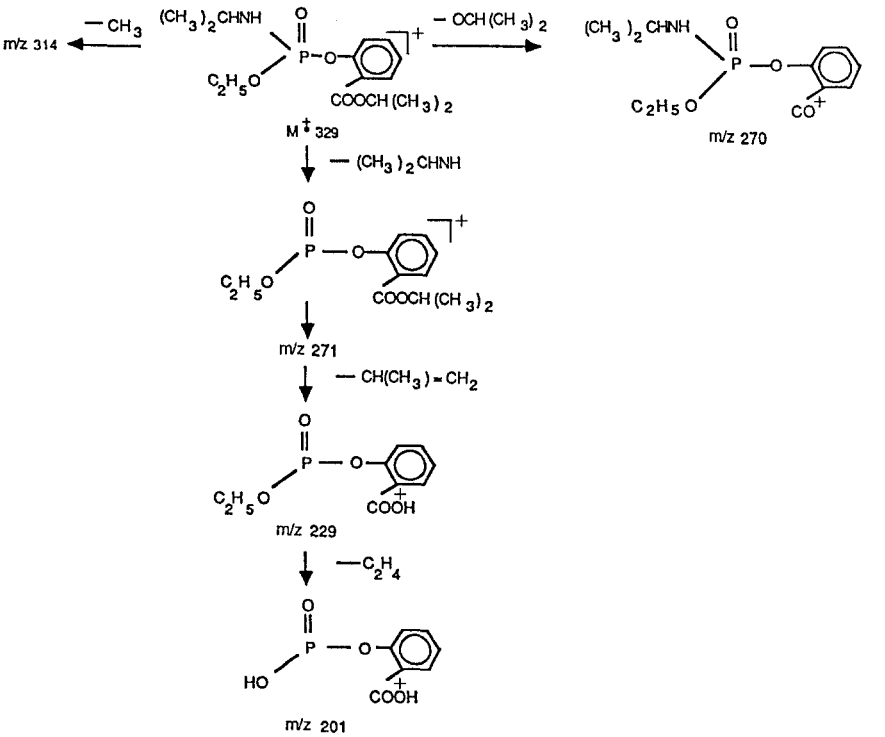

Figure 4. Major fragmentation pathways of isofenphos oxon

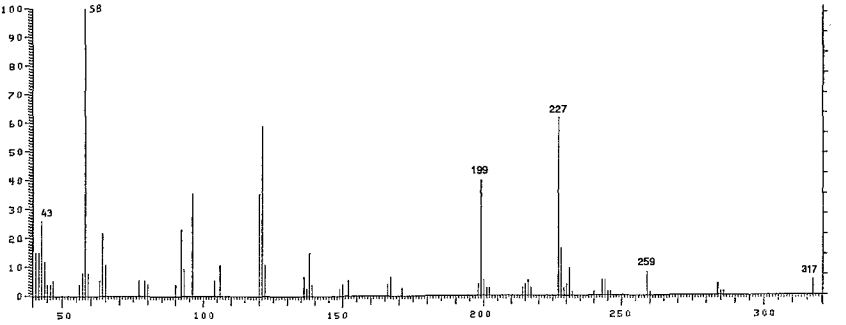

Figure 5. Mass spectrum of methyl-isofenphos

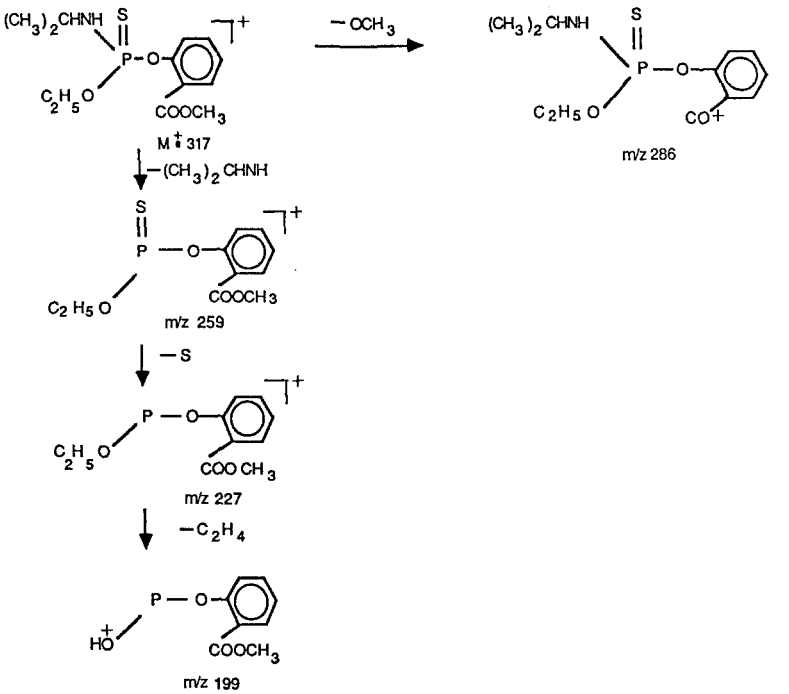

Figure 6. Major fragmentation pathways of methyl-isofenphos

Table 1. The 70 eV characteristic ions and GC retention time of isofenphos, isofenphos oxon and methyl-isofenphos.

Characteristic ions	Isofenphos	Isofenphos oxon	Methyl-isofenphos
	m/z (percent)		
M.+	345 (10)	329 (3)	317 (5.1)
M-15	-	314 (38)	-
M-43	302 (2)	286 (1)	-
M-58	287 (4)	271 (43)	259 (7.7)
M-59	286 (12)	270 (15)	-
M-90	255 (65)	-	227 (62)
229	-	(100)	-
213	(100)	-	-
201	-	(95)	-
199	-	-	(40)
185	(68)	-	-
RT (min)	12.4	10.5	11

corresponding rearrangement ion is absent from the methyl-isofenphos spectrum. Its absence suggests a functional group of the benzene ring on the unknown component that differs from those of isofenphos and isofenphos oxon.

The m/z 43 alkyl ion of methyl-isofenphos is less intense than those of isofenphos or isofenphos oxon (Figures 1, 3, and 5). This difference might be expected if the molecular structure of methyl-isofenphos contains only one methylethyl group, as opposed to the two methylethyl groups in both isofenphos and isofenphos oxon. This observation provided further support for our suggested structure of methyl-isofenphos.

The unknown was also examined by chemical ionization mass spectrometry. The appearance of a protonated molecular ion and the adduct ions at m/z 318, 346, and 358 representing $[M+H]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$, respectively. The CI and EI spectra of the unknown were identical to those of an authentic sample of methyl-isofenfos.

Based on our comparative interpretation of the mass spectra of isofenphos, isofenphos oxon, and the unknown component, we conclude that the unknown is a methyl derivative of isofenphos. Since this compound is present in commercial Amaze formulations and is transiently present in the environment, further studies are needed to characterize its toxicity.

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