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Identification of the Herbicide 2,4,6-Trichlorophenyl p⁴-Nitrophenyl Ether in Imported Rainbow Trout

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A sample of frozen rainbow trout imported from Japan was examined for pesticide residues. The sample extract, after elution from a Florisil column (AOAC, 29.001, 1975), exhibited an electron capture glc response having relative retention times (aldrin = 1.0) of 2.8 and 4.0 on 10% OV-101 and on 10% OV-101/15% QF-1, respectively. The electrolytic conductivity detector (chloride mode) and the microcoulometric detector provided similar responses, indicating that the compound of interest contained chlorine. The unknown compound could not be identified as a common halogenated pesticide or industrial chemical using our available retention time data. This paper reports the structural elucidation, glc characteristics, and recovery data for this compound.

MATERIALS AND METHODS

GC-MS-COM System: Pye 104 gas chromatograph interfaced to an AEI MS-30 Double Beam Mass Spectrometer by means of a silicone membrane separator. Data were acquired using an AEI DS-50DB data system. The mass spectrometer was operated at 70 eV electron energy, 4kV accelerating voltage, 100 uA trap current, 250°C source temperature, 3 sec./decade scan speed, 1000 resolution for low resolution scans and 3000/3000 resolution for double beam accurate mass measurements. The gas chromatograph was equipped with a 5 ft x 4 mm i.d. glass column packed with 3% OV-101 on Chromosorb WHP, 80/100 mesh, and was operated at column and injection temperatures of 190 and 215°C, respectively, and a helium flow rate of 40 ml/min. The membrane separator and transfer lines were maintained at 205 and 210°C, respectively.

GLC (for retention time and recovery data): Tracor Model 222 gas chromatograph equipped with a ^{63}Ni electron capture detector and 6 ft x 4 mm i.d. glass columns packed with (A) 10% OV-101 on Chromosorb WHP, 80/100 mesh, and (B) a 1:1 mixture of 10% OV-101 and 15% QF-1 on the same support. Column temperature (ca. 200°C) was adjusted to permit elution of p,p'-DDT at 3.03 and 3.28 (retention time relative to aldrin) on columns A and B, respectively. The detector response was adjusted to provide one-half full scale deflection for one nanogram of heptachlor epoxide.

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Sample clean-up: The 15% ether in petroleum ether fraction was streaked on an E. Merck 0.2 mm precoated aluminium oxide F-254 neutral (type E) tlc sheet that had been pre-washed with a solution of n-heptane-acetone (98:2) and activated at 85°C for 15 min. The unknown material was separated by elution with this same solution and visualized by spraying the sides of the sheet with a solution of silver nitrate and 2-phenoxyethanol in acetone with subsequent exposure to UV light (MITCHELL 1961). The protected portion of the tlc sheet was scraped and the compound of interest eluted with acetone.

RESULTS AND DISCUSSION

Low resolution gc-ms analysis of the sample extract after tlc clean-up was used to determine that the molecular weight of the unknown compound was 317, that it contained three chlorine atoms and that it contained an odd number of nitrogen atoms (Fig. 1). The fragmentation pattern was similar to that of the herbicide nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether), $C_{12}H_7Cl_2NO_3$ (Fig. 2), suggesting a similar structure. The unknown component was tentatively identified as a trichloro-nitro-phenyl ether ($C_{12}H_6Cl_3NO_3$). The structure of the unknown was confirmed by obtaining accurate mass measurements and elemental composition data (Table 1) using double beam gc-ms. A search of the literature led to references on a relatively new herbicide, 2,4,6-trichlorophenyl-p-nitrophenyl ether, tradenamed MO^R. FORMIGONI and CASTAGNA (1972) reported that the herbicide has a spectrum of action similar to that of the chemically related herbicide nitrofen. AKAHIRA et al. (1973) suggested its use for weed control in rice fields. YAMADA (1975) reported finding residues of both MO^R and nitrofen in soil from rice fields several months after application.

A reference standard of this compound was obtained from the manufacturer, and the identity of the unknown component in the sample was confirmed by retention time and mass spectral data. Quantitation by electron capture glc showed that the level of 2,4,6-trichlorophenyl-p-nitrophenyl ether in the original trout sample was 2.2 ppm in the edible portion. Two subsequent samples of rainbow trout from Japan were found to contain residue levels of 0.6 and 0.2ppm in the edible portion.

Preliminary recovery studies (Table 2) indicate that 2,4,6-trichlorophenyl-p-nitrophenyl ether can be recovered by the official pesticide multiple residue procedures for fatty foods (AOAC, 29.014, 1975) and for non-fatty foods (AOAC, 29.011a, 1975). Elution of the herbicide through Florisil (AOAC, 29.015, 1975) with fatty foods is variable; more than half is recovered in the 6% ether in petroleum ether fraction, and the remainder is recovered in the 15% ether in petroleum ether fraction. The herbicide is recovered quantitatively from non-fatty commodities (less than 2% fat content) in the 15% ether in petroleum ether fraction.

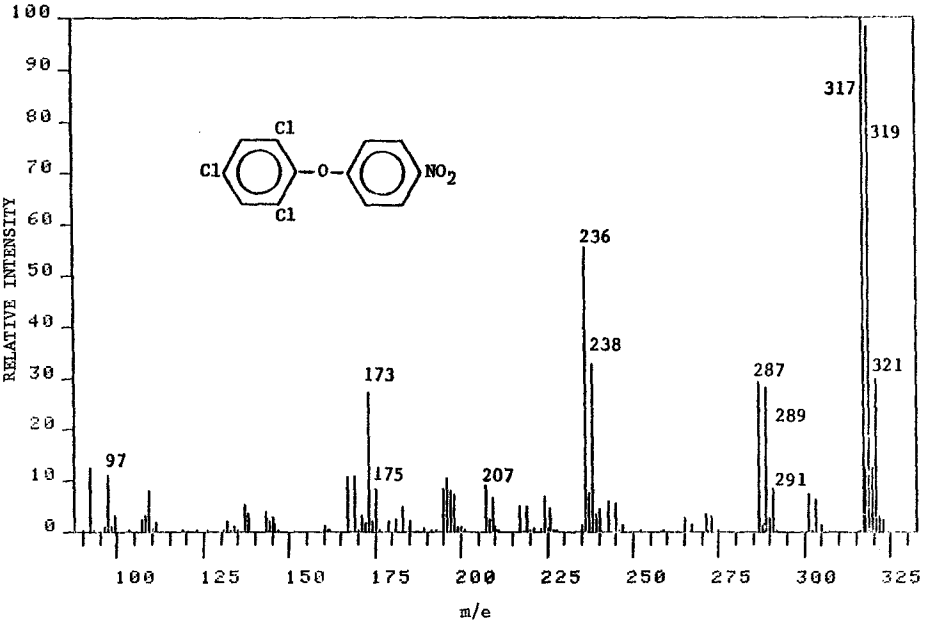

Fig. 1. Mass spectrum of 2,4,6-trichlorophenyl-p-nitrophenyl ether

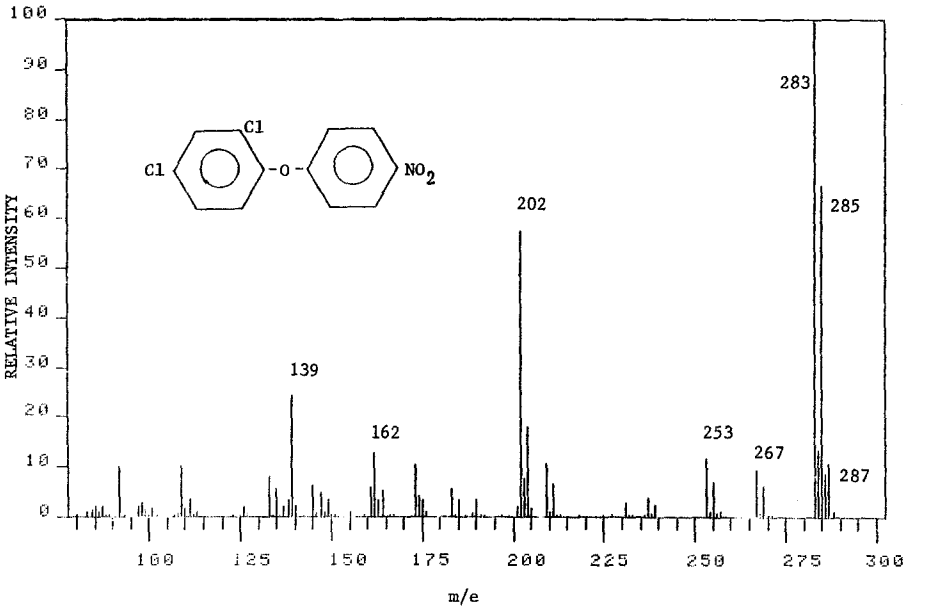

Fig. 2. Mass Spectrum of 2,4-dichlorophenyl-p-nitrophenyl ether

TABLE 1

Accurate Mass Measurements and Elemental Composition Data for 2,4,6-trichlorophenyl-p-nitrophenyl ether

ISOTOPE CLUSTER	MEAS MASS	DEV.
$C_{12}H_6NO_3{}^{35}Cl_3$	316.9397	-1.6
$C_{12}H_6NO_3{}^{35}Cl_2{}^{37}Cl_1$	318.9363	-2.1
$C_{12}H_6NO_3{}^{35}Cl_1{}^{37}Cl_2$	320.9344	-1.0
$C_{12}H_6NO_2{}^{35}Cl_3$	300.9492	2.8
$C_{12}H_6NO_2{}^{35}Cl_2{}^{37}Cl_1$	302.9439	0.5
$C_{12}H_6NO_2{}^{35}Cl_1{}^{37}Cl_2$	304.9400	-0.5
$C_{12}H_6O_2{}^{35}Cl_3$	286.9440	0.6
$C_{12}H_6O_2{}^{35}Cl_2{}^{37}Cl_1$	288.9409	0.5
$C_{12}H_6O_2{}^{35}Cl_1{}^{37}Cl_2$	290.9385	0.3
$C_{12}H_6O{}^{35}Cl_2$	235.9831	3.6
$C_{12}H_6O{}^{35}Cl_1{}^{37}Cl_1$	237.9739	-2.8
$C_{12}H_6O{}^{37}Cl_2$	239.9758	2.1
$C_{11}H_5{}^{35}Cl_2$	206.9783	1.5
$C_{11}H_5{}^{35}Cl_1{}^{37}Cl_1$	208.9755	1.7
$C_{11}H_6{}^{35}Cl_1$	173.0160	0.2
$C_{11}H_6{}^{37}Cl_1$	175.0155	2.7
C_6H_4O	92.0279	1.6

* Deviation is the difference between the actual measured mass and the theoritical mass expressed in millimass units.

TABLE 2

Recovery Data for 2,4,6-trichlorophenyl-p-nitrophenyl ether through AOAC Pesticide Multiple Residue Methodology

Commodity	Method (AOAC, 1975)	Spiking Level (ppm)	% Recovery
Cheese Fat	Fatty Food	0.64	85.6*
		0.64	94.4**
Raw Shrimp	Non-Fatty Food	0.94	95.2
		0.94	99.8

*64.1% recovered in 6% ether in petroleum ether fraction
21.5% recovered in 15% ether in petroleum ether fraction

**58.6% recovered in 6% ether in petroleum ether fraction
35.8% recovered in 15% ether in petroleum ether fraction

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