FENITROTHION, DERIVATIVES, AND ISOMERS: HYDROLYSIS, ADSORPTION, AND BIODEGRADATION

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ABSTRACT - Ultraviolet spectra of fenitrothion and related compounds were determined. Hydrolysis of fenitrothion and fenitrooxon, adsorption of fenitrothion on sediments, and behaviour of fenitrothion in aerated and non-aerated mixtures of river water and sediment were studied. Aminofenitrothion was prepared and characterized. The purity of technical fenitrothion was determined.

The hydrolysis and adsorption were studied by an ultraviolet spectrophotometric method enabling a simultaneous determination of fenitrothion or fenitrooxon and 3-methyl-4-nitrophenol. A gas chromatographic method was used to determine fenitrothion in river water. Aminofenitrothion was prepared by reduction of fenitrothion in methanolic hydrogen chloride by iron filings. Technical fenitrothion was analyzed by thin-layer chromatography.

The hydrolysis of fenitrothion and fenitrooxon and the formation of 3-methyl-4-nitrophenol proceeds only very slowly under environmental conditions. Fenitrothion is extensively adsorbed on sediments. Microbiological processes are probably the main cause of fenitrothion disappearance from river water and aminofenitrothion is an intermediate degradation product under anaerobic conditions. The concentration of fenitrothion in river water follows a first-order kinetic equation with a half-life of 30-50 h. The purity of technical fenitrothion varies from preparation to preparation. O,S-dimethyl-(3-methyl-4-nitrophenyl)-phosphorothioate (S-methyl fenitrothion) was identified in two, O,O-dimethyl-S-(3-methyl-4-nitrophenyl)-phosphorothioate (S-phenyl fenitrothion) in one technical fenitrothion preparation. It is suggested that more attention should be given to the purity of technical fenitrothion and the properties of fenitrothion isomers should be determined.

INTRODUCTION

Fenitrothion (O,O-dimethyl-O-(3-methyl-4-nitrophenyl)-phosphorothioate) is dissipated in the environment by several routes. A significant amount of fenitrothion may evaporate and be deposited well outside the application area. Droplets of fenitrothion, 10, 50, 100, and 500 μ in diameter, deposited on cardboard, evaporated completely in 5, 25, 50, and 100 h at a wind velocity of 3 m/sec (6.7 mph, Murai and Tanaka 1970). The evaporated fenitrothion will be of course highly diluted and very likely will not cause detectable effects in non-target areas. The evaporation may partly account for the initially rapid disappearance of fenitrothion from foliage. Foliage, containing 2-4 μg/g of fenitrothion lost 50% of the residue during the first 4 days and 70-85% during two weeks (Yule and Duffy 1971). A large amount of fenitrothion is probably also absorbed by the foliage. According to Miyamoto (1972), 50% of the deposited fenitrothion (12 μg/g) penetrated the tissue of rice plants. The concentration in the plant tissues decreased to less than 5% by 7 days, and only traces (0.07 ng/g) were present after 46 days. In the plant itself, fenitrothion is probably hydrolyzed.

The half-life of fenitrothion in soils is 3-6 days. Fenitrothion is quite strongly adsorbed and hydrolyzed to 3-methyl-4-nitrophenol, and small amounts of aminofenitrothion and demethylfenitrothion are also detectable (Anonymous 1973a). Yule and Duffy (1971) found only a low concentration of fenitrothion in forest soil. A structurally closely related pesticide, parathion, is more stable with a half-life of 8-25 days in the soil in the tropics (Sethunathan 1973a, 1973b; Sethunathan and Yoshida 1973). The main degradation product of parathion is aminoparathion.

The solubility of fenitrothion in water is of the order of 20 mg/ℓ. Fenitrothion is rapidly adsorbed on suspended solids (Peterson and Zitko 1974) and probably removed by sedimentation. Little is known about its fate in the sediment, but it is likely that, similarly to parathion, the amino derivative is the first degradation product. The fate of parathion in sediments was studied by Graetz et al. (1970). Microbiological activity rather than a purely chemical process was responsible for the degradation of parathion. From 0.15 to 0.18% of initially present parathion was decomposed per day. Aminoparathion was the degradation intermediate. Some other compounds, formed from parathion by bacteria, were detected, but not identified.

Little is known about the degradation of fenitrothion, dissolved in water. A solution of fenitrothion in distilled water is stable whereas fenitrothion dissolved in "polluted water" disappears in approximately 3 days (Anonymous 1973a). As can be expected, the rate of hydrolysis of organophosphates generally increases with increasing pH and at a constant pH the hydrolysis follows a pseudo first-order kinetics. O-methyl organophosphates are hydrolyzed faster than the corresponding O-ethyl compounds. For example, only 26% of methyl parathion remained dissolved in distilled water after 3 weeks as compared to 56% of parathion (Cowart et al. 1971). The half-lifes of several organophosphate pesticides, determined by Ruzicka et al. (1967) are summarized in Table 1. Due to the experimental conditions the data are not directly applicable to a natural situation, however they give a good idea about the relative stability of these compounds.

TABLE 1. Rate of hydrolysis of organophosphate pesticides (ethanol-buffer pH = 6.0, 20:80, 70°, Ruzicka et al. 1967).

Pesticide	Half-life, h
Dichlorvos	1.35
Malaoxon	7.0
Malathion	7.8
Methyl parathion	8.4
Phosphamidon peak I	10.5
Fenitrothion	11.2
Phosphamidon peak II	14.0
Fenthion	22.4
Paraoxon	28.0
Parathion	43.0
Chlorfenvinphos	93.0
Carbophenothion	110.0
Dimefox	212

The data of Truchlik et al. (1972) on the hydrolysis of fenitrothion are given in Table 2.

TABLE 2. Rate of hydrolysis of fenitrothion at 20° (Truchlik et al. 1972).

Conditions	Half-life
1M sodium hydroxide	5.3 min
0.1M sodium hydroxide	41 min
Buffer pH = 12.3	192 min
Buffer pH = 9.2	150 days
Water pH = 7.0	12.6% hydrolysis at 390 days

The rate of disappearance of fenitrothion from water samples was studied by Sundaram (1973) and by Peterson and Zitko (1974). The hydrolysis product, 3-methyl-4-nitrophenol was however not measured and it is not certain whether the decrease of fenitrothion concentration was due to hydrolysis or some other causes such as adsorption on container walls.

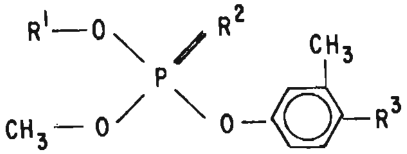
Quentin et al. (1969) investigated the hydrolysis of parathion in distilled and tap water. The hydrolysis was slow and only a small amount of 4-nitrophenol was generated. Gomaa and Faust (1972) determined the hydrolysis of parathion and paraoxon (10^{-5}-10^{-4}M) in 0.2M phosphate buffers. The hydrolysis proceeded according to the first-order kinetics. Parathion and paraoxon were determined by gas chromatography and the results are summarized in Table 3.

TABLE 3. Hydrolysis of parathion and paraoxon (Gomaa and Faust 1972).

pH	Temperature, °C	Half-life, h	
		parathion	paraoxon
3.1	20	4182	4726
5.0	20	3670	4156
7.4	20	2594	3450
9.0	20	523	70
10.4	20	33	6
3.1	10	13327	13832
3.1	40	808	861
3.1	60	181	172
9.0	10	1281	181
9.0	40	127	18
9.0	60	26	6

Relatively little attention has been paid to degradation products of fenitrothion and to impurities present in commercial fenitrothion. The structural formulae of compounds discussed below are given in Figure 1. Fenitrooxon is generally assumed to be the biologically active transformation product of fenitrothion, responsible for its insecticidal activity. Methods for the determination of fenitrooxon are available (see for example, Bowman and Beroza 1969; Hladka and Kovac 1973). Only low levels of fenitrooxon are usually found, and Yule and Duffy (1971) reported that fenitrooxon may be generated as an artifact during thawing and refreezing of samples. The detection of fenitrooxon by gas chromatography is much less sensitive than that of fenitrothion. The reasons for this are not clear. It is possible that fenitrooxon as well as oxons of other organophosphates decompose on the column. Aminofenitrothion is the first intermediate in the reductive degradation of fenitrothion by bacteria. *Bacillus subtilis* converts 65% of fenitrothion into aminofenitrothion within 48 h. In addition, a

I



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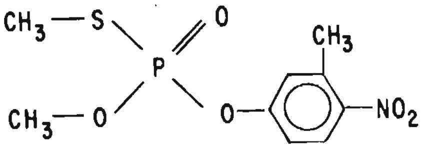

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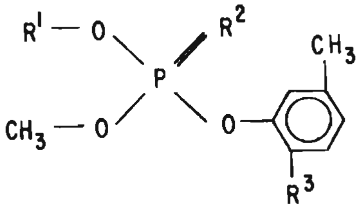

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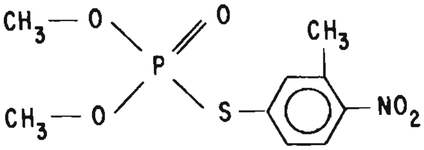

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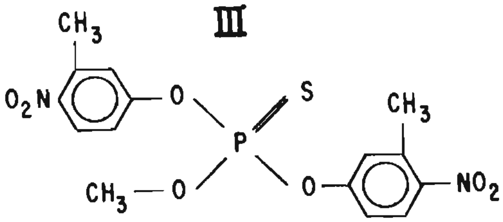

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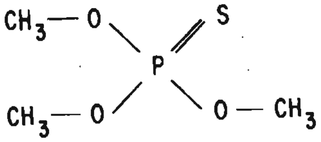

Fig. 1. Structural formulae of fenitrothion and related compounds.

I. $R^1 = CH_3$, $R^2 = S$, $R^3 = NO_2$: fenitrothion
$R^1 = H$, $R^2 = S$, $R^3 = NO_2$: demethylfenitrothion
$R^1 = CH_3$, $R^2 = O$, $R^3 = NO_2$: fenitrooxon
$R^1 = CH_3$, $R^2 = S$, $R^3 = NH_2$: aminofenitrothion

II. $R^1 = CH_3$, $R^2 = S$, $R^3 = NO_2$: O,O-dimethyl-O-(3-methyl-6-nitrophenyl)-phosphorothioate

III. O-methyl-O,O-di-(3-methyl-4-nitrophenyl)-phosphorothioate

IV. O,S-dimethyl-O-(3-methyl-4-nitrophenyl)-phosphorothioate (S-methyl fenitrothion)

V. O,O-dimethyl-S-(3-methyl-4-nitrophenyl)-phosphorothioate (S-phenyl fenitrothion)

VI. O,O,O-trimethylphosphorothioate

small amount of demethyl fenitrothion and demethyl aminofenitrothion are also formed (Miyamoto 1972). Bacterial action is also responsible for the occurrence of aminofenitrothion in milk of fenitrothion-treated cows (Miyamoto et al.). The preparation and properties of aminofenitrothion were described while this report was in preparation (Anonymous a). Aminofenitrothion was separated from the parent compound and from other products by two-dimensional TLC using the mixtures of benzene-ethyl acetate 4:1, and toluene-ethyl formate-formic acid 5:7:1. Both fenitrooxon and aminofenitrothion were reported to be unstable on TLC plates. A good gas chromatographic separation of fenitrothion, fenitrooxon, and aminofenitrothion was achieved on a 1.5 m x 4 mm column, containing 5% DC 200 and 10% DCQF 1 on Chromosorb W. The column was operated at 210° and the retention times relative to fenitrothion were 0.79 and 1.19 for aminofenitrothion and fenitrooxon, respectively (Anonymous b).

Some fenitrothion preparations, particularly the Russian "methylnitrophos" are mixtures of fenitrothion and O,O-dimethyl-O-(3-methyl-6-nitrophenyl)-phosphorothioate and, consequently, both 3-methyl-4-nitrophenol and 3-methyl-6-nitrophenol occur among the hydrolysis products (Novikova and Leshchinskaya 1972). According to Suzuki the latter phosphorothioate is much less toxic than fenitrothion. Suzuki also reviews the toxicity of other compounds, structurally related to fenitrothion.

As a result of side reactions during the preparation of fenitrothion, the preparations may contain several impurities, such as O-methyl-O,O-di-(3-methyl-4-nitrophenyl)-phosphorothioate, O-methyl-O,O-di-(3-methyl-4-nitrophenyl)-phosphate, and isomers of fenitrothion, O,S-dimethyl-O-(3-methyl-4-nitrophenyl)-phosphorothioate and O,O-dimethyl-S-(3-methyl-4-nitrophenyl)-phosphorothioate, and O,O,O-trimethylphosphorothioate. The separation of these compounds by thin-layer or paper chromatography was described. Kovac (1963) used thin-layer chromatography on silica and 1.4% acetone in petroleum ether as the developing solvent. Individual compounds were determined by polarographic reduction of the nitro group. Gruca and Mosinska (1970) used thin-layer chromatography on silica and a mixture of hexane-benzene-acetone (18:3:1) to separate some of the impurities in fenitrothion preparations. Up to 6% of O-methyl-O,O-di-(3-methyl-4-nitrophenyl)-phosphorothioate and 3% of 3-methyl-4-nitrophenol were found in some preparations. Polarography was used for quantitation. Paper chromatography on a paper impregnated with 25% formamide in acetone and elution with hexane provides a good separation of fenitrothion from its S-methyl isomer and from fenitrooxon. Thin-layer chromatography on silica and elution with 10% acetone in hexane may also be used (Kovacicova et al. 1971). The authors describe the preparation of analytical fenitrothion standards. Commercial fenitrothion is treated with alkali to remove most of the impurities which are easier hydrolyzable than fenitrothion. Volatile impurities and hydrolysis products are removed by steam distillation.

The World Health Organization published specifications for fenitrothion used in public health programs (Anonymous 1973b). Technical fenitrothion must contain a minimum of 95% of fenitrothion, as determined by reduction of the nitro group and titration with sodium nitrite after removing any 3-methyl-4-nitrophenol by extraction with alkali. Other impurities present in fenitrothion preparations are not specifically mentioned and it is not known to what extent would the above-mentioned compounds be hydrolyzed during the removal of 3-methyl-4-nitrophenol. Other WHO specifications include acidity, acetone-insoluble compounds, and water, present in maximum concentrations of 0.3, 0.3, and 0.5%, respectively,and a minimum specific gravity of 1.3.

The S-methyl isomer of fenitrothion (Fig. 1, IV) is formed, for example, by thermal isomerization of fenitrothion. The yield after 10 h heating at 130° is 45% (Truchlik et al. 1972). The isomerization may also take place as a result of irradiation. The photochemical reactions of fenitrothion have not been studied, however, Joiner and Baetcke (1973) detected S-ethyl and S-(4-nitrophenyl) isomers of parathion among its photoalteration products. The products were analyzed by thin-layer chromatography on silica, using a mixture of petroleum ether-ether-glacial acetic acid (80:15:5). Photolysis of parathion in aqueous tetrahydrofuran or ethanol yielded O,O,S-triethylphosphorothioate, and smaller amounts of O,O,O-triethylphosphorothioate and triethyl phosphate (Grunwell and Erickson 1973). Infrared spectra of photoalteration products of parathion, including O,S-diethyl-O-(4-nitrophenyl)-phosphorothioate (S-ethyl parathion, SEP), O,O-diethyl-S-(4-nitrophenyl)-phosphorothioate (S-phenyl parathion, SPP), O,O-diethyl-O-phenyl-phosphorothioate (DEPT), diethyl phenyl phosphate (DEPP), O-ethyl-O,O-di-(4-nitrophenyl)-phosphorothioate (EBNTP), and ethyl di-(4-nitrophenyl)-phosphate (EBNPP) were recently published (Joiner and Baetcke 1974).

Little is known about the toxicological properties of these compounds. S-methyl fenitrothion is a stronger acetylcholine esterase inhibitor than fenitrothion (Kovacicova et al. 1971), and O,O,S-triethyl phosphorothioate may be synergistic with parathion (Grunwell and Erickson 1973). The toxicity of some organophosphate pesticides, such as malathion, to warm-blooded animals was potentiated by O-methyl-S,S-dimethyl-phosphorodithioate, O,O-dimethyl-S-methyl-phosphorothioate, and O,O-dimethyl-S-methyl-phosphorodithioate, present as impurities in commercial organophosphate pesticides (Pellegrini and Santi 1972).

Ultraviolet spectrophotometry was used in this report to characterize nitrophenols and to study the hydrolysis of fenitrothion. Related spectrophotometric studies are therefore briefly reviewed. UV spectrophotometry has not been used previously to determine fenitrothion. However, the determination of parathion in formulations by this technique has been described (Carrasquero 1968). Absorption maximum of parathion is at 270 nm and the molar absorptivity is 9360-9472. Aly et al. (1971) reported the absorption maxima of parathion and paraoxon in ethanol at 274 and 268 nm, respectively. The absorption maximum of parathion in hexane is at 268 nm (Gore et al. 1971). Fenitrothion was determined after hydrolysis by quantitation of 3-methyl-4-nitrophenol using UV spectrophotometry. The method was reported for plant material (vegetables) and the sensitivity was 12 μg/kg (Golacka 1971). In cereal extracts, concentrations as low as 5 μg/kg could be determined (Das et al. 1970). In human urine, 3-methyl-4-nitrophenol was detected spectrophotometrically with a sensitivity of 10 μg/ℓ (Hladka 1969), and a similar method was described for 4-nitrophenol (Cranmer 1970).

EXPERIMENTAL

Chemicals

All chemicals were used as received. Fisher Scientific buffer salts were used to prepare buffers. Fenitrothion was obtained from Forest Protection Limited, Fredericton, New Brunswick, in 1971 and in 1973. The 1971 sample was a product of Bayer, the origin of the 1973 sample is not known. An analytical standard of fenitrothion from Analabs Inc. was also tested. Fenitrooxon was a gift from the Sumitomo Chemical Company.

Instruments

Ultraviolet and infrared spectra were recorded on a Beckman DK-2A and a Perkin-Elmer Model 700 spectrophotometer, respectively. Gas chromatographic determinations were carried out on a Perkin-Elmer Model 990 gas chromatograph, equipped with a Melpar flame photometric detector, operated in the phosphorus mode. A glass column (0.08 in I.D. x 6 ft), containing 4% SE-30 on Chromosorb W was used. The operating temperatures were 172, 163, 210, and 160° in the injection port, column, manifold, and detector, respectively. The carrier gas was nitrogen at 80 mℓ/min, the flow rates of hydrogen and air were 175 and 100 mℓ/min.

Determination of UV spectra

Nitro and aminophenols and an equimolar amount of sodium hydroxide were dissolved in distilled water to give a final concentration of 100 mg/ℓ. These stock solutions were diluted as required by tap water or buffer, and UV spectra were recorded. Stock solutions of fenitrothion, fenitrooxon, and aminofenitrothion were prepared in ethanol (20 mg/mℓ). Diluted solutions in tap water, river water or buffers were prepared by adding slowly an aliquot of the ethanolic solution to a vigorously stirred diluent.

Adsorption of fenitrothion on sediment

Coarse (sandy) and fine (muddy) sediment were obtained from a nearby stream. One-gram samples of sediments were added to 100 mℓ samples of fenitrothion solutions in tap water (fenitrothion concentration 5, 10, and 15 mg/ℓ) in 250-mℓ Erlenmeyer flasks, and the mixtures were shaken for 30 min on a wrist-action shaker. The mixtures were then centrifuged and concentration of fenitrothion in the supernatant was determined spectrophotometrically.

Hydrolysis of fenitrothion and fenitrooxon

The solutions of fenitrothion and fenitrooxon were prepared as described above. Samples were taken periodically and their UV spectra were recorded. The concentration of fenitrothion and of 3-methyl-4-nitrophenol was calculated from the absorbance at 285 and 360 nm using the equations:

$$A_{285} = E^{F}_{285}C_{F} + E^{M}_{285}C_{M}$$

$$A_{360} = E^{F}_{360}C_{F} + E^{M}_{360}C_{M} ,$$

where A_{285}, A_{360} = absorbance at 285 and 360 nm, respectively,

E^{F}_{285}, E^{F}_{360} = absorption coefficient of fenitrothion at 285 and 360 nm, respectively,

E^{M}_{285}, E^{M}_{360} = absorption coefficient of 3-methyl-4-nitrophenol at 285 and 360 nm, respectively,

and C_{F}, C_{M} = concentration of fenitrothion and 3-methyl-4-nitrophenol, respectively. The units of concentration depend on the units of the absorption coefficients.

An analogous set of equations was used for mixtures of fenitrooxon and 3-methyl-4-nitrophenol.

Fate of fenitrothion in river water

Water and sediment samples were obtained from the Digdeguash River (Charlotte County, N.B.). A 3.8 cm layer of the sediment was placed in a 3-liter Erlenmeyer flask and 2 liters of a fenitrothion solution in river water were added slowly. The flask was stoppered and kept at room temperature. Three concentrations of fenitrothion (2.5, 5.0, and 10.0 mg/ℓ) were tested. In another flask a mixture of sediment and river water, containing fenitrothion at 10 mg/ℓ, was kept aerated using a pump and an airstone. The presence of UV-absorbing interferences and low concentrations of fenitrothion made its determination by spectrophotometry impossible, and fenitrothion was determined by gas chromatography. At times, aliquots were withdrawn from the flasks and extracted as described previously

(Peterson and Zitko 1974).

Preparation of aminofenitrothion

Several unsuccessful attempts to reduce fenitrothion to aminofenitrothion on a preparative scale were made. These included reduction by metallic tin in a mixture of concentrated hydrochloric acid and ethanol, and, under similar conditions, iron filings and hydrochloric acid. Major problem was the solubility of fenitrothion in the reaction medium. Iron filings and ethanolic hydrochloric acid (hydrogen chloride gas dissolved in ethanol) gave the desired product, but in addition a large amount of an ethanol-transesterified product was formed, and in the final version of the method ethanol was replaced by methanol.

Fenitrothion (1 g) was dissolved in methanol (30 mℓ), iron filings (5 g) were added, followed by methanolic hydrogen chloride (25 mℓ of 10% methanolic hydrogen chloride, added slowly in small portions), and the mixture was stirred for 2 h at room temperature. The mixture was then filtered, the filtrate was neutralized with solid sodium carbonate and extracted with chloroform (3 x 50 mℓ). The combined chloroform extracts were washed twice with distilled water, dried with anhydrous sodium sulfate, and evaporated to dryness on a rotatory evaporator at 36°. The product was characterized by its infrared spectrum, by gas and thin-layer chromatography on 0.25 mm layers of alumina (Camag DSFS), using a mixture of cyclohexane and ethyl acetate (Yule and Duffy 1971) as the developing solvent. The developed plates were examined under a UV light, and sprayed with a 0.5% solution of sulfanilic acid and sodium nitrite in 1M hydrochloric acid, followed by 1M potassium hydroxide. Amino derivatives were also fluorigenically labelled with dansyl chloride (Lawrence and Frei 1972). The solvent system benzene-chloroform 2:1 was used.

Qualitative determination of impurities in technical fenitrothion

Fenitrothion preparations were examined by thin-layer chromatography on 0.25 mm silica layers (Camag DFS) using pesticide-grade hexane-ethyl acetate 3:1 as the developing solvent. The 1971 fenitrothion sample was separated preparatively, the impurities were eluted with acetone and examined by IR spectrophotometry. A small amount of the 1973 sample was thermally isomerized in a 0.3-mℓ ReactiVial for 2 h at 150°.

RESULTS AND DISCUSSION

UV spectra of nitro- and aminophenols, fenitrothion, fenitrooxon, and aminofenitrothion are summarized in Table 4 in terms of absorption maxima and molar absorptivities. As can be seen, in some cases isomers could be determined in mixtures, but generally

TABLE 4. UV spectra.

Compound	pH	Absorption Maxima (nm)	Molar Absorptivity $(x\ 10^{-3})$
2-nitrophenol	7.2	210,275	11.00,5.52
3-nitrophenol		255,275(10.7 mg/ℓ) 225,275(4 mg/ℓ)	8.92,7.90,5.51
4-nitrophenol		225,325	8.71,7.80
2-aminophenol		285,335	2.85,2.58
3-aminophenol		230,282	7.46,1.93
4-aminophenol		225	5.80
3-methyl-4-nitrophenol		280,320	5.64,6.41
3-methyl-6-nitrophenol		292	6.72
3-methyl-4-aminophenol		260,290	8.64,5.36
fenitrothion		270	6.62
fenitrooxon		267	6.45
aminofenitrothion		234,285	11.76,2.45
3-methyl-4-nitrophenol	10.4	230,260	5.73,3.88
3-methyl		278	5.79
fenitrothion		270	7.11
fenitrooxon		267	6.34
aminofenitrothion		234,285	9.78,2.23
3-methyl-4-nitrophenol	12.45	230,260	7.39,4.95
3-methyl-4-aminophenol		278	4.99
fenitrothion		270	7.42
fenitrooxon		267	7.34

the differences between isomers and between the respective amino and nitro derivatives are quite small. There is no significant difference between the spectra of fenitrothion and fenitrooxon. However, the maximum of aminofenitrothion (285 nm) is distinctly different from those of the two previous compounds. The spectra of fenitrothion, fenitrooxon, and aminofenitrothion are also sufficiently different from those of 3-methyl-4-nitrophenol and 3-methyl-4-aminophenol to allow a spectrophotometric analysis of their mixtures. The equations for the analysis of mixtures of fenitrothion and 3-methyl-4-nitrophenol, and fenitrooxon and 3-methyl-4-nitrophenol are given in the Experimental part. Absorbancies in these cases are not measured at the maxima of these compounds, but at wavelengths where the differences between the absorptivities are as large as possible (285 and 360 nm).

The results of analyses of mixtures of fenitrothion, fenitrooxon and 3-methyl-4-nitrophenol are summarized in Table 5. The agreement of "Added" and "Found" concentrations is good and the method is suitable for the determination of hydrolysis of fenitrothion and fenitrooxon. However, UV spectrophotometry is not as sensitive as gas chromatography and the method is limited to concentrations several orders of magnitude higher than those found normally in the environment after an operational application of fenitrothion. For model experiments, carried out at higher concentrations, the spectrophotometric method has the advantage that it determines simultaneously both fenitrothion and 3-methyl-4-nitrophenol.

TABLE 5. Spectrophotometric analysis of mixtures of fenitrothion and 3-methyl-4-nitrophenol.

mg/ℓ

Added		Found	
Fenitrothion	3-methyl-4-nitrophenol	Fenitrothion	3-methyl-4-nitrophenol
15.0	5.00	15.4	5.50
15.0	4.00	15.6	3.85
15.0	2.00	15.8	1.87
15.0	1.00	15.7	0.94
15.0	0.50	15.7	0.43
10.0	5.00	9.51	4.98
10.0	3.00	10.6	2.74
10.0	2.00	9.95	1.86
10.0	1.00	10.1	0.89
10.0	0.75	10.3	0.63
10.0	0.50	9.91	0.42
10.0	0.25	10.1	0.22
5.0	7.50	4.22	7.95
5.0	5.00	4.28	5.19
Fenitrooxon		Fenitrooxon	
10.0	5.00	11.6	5.59
10.0	3.00	11.9	3.14
10.0	1.00	10.7	1.13

The degree of hydrolysis of fenitrothion is then immediately apparent. If the determination of fenitrothion is carried out by gas chromatography, it is not obvious whether a decrease in fenitrothion concentration is due to hydrolysis or to adsorption of fenitrothion on suspended matter or walls of the container.

Adsorption of fenitrothion on sediment

The extent of fenitrothion adsorption on coarse and fine sediment is presented in Table 6. The adsorption was followed over a number of days. Within the experimental error the equilibrium was established during the first shaking of fenitrothion solutions with the sediment and no hydrolysis of fenitrothion was detectable. As can be expected, fine sediment with larger surface area adsorbed more fenitrothion than the coarse sediment. The adsorption on fenitrothion on the coarse sediment is only slightly lower than reported by Peterson and Zitko (1974) at a much lower concentration of fenitrothion (0.212 mg/ℓ). In the case of the fine sediment the data of Peterson and Zitko show a pronounced increase of adsorption during a period of 4 days (33% and 83% adsorption on day 1 and day 4, respectively). It is possible that with low concentrations of fenitrothion the adsorption equilibrium is established more slowly than in the case of higher fenitrothion concentrations.

TABLE 6. Adsorption of fenitrothion on sediment.

Concentration of fenitrothion, mg/ℓ	15		10		5	
	Adsorbed mg/g	%	Adsorbed mg/g	%	Adsorbed mg/g	%
Coarse sediment	0.205	13.7	0.125	12.5	0.097	19.4
Fine sediment	0.453	30.0	0.570	57.0	0.370	74.0

Rate of hydrolysis of fenitrothion and fenitrooxon

Hydrolysis of fenitrothion in tap water (pH approximately 7.0) was not detectable over a period of 45 days. The generation of 3-methyl-4-nitrophenol from fenitrothion at pH = 9.8 and from fenitrooxon at pH = 7.2, 10.4, and 12.45 is presented in Figure 2. A concentration of 6.0 mg/ℓ of 3-methyl-4-nitrophenol corresponds to 100% hydrolysis. It can be seen that fenitrooxon hydrolyzes very slowly, but measurably at pH = 7.2, whereas the hydrolysis at pH = 12.45 is practically completed within 4 hours. Measurable generation of 3-methyl-4-nitrophenol from fenitrothion occurred at pH = 9.8. None of the curves in Figure 2 corresponds particularly well to a first-order kinetic equation (straight line in semi-log coordinates). While deviations are common as

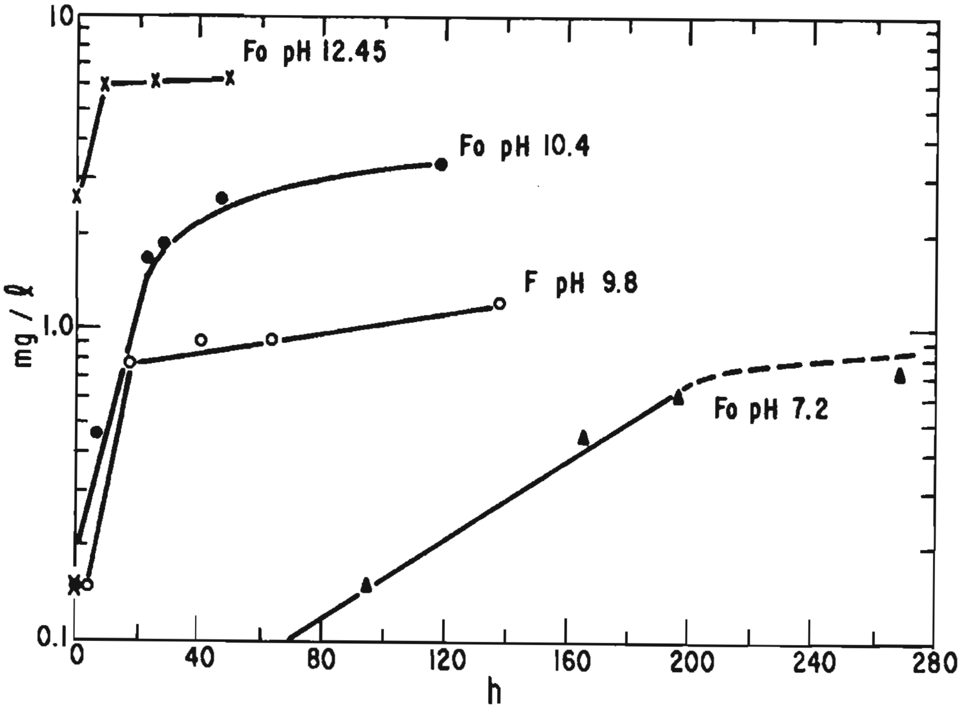

Fig. 2. Hydrolysis of fenitrothion and fenitrooxon. Concentration of 3-methyl-4-nitrophenol (mg/ℓ) plotted against time (h).

F = fenitrothion, FO = fenitrooxon

Initial concentration of fenitrothion and fenitrooxon was 10 mg/ℓ.

the reaction nears completion, the fast initial hydrolysis may be at least in the case of fenitrothion due to impurities which hydrolyze faster than fenitrothion itself (see section on impurities).

Fate of fenitrothion in river water

The disappearance of fenitrothion from the aerated water sample is presented in Figure 3, from the non-aerated samples in Figure 4. Since in all these cases fenitrothion was determined by gas chromatography, it is not known how much of the observed decrease in fenitrothion concentration is due to hydrolysis. However, as mentioned already in the previous paragraph, chemical hydrolysis at this pH is negligible. Some loss of fenitrothion is due to adsorption on the sediment. The adsorption equilibrium was established fast in the aerated sample because of stirring the flask content by aeration. In the non-aerated samples the adsorption equilibrium must have been reached much more slowly. Most of the observed loss of fenitrothion is very likely caused by microbiological decomposition. An intermediate product of the decomposition in the non-aerated samples is aminofenitrothion which was detectable by gas chromatography after 5 days. Its presence was also confirmed by thin-layer chromatography. Another unidentified peak was also observed by gas chromatography (Table 7). The disappearance of fenitrothion from river water samples follows quite well the first-order kinetic equation with a half-life of approximately 50 h in the aerated sample (not considering the fast initial drop in concentration) and 30-40 h in the non-aerated samples.

TABLE 7. Retention times on gas chromatography.

Fenitrothion	1.00
Aminofenitrothion	0.91
Aminofenitrothion decomposition product	1.13
River water experiment peak	0.92
River water experiment decomposition peak	1.13
Commercial dimethyl phosphate	0.35

Aminofenitrothion was isolated as a dark brown sirup. It gave a single spot on thin-layer chromatography, which turned dark brown after spraying with sulfanilic acid and sodium nitrite, and showed a yellow-brown fluorescence after the reaction with dansyl chloride and thin-layer chromatography. Its retention time on gas chromatography is given in Table 7.

The infrared spectrum of aminofenitrothion is reproduced

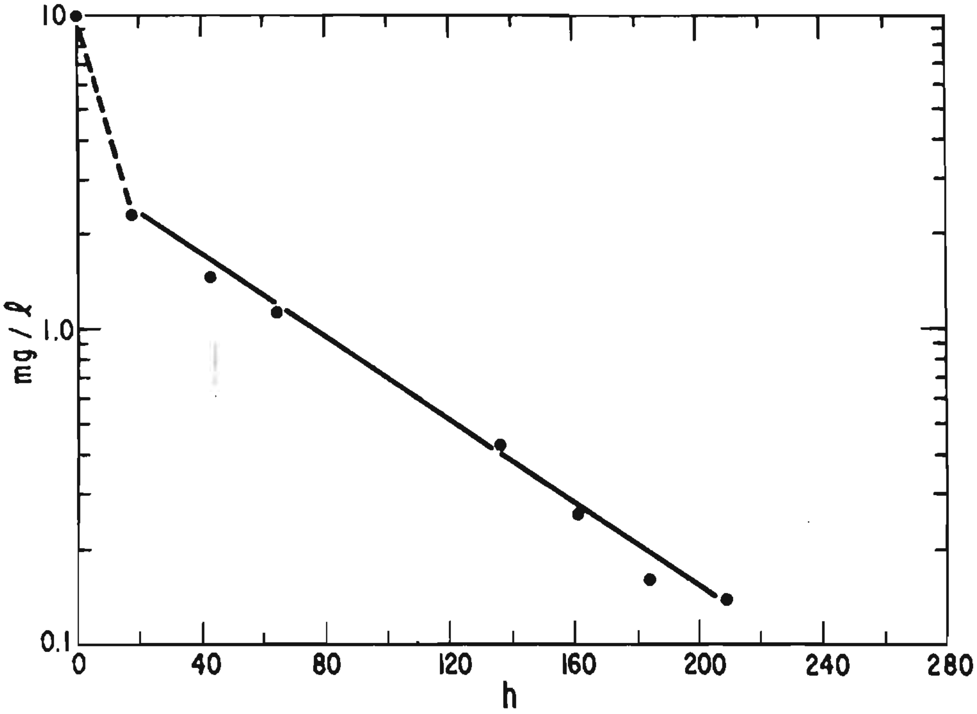

Fig. 3. Decomposition of fenitrothion in the aerated sample of river water and mud. Concentration of fenitrothion (mg/ℓ) plotted against time (h).

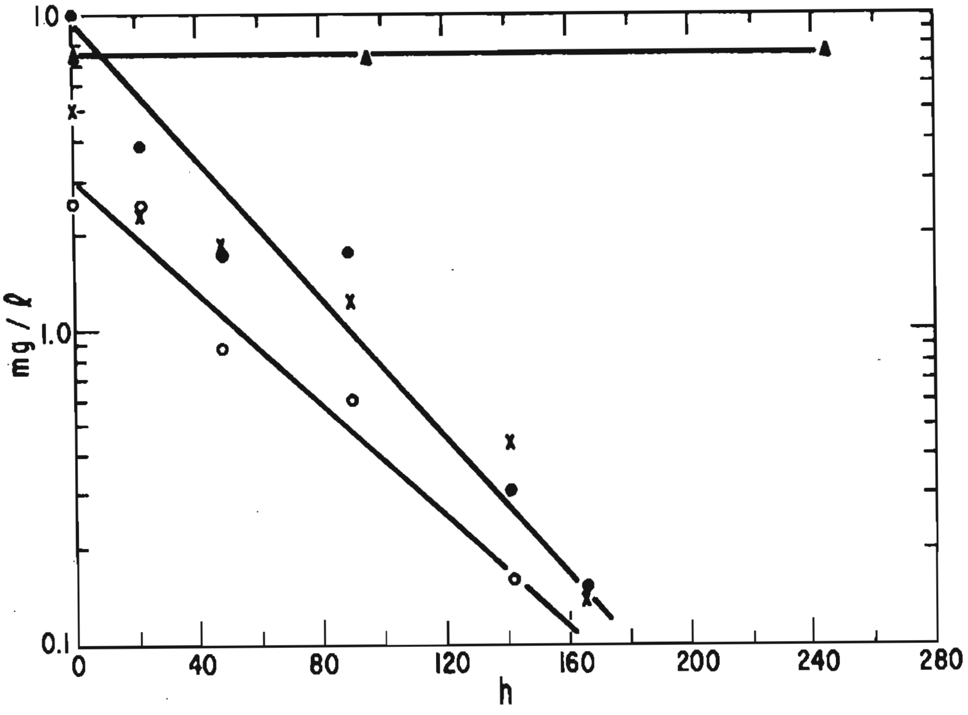

Fig. 4. Decomposition of fenitrothion in the non-aerated samples of river water and mud.

Concentration of fenitrothion (mg/ℓ) plotted against time (h).

The time dependence of fenitrothion concentration in tap water is added for comparison.

in Figure 5. The absorption maximum at 3350 cm^{-1} and a maximum at 1630 cm^{-1}, not fully resolved from the benzene ring C=C stretching at 1610 cm^{-1} indicate the presence of an aromatic amino group. An intensive P=S band at 830 cm^{-1} and P-O-ϕ band at 1230 cm^{-1}, and a weak absorption at 1280 cm^{-1} indicate that no significant isomerization (see below) took place during the preparation. The NO_2 bands of fenitrothion (1585, 1525, and 1355 cm^{-1}) are absent from the spectrum.

Aminofenitrothion is unstable. The same unidentified decomposition product (Table 7) was observed on standing in the synthetic aminofenitrothion and in the non-aerated river water.

Impurities in technical fenitrothion

None of the available samples of fenitrothion was found pure, when analyzed by thin-layer chromatography. Even the analytical standard contained a number of impurities, and the 1973 technical product was the purest fenitrothion preparation. The TLC characteristics of the three fenitrothion preparations are given in Table 8.

TABLE 8. TLC characteristics of fenitrothion preparations (0.25 mm silica, pesticide-grade hexane-ethyl acetate 3:1).

R_f				Reaction with 10% ethanolic sodium hydroxide	Identification
Sample 1971	1973	Fenitrothion standard	Standard		
0.42	0.42	0.45		slow	fenitrothion
		0.30		fast	
			0.25	"	3-methyl-4-nitrophenol
0.23		0.25		"	
		0.18			
0.15		0.14		no reaction	V*
0.11	0.11		0.11	fast	IV*
			0.08	"	fenitrooxon

*See Fig. 1.

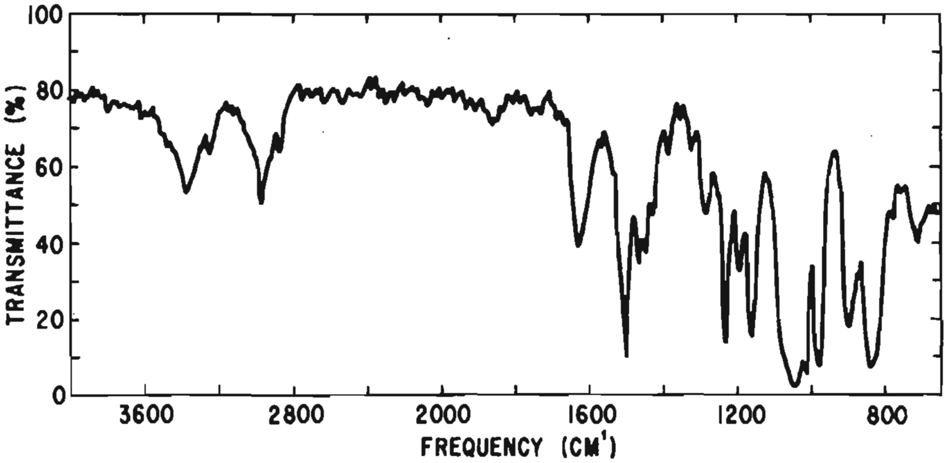

Fig. 5. Infrared spectrum of aminofenitrothion (neat).

Infrared spectra were used to identify two of the impurities, isolated from the 1971 fenitrothion preparation.

The infrared spectrum of fenitrothion, purified by thin-layer chromatography, is presented in Figure 6, where also some of the absorption bands are identified. The infrared spectrum of fenitrooxon is reproduced in Figure 7. It can be seen that the P=S absorption of fenitrothion at 840 cm^{-1} is absent from the spectrum of fenitrooxon, which in turn contains a strong P=0 absorption at 1280 cm^{-1}. Spectra of both compounds contain a peak at 1240-1250 cm^{-1}, which from comparison with the data of Joiner and Baetcke (1974) on parathion and its isomers, was assigned to the P-O-Ø vibrations.

The infrared spectrum of S-methyl fenitrothion (IV, Fig. 1) is given in Figure 8. Strong P=S absorption at 830 cm^{-1} is absent and the compound is absorbing in the P=0 region (1280 cm^{-1}). It should be, however, noted that this preparation was not purified and contained a number of impurities, but only a trace of fenitrothion.

The infrared spectrum of the compound R_f 0.11 is reproduced in Figure 9. The spectrum quite closely resembles that of S-methyl fenitrothion (Fig. 8). Since also the R_f values of both compounds are identical, it is concluded that S-methyl fenitrothion is present in both 1971 and 1973 fenitrothion preparations. Its concentration is much higher in the former than in the latter. The infrared spectrum of the compound R_f 0.15 is presented in Figure 10. It shows a weak absorbance in the P=S region (830 cm^{-1}), and in the P-O-Ø region and a relatively strong P=0 absorbance. According to the spectrum, this compound is probably S-phenyl fenitrothion (V, Fig. 1). The compound does not give yellow color with ethanolic sodium hydroxide in spite of the fact that benzene ring and a nitro group are present (Fig. 10).

The compound with R_f 0.23 was present in the 1971 fenitrothion preparation in a concentration too low to obtain an infrared spectrum. On TLC this compound moved close to 3-methyl-4-nitrophenol, but its R_f was consistently slightly lower than that of the phenol.

The environmental fate of S-methyl and S-phenyl fenitrothion is not known. S-methyl fenitrothion hydrolyzes on TLC plates approximately as fast as fenitrooxon and it is possible that the hydrolysis rates of these compounds in water are also similar. Except for the reference, mentioned in Introduction, that S-methyl fenitrothion is a stronger acetycholine esterase inhibitor than fenitrothion, nothing is known about its toxicity.

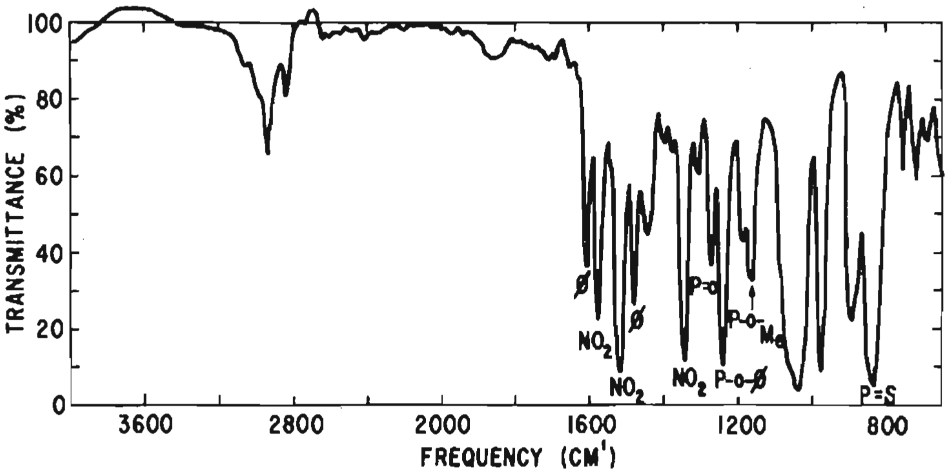

Fig. 6. Infrared spectrum of fenitrothion (neat).

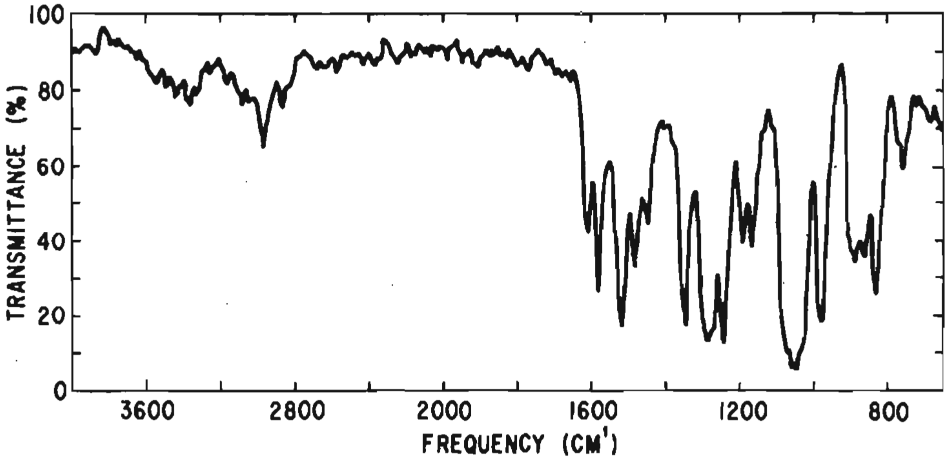

Fig. 7. Infrared spectrum of fenitrooxon (neat).

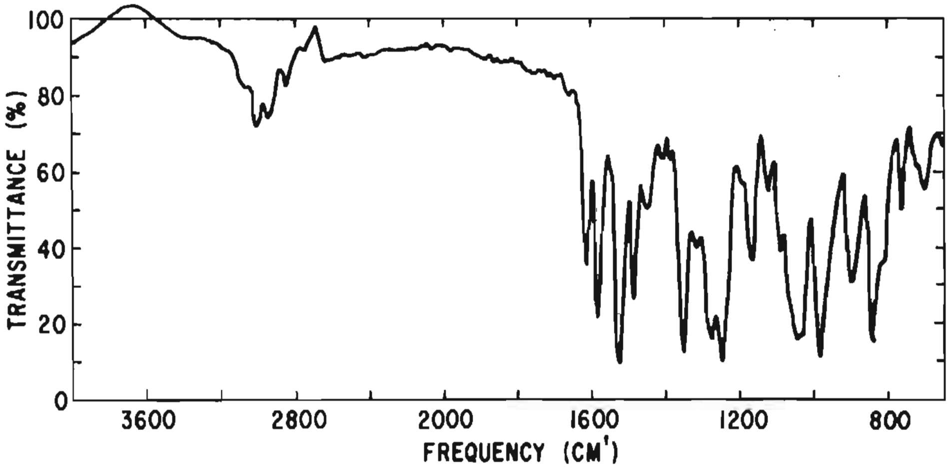

Fig. 8. Infrared spectrum of S-methyl fenitrothion (neat).

For structure see IV, Fig. 1.

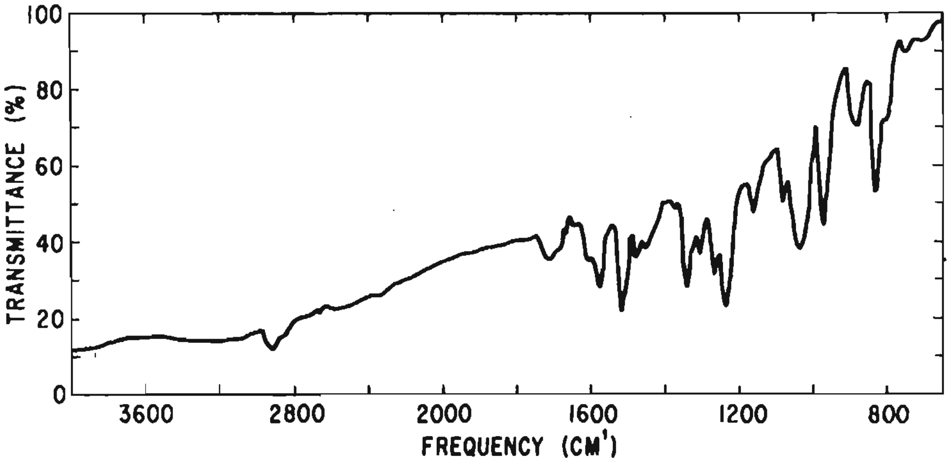

Fig. 9. Infrared spectrum of compound $\underline{R}_f$ 0.11 (KBr).

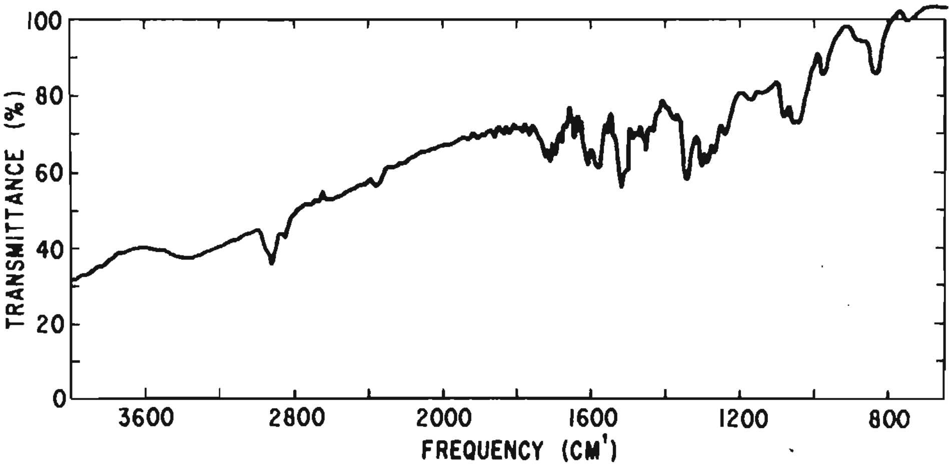

Fig. 10. Infrared spectrum of compound $\underline{R}_f$ 0.15 (KBr).

It is not known whether S-methyl fenitrothion would be seen under the analytical conditions used to determine fenitrothion and fenitrooxon.

As this report demonstrated, the purity of technical fenitrothion preparations is quite variable. The environmental effects of the impurities present in some fenitrothion preparations are unknown and may be insignificant in comparison with fenitrothion. In spite of this it may be wise to adopt some quality standard for fenitrothion, similar to that used by WHO, or at least check the purity of different batches by thin-layer chromatography.

At the same time, the formation of fenitrothion isomers under environmental conditions should be studied. It is possible that these compounds are intermediates in the degradation of fenitrothion, but more toxic than fenitrothion itself.

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