Confirmation of Organophosphorus Insecticides by Chemical Reduction

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The requirement to confirm the identity of pesticide residues in the nanogram or picogram range is becoming increasingly important in environmental studies (McCULLY 1969). Various methods have been employed including thin layer chromatography (KOVACS 1963, MINYARD and JACKSON 1965); extraction p-values (BOWMAN and BEROZA 1965); gas chromatography - mass spectrometry combination (DAMICO et al. 1970) and multiple gas-liquid chromatography column methods (FAHEY and SCHECTER 1961). Chemical derivative formation followed by electron capture gas chromatography has been employed in a number of laboratories performing routine analyses and has found wide acceptance (McCULLY 1969, WOODHAM et al. 1972; COCHRANE and CHAU 1970). The latter has the advantage of being convenient and sensitive. The identification of organophosphorus insecticides can be made more specific by the use of a flame photometric or an alkali flame ionisation detector rather than an electron capture detector. Even so, the response of these detectors is not unequivocal evidence for the presence of a specific insecticide (ASKEW et al. 1969, GIUFFRIDA 1964, BRODY and CHANEY 1966).

In these laboratories, it has been found that a number of organophosphorus pesticides elute at the same retention time on various GLC columns both in the isothermal and temperature programmed modes. Confirmation is normally required and an unambiguous technique is necessary.

EXPERIMENTAL

Materials:

(a) Zinc Dust - (Fisher Scientific Co.) reagent grade
(b) Hydrochloric Acid - (Fisher Scientific Co.) reagent grade
(c) Benzene - Pesticide Grade (Burdick and Jackson Laboratories) used without additional purification.
(d) Sodium Bicarbonate - (Canlab) ACS grade used after washing with Pesticide grade acetone.

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(e) Aqueous Chromous chloride, Titanous chloride and Palladium(ous) chloride - (Fisher Scientific Co.) used without further purification.

(f) Cobalt(ous) chloride, Nickel(ous) chloride - (Fisher Scientific Co.) and Ferrous chloride - (Merck) used as 1 N aqueous solutions.

(g) Pesticide Standards - Parathion (99.1%); Methyl Parathion (99.4%); Fenitrothion (99.1%); Thimet (97.6%) and Malathion (99.0%) were all obtained from American Cyanamid Co. Ltd. Captan (98.8%) and Trithion (95.0%) were obtained from Stauffer Chemical Ltd. Ronnel (99%) was obtained from Dow Chemical Ltd. Ruelene (92%) was obtained from Poly Science Corp.; Gardona (98%) was obtained from Shell Chemicals; Diazinon (97.6%) was obtained from Fisons Canada Ltd.; EPN was obtained from E.I. Dupont de Nemours; Surecide was obtained from Sumitomo Chemical Co. Ltd., Japan.

(h) Stock Solutions - Dissolve 100 mg of the analytical grade pesticide in 100 ml of hexane or benzene.
Injection Standard - Transfer 100μl of stock solution to a volumetric flask and dilute to 100 ml with hexane (1 nanogram/μl).

Apparatus:

Gas Chromatographs

(a) Tracor MT 220 with dual Ni^{63} EC detectors and Dual Flame Photometric Detectors (Melpar). Equipped with 6' x 1/4" OD glass columns packed with 3% OV-1 or 4% SE-30, 6% QF-1 on 80-100 mesh, acid-washed, Chromosorb W, HMDS.
Operating Conditions: column temp. 200°C, EC detector 265°C, FPD detector 200°C, injection block temp. 225°C; nitrogen carrier gas 60 ml/min., oxygen at 20 ml/min., hydrogen 150 ml/min. and air 40 ml/min. Flame photometric detector range and attenuation 10^4 and 16 respectively; EC range and attenuation 10^2 and 16 respectively.

(b) Pye Series 104, Model 154 equipped with an alkali flame ionisation detector which had a rubidium chloride annulus. A glass column, 3' x 1/4" packed with 4% SE-30, 6% QF-1 on 80/100 mesh, Chromosorb W, HP, was used.
Operating Conditions: column, injection block and detector temp. 225-250°C. Nitrogen carrier gas flow was 40 ml/min.

Mass spectral data were obtained using a Dupont 490 mass spectrometer interfaced to a Pye 104 gas chromatograph. Infra red spectra were determined on a Perkin Elmer Model 457 infra red spectrometer.

Procedure:

(a) Metal Chloride Reductions (Residue Scale)

A suitable sample of pesticide standard (20μg) dissolved in 2 ml benzene was treated in a 15 ml glass stoppered graduated centrifuge tube. While passing a gentle stream of nitrogen into the open tube, 2 ml of the metal chloride solution was added and the contents were tightly stoppered under a nitrogen

atmosphere with a spring-loaded clamp. The tube was transferred to a water bath and maintained at 60°C for 10 min. with frequent shaking. After complete reaction the tube was cooled to room temperature and 5 ml of glass distilled water was added. The upper benzene layer was then examined by GLC.

(b) Zinc Hydrochloric Acid Conversion Method (Residue Scale)

Suitable aliquots of standards of fenitrothion, methyl parathion and parathion were dissolved in 3 ml of ethanol (usually 5 micrograms of each pesticide, equivalent to 5.0 ppb in one litre of water). To this solution in a 15 ml centrifuge tube, 3 ml of glass distilled water, 1 ml of 5 N HCl and 0.2 g of zinc dust were added. The tubes were heated on a steam bath and the length of time required for complete reaction was monitored at 5, 10, and 15 minute intervals. The tubes were removed from the steam bath, cooled to room temperature and the contents neutralized with sodium bicarbonate. Two 2.0 ml portions of benzene were added and after shaking, and subsequent separation of the two layers, the benzene portions were combined, reduced under a gentle stream of dry nitrogen and analysed by gas-liquid chromatography.

(c) Preparation of Aminoparathion using Aqueous Chromous Chloride (Macro Scale)

Parathion (1.0 gram) was dissolved in 10 ml of acetone in a 125 ml glass-stoppered Erlenmeyer Flask. Ten ml of aqueous chromous chloride was added under an atmosphere of dry nitrogen and the mixture was maintained at 60°C in a water bath for three hours. After the reaction was completed, the mixture was cooled to room temperature, transferred to a 500 ml separatory funnel and 300 ml of glass distilled water was added. The aqueous mixture was extracted with three 100 ml portions of benzene and the combined benzene extracts were dried over anhydrous sodium sulfate. The benzene was then removed under vacuum on a rotary evaporator.

Parathion (1 gram) yielded 0.83 g (93%) of amber liquid having a characteristic amine odour. An IR analysis confirmed the presence of an amine group. The compound gave a response at the same retention time as the product obtained on the residue scale. The product could be distilled using a molecular still: bp 120-125°C/0.01 mm.

RESULTS AND DISCUSSION

Metal chlorides, especially chromous chloride have been used extensively for the confirmation of organochlorine insecticides at the residue level (COCHRANE and FORBES 1971). Their reactivity towards Heptachlor was found to decrease in the order Cr^{2+}, Pd^{2+}, Ti^{3+}, Ni^{2+}, Co^{2+} and Fe^{2+}. These reagents were applied to the reduction of parathion, fenitrothion, their oxons, EPN and methyl parathion, all of which contain an aryl nitro group. The nitro

group is known to be reduced under a variety of conditions (MIYAMOTO et al. 1966, AVERELL and NORRIS 1948, YULE and DUFFY 1972). Surecide, with a cyano group in place of a nitro group was included in this study, because this moiety is also subject to reduction.

When the reduction was carried out in benzene, chromous chloride was the only metal chloride to give any reaction with the insecticides. A single product was formed, and in the case of fenitrothion, it had a relative retention time of 0.66 with respect to the parent insecticide on an SE-30/QF-1 column. Figure 1 shows a gas chromatogram before and after reduction with chromous chloride.

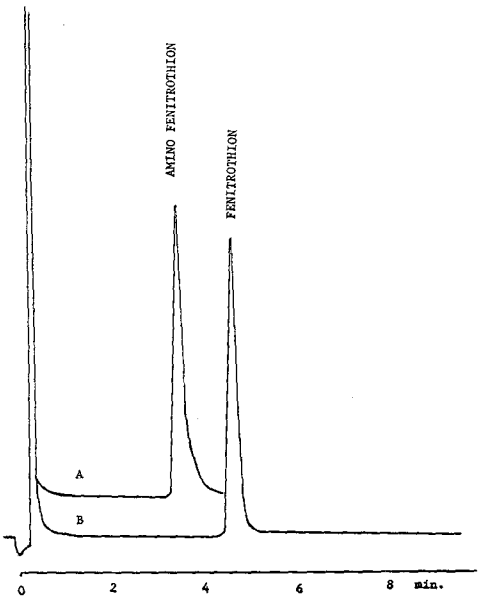
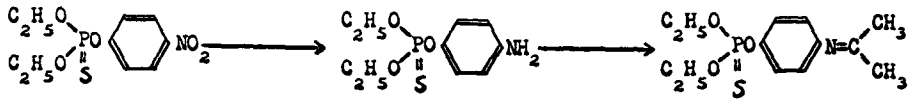


Figure 1 Chromatogram of Fenitrothion before and after chromous chloride treatment

A GC/MS of this compound indicated it to be the amino derivative, showing a parent ion at m/e 247 and an ion at m/e 125 corresponding to $(CH_3O)_2$ P=S and another at m/e 109. The absence of an ion at m/e 230 corresponding to (M-17) is indicative of the replacement of the nitro by an amino group, which is unable to lose OH. All the products from the reductions of the insecticides had retention times less than those of the parent compounds as shown in Table I.

The chromous chloride reaction must be carried out under an inert atmosphere due to the extreme reactivity of the reagent. The solution must be blue in colour if good results are to be obtained. The solvents used for the reaction can be either benzene or acetone, however, some anomolous results have been obtained with the latter solvent, in the form of multiple products. With parathion, for example, a second product was obtained with a relative retention time of 0.81. Its mass spectrum indicated a molecular weight of

301 (base peak), second most abundant ion at m/e 148, and peaks at m/e 109 and m/e 97. These peaks are consistent with those expected from 0,0-diethyl-0-(isopropylideneamino)-phosphorothioate resulting from condensation of the amino group with acetone. A proposed reaction scheme is shown in Figure 2.



M.W. = 291

m/e = 109
125

M.W. = 301

m/e = 109
148

Figure 2 Suggested products from the reduction of Parathion by chromous chloride in acetone

TABLE I

Relative retention times of EPN, Fenitrothion, Methyl Parathion and their amino derivatives

	Retention Time Rp	
	Insecticide	Amino Derivative
Parathion	1.0	0.66
Fenitrothion	0.85	0.64
EPN	3.46	2.42
Methyl Parathion	0.76	0.51

4% SE-30, 6% QF-1 column

The effect of chromous chloride on eight other pesticides which do not contain a nitro group was studied. These were Thimet, Diazinon, Ronnel, Malathion, Captan, Crufomate, Gardona and Trithion. In all cases, except for Captan, no reaction occurred even after 24 hrs. Captan, however, was completely decomposed within 5 min. The results of reactions of chromous chloride are summarized in Table II. It can be seen that the phosphorothioates containing a nitro group are reduced in high yields. On the other hand, the oxon compounds (i.e. phosphates) of fenitrothion and parathion are decomposed. Chromous chloride does not reduce the cyano moiety of Surecide and compounds not having a nitro group are unaffected.

In Table II are also shown the results of reduction with zinc and hydrochloric acid at the residue level. This method is the basis of the first analytical method for parathion reported by AVERELL and NORRIS (1948). In all cases the yields are lower than those obtained by chromous chloride. However, this method will reduce the oxon derivatives. From these results, chromous chloride reduction appears to be the method of choice. It is a more facile reaction, taking place at 60°C and going to completion in less than 5 min. as compared with 15 min. for the zinc/hydrochloric acid method.

TABLE II

Reaction of some organophosphorus pesticides with zinc/hydrochloric acid and chromous chloride

	% Yield	
	Zn/HCl	$CrCl_2$
Parathion	68	98
Fenitrothion	39	95
EPN	45	97
Surecide	10	NR*
Paraoxon	24	decomp.
Fenitrooxon	32	decomp.

Diazinon, Ronnel, Malathion, Thimet, Crufomate, Gardona and Trithion - no reaction after 24 hours.

Captan - decomposed in 5 minutes

* no reaction

In most cases, reaction of the pesticide with zinc/hydrochloric acid produced more than one product. These compounds were thought to be the intermediate reduction products such as the nitroso and hydroxylamino derivatives, formed in the reaction (ROBERTS and CASERIO 1965).

$$ArNO_2 \longrightarrow Ar\text{-}N{=}O \longrightarrow Ar\text{-}NH\text{-}OH \longrightarrow Ar\text{-}NH_2$$

The reaction between parathion and chromous chloride also proceeds in high yield on the macro scale, although it is very exothermic. Again, only one product was obtained, which could be purified by distillation under high vacuum. With this compound, the response factors of the various detectors, i.e. EC, AFID and FPD, to parathion and amino parathion were determined. The values obtained are given in Table III, expressed as the least detectable amounts at 2 x noise level.

It can be seen that the amino parathion is less sensitive

than parathion by a factor of three on the AFID and the FPD detectors. This may be partly accounted for by the peak shape, the amino parathion peak being broader. Although the EC is the most sensitive detector of the three for parathion, its sensitivity for the amino parathion is markedly less, by a factor of over 400. Thus, the reduction reaction as a confirmatory test for pesticides containing an aryl nitro group is best applied using the two specific phosphorus detectors.

TABLE III

The response of electron capture, flame photometric and alkali flame ionisation detectors to parathion and amino parathion

	Least Detectable Amounts (x 10^{-12} g/sec.)		
	EC	AFID	FPD
Parathion	0.24	0.5	2.1
Aminoparathion	93	1.5	6.4

Both fenitrothion and malathion are widely used in the environment. They are not very clearly resolved by GLC when an OV-1 column is used as shown in Figure 3A. Even so, the presence of fenitrothion can be readily confirmed by reduction with chromous chloride as shown in Figure 3B. The fenitrothion is reduced to amino fenitrothion, whereas malathion remains unchanged.

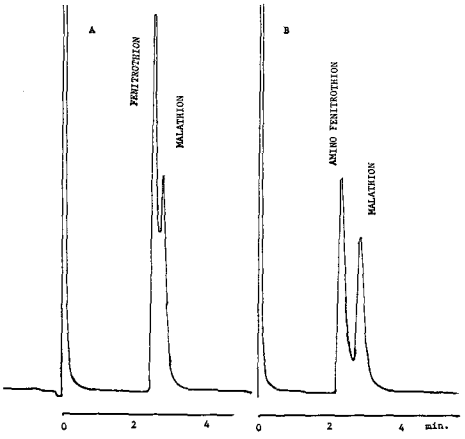


Figure 3 Chromatogram of a mixture of Malathion and Fenitrothion before and after treatment with chromous chloride

In summary, reduction by chromous chloride provides a sensitive, rapid and reliable method which can be used advantageously to confirm the presence of organophosphorus insecticides containing a nitro group. In this study, as little as 50 ng/l in water or 50μg/kg in fish or sediment have been confirmed. Other organophosphorus compounds present were not destroyed during the reaction.

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References

ASKEW, J., RUZICKA, J.H. and WHEALS, B.B., Analyst, 94, 275 (1969)
AVERELL, P.R. and NORRIS, M.V., Anal. Chem., 20, 753 (1948)
BOWMAN, M.C., BEROZA, M., J. Ass. Offic. Agr. Chem., 48, 943 (1965)
BRODY, S.S. and CHANEY, J.E., J. Gas Chromat., 42 (1966)
COCHRANE, W.P. and CHAU, A.S.Y., Bull. Envir. Cont. Toxicol., 5, 251 (1970)
COCHRANE, W.P. and FORBES, M.A., Pesticide Chemistry Vol. IV, p. 385 (1971) Editor: A.S. Tahori, Gordon & Breach, London
DAMICO, J.N., BARRON, R.P. and RUTH, J.M., Org. Mass Spectrom., 3, 51 (1970)
FAHEY, J.E., SCHECTER, M.S., J. Agr. Food Chem., 9, 192 (1961)
GIUFFRIDA, L., J. Ass. Offic. Agr. Chem., 47, 293 (1964)
KOVACS, M.F. Jr., J. Ass. Offic. Agr. Chem., 46, 884 (1963)
McCULLY, K.A., World Rev. Pest. Contr., 8, 59 (1969)
MINYARD, J.P. and JACKSON, E.R., J. Agr. Food Chem., 13, 150 (1965)
MIYAMOTO, Junshi, KITAGAWA, Kimiko and SATO, Yashishige, Japan J. Ex. Med., 36, 211 (1966)
ROBERTS, John and CASERIO, Marjorie, Basic Principles of Organic Chem., p. 867, W.A. Benjamins Inc., New York (1965)
WOODHAM, D.W., LOFTIS, C.D. and COLLIER, C.W., J. Agr. Food Chem., 20, 163 (1972)
YULE, W.N. and DUFFY, J.R., Bull. Envir. Cont. Toxicol., 8, 12 (1972)