Confirmation of Some NO_2-Containing Pesticides by Chemical Reduction and Gas Chromatography with Electrolytic Conductivity Detection

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Confirmation of a number of NO_2-containing herbicides and fungicides has been carried out by reduction of the NO_2 group to $-NH_2$ with aqueous chromous chloride. The pesticide residue in 1 mL of benzene was shaken with 0.5 mL of the reagent for about 1 min at room temperature to effect complete conversion to the amino product. The aqueous phase was made basic and the products extracted with benzene for analysis. Analysis of the products by GLC with electrolytic conductivity detection (nitrogen mode) showed that most were about as sensitive as the parents. Generally, the derivatives eluted with retention times of 0.4–0.9 relative to the parent compounds on an SE-30/SP-2401 column. Applications of this method to the confirmation of trifluralin spiked in extracts of potato and dinoseb in peas were successful at levels of 0.5–1.0 ppm.

Confirmatory tests play an important role in pesticide residue analysis. While there are several approaches to confirmation including instrumental (spectrophotometry, NMR, mass spectrometry, etc.), physical (retention behavior on different columns, partition values between two immiscible solvents), biological (bioassay, enzyme inhibition), and chemical techniques, it is the latter which has the most potential for routine application for several reasons. Chemical derivatization is usually simple, rapid, and selective. Positive confirmation is evident when a known product is derived from a suspected compound. Normally, the derivatives can be analyzed by the same system as was the original compound which makes need for additional instrumentation unnecessary. Chemical confirmation normally provides more specific information about an unknown than the physical or biological techniques mentioned above. Thus, chemical reactions are often used for confirmation of pesticide residue identity. The use of chromous chloride for confirmation of many organochlorine pesticides has proven to be an asset for residue determinations (Cochrane and Forbes, 1971). Chromous chloride was evaluated along with three other reducing agents for the confirmation of selected organophosphate insecticides which contained the $-NO_2$ group (Forbes et al., 1975). It was found that while the parent compounds had strong electron-capture sensitivity, the derivatives, because of the loss of the $-NO_2$, were less sensitive by a factor of about 400. Thus other detectors such as the flame photometric (phosphorus) or nitrogen selective alkali-flame detectors were suggested for best application of this confirmatory technique.

The present work describes the application of chromous chloride reduction for the confirmation of several NO_2-containing herbicides and fungicides by gas chromatography with electrolytic conductivity detection (nitrogen mode). Application of this approach to the confirmation of trifluralin and dinoseb in potatoes and peas, respectively, illustrates the potential of this technique.

EXPERIMENTAL SECTION

Apparatus. A Tracor Microtek MT220 gas chromatograph equipped with a Coulson conductivity detector in the nitrogen mode was employed for the analyses. One column (100 cm × 4 mm i.d.) was packed with 4% Se-30/6% SP-2401 while a second column (120 cm × 4 mm i.d.) was packed with 3% OV-1, both on chromosorb W/HP (80–100 mesh). Operating conditions were: carrier gas (helium), 40 mL/min; helium sweep, 40 mL/min; hydrogen, 31 mL/min; injection port, 220 °C; pyrolysis furnace, 825 °C; transfer line, 235 °C, D.C. voltage 30 V. Column temperatures were varied for the pesticides analyzed.

Reagents. The herbicides studied are listed with their chemical names in Table I. Stock solutions of these were prepared at 1 mg/mL in benzene. Working solutions were prepared by appropriate dilution of the stock solutions with benzene.

Aqueous chromous chloride solution was purchased from Fisher Scientific and determined to be approximately 1 N. The dark-blue solution was covered with a layer of paraffin oil about 0.25 in. thick to prevent contact with the air. All test tubes were flushed with a stream of nitrogen before addition of the reagent. The reagent bottle was flushed with nitrogen each time before storage at 4–5 °C. With this treatment the reagent remained useful for a year or more. All organic solvents were glass-distilled, residue-free materials.

Chemical Reduction. One milliliter of a benzene solution of the pesticide was added to a 20-mL screw-cap test tube with teflon-lined screw-cap. The tube was flushed with nitrogen for a few seconds, then 0.5 mL of chromous chloride solution was added by volumetric pipet. The screw-cap was tightly replaced and the contents shaken vigorously for 1 min. After this, 10 mL of distilled water followed by 5.0 mL of 6.0 N NaOH were added to ensure that the aqueous phase was strongly basic. The mixture was shaken for 1 min and then centrifuged at 2000 rpm. An aliquot of the benzene layer was injected directly (or after appropriate dilution) into the gas chromatograph.

Sample Extraction. Samples of potato and peas were extracted and cleaned up according to a procedure described earlier (Lawrence and McLeod, 1977). The sample extracts were spiked with trifluralin or dinoseb. Trifluralin was eluted from the 2% deactivated Florisil column with 100 mL of 30% methylene chloride in hexane. This fraction was analyzed by GLC and then subjected to the confirmation procedure. The pea extract containing added dinoseb was methylated with diazomethane (Yip and Howard, 1968) before Florisil cleanup. The methylated extract was then passed through the Florisil column. Dinoseb methyl ether was eluted with 100 mL of 15% acetone in hexane and analyzed directly by GLC. An aliquot of this fraction was then evaporated just to dryness, dissolved in 1.0 mL of benzene, and subjected to reduction

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Table I. Chemical Names and Use of the Pesticides Studied

Common or trade name	Chemical name	Use
Binapacryl	2-*sec*-Butyl-4,6-dinitrophenyl-3-methyl-2-butenoate	Acaricide
Dicloran	2,6-Dichloro-4-nitroaniline	Fungicide
Dinobuton	2-*sec*-Butyl-4,6-dinitrophenyl isopropyl carbonate	Fungicide
Dinoseb	2-*sec*-Butyl-4,6-dinitrophenol	Herbicide
Dinoseb acetate	2-*sec*-Butyl-4,6-dinitrophenyl acetate	Herbicide
Dinoterb	2-*tert*-Butyl-4,6-dinitrophenol	Herbicide
Dinoterb acetate	2-*tert*-Butyl-4,6-dinitrophenyl acetate	Herbicide
DNOC	2-Methyl-4,6-dinitrophenol	Herbicide
Fenitrothion	*O,O*-Dimethyl-*O*-(4-nitro-*m*-tolyl) phosphorothioate	Insecticide
Methyl parathion	*O,O*-Dimethyl-*O*-*p*-nitrophenyl phosphorothioate	Insecticide
Nitralin	4-(Methylsulfonyl)-2,6-dinitro-*N,N*-propylaniline	Herbicide
Nitrofen	2,4-Dichlorophenyl 4′-nitrophenyl ether	Herbicide
Parathion	*O,O*-Diethyl-*O*-*p*-nitrophenyl phosphorothioate	Insecticide
Quintozene	Pentachloronitrobenzene	Fungicide
Tecnazine	1,2,4,5-Tetrachloro-3-nitrobenzene	Fungicide
Trifluralin	$\alpha\alpha\alpha$-Trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine	Herbicide

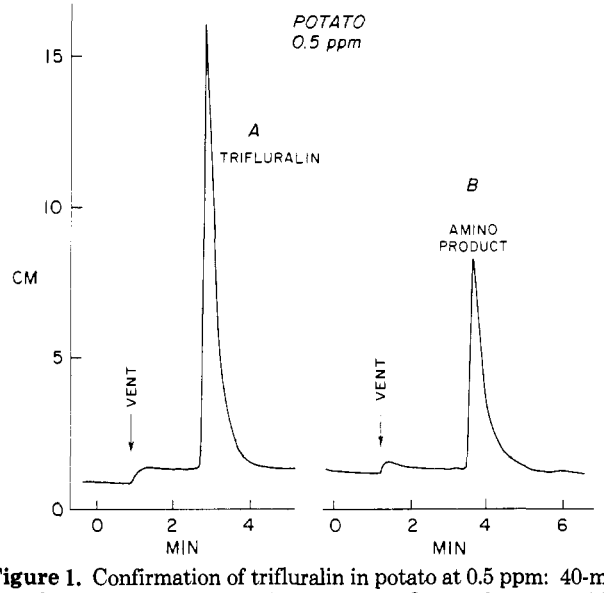

Figure 1. Confirmation of trifluralin in potato at 0.5 ppm: 40-mg sample injected, 2× attenuation, 30 V; 3% OV-1 column, 180 °C, 40 mL/min, helium; A = extract containing trifluralin, B = after reduction.

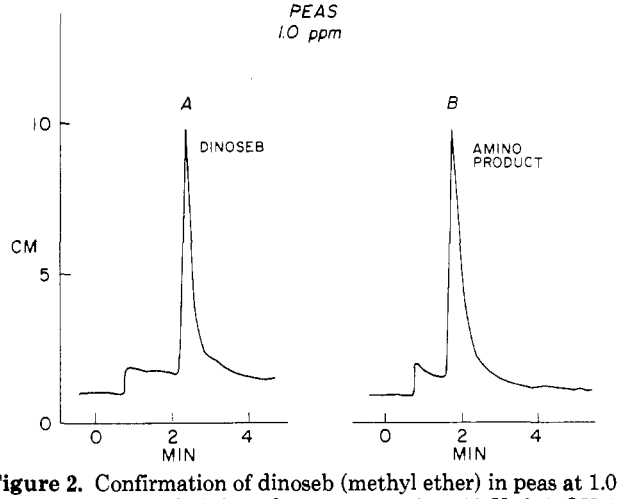

Figure 2. Confirmation of dinoseb (methyl ether) in peas at 1.0 ppm: 45-mg sample injected, 1× attenuation, 30 V; 3% OV-1 column, 200 °C, 40 mL/min, helium; A = extract containing dinoseb (methyl ether), B = after reduction.

with chromous chloride for confirmation.

RESULTS AND DISCUSSION

Table II summarizes the results obtained on the mixed column (SE-30/SP-2401) for the confirmation of the pesticides studied. The OV-1 column was also useful for most compounds and results are illustrated in Figures 1 and 2 for trifluralin and dinoseb. The organophosphates were run to compare our electrolytic conductivity results with those obtained earlier by flame photometric (phosphorus) and alkali flame ionization detection under slightly different reaction conditions (Forbes et al., 1975). The conductivity results (Table II) showed that the reduction products were equally or more sensitive than the parent organophosphates and they chromatographed with similar peak shapes. These results appear to agree with fenitrothion by alkali flame or flame photometric detection but differ from parathion where the amino derivative was about one-third as sensitive as the parent on these two detectors. It is probable that chromatography and detection mechanisms account for these differences since we have found that tailing of the amino products is significantly affected by column type and temperature.

The sensitivity of the other amino products relative to the parents varied from 0.25–2 for the different compounds studied. The difference would not be expected to be great since both parent and product retain the same number of nitrogen atoms which is the basis for detection by electrolytic conductivity in the nitrogen mode. The observed differences in sensitivity between parents and products might be generally caused by differences in chromatographic behavior and losses upon extraction. All nitro groups in the parent molecules appeared to be converted to amino groups. Thus it was probable that the mononitro pesticides were reduced to their monoamino derivatives, and the dinitro compounds were converted to the diamino products. This was confirmed by mass spectrometry for trifluralin (diamino product, m/e 275) and tecnazine (amino product, m/e 231).

Table II. Chromatographic and Detector Characteristics of the Pesticides and Their Reduction Products

Pesticide	Retention time		Sensitivity[a]		Glc conditions:[b] temp, °C
	Parent	Product	Parent	Product	
Binapacryl[c]	5.8	4.0	6	20	200
Dicloran	6.6	3.9	10	40	180
Dinobuton[d] (methyl ether)	2.0	1.5	5	20	215
Dinoseb (methyl ether)	3.6	1.8	4	4	200
Dinoterb (methyl ether)	3.6	1.8	4	4	200
Dinoterb acetate	5.1	1.8	12	24	200
DNOC (methyl ether)	5.4	2.0	1.5	3	175
Fenitrothion	5.8	4.5	10	9	215
Methyl parathion	5.2	3.6	10	10	215
Nitralin[e]	4.5	7.5	100	200	225
Nitrofen	4.8	3.2	20	30	215
Parathion	6.6	4.6	15	7	215
Quintozene	5.1	6.2	10	16	160
Tecnazine	5.7	5.3	5	4	170
Trifluralin	5.7	4.4	5	10	180
Trifluralin[f]	2.8	3.6	2	4	180

[a] Nanograms required to produce a 1 cm peak at 1× attenuation. [b] 4% SE-30/6% SP-2401 was used for the analyses. [c] Carrier flow rate, 90 mL/min. [d] Carrier flow rate, 100 mL/min. [e] Carrier flow rate, 80 mL/min. [f] 3% OV-1 column was used.

The addition of the strong alkali after the reduction reaction was required to help force the amino products into the benzene phase. The chromous chloride solution, being acidic, tended to hold the amino products to varying degrees. Neutralizing the aqueous phase increased the extraction yield for most compounds, but for some such as DNOC and dinobuton (methyl ethers) and others, strongly basic conditions were required. It is possible that this extraction problem was associated with the complexation of the diamino products with chromium ion and that strong base was required to release them. For a standard reaction procedure the addition of strong alkali solution was included. This had no adverse effects on those products which were normally extracted under neutral conditions.

Since no macro-scale reactions were carried out, the percent yields were not calculated. However, in all cases, the parent pesticide was completely absent from the chromatograms after the reduction reaction. Only one peak appeared for the reduced compounds in all cases. The reaction appeared to proceed faster in the present work than described earlier (Forbes et al., 1975), since in the latter, 5 min at 60 °C was required for best conversion. This is possibly a reflection on the condition of the chromous chloride solution used, since it is very reactive and decomposes upon contact with air.

The retention times of the products on the SE-30/SP-2401 column were shorter than the parents in all cases except for quintozene and nitralin. The sensitivity of nitralin was by far the worst of the compounds studied. Its amino reduction product tailed very badly and would likely be undetectable in crop samples below 1 ppm. All other amino products chromatographed well with slightly more tailing than the parent compounds. Dinoseb and dinoterb (methyl ethers) could not be separated under the conditions used on either column examined. The reduction products also could not be separated from one another. Attempts at separation of these parents or products were not carried out.

Application of this confirmatory test to crop sample extracts spiked with trifluralin and dinoseb indicates its usefulness. Figures 1 and 2 show chromatograms obtained before and after reduction. Both compounds were easily confirmed at 0.5–1.0 ppm. No interfering peaks were observed for either parent or product at these levels. Trifluralin was also easily confirmed at 0.1 ppm in spiked potato extract. The amino product of trifluralin chromatographed later than the parent on OV-1 as opposed to earlier on the SE-30/SP2401 column. The pea extract was not as clean as potato and prevented confirmation of dinoseb at levels less than 0.1 ppm.

We found in some cases where an extract contained significant crop material, some emulsion appeared at the organic–aqueous interface during the extraction of the product from the reduction mixture. While most of this was removed upon centrifugation, the addition of 2–5 mL of benzene, or removing most of the aqueous phase and partitioning with 10 mL of distilled water greatly improved phase separation and thus facilitated removal of an aliquot for GLC analysis.

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