# Confirmation of Some Organonitrogen Herbicides and Fungicides by Chemical Derivatization and Gas Chromatography

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The confirmation of residue levels of eight organonitrogen herbicides and two fungicides was carried out by alkylation, methoxylation, and/or trifluoroacetylation. The pesticides included members of the triazine, urea, *N*-phenylcarbamate, thiocarbamate, substituted aniline, and uracil groups. The parents and derivatives were analyzed by gas–liquid chromatography with electrolytic conductivity detection (nitrogen mode). In most cases, conversion of the pesticides to their derivatives occurred in high yields after reaction periods of 1 h or less at 20–100 °C depending upon pesticide and reagent. Identification of the products was aided by mass spectrometry. These confirmation techniques were applied to the analysis of several of the herbicides and fungicides in corn at 0.1 ppm.

Chemical derivatization can be a very useful approach for pesticide confirmation by gas–liquid chromatography (GLC). Usually, the reagents are inexpensive and products can normally be run on the same GLC system as the parent compounds. Derivatization also serves a useful purpose when there is no access to equipment such as a mass spectrometer for unequivocal identification of the compound. Since GLC has proven to be such a useful tool for trace organic analysis, much effort has been put into the formation of stable derivatives of compounds which otherwise could not be analyzed by GLC. Evidence of such work appears in recent reviews on chemical derivatization in gas chromatography (Cochrane, 1975; Drozd, 1975; Khan, 1975). These papers discuss many reactions for forming derivatives suitable for GLC both in terms of chromatographic behavior and detector sensitivity.

The approach taken in the present work is directed primarily at the confirmation of pesticides by forming a derivative with a different retention time than the parent. Since a nitrogen selective detector is used and since the reagents contain no nitrogen, the sensitivities of the products are of the same order as the parent compounds. The pesticides included herein were taken on the basis of their importance in Canada without regard to class. The reactions described should be applicable to other pesticides similar in nature to the pesticides studied. This work is part of an attempt to establish a multi-residue screening method with confirmation for organonitrogen herbicides and similar fungicides in foods. The GLC of these compounds and their sensitivities in both nitrogen and halogen modes have been reported (Lawrence, 1976).

## EXPERIMENTAL SECTION

**Apparatus.** A Tracor MT220 gas chromatograph equipped with a Coulson conductivity detector (CCD) (nitrogen mode) was used for the analyses. This detector is no longer being sold by Tracor, but the Hall conductivity detector should provide greater sensitivity and better peak shape. The columns consisted of 120 cm × 4 mm i.d. borosilicate glass U-tubes packed with either 4% SE-30/6% SP-2401 or 20% Carbowax 20M on Chromosorb W/HP (80–100 mesh). Column temperatures varied with pesticides and derivatives. Operational parameters were as follows: carrier gas (helium), 40–130 ml/min depending upon derivative; helium sweep, 40–130 ml/min (set to match the carrier gas flow rate); hydrogen, 50 ml/min; injection port, 240 °C; transfer line, 215 °C; furnace, 820 °C; dc voltage, 30 V. A 0.004-in. diameter stainless steel wire was inserted into the capillary water entrance to the mixing chamber of the Coulson cell to reduce the flow so that no water escaped through the vent tube (Lawrence and Sen, 1975).

**Reagents.** The pesticides studied were: atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine), chlorpropham (isopropyl *N*-(3-chlorophenyl)carbamate), diallate (*S*-2,3-dichloroallyl-*N*,*N*-diisopropyl thiolcarbamate), dichlobenil (2,6-dichlorobenzonitrile), dichloran (2,6-dichloro-4-nitroaniline), dyrene (2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine), EPTC (ethyl-*N*,*N*-di-*n*-propylthiolcarbamate), linuron (*N*-3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea), propanil (*N*-(3,4-dichlorophenyl)propionamide), and terbacil (3-*tert*-butyl-5-chloro-6-methyluracil). Stock solutions of these were prepared at 1 mg/ml in acetone. Working solutions were prepared by appropriate dilution of aliquots of the stock solutions with acetone.

The derivatization reagents used for the confirmation reactions were used as obtained from the suppliers. For methoxylation, a 25% solution of sodium methoxide in methanol (Anachemia Chemicals Ltd.) was used. For alkylation dry dimethyl sulfoxide (BDH Chemicals), methyl iodide (Fisher), and sodium hydride (obtained as a 50% oil dispersion, Baker) were used. About 10 g of the sodium hydride was washed with 50 ml of hexane before use and stored in a tightly sealed vial. Care must be exercised in working with this reagent since it reacts with water and alcohols to form flammable hydrogen gas. Trifluoroacetic anhydride (Eastman) was used for the trifluoroacetylation reactions. All organic solvents were glass-distilled residue free materials.

**Sample Extraction.** The extraction of corn was carried out as described earlier (Lawrence and McLeod, 1976). The final corn extract was spiked with a pesticide just before 2% deactivated Florisil column cleanup (15 g of Florisil in a 1.5-cm diameter column). Elution of the pesticides from the column was accomplished with 100 ml each of 30% methylene chloride/hexane followed by 15% acetone/hexane and 50% acetone/hexane. Each fraction was collected separately and reduced to ca. 0.25 ml with a rotary evaporator. The residue was quantitatively transferred to a 5-ml centrifuge tube and brought to 1.0 ml with acetone. An aliquot of this was directly analyzed by gas chromatography for the parent pesticides.

**Methoxylation.** A 0.5-ml volume of the extract containing the pesticide from above was transferred to a 20-ml screw-capped test tube. The solvent was evaporated under

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Table I. Chemical Derivatization Results

| Pesticide | Product | % yield | Ret. time, min | | GLC conditions[a] | | |
|---|---|---|---|---|---|---|---|
| | | | Parent | Product | Column | Temp, °C | Flow, ml/min |
| Atrazine | Alk. | 100 | 14.0 | 3.8 | 2 | 200 | 60 |
| | Methox. | 100 | 10.9 | 7.9 | 2 | 220 | 130 |
| Chlorpropham | Alk. | 100 | 3.3 | 2.3 | 1 | 175 | 50 |
| | TFA | 90 | 3.3 | 2.4 | 1 | 175 | 50 |
| Diallate[b] | Methox. | 100 | >30.0 | 1.4 | 2 | 170 | 50 |
| Dichlobenil | Methox. | 90 | 2.1 | 4.3 | 1 | 155 | 60 |
| Dichloran | Alk. | 100 | 5.3 | 3.6 | 1 | 180 | 40 |
| Dyrene | Alk. | 100 | 3.1 | 4.3 | 1 | 200 | 60 |
| | Methox. | 75 | 2.3 | 3.4 | 1 | 220 | 60 |
| | TFA | 75 | 4.6 | 2.6 | 1 | 200 | 50 |
| EPTC | Methox. | 60 | 5.8 | 2.0 | 2 | 170 | 50 |
| Linuron | Alk. | 90 | 6.0 | 3.8 | 1 | 190 | 60 |
| Propanil | Alk. | 100 | 3.4 | 2.2 | 1 | 200 | 60 |
| | TFA | 70 | 6.5 | 2.4 | 1 | 185 | 50 |
| Terbacil | Alk. | 100 | 2.1 | 8.9 | 1 | 210 | 70 |

[a] Column 1 = 4% SE-30/6% SP-2401; column 2 = 20% Carbowax 20M. [b] Diallate (parent) was analyzed on column 1 at 185 °C, 60 ml/min, retention time, 2.1 min.

a stream of nitrogen and 0.5 ml of 25% sodium methoxide in methanol was added. The contents were gently swirled and the test tube tightly capped and heated for 1 h at 100 °C by immersing the bottom 1 in. of the tube in an oil or sand bath. The tube was cooled and 2 ml of ethyl acetate was added followed by 5 ml of $H_2O$. The mixture was then shaken for 1 min. The ethyl acetate was removed to a 5-ml centrifuge tube by pasteur pipet and the aqueous layer extracted twice more with 1.0-ml volumes of ethyl acetate. The combined organic layers were reduced to 0.5 ml under nitrogen for GLC analysis. This reaction was suitable for confirmation of atrazine, diallate, dichlobenil, dyrene, and EPTC.

**Alkylation.** A 0.5-ml volume of the cleaned-up corn extract was evaporated with a stream of nitrogen just to dryness in a 20-ml test tube. Then, 0.5 ml of benzene, 0.5 ml of dimethyl sulfoxide, and 0.5 ml of methyl iodide were added followed by ca. 20–30 mg of sodium hydride. The test tube was capped, gently swirled, and placed in a test tube rack for 30 min at room temperature. Following this, 3.0 ml of hexane was added and the contents vigorously shaken for 1 min. A 10-ml volume of distilled water was added dropwise to destroy excess sodium hydride (caution, sodium hydride reacts rapidly with $H_2O$ evolving $H_2$). When effervescence ceased the tube was capped and shaken vigorously for 30 s. After the phases separated the aqueous layer was removed by pasteur pipet and the organic layer washed with a second 10-ml volume of $H_2O$. The hexane layer was then removed by pasteur pipet and evaporated to dryness in a 5.0-ml centrifuge tube. The residue was dissolved in 0.5 ml of hexane for GLC analysis. This reaction was suitable for the confirmation of atrazine, chlorpropham, dichloran, dyrene, linuron, propanil, and terbacil.

**Trifluoroacetylation.** A 0.5-ml volume of the extract containing the pesticide was evaporated with a stream of nitrogen just to dryness in a 20-ml test tube. To the residue was added 1.0 ml of dry ethyl acetate and 0.5 ml of trifluoroacetic anhydride. The tube was tightly capped and heated at 80 °C for 30 min. After cooling the tube to room temperature, the solution was evaporated to 0.1 ml to remove excess reagent. The remainder was then diluted to 0.5 ml with hexane for GLC analysis. This reaction is suitable for confirmation of chlorpropham, dyrene, and propanil.

## RESULTS AND DISCUSSION

Table I lists the types of derivatives formed together with their retention times and percent conversion of their

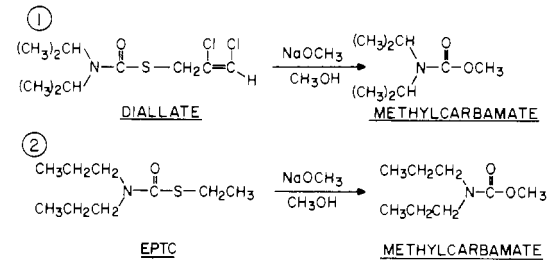


Figure 1. Methoxylation reaction for diallate and EPTC.

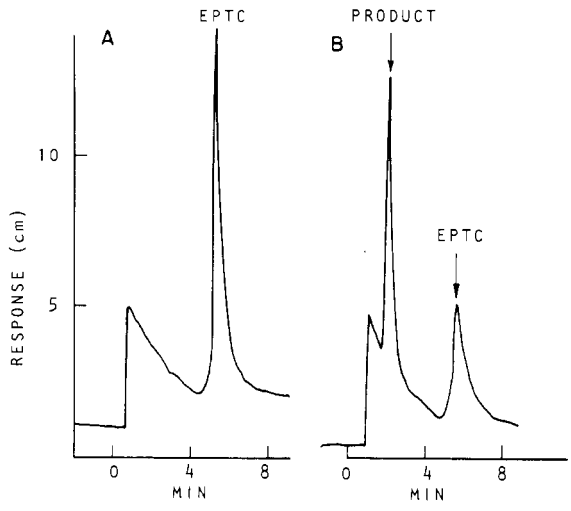


Figure 2. Confirmation of EPTC in corn (0.1 ppm) by methoxylation: (A) 350-mg sample injected before reaction; (B) same extract after derivatization. Conditions are as described in Table I; attenuation 2×.

respective parents. Yields were calculated on the quantity of parent remaining after the reaction, as well as the derivative response based on nitrogen content. The yields proved to be consistent over more than ten derivatization attempts for each compound.

The methoxylation reaction involved the replacement of the chlorine atoms on the triazine ring of dyrene and atrazine with methoxyl groups. This reaction was examined earlier for other chloro-*s*-triazines (Lawrence, 1974). Mass spectrometry indicated that both chloro substituents on the triazine ring of dyrene were replaced (molecular ion at $m/e$ 266 for product). No monomethoxylated compound was found (no $m/e$ at 271.5) as was under other methoxylation conditions (Mendoza et al.,

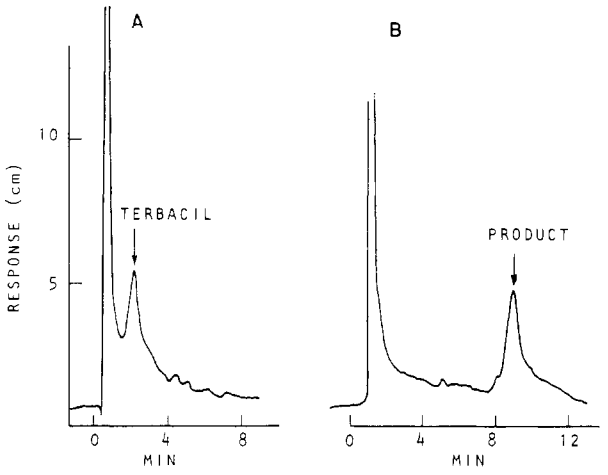


Figure 3. Confirmation of terbacil in corn (0.1 ppm) by alkylation: (A) 400-mg sample injected before reaction; (B) same extract after alkylation. Conditions are as described in Table I; attenuation 2×.

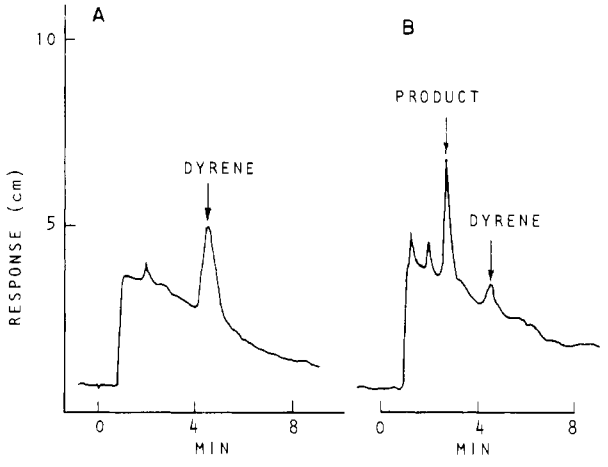


Figure 4. Confirmation of dyrene in corn (0.1 ppm) by trifluoroacetylation: (A) 400-mg sample injected before reaction; (B) same extract after acetylation. Conditions are as described in Table I; attenuation 2×.

1971). Figure 1 shows the reaction scheme for the methoxylation of the thiolcarbamates, EPTC, and diallate. The thiocarbamate linkage was broken and the corresponding methylcarbamates were formed which eluted from the GLC much faster than the parents. The methoxylation of dichlobenil resulted in both ring chlorine atoms being replaced with methoxyl substituents (*m/e* parent, 172; *m/e* product, 163).

The alkylation reactions proceeded well for atrazine, chlorpropham, dichloran, dyrene, linuron, propanil, and terbacil. The N–H substituent in all cases was converted to N–$CH_3$. The reaction mechanism has been discussed earlier for N–H containing compounds in general (Greenhalgh and Kovacicova, 1975; Lawrence and Laver, 1975).

Trifluoroacetylation was carried out as described in the literature (Drozd, 1975; Cochrane, 1975; Khan, 1975). The reactions proceeded in the normal manner resulting in the N–H moiety being converted to N–CO–$CF_3$. The derivatives eluted earlier than the parents in all cases.

The confirmation techniques were applied to sample extracts of corn spiked at 0.1 ppm. Figures 2–4 compare sample extracts containing three of the herbicides studied before and after derivatization. All pesticides eluted from the Florisil in the 15% acetone/hexane fraction with the exceptions of terbacil (50% acetone/hexane fraction) and diallate and dichlobenil which eluted in the 30% methylene chloride fraction. All could be confirmed at 0.1 ppm in corn by at least one of the derivatization reactions described herein.

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# Induction of Paraoxon Dealkylation by Hexachlorobenzene (HCB) and Mirex

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The induction of paraoxon dealkylation activity was measured in the microsomal fraction from livers of rats fed for 2 weeks on diets containing hexachlorobenzene (HCB) or Mirex. Formation of deethylparaoxon was increased significantly at 2 ppm of HCB and 1 ppm of Mirex. Other parameters, body weight gain, liver weight, microsomal protein, $P_{450}$, and aminopyrine *N*-demethylase, were not significantly affected by these dose levels, but they were increased after feeding 10 and 40 ppm of HCB or 5 ppm of Mirex.

Dealkylation of organophosphate insecticides is believed to occur via the microsomal mixed function oxidase system and a cytoplasmic glutathione transferase (Appleton and Nakatsugawa, 1972). Ku and Dahm (1973) have reported that O-dealkylation is increased considerably after administration of phenobarbital and other inducers, while the glutathione-requiring system is unaffected. Donninger (1971) also reported a 600-fold increase in specific activity

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