fully understood, but Akoyunoglou *et al.* (1963) had difficulty determining the equilibrium constants of the nitrogenous base hemochromes they studied, and Fox *et al.* (1975) could not determine the rate constants for formation of substituted-pyridine, SLS-denatured globin hemochromes. Since the pigments are hemochromes (ferrous), reductants are necessary, but the addition of reductants will not necessarily improve color, as Howard *et al.* (1973) also observed. Reductants do, however, increase stability of the pyridine hemochrome. The role of reductants in heme pigment chemistry is ambiguous for there are a number of green heme pigments that are specifically produced by oxidation in the presence of reductants, choleglobin with ascorbate and sulfmyoglobin and sulfhemoglobin with cysteine (Lemberg and Legge, 1949).

Reflectance spectrophotometry is a useful tool in corroborating visual observations and identifying specific pigments. The comparatively small area which the microspecular attachment examines has been particularly useful in determining the spectra of pigments that occur only over a limited area, such as the discolored rings observed in the frankfurters of this study. Although the reflected intensities of light from this device are low, the spectra are sufficiently distinctive to allow positive identification of specific pigments.

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A Chemical Confirmatory Test for Organophosphorus and Carbamate Insecticides and Triazine and Urea Herbicides with Reactive NH Moieties

Roy Greenhalgh* and Jana Kovacicova[1]

The effects of different solvents and temperatures on the base-catalyzed reaction of *O,O*-diethyl *N*-methyl phosphoramidothioate and methyl iodide were studied. The *N*-methyl derivative was obtained in high yield in dimethyl sulfoxide at 50° for 10 min with sodium hydride as the base. This alkylation procedure was adapted to the microgram level and applied to 16 insecticides and herbicides which have an NH or NH_2 moiety. With carbamates, the best yields were obtained when the reaction was carried out at room temperature. The alkylated derivatives of organophosphorus and carbamate insecticides and triazine and urea herbicides all had better gc characteristics than the parent compounds. Thus, the alkylureas readily chromatographed on SE-30/QF-1 under conditions where the parent compounds decomposed. On this column, the alkylated phosphoramidothioates and carbamates also had shorter retention times. Alkylation of crude extracts of soil, plants, and blood plasma containing herbicides and insecticides illustrated the ability of the sodium hydride–methyl iodide–dimethyl sulfoxide procedure to confirm the identity of residues at the sub parts per million level.

Although mass spectrometry is the ideal technique for confirmation of the identity of insecticide residues, its use is often limited by availability and cost of instrumention. Chemical derivatization, on the other hand, is a simple, quick, and cheap technique, which can be used as an alternative procedure for insecticide confirmation.

Derivatization of insecticides and herbicides reported in the literature includes reactions with both parent compounds as well as their hydrolytic products. For confirmatory purposes, the former reactions are preferred since they yield specific derivatives. Examples of these reactions are the ones developed by Cochrane *et al.* (Cochrane and Chau, 1971; Cochrane and Maybury, 1973; Cochrane and Purkayastha, 1973) for the cyclodienes and BHC. A general method for organophosphorus insecticides was reported by Shafik *et al.* (1971), which consists of hydrolysis followed by alkylation to identify the type of substitution on phosphorus. A more specific test for parathion and fenitrothion however exists, involving reduction of the nitro group (Forbes *et al.*, 1975). Several methods for the derivatization of carbamates were referenced by Lorah and Hemphill (1974); of these, perfluoroacylation is probably the most suitable for a specific confirmatory test (Seiber, 1972). In the case of triazine and urea herbicides, derivatives of the reactive NH group can be used for confirmatory tests. The silyl derivatives of hydroxyatrazine were prepared by Flint and Aue (1970) and both atrazine and fenuron have been alkylated at the residue level (micrograms/milliliter) (Greenhalgh and Kovacicova, 1975). Recently, Saunders and Vanatta (1974) showed that both the trifluoroacyl and alkyl derivatives of four urea herbicides are thermally stable. In this case, potassium *tert*-butoxide and methyl iodide were used to alkyl the herbicides (milligrams/milliliter level).

This paper examines the feasibility of using base-catalyzed alkylation as a general reaction for the confirmation of the identity of insecticides and herbicides which have

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Table I. Amount of Alkylation of *O,O*-Diethyl *N*-Methyl Phosphoramidothioate by Methyl Iodide in Different Solvents[a]

Solvent	Av yield, %[b]	
	t-BuOK	NaH
Hexane	18.2	42.6
Benzene	43.5	57.2
Acetone	22.6	60.1
Ethyl acetate	14.3	
THF	60.7	64.3
tert-Butyl alcohol	60.4	
Acetonitrile	72.1	84.6
DMSO	92.6	95.2
10% DMSO in hexane	81.2	93.1

[a] Reaction conditions: room temperature/1 hr; DEMPAT concentration, 1 mg/ml. [b] Duplicate reactions.

NH or NH_2 moieties. This includes phosphoramidothioates, $R_1R_2P(S)NHR_3$; phosphoramidates, $R_1R_2P(O)NHR_3$; and carbamates, $R_1NHC(O)OR_2$; as well as the ureas, $R_1NHC(O)NR_2R_3$; and triazines, R_1NHR_2. *O,O*-Diethyl *N*-methyl phosphoramidothioate (DEMPAT) was used as a model compound for a study of some reaction parameters.

EXPERIMENTAL SECTION

Apparatus and Chemicals. A Pye chromatograph Model 134 equipped with an alkali flame ionization detector (AFID) was used with a RbCl annulus. In the model studies, a 2.3 m × 4 mm glass column was employed, packed with 100–120 mesh Chromosorb W coated with 5% Reoplex. For the organophosphorus and carbamate insecticides, triazine and urea herbicides, the column was packed with 100–120 Gas-Chrom Q coated with 4% SE-30/6% QF-1. The column flow was maintained at 40 ml/min of nitrogen and the column temperature selected to give retention times of 2–5 min.

All the insecticides and herbicides used were analytical grade, obtained from the respective manufacturers. Sodium hydride was purchased from Alfa Inorganics, Beverly, Mass., as a 57% oil dispersion. Potassium *tert*-butoxide was obtained from Aldrich Chemical Co., Cedar Knolls, N.J. *O,O*-Diethyl *N*-methyl phosphoramidothioate was prepared by passing methylamine into *O,O*-diethyl phosphorothiochloridate dissolved in benzene. The product was washed with water, dried, and distilled *in vacuo*, and characterized by mass spectrometry (ms) and nuclear magnetic resonance [bp 43–46° (0.05 mm)]. The oxygen analog was prepared in a similar manner [bp 78° (0.05 mm)] (McCombie *et al.*, 1945).

The solvents used were pesticide grade except for *t*-BuOH, which was distilled from calcium hydride. Both DMSO and *t*-BuOH were stored over Linde molecular sieve, type 5A.

Procedures. (*A*) *Model Studies.* (*1*) *Alkylation with Potassium tert-Butoxide–Methyl Iodide* (*t-BuOK–MeI*). DEMPAT (10 mg) was dissolved in the appropriate solvent (10 ml) and a solution of *t*-BuOK in *tert*-butyl alcohol (0.2 ml, 55 mg/ml) and methyl iodide (0.1 ml) added. The reaction vessel was sealed and left to stand at room temperature for 60 min. Samples (1 ml) were taken, diluted with acetone (9 ml), and analyzed by gc.

(*2*) *Alkylation with Sodium Hydride–Methyl Iodide* (*NaH–MeI*). Sodium hydride (20 mg) was washed twice with hexane; the appropriate solvent (10 ml) containing the DEMPAT (10 mg) was added followed by methyl iodide (0.1 ml). The reaction mixture was kept at the desired temperature for 60 min, after which it was centrifuged, and an aliquot (1 ml) was removed and diluted with acetone to 10 ml for gc analysis.

(*B*) *Small Scale.* (*1*) *Alkylation with Sodium Hydride–Methyl Iodide–Dimethyl Sulfoxide* (*NaH–MeI–DMSO*). Sodium hydride (10 mg) was placed in a centrifuge tube and washed as before. Hexane solution (1 ml), insecticide or herbicide (concentration, 2 μg/ml), was added to the tube, followed by DMSO (0.1 ml) and CH_3I (0.1 ml). The tube was sealed with a screw cap and heated at 50° for 10 min. The tube was cooled, water (1 ml) added, and the reaction mixture extracted with ether (3×). The ether extracts were combined, taken to near dryness by a stream of dry nitrogen, and made up for gc analysis with acetone (1 ml).

RESULTS AND DISCUSSION

Reaction of phosphoramidothioates with methyl iodide normally takes place at elevated temperatures to give the S-alkylated derivative (Burns and Cadogen, 1961). Miller and O'Leary (1962) later showed that in the presence of a strong base, DEMPAT reacted with various alkylating agents to give the *N*-methyl derivative exclusively according to the following equation:

$$(C_2H_5O)_2P(S)\text{-}NHCH_3 \xrightarrow{\text{base}} (C_2H_5O)_2P(S)\text{-}\bar{N}CH_3 \xrightarrow{RI} (C_2H_5O)_2P(S)\text{-}NRCH_3$$

This reaction took place at room temperature and neither the base nor the solvent appeared to be critical.

The alkylation of DEMTAP (milligrams/milliliter concentration) by MeI was examined in various solvents with *t*-BuOK and NaH as the bases. The ratio of reactant, base, and MeI was 1:2:30. The yields of N-alkylated product obtained are given in Table I, based on peak area compared with that of an *O,O*-diethyl *N,N*-dimethyl phosphoramidothioate standard.

The results show that the amount of alkylation which took place in a given time at room temperature is related to the polarity of the solvent, DMSO being the best solvent for both bases. This may be partially accounted for by the known ability of DMSO to stabilize anions. NaH gave slightly better yields of alkylated product and efforts were concentrated on this procedure. DMSO appeared to have some catalytic effect, since alkylation proceeded quite readily in 10% DMSO in hexane. The effect of prereacting NaH with DMSO to form sodium methyl methide sulfoxide was tried, but this procedure offered no advantage over mixing the reactants *in situ*.

The effect of different temperatures (room temperature, 30, 40, and 50°) on the alkylation of DEMPAT with NaH–MeI–DMSO was examined. The best results were obtained at 50°, when all the DEMPAT had reacted within 10 min to give the alkylated product in a 92% yield. These reaction conditions were adopted as a standard for this procedure. The oxygen analog of DEMPAT, *O,O*-diethyl *N*-methyl phosphoramidate, was found to be far more reactive than the parent compound, alkylation having gone to completion in 15 min at room temperature. This reflects the greater acidity of the NH hydrogen when activated by a P=O bond as compared with a P=S bond.

The reaction products were determined by gc–AFID; this detector has a preferential response to phosphorus and nitrogen compounds. On adapting the NaH–MeI–DMSO procedure to the residue level, DMSO was found to affect the response of the AFID in the form of large solvent peaks. This necessitated changing the reaction solvent to 10% DMSO in hexane which proved quite satisfactory, allowing direct injection of the hexane layer. For gc systems or detectors fitted with solvent venting valves, the use of DMSO offered no problem. A second change involved the addition of water to stop the reaction by decomposing the excess NaH. Simultaneously, the water

Table II. Yields and Gc Retention Times of Some Organophosphorus, *N*-Phenyl- and *N*-Methylcarbamates Insecticides, Triazine and Urea Herbicides Alkylated with NaH-MeI-DMSO

Compound	Yield,[a] %	Col. temp, °C	Product ret. time, min	Rel. ret. time[b]
Crufomate	85	228	3.56	0.67
Bay 93820	79	234	2.60	0.78
Bay 93820[c] oxon		234	2.36	
Dimethoate	100	215	2.54	0.94
Prometone	95	204	3.39	1.19
Atrazine	96	204	3.81	1.11
Atratone	97	200	2.87	1.07
Prometryne	98	212	3.94	1.31
Linuron		210	4.29	
Fenuron		178	3.36	3.04
Monuron		200	3.98	3.37
Diuron		210	5.12	3.8
Propham	94	192	1.7	0.70
Methiocarb		192	1.6	
Terbutol	83	210	1.59	0.93
Solan	97	234	1.46	0.56

[a] Product peak area × 100/parent compound peak area. [b] Relative to parent compound on 4% SE-30/6% QF-1. [c] Could not be chromatographed.

dissolved the precipitated sodium iodide and caused the hexane layer to separate. It was found with the more polar compounds such as crufomate that ether was a better solvent for extracting the alkylated product than hexane. The small scale alkylation involved 2 μg/ml of reactant (0.1 μM) together with 0.4 mM NaH and 0.8 mM MeI. The large excess of base and alkylating agent appeared to have no detrimental effects on the reaction.

The NaH-CH_3I-DMSO alkylation procedure was applied to some organophosphorus insecticides (Table II). All the reactions went to completion using the standard conditions of 50°/10 min and gave a single product, except in the case of dimethoate, which gave a higher yield of alkylated product when alkylated at room temperature for 15 min. This can be attributed to the fact that an amidic hydrogen is alkylated, which is more acidic than that of a phosphoramidic hydrogen present in the other organophosphorus compounds. The other compounds also reacted at room temperature with the exception of Bay 93820, which gave a mixture of two products, identified as the mono- and dimethyl derivatives by gc/ms. Bay 93820 oxon was difficult to chromatograph by gc and hence no yield could be calculated. It is normally analyzed by deamination, followed by methylation with diazomethane (Thornton and Stanley, 1971).

The yields in Table II are based on the relative peak

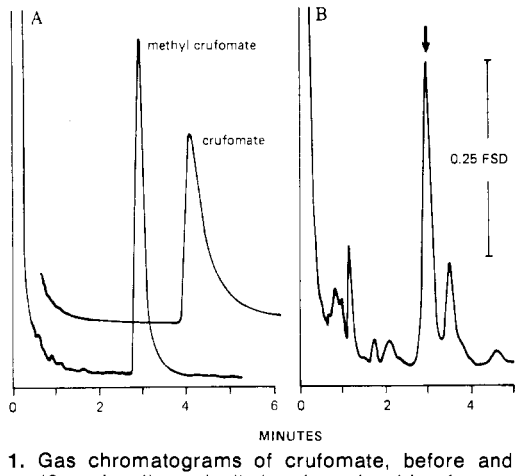


Figure 1. Gas chromatograms of crufomate, before and after alkylation (2-μg level), and alkylated crude chloroform extract of bovine blood (0.42 ppm of crufomate): (A) 15 ng of crufomate, before and after alkylation; (B) alkylated extract of bovine blood.

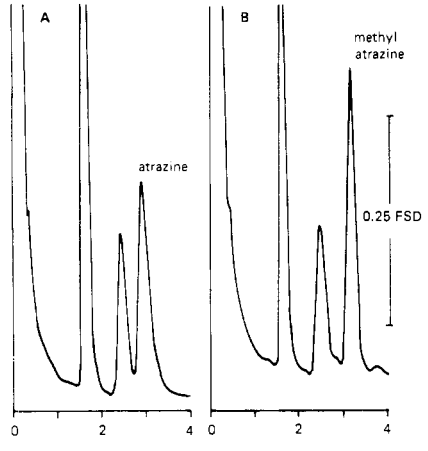


Figure 2. Gas chromatograms of crude methanol extract of organic soil (0.26 ppm of atrazine) before and after alkylation: (A) crude soil extract; (B) alkylated soil extract.

areas of the starting material and its alkylated product. The assumption is made that the AFID response is similar for the two compounds; the selective nature of the AFID being such that it is least sensitive to changes in the number of carbon atoms in a particular molecule.

Alkylation of crufomate resulted in a greatly improved peak shape as shown in Figure 1A. This phenomenon of improved peak shape on going from R_1R_2NH to $R_1R_2R_3N$ was also noticed by Seiber with *N*-methylcarbamates and their trifluoroacetyl derivative and Saunders and Vanatta with the alkylated ureas.

A gc/ms analysis of alkylated crufomate showed a parent ion *m/e* 305 and a base ion *m/e* 122, attributed to the $[N(CH_3)_2{\cdot}P(O){\cdot}OCH_3]^+$ ion. Comparison with the ms of crufomate, which has a base ion *m/e* 108, indicated alkylation of the nitrogen had taken place. Other ions corresponding to the loss of CH_2 (M − 14) from the *tert*-butyl group and chlorine (M − 35) from the aromatic ring were present in both spectra.

The NaH-MeI-DMSO alkylation procedure was tried on a crude chloroform extract of bovine blood plasma containing 0.42 ppm of crufomate. A chromatogram of the reaction products, Figure 1B, shows a peak corresponding to the *N*-methyl derivative, relative retention time 0.74, confirming the presence of crufomate.

In addition to organophosphorus compounds, the NaH-MeI-DMSO procedure has been applied to other insecticides and herbicides which possess an NH moiety. The

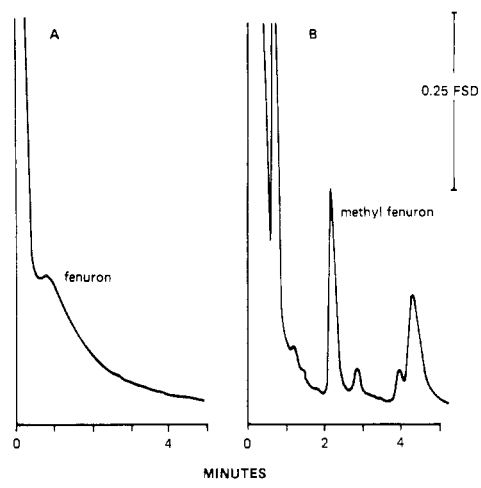
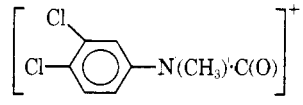


Figure 3. Gas chromatograms of fenuron and alkylated fenuron (0.5-μg level): (A) 63 ng of fenuron; (B) 11 ng of alkylated fenuron.

triazines listed in Table II were alkylated with yields in the range 86–91% at a concentration of 2 μg/ml with the standard reaction conditions. A gc-ms of alkylated atrazine showed a parent ion *m/e* 243, with a strong ion *m/e* 228 (M − 15) corresponding to the loss of a methyl group from the isopropyl group. The spectrum indicates that both the NH ethyl and NH isopropyl groups of atrazine were alkylated. In contrast, silylation with BSTFA has been found to react predominantly with the NH ethyl group of atrazine. Hydroxyatrazine can also be alkylated by this procedure to give a trimethyl derivative, which is identical with the product obtained on alkylation of atratone.

An extract of field treated soil, containing 0.26 ppm of atrazine, was alkylated by the NaH-MeI-DMSO procedure and the gc before and after alkylation are shown in Figure 2. The atrazine peak disappeared on alkylation with the formation of a peak corresponding to the alkylated product with no effect on the other impurities present in the extract. The separation of the atrazine and dimethylatrazine peaks can be increased by the use of a more polar column substrate, such as Carbowax 20M.

The difficulty of chromatographing urea herbicides and their low sensitivity on gc have been extensively reported (Cochrane and Purkayastha, 1973). Linuron decomposes rapidly above 170° and the use of short gc columns together with on-column injection is recommended to reduce thermal decomposition during gc analysis. Linuron, along with the other urea herbicides in Table II, gave a single product under the standard alkylation conditions. The alkyl derivatives of urea herbicides are thermally stable, in agreement with the results of Saunders and Vanatta (1974), and gave sharp peaks when chromatographed on a SE-30/QF-1 column. The gc-ms of alkylated linuron had a base ion *m/e* 202 corresponding to the fragment



and a parent ion *m/e* 263, as required for *N*-methyllinuron. Using the general reaction conditions, alkylation of the urea herbicides can be carried out at a 0.5 μg/ml level as shown for fenuron, Figure 3, which lowers the detection limits of these compounds.

Carbamate insecticides, like the urea herbicides, are difficult to chromatograph directly by gc due to decomposition. The use of short columns (Lewis and Paris, 1974) or highly deactivated supports (Lorah and Hemphill, 1974) has been advocated to overcome this problem. How-

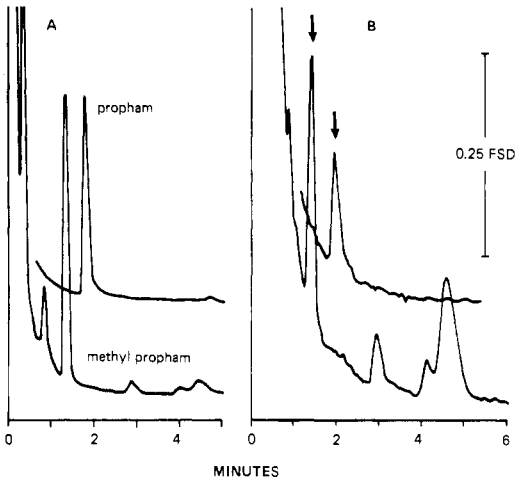


Figure 4. Gas chromatograms of propham and alkylated propham (2- and 0.48-μg levels): (A) 24 ng of propham, before and after alkylation (2 μg); (B) 24 ng of propham, before and after alkylation (0.48 μg).

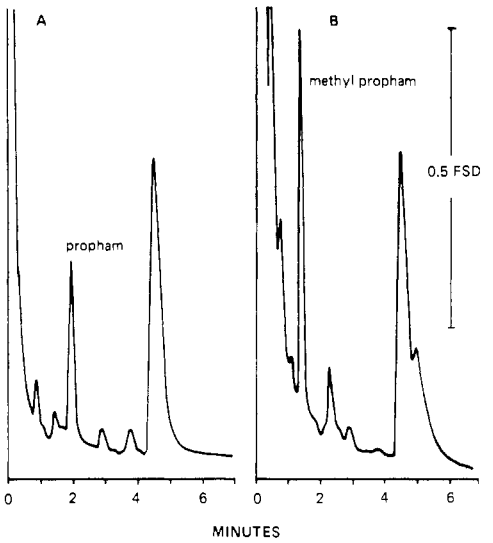


Figure 5. Gas chromatograms of crude chloroform extract of potatoes fortified with propham (0.5 ppm), before and after alkylation: (A) crude potato extract; (B) alkylated potato extract.

ever, derivatives formed by perfluoracylation or alkylation are quite thermally stable. With the standard NaH-MeI-DMSO alkylation conditions, little or no product was obtained for the carbamates listed in Table II due to decomposition, but at room temperature for 5 min they all gave a single product. A mass spectrum of the product from propham indicated it to be methyl propham with a parent ion *m/e* 193. Propham was alkylated at two concentrations, 2.2 and 0.44 μg/ml, and the gas chromatograms are shown in Figure 4A and B, respectively. At the lower concentration, background impurities with a retention time less than 4 min are observed, which may interfere with the detection of some compounds. It suggests that this is the lowest level at which the reaction can be used for confirmation purposes with the present experimental conditions. The use of a more specific gc detector for nitrogen compounds such as the Coulson electrolytic conductivity detector could further reduce the level at which residues can be confirmed.

A crude chloroform extract of potatoes, fortified with propham at the 0.44-ppm level, was alkylated with the NaH-MeI-DMSO procedure at room temperature for 5 min. The gas chromatograms before and after alkylation are shown in Figure 5A and B. The complete disappearance of propham and the formation of the alkyl propham in high yield indicate the ease of confirming residues with this method. Alkylation of the impurities present in the extract appeared to be minimal, illustrating the specificity of the reaction.

In order for a chemical reaction to be suitable as a confirmation test for a pesticide or herbicide, it should be easy to carry out and give a single specific product in high yield at the residue level. Alkylation by the NaH-MeI-DMSO procedure meets these criteria. It has general application to organophosphorus and carbamate insecticides as well as urea and triazine herbicides with NH moieties, giving thermally stable products with good gc characteristics. Standard reaction conditions of 50°/10 min give high yields of alkylated derivatives for all types of compounds except the carbamates, where much milder reaction conditions must be used. The order of reactivity of the compounds with NH moieties appears to be carbamates > phosphoramidates-amides > phosphoramidothioates > triazines > ureas. Finally, it has been demonstrated that the NaH-MeI-DMSO alkylation procedure can confirm insecticide and herbicide residues present in crude plant and soil extracts at the sub parts per million level.

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