Confirmation Techniques for Triazine Herbicides by Gas Chromatography with Electrolytic Conductivity Detection

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Three chemical derivatization techniques for triazine herbicides were examined for the confirmation of these compounds in plant material. Methoxylation of chloro-*s*-triazines carried out in sodium methoxide–methanol quantitatively yielded the corresponding methoxy analogs. Thus, atrazine, simazine, and propazine were converted to atratone, simetone, and prometone, respectively. The methylation of triazines containing secondary amino substituents was readily accomplished with methyl iodide–sodium hydride in dimethyl sulfoxide. The methylated products were easily separated from their parents on a 5% Reoplex 400 column. The hydrolysis of the ring amino substituents from a number of triazines for subsequent coupling with 2,4-dinitro-1-fluorobenzene offered a third approach to triazine herbicide confirmation. The resulting dinitrophenylamines were then used to identify the parent triazine. These derivatization reactions were applied to residue analysis at concentrations of less than 0.05 ppm.

The selectivity of the Coulson electrolytic conductivity detection system for the gas–liquid chromatographic (glc) determination of triazine herbicides in crops and soil is well documented (Purkayastha and Cochrane, 1973; Westlake *et al.*, 1970; Hörmann *et al.*, 1972; Lawrence, 1974; Lawrence and McLeod, 1974; Sirons *et al.*, 1973; Young and Chu, 1973). The sample cleanup with such a detector system can be significantly reduced thus decreasing analysis time. To date, no confirmation techniques have been reported for triazine herbicides using electrolytic conductivity detection. However, Cochrane and Greenhalgh (1973) reported on the confirmation of atrazine in chicken feces by silylation with AFID detection. The present paper reports on three chemical derivatization techniques for the confirmation of triazine herbicides by glc with a Coulson conductivity detector. The methoxylation of chloro-*s*-triazines is similar to that reported for the fungicide dyrene (2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine) (Mendoza *et al.*, 1971). The methylation reaction is a modification of the technique recently mentioned by Cochrane and Greenhalgh (1973) for atratone, linuron, and some organophosphates. The actual reaction conditions are based upon the work of Greenhalgh and Kovacicova (1973). The hydrolysis reaction is based on the method of Cee and Gasparic (1972) for the determination of the *s*-triazine ring in organic compounds.

MATERIALS

Apparatus. An Aerograph Model 600C gas chromatograph equipped with a Coulson conductivity detector (Tracor Inc., Austin, Tex.) set up for nitrogen determination was used for the study. A 6 ft × 6 mm o.d. coiled glass column was packed with 4% SE 30, 5% SP 525, or 5% Reoplex 400 on Chromosorb W H/P, 80–100 mesh. Operating conditions were: pyrolysis furnace temperature, 780°; transfer unit temperature, 210°; helium sweep flow, 60 ml/min; hydrogen flow, 50 ml/min; d.c. bridge potential, 30 V. Column temperatures and helium carrier flow rates varied with the types of derivatives being determined. The glc effluent was vented to the atmosphere for 1–2 min after injection of the samples before being directed to the Coulson furnace.

Reagents. The chemicals for the confirmation reactions were used as obtained from the suppliers. The sodium hydride, obtained in an oil dispersion, was washed with hexane before use. All organic solvents were distilled in glass analytical grade materials. The triazines included in this study were: atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine), propazine (2-chloro-4,6-bis(isopropylamino)-*s*-triazine), simazine (2-chloro-4,6-bis(ethylamino)-*s*-triazine), atratone (2-methoxy-4-ethylamino-6-isopropylamino-*s*-triazine), prometone (2-methoxy-4,6-bis(isopropylamino)-*s*-triazine), simetone (2-methoxy-4,6-bis(ethylamino)-*s*-triazine, and sencor (4-amino-6-*tert*-butyl-3-methylthio-*as*-triazin-5-(4*H*)-one). Solutions of these were prepared in methanol. The crops examined were corn, parsnips, beets, peas, and turnips.

Sample Extraction. The extraction procedure was that described by Lawrence (1974) for triazines in root crops. The combined organic extracts were reduced to about 0.5 ml by rotary vacuum evaporation. The concentrate was then transferred to a glass-stoppered centrifuge tube and brought to 1.0 ml for injection into the gas chromatograph. For confirmation by methylation and methoxylation, the solvent was evaporated to dryness under a stream of nitrogen.

Methylation. To the residue in the centrifuge tube was added 0.5 ml of dry dimethyl sulfoxide (Me_2SO) (BDH Chemicals) followed by about 10 mg of NaH (Baker) and 0.1 ml of methyl iodide (Fisher). The tube was stoppered and heated at 45° for 10 min. After cooling to room temperature, 1 ml of benzene was added followed by the careful dropwise addition of 2 ml of distilled water (NaH rapidly evolves hydrogen gas upon contact with water). When evolution of hydrogen was complete, the tube was stoppered and shaken. The benzene layer was transferred by pasteur pipet to a clean centrifuge tube. The extraction was repeated with second and third volumes of benzene. The combined extracts were evaporated under a stream of nitrogen to 1 ml for gas chromatography.

Methoxylation. A 0.1-ml volume of 25% sodium methoxide in methanol (Anachemia Chemicals Ltd.) was added to the residue. The tube was stirred on a Vortex Jr. mixer for 1 min. Following this, 1 ml of ethyl acetate and 1 ml of distilled water were added and the tube was stirred once more. The ethyl acetate layer was removed, and the aqueous portion extracted twice more with 1-ml volumes of ethyl acetate. The combined organic layers were dried over sodium sulfate and reduced under a stream of nitrogen to 1 ml for glc analysis.

Hydrolysis and DNFB Reaction. The chloroform solution containing the residue was transferred to a 15-ml test tube with a Teflon-lined screw cap. The solvent was evaporated under a gentle stream of nitrogen. After this 1 ml of 1 *N* HCl was added to the residue and the cap placed tightly on the tube. The test tube was then heated in an oven overnight (16–18 hr) at 150°, cooled, and opened. A

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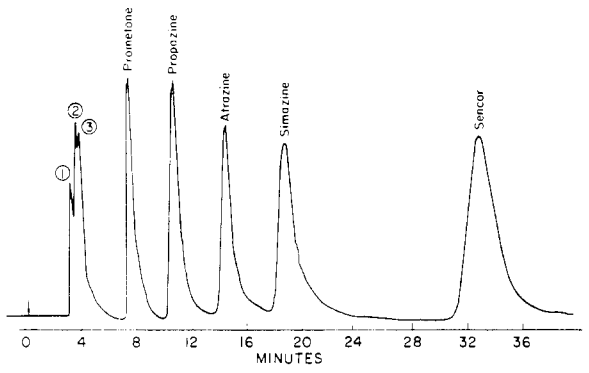


Figure 1. Chromatogram of parent triazines and their methylated products separated on 5% Reoplex 400 at 196° and at a flow rate of 60 ml/min: (1) methylated sencor; (2) methylated simazine and methylated atrazine; (3) methylated propazine and methylated prometone. From 30 to 40 ng of each was injected.

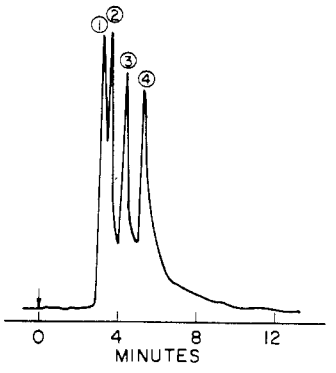
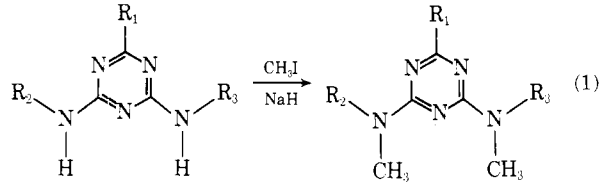


Figure 2. Separation of methylated triazines on 4% SE 30 at 175° and a flow rate of 60 ml/min: methylated (1) sencor, (2) simazine, (3) atrazine, and (4) propazine and prometone. About 25 ng of each was injected.

1-ml volume of 1 *N* NaOH was added followed by 2 ml of 5% borax solution and 1 ml of a 1 mg/ml solution of 2,4-dinitrofluorobenzene (DNFB) (Eastman) in acetone. The mixture was shaken and heated at 85° for 15 min. A second 1-ml portion of 1 *N* NaOH was added and the contents heated for a further 10 min. After this time, 2 ml of benzene was added and the test tube shaken. The benzene layer was retained and the aqueous mixture extracted twice more with 1-ml volumes of benzene. The combined organic extracts were dried over sodium sulfate and reduced to 1 ml under a stream of nitrogen for glc analysis.

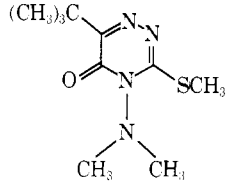
RESULTS AND DISCUSSION

Methylation. The overall reaction involved in the methylation of the triazines is shown in eq 1, where R_1 = Cl, OCH_3, or SCH_3 and R_2,R_3 = isopropyl or ethyl.



(1)

The structures of the methylated products were verified by glc–mass spectrometry which indicated that the dimethyl derivative is formed exclusively. Cochrane and Greenhalgh (1973) also found the dimethyl derivative of atratone after treatment with methyl iodide–sodium hydride. The alkylated product of sencor was found to be



as indicated by mass spectrometry.

Figure 1 shows a chromatogram of several triazines and their methylated products. All methyl derivatives appeared within 5 min after injection and were not separated from each other under the conditions used for the parent compounds. The derivatives were separated using 4% SE 30 and are depicted in Figure 2. The chromatographic pattern of the derivatives was the reverse of the parents. Methylated sencor, for example, appeared first whereas sencor itself was last of the series of parent triazines.

Figure 3 shows the application of this technique to the analysis of atrazine in turnips at 0.05 ppm. The triazine was quantitatively converted to its methyl derivative. Similar results were obtained for triazines in peas, beets, and parsnips.

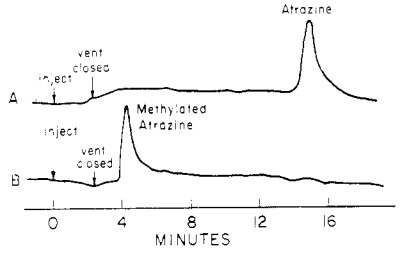


Figure 3. Confirmation of atrazine in turnip at 0.05 ppm by methylation: (A) crop extract; (B) methylated crop extract. Glc conditions are as in Figure 1. The equivalent of a 40-mg sample was injected.

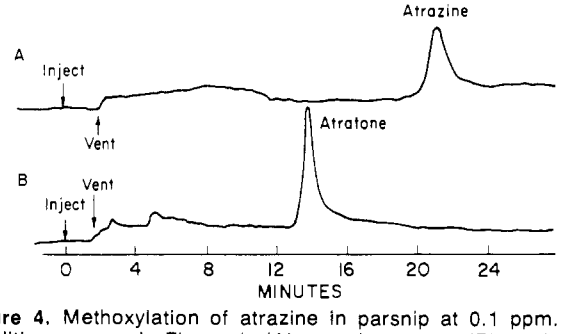
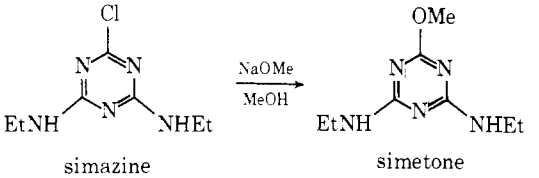


Figure 4. Methoxylation of atrazine in parsnip at 0.1 ppm. Glc conditions are as in Figure 1: (A) parsnip extract; (B) methoxylated parsnip extract. The equivalent of a 40-mg sample was injected.

Methoxylation. The methoxylation reaction went to completion with quantitative yields of products for the three chloro-*s*-triazines studied. The reaction was simply the replacement of the labile chlorine atom of the triazine with OCH_3. Ethoxide would replace the chlorine atom equally as well. The equation for the conversion is shown in eq 2.



(2)

The chromatographic separation of the chloro-*s*-triazines from their methoxy analogs can be accomplished with the column mentioned in Figure 1. The application of this confirmation technique to atrazine in parsnip at 0.1 ppm is shown in Figure 4. The conversion to atratone was quantitative. No interfering peaks were observed from reaction side products or coextractives.

Hydrolysis and DNFB Reaction. The hydrolysis rates of the triazines varied with amino substituents. Simazine,

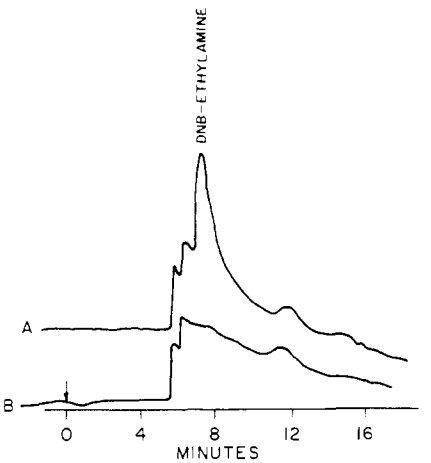
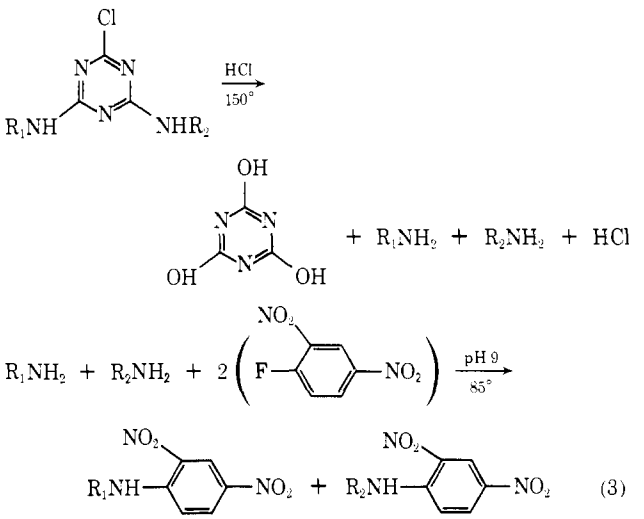


Figure 5. Confirmation of simazine in corn at 0.1 ppm by hydrolysis and DNFB reaction: (A) spiked sample, (B) blank sample. The equivalent of a 40-mg sample was injected.

for example, was completely hydrolyzed in 6–8 hr compared to propazine which required 16–18 hr. The difference was attributed to the greater steric hindrance caused by the isopropylamino groups (Lawrence and Laver, 1974). The reaction scheme for hydrolysis and DNFB reaction is shown in eq 3, where R_1 and R_2 = ethyl or isopropyl.



(3)

Both hydrolysis and DNFB reaction yielded the dinitrophenylamine derivatives in high (80–90%) yields. The major source of interference was in unreacted DNFB which tails badly in the Coulson system causing high background. The addition of 1 ml of 1 *N* NaOH and heating for 10 min after derivative formation successfully hydrolyzed the excess reagent to the water-soluble phenol. Baba *et al.* (1974) have separated a large number of dinitrophenylamines using temperature programming and a 10% SE 30 column. The application of this technique to simazine confirmation in corn at 0.1 ppm is shown in Figure 5. The high background encountered after venting was due to unhydrolyzed DNFB. The sample was not treated with NaOH after derivative formation as mentioned above.

Comparison of Reactions. For confirmation of chloro-*s*-triazines, the methoxylation reaction was the simplest and fastest method. However, in the presence of methoxy-*s*-triazines, the methylation reaction with methyl iodide would be preferred. The hydrolysis–DNFB reaction was found useful for further characterization of the triazines by determination of the ring amino groups.

The above reactions may also be used for confirmation in a stepwise manner. Atrazine, for example, could be first verified by the conversion to atratone. The atratone could then be treated with methyl iodide to give the methylated product or it could be hydrolyzed to confirm the presence of the ring amino groups. All reactions were found capable of determining triazine residues at 0.05 ppm or less in the foods examined.

LITERATURE CITED

Baba, S., Hashimoto, I., Ishitoya, Y., *J. Chromatogr.* **88**, 373 (1974).
Cee, A., Gasparic, J., *Mikrochim. Acta*, 823 (1972).
Cochrane, W. P., Greenhalgh, R., presented at the 166th National Meeting of the American Chemical Society, Chicago, Ill., 1973.
Greenhalgh, R., Kovacicova, J., unpublished results (1973).
Hörmann, W. D., Formica, G., Ramsteiner, K., Eberle, D. O., *J. Ass. Offic. Anal. Chem.* **55**, 1031 (1972).
Lawrence, J. F., *J. Agr. Food Chem.* **22**, 137 (1974).
Lawrence, J. F., Laver, G. W., *J. Chromatogr.* submitted for publication (1974).
Lawrence, J. F., McLeod, H. A., *Bull. Environ. Contam. Toxicol.*, in press (1974).
Mendoza, C. E., Wales, P. J., Hatina, G. V., *J. Agr. Food Chem.* **19**, 41 (1971).
Purkayastha, R., Cochrane, W. P., *J. Agr. Food Chem.* **21**, 93 (1973).
Sirons, G. J., Frank, R., Sawyer, T., *J. Agr. Food Chem.* **21**, 1017 (1973).
Westlake, W. E., Westlake, A., Gunther, F. A., *J. Agr. Food Chem.* **18**, 683 (1970).
Young, H. Y., Chu, A., *J. Agr. Food Chem.* **21**, 711 (1973).

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