Pesticide Residue Analysis in Human Tissue by Combined Gas Chromatography–Mass Spectrometry

Francis J. Biros and Annita C. Walker

Pesticide residues in human adipose tissue and liver tissue samples were identified by mass spectrometry coupled with gas chromatography. An extensive extraction and cleanup procedure was adapted from existing analytical methods to separate pesticide residues from the tissue samples. Concentration of pesticides in tissue ranged from 0.073 to 28.7 ppm. Mass spectral data were computer-enhanced to identify heptachlor epoxide. Other pesticides confirmed included the β-isomer of 1,2,3,4,5,6-hexachlorocyclohexane (HCH), several isomers and metabolites of *p,p'*-DDT, and dieldrin. Advantages, limitations, and suggested modifications for improving the combined gas chromatography—mass spectrometry technique for pesticide residue analysis are discussed.

Mass spectrometry coupled with gas chromatography is a particularly useful approach to the analysis of pesticide residues and their metabolites because positive identification of the components of a mixture can be made without prior separation and at a sensitivity compatible with the limited quantities of residues encountered. Mass spectrometry is widely employed for analysis of individual residues and metabolites isolated by conventional separation techniques such as thin-layer chromatography, liquid chromatography, paper and column chromatography, and gas-liquid chromatography; yet few reports can be found in the literature on the utility of the combined gas chromatography—mass spectrometry method for residue analysis.

Several specialized techniques are available for efficient trapping and collection of individual gas chromatographic peaks for subsequent mass spectral analysis by the direct probe introduction method (Amy *et al.*, 1965; Damico *et al.*, 1967; Muller, 1963; Mumma and Kantner, 1966). These methods, although practical and reliable, are time-consuming and may risk sample loss by oxidation or hydrolysis. Amy *et al.* (1965) previously listed a number of disadvantages of the direct technique, such as memory effects associated with molecular separators and ion source pressure fluctuations due to flow of carrier gas into the mass spectrometer system. These disadvantages, however, are no longer a serious problem in view of the improvements made in commercially available instruments. In addition, decomposition of the components of gas chromatographic effluents can be minimized by conversion to all-glass inlet systems or by use of glass capillary columns of sufficiently small inner diameter to allow direct entry to the ion source (McFadden, 1966).

Recent reports have focused attention on the need for confirming pesticide residues, particularly those isolated from environmental samples (Elgar, 1967; Schechter, 1968) and for chemical identity in ultramicroanalysis in general (Robinson *et al.*, 1966). In this area the combined technique of gas chromatography–mass spectrometry offers many advantages over other forms of spectroscopic techniques and physical measurements. These advantages include an increase in the speed of analysis, elimination of the necessity for isolating minute quantities of pure sample, and greater certainty in identification of the eluted component than that achieved by gas chromatographic retention times alone (Watson and Biemann, 1965), an advantage of particular importance for residue confirmation. Mass spectrometry allows direct determination of molecular weight and, frequently, the number and kinds of heteroatoms present in the molecule. Additionally, full identification and structural information can be obtained by a thorough analysis of the spectrum.

This communication reports the practical application of gas chromatography–mass spectrometry to residue analysis of several chlorinated hydrocarbon residues in human tissue samples. The mass spectrum of each pesticide eluted from the gas chromatographic column was obtained and compared with those available in the literature (Damico *et al.*, 1968; Jörg *et al.*, 1966; Kantner and Mumma, 1966) or from standard pesticidal compounds. These pesticides all gave easily recognizable molecular ion peaks and characteristic ion fragments. The number of chlorine atoms in each fragment ion, including the parent ion, was readily determined by the specific isotopic distribution pattern (Beynon, 1960). Initially, the objectives of this endeavor were to determine which available analytical procedure offered the best approach to sample extraction and cleanup with minimum gas chromatographic and mass spectrometric interferences; to ascertain the lower practical limit of identification of pesticide residues; and to establish the ability of the combined gas chromatograph-mass spectrometric method to distinguish among configurational isomers (*e.g.*, the isomers of 1,2,3,4,5,6-hexachlorocyclohexane, HCH) and structural isomers [*e.g.*, 1,1-bis-(*p*-chlorophenyl)-2,2,2-trichloroethane and 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2,2-trichloroethane; *p,p'*-DDT and *o,p'*-DDT, respectively].

Perrine Primate Research Branch, Division of Pesticides, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Perrine, Fla. 33157

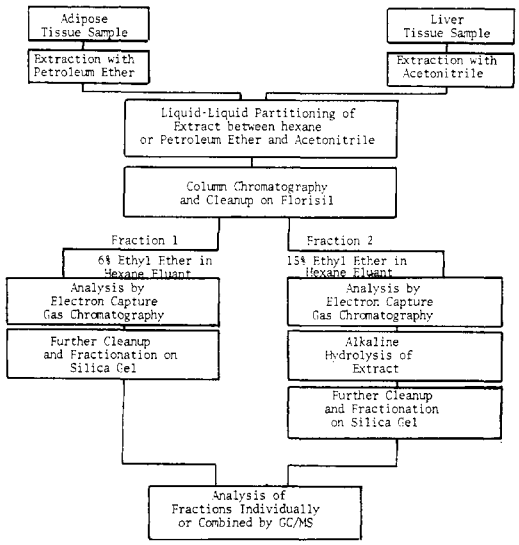

Figure 1. Analytical scheme for isolation of pesticide residues from adipose tissue and liver tissue

EXPERIMENTAL

Extraction and Cleanup. Human adipose tissue and liver tissue obtained from the Dade County Medical Examiner's office were used as working samples in this investigation. It was desired to obtain tissue samples containing a relatively large variety of chlorinated hydrocarbon residues and in as wide a concentration range as possible. To provide a reasonably thorough and extensive cleanup procedure, several available methods were combined in the analytical procedure. The final scheme used in this work is shown in Figure 1. For each determination, 5 g of tissue was analyzed. Methods described in the Pesticide Analytical Manual (Food and Drug Administration, 1966) were used for initial extraction, liquid-liquid partitioning, Florisil column cleanup, and electron capture gas chromatographic analysis of the residues. An alkaline hydrolysis was performed on the extract obtained from fraction 2 of the Florisil column cleanup because of the large quantities of lipids and other tissue extractives co-eluted with the pesticide residues.

The individual extracts from fraction 1 and fraction 2 of the Florisil chromatographic step were then concentrated and subjected to further cleanup and pesticide residue fractionation by silica gel column chromatography, employing a published procedure (Kadoum, 1967). The concentrated extracts were chromatographed on 10 g of silica gel with the solvents and solvent volumes reported previously (Kadoum, 1968). The pesticide residues were thus distributed among 10 column chromatographic eluent fractions. The order of elution of the individual pesticides has been elaborated by Kadoum.

Selected chromatographic fractions containing the desired pesticide residues were then combined to form two larger fractions, the first of which contained isomers of DDT and DDE, and the second contained DDD, isomers of HCH, and the cyclodiene-type pesticides. Considerable care was used in the various solvent concentration steps to avoid loss of pesticides and permit injection of virtually the entire extract into the gas chromatograph after the final column chromatographic step. Thus, solvent volumes were reduced to 0.5 ml by Kuderna-Danish evaporative concentrators. This volume of solvent containing the pesticide residues was then transferred quantitatively to a 2-ml concentrator tube and evaporated using a stream of clean, dry nitrogen

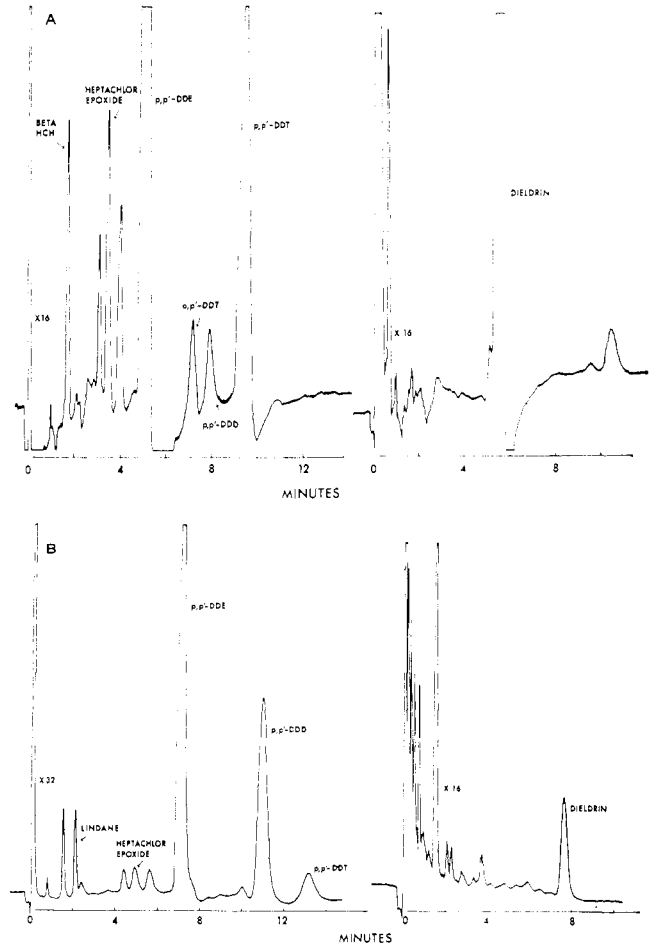

Figure 2. Electron capture gas chromatograms of pesticides isolated from human tissue

Column, mixed phase 1.5% OV-17/1.95% QF-1 on Chromosorb W (HP); column temperature 200° C; detector temperature 215° C; injector temperature 225° C

A. Upper trace. Adipose tissue extracts, fractions 1 and 2 immediately after Florisil column cleanup, nitrogen gas flow 45 ml/min, chart speed ¼ in./min

B. Lower trace. Liver tissue extracts, fractions 1 and 2 immediately after Florisil column cleanup, nitrogen gas flow 70 ml/min, chart speed ½ in./min

to a final volume of 5 μl, which was then analyzed by gas chromatography–mass spectrometry.

The silica gel column chromatographic procedure was utilized in an attempt at further cleanup of the sample extract and as a pesticide fractionation step designed to allow the analyst to choose residues or groups of residues for analysis by gas chromatography–mass spectrometry. The relatively large diameter and short length of the gas chromatography columns used in the mass spectrometric analysis resulted in somewhat low separation efficiencies for several of the pesticides found in the tissue samples, and consequently, prior separation of some of the pesticides was required.

Electron Capture Gas Chromatography Determination. The individual fractions collected from the Florisil column step were analyzed by gas-liquid chromatography using electron capture detection. Minimum sample loss occurred at this step. A Micro-Tek MT 220 gas chromatograph equipped with a ^{3}H electron capture detector was used. The conditions under which the analytical separation were carried out are given in Figure 2. Table I lists the chlorinated hydrocarbon residues found and their respective concentrations

Table I. Identity and Concentration of Pesticide Residues Found in Human Tissue Samples

A. Adipose Tissue		B. Liver Tissue	
Pesticide	Concentration (ppm)	Pesticide	Concentration (ppm)
β-HCH	0.96	Lindane	0.40[a]
Heptachlor epoxide	0.52	Heptachlor epoxide	0.07
p,p'-DDE	28.71	*p,p'*-DDE	3.56
Dieldrin	1.04	Dieldrin	0.34
p,p'-DDD	0.30	*p,p'*-DDD	2.00
o,p'-DDT	0.63	*p,p'*-DDT	0.45
p,p'-DDT	7.65		

[a] A total of 2 μg of lindane was added to the liver tissue sample prior to extraction and cleanup.

based on relative retention and peak height data obtained from reference standard pesticide chromatograms. Since no recovery studies were performed utilizing the combined extraction, liquid-liquid partitioning, Florisil column cleanup, and silica gel chromatographic fractionation methods, it should be pointed out that the quantitative data given in Table I represent approximations of the actual concentrations of residues in the tissue extract finally analyzed by gas chromatography–mass spectrometry. However, recovery data for the individual steps of the described method are well documented in the original literature references and are uniformly acceptable for the individual pesticide residues found in the human tissue samples studied.

Gas Chromatography–Mass Spectrometry. The mass spectrometric analyses were performed with a double focusing, medium resolution mass spectrometer (Model 270, Perkin-Elmer Corp., Norwalk, Conn.) coupled through a Watson-Biemann type of molecular separator with a gas chromatographic system. The coiled glass gas chromatographic column was 4 ft long by 1/8 in. o.d., and was packed with 3% OV-210 on 80 100 mesh Chromosorb W (HP) obtained from the Pierce Chemical Co., Rockford, Ill. Programmed temperature analyses were made with the oven initially at 150° C for 1 min, followed by a 5°/C min increase to 200° C; finally, the oven was held isothermally at 200° C. The molecular separator and gas inlet temperatures were maintained at 150 and 210° C, respectively. All mass spectra were recorded at 80 e.v. electron energy with 2300 V accelerating voltage; the filament emission current was 100 μa. Chromatograms were recorded from the total ion-current monitor located between the electrostatic and magnetic analyzer sectors. Helium carrier gas flow rate was 10 ml per min. Injector temperature was 165° C. Mass spectra were scanned magnetically over the range *m/e* 5 to 500 in 6 sec.

In the initial phases of this work considerable decomposition of pesticides injected into the gas chromatographic unit occurred in the stainless steel restricter line, transfer line, and fritted glass molecular separator of the manifold system. This degradation was manifested as a diminished intensity molecular ion peak for several pesticidal compounds examined, including *p,p'*-DDT and lindane. In some cases, pesticide identity was confused, particularly at concentrations below 1 μg. The following instrumental modification and silanization treatment greatly alleviated this problem. The stainless steel transfer line, restricter line, and Kovar glass-to-metal seal portion of the molecular separator were removed and replaced with glass capillary tubing to minimize contact of eluted pesticides with hot metal surfaces. The entire glass separator assembly was then washed with hot nitric acid, water, acetone, benzene, and hexane, silanized with N,O'-bis-trimethyl silyl acetamide (Pierce Chemical Co., Rockford, Ill.), and reinstalled. In addition, polar chemisorption was further deactivated by silanization *in situ* of the gas chromatographic column, fritted glass molecular separator, and transfer lines by using a published procedure (MacLeod and Nagy, 1968).

Heptachlor epoxide (1,4,5,6,7,8,8-heptachloro-2,3-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene) was determined by a recently developed procedure for enhancing mass spectral data by means of a time averaging computer (Biros, 1970). Repetitive rapid scanning and spectrum accumulation in the region of the parent molecular ion for heptachlor epoxide produced a considerably enhanced isotopic distribution pattern characteristic of a molecule containing seven chlorine atoms and having a molecular weight of 386.

RESULTS AND DISCUSSION

Mumma and Kantner (1966) reported that 0.1 ppm of halogenated pesticides in alfalfa and wheat samples gave sufficient residues for analysis by mass spectrometry. These workers utilized a gas chromatographic trapping technique in conjunction with mass spectrometric analysis by a direct introduction probe. Residues as small as 0.1 μg were determined in this fashion. Because of the relatively large diameter of the gas chromatographic columns utilized in this work and the probability that up to 60% of the sample is lost by using a fritted glass tube molecular separator (Grayson and Wolf, 1967), it was anticipated that the minimum sample required for analysis would be of the order of 0.2 to 0.3 μg, corresponding to 0.04 to 0.06 ppm of the pesticide residue based on the quantity of tissue analyzed. Examination of the electron capture gas chromatogram (Figure 2) of the adipose tissue sample reveals that seven pesticide residues were present in concentrations ranging from 0.30 to 28.7 ppm (Table I). By relative retention time data, these were determined to be the β-isomer of HCH; heptachlor epoxide; 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene (*p,p'*-DDE), 1,2,3,4,10,10 - hexachloro - 6,7 - epoxy - 1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene (dieldrin), 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethane (*p,p'*-DDD), *o,p'*-DDT, and *p,p'*-DDT. The pesticide residues were conveniently separated by silica gel chromatography into two fractions, each of which contained pesticides eluted with sufficient resolution for proper identification. The total ion current traces of the adipose tissue extracts are shown in Figure 3. The mass spectra of authentic reference compounds were compared with those of the components of the extract to identify the following pesticides. (Discussions of fragmentation modes, mass spectral peak intensities, and identities of pesticides under electron impact-induced ioniza-

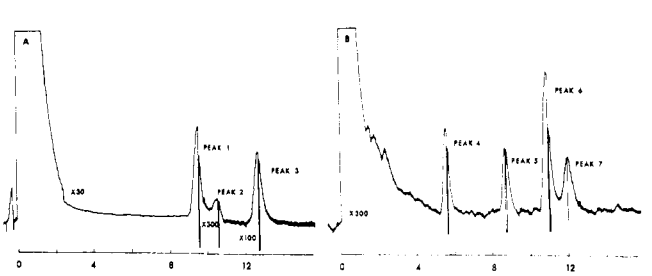

Figure 3. Total ion current traces of extracts containing pesticide residues isolated from adipose tissue (A) fraction 1; (B) fraction 2 (see text)

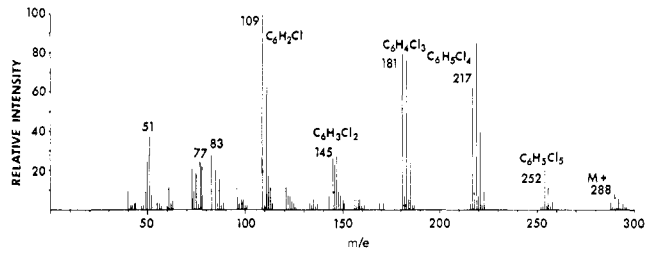

Figure 4. Mass spectrum of the β-isomer of 1,2,3,4,5,6-hexachlorocyclohexane isolated from adipose tissue

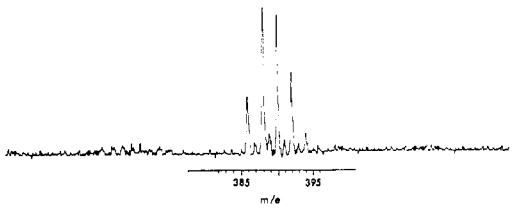

Figure 6. Computer time averaged spectrum (10 scans) of the molecular ion region of heptachlor epoxide, *m/e* 380 to 400, isolated from liver tissue

tion have been reported by Damico *et al.*, 1968; Jörg *et al.*, 1966; and Kantner and Mumma, 1966).

Peak 1. *p,p'*-DDE. *m/e* 246 (100%), 318 (83%), 316 (molecular ion, 66%), 248 (58%), 320 (41%), 176 (41%), 210 (16%).

Peak 2. *o,p'*-DDT. *m/e* 235 (100%), 237 (70%), 165 (40%), 75 (20%), 199 (18%), 246 (18%), 352 (molecular ion, 3%).

Peak 3. *p,p'*-DDT. *m/e* 235 (100%), 237 (72%), 165 (48%), 75 (22%), 50 (18%), 51 (9%), 352 (molecular ion, 2%).

Peak 4. β-HCH. *m/e* 109 (100%), 219 (85%), 181 (78%), 183 (80%), 111 (62%), 193 (62%), 288 (molecular ion, 4%).

Peak 5. Heptachlor epoxide. *m/e* 81 (100%), 353 (84%), 355 (76%), 351 (48%), 357 (35%), 237 (33%), 386 (molecular ion, 8%).

Peak 6. Dieldrin. *m/e* 79 (100%), 108 (19%), 263 (18%), 277 (19%), 279 (16%), 345 (7%), 378 (molecular ion, 3%).

Peak 7. *p,p'*-DDD. *m/e* 235 (100%), 237 (66%), 165 (58%), 75 (21%), 82 (16%), 88 (16%), 318 (molecular ion, 5%).

As expected, the mass spectrum of β-HCH (Figure 4) at low resolution and at the concentrations encountered in the analysis was indistinguishable from spectra of reference samples of the three remaining isomers (α-, γ-, and δ-HCH) occasionally found as residues in human tissue. Consequently, identification of this pesticidal compound as β-HCH must be corroborated by gas chromatographic retention time data. A similar difficulty arose with *o,p'*-DDT and *p,p'*-DDT. At high concentrations and under controlled conditions, these configurational and structural isomers can be differentiated by ion intensity ratios of characteristic ion fragments (Damico, 1969). However, at the lower concentrations at which they are encountered as residues and under the conditions of the analysis, the mass spectral properties of *o,p'*-DDT and *p,p'*-DDT are too similar to allow identification on this basis alone, and their identification requires gas chromatographic retention time data in addition to mass spectral fragmentation patterns, peak intensities, and molecular weights. Adequate and definitively characteristic mass spectral data were, however, obtained for the remaining pesticide residues isolated from the adipose tissue sample.

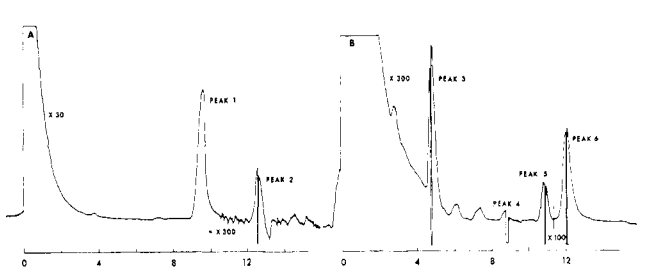

Figure 5. Total ion current traces of extracts containing pesticide residues isolated from liver tissue (A) fraction 1; (B) fraction 2 (see text)

The total ion current traces of the residue extracts of the liver tissue sample are presented in Figure 5. Five pesticides were isolated from the liver tissue, as shown in Table I. Lindane at 0.4 ppm was added to the tissue sample prior to extraction. Pesticides were identified on the basis of the following mass spectral data.

Peak 1. *p,p'*-DDE. *m/e* 246 (100%), 318 (80%), 316 (molecular ion, 63%), 248 (59%), 320 (39%), 176 (44%), 210 (18%).

Peak 2. *p,p'*-DDT. *m/e* 235 (100%), 237 (69%), 165 (51%), 75 (25%), 50 (20%), 51 (12%), 352 (molecular ion, 3%).

Peak 3. Lindane. *m/e* 109 (100%), 111 (79%), 181 (96%), 183 (96%), 219 (93%), 217 (80%), 51 (46%), 288 (molecular ion, 3%).

Peak 4. Heptachlor epoxide. Molecular ion isotope group at *m/e* 386, 388, 390, 392, 394 (Figure 6).

Peak 5. Dieldrin. *m/e* 79 (100%), 108 (16%), 263 (15%), 277 (18%), 279 (16%), 345 (8%), 378 (molecular ion, 3%).

Peak 6. *p,p'*-DDD. *m/e* 235 (100%), 237 (69%), 165 (52%), 75 (18%), 82 (15%), 88 (15%), 318 (molecular ion, 6%).

Relative ion intensity data of the mass spectra of reference samples of the four isomers of HCH were compared with the mass spectrum of Peak 1.

Data again showed too wide a discrepancy to allow confirmation of lindane on this basis alone. Gas chromatographic retention time data, however, permitted definitive identification. Heptachlor epoxide was identified by accumulating 10 scans of the mass spectrum of Peak 4 in the region of *m/e* 380 to 400. Figure 6 illustrates the results of the computer scans plotted in analog form on an X-Y recorder. The characteristic isotopic distribution pattern for seven chlorine atoms is clearly recognizable. These data, along with the molecular weight obtained from this group of mass spectral peaks (386 amu), and gas chromatographic retention time data, identified Peak 4 as heptachlor epoxide. The remaining pesticides were directly identified by comparing mass spectral data with those of reference standard materials and literature mass spectra.

CONCLUSIONS

The combined gas chromatography–mass spectrometry technique has been successfully applied to the identification and confirmation of pesticide residues isolated from human adipose and liver tissue samples. A general extensive extraction and cleanup technique adapted from existing methods was used to isolate and purify residues. Pesticides were separated by a silica gel chromatographic step into two fractions with sufficient resolution on the gas chromatographic column for identification of seven pesticide residues from the adipose tissue sample and six pesticide residues from the liver tissue sample. These include: β- and γ-HCH, heptachlor epoxide, dieldrin, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT, and *p,p'*-DDT.

Instrumental sensitivity was sufficient to identify residues at concentrations of 0.30 ppm. Recently developed computer enhancement techniques permitted identification of heptachlor epoxide residues at a level of 0.073 ppm. Definitive confirmation of pesticide residues was obtained by comparison of parent and fragment ion intensities and mass numbers of eluted residues and reference pesticides. Some difficulties were encountered in the absolute confirmation of configurational and structural isomers where mass spectral peak intensities and mass values of characteristic fragment ions were too similar under the conditions of the analysis. In these instances, however, identification was made readily on the basis of gas chromatographic retention times. The estimated lower limit of detectability of pesticide residues by the analytical scheme and instrument configuration reported is approximately 0.05 to 0.1 ppm. This can be improved considerably by instrumental modifications utilizing either glass capillary gas chromatographic columns with direct entry to the ion source or possibly other types of molecular separators such as the membrane type, which provide somewhat greater sample recoveries. In addition, the inclusion of an electron capture detector and splitter arrangement in tandem with the molecular separator would allow simultaneous gas chromatographic recording of pesticide effluents with minimum sacrifice of sample. Thus mass spectrometric identification and electron capture detection and quantitation of pesticide residues from a single gas chromatographic injection would be possible. Work is currently in progress on these and related areas of pesticide residue analysis and confirmation by GC-MS.

LITERATURE CITED

Amy, J. W., Chait, E. M., Baitinger, W. E., McLafferty, F. W., *Anal. Chem.* **37,** 1265 (1965).
Beynon, J. H., "Mass Spectrometry and Its Applications to Organic Chemistry," p. 298, Elsevier Publishing Co., New York, 1960.
Biros, F. J., *Anal. Chem.* **42,** 537 (1970).
Damico, J. N., Food and Drug Administration, personal communication, 1969.
Damico, J. N., Barron, R. P., Ruth, J. M., *Org. Mass Spectrom.* **1,** 331 (1968).
Damico, J. N., Wong, N. P., Sphon, J. A., *Anal. Chem.* **39,** 1045 (1967).
Elgar, K. E., *J. Ass. Offic. Anal. Chem.* **50,** 1067 (1967).
Food and Drug Administration, Pesticide Analytical Manual, Vol. I, Sec. 211, U.S. Dept. of Health, Education, and Welfare, Washington, D.C., 1966.
Grayson, M. A., Wolf, C. J., *Anal. Chem.* **39,** 1438 (1967).
Jörg, J., Houriet, R., Spiteller, G., *Monatsh. Chem.* **97,** 1064 (1966).
Kadoum, A. M., *Bull. Environ. Contam. Toxicol.* **2,** 264 (1967).
Kadoum, A. M., *Bull. Environ. Contam. Toxicol.* **3,** 65 (1968).
Kantner, T. R., Mumma, R. O., in "Residue Reviews," Vol. 16, p. 138, F. A. Gunther, Ed., Springer-Verlag, New York, 1966.
MacLeod, W. D., Nagy, B., *Anal. Chem.* **40,** 841 (1968).
McFadden, W. H., *Separ. Sci.* **1,** 723 (1966).
Muller, D. O., *Anal. Chem.* **35,** 2033 (1963).
Mumma, R. O., Kantner, T. R., *J. Econ. Entomol.* **59,** 491 (1966).
Robinson, J., Richardson, A., Elgar, K. E., Abstracts, 152nd National Meeting ACS, New York, Sept. 1966, Div. of Agricultural and Food Chemistry, No. A-75.
Schechter, M. S., *Pestic. Monit. J.* **2,** 1 (1968).
Watson, J. T., Biemann, K., *Anal. Chem.* **37,** 844 (1965).

Received for review November 28, 1969. Accepted March 23, 1970. Presented in part at the 158th National Meeting of the American Chemical Society, N.Y., September, 1969. Division of Pesticide Chemistry. Commercial sources and trade names are provided for identification only. Their mention does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.