Environ. Sci. Technol. **1998**, *32,* 3213−3217

ECD-Dual-Column Pesticide Method Verification by Ion Trap GC/MS and GC/MS/MS

STEVEN M. PYLE,*
ALVIN B. MARCUS,† AND
GARY L. ROBERTSON

United States Environmental Protection Agency, National Exposure Research Laboratory, Environmental Science Division, 944 East Harmon Avenue, Las Vegas, Nevada 89119-6748

Soil extracts from five Superfund Contract Laboratory Program (CLP) laboratories were analyzed for organochlorine pesticides using gas chromatographic separation with ion trap mass spectrometric detection in both electron impact (GC/EIMS) and tandem mass spectrometry (GC/MS/MS) modes. These results were compared with those from the standard CLP dual-column gas chromatography electron capture detection (GC/ECD) pesticide method. This was accomplished to (a) determine the number of false positives and false negatives in the CLP data, (b) evaluate ion trap GC/MS/MS as a potential technique to replace/augment conventional ECD-dual-column methodology, and (c) to compare conventional ion trap GC/EIMS with the relatively new ion trap GC/MS/MS. In all, 16 pesticide extracts from five CLP laboratories were analyzed for 20 pesticides by GC/EIMS and GC/MS/MS, and the data were compared with the results from the CLP method (ECD-dual-column pesticide method). Of a possible 960 parameters (20 analytes × 16 samples × 3 data sets), there were 253 detections with concentrations ranging from 1 pg/μL to 77 ng/μL. The respective number of false positives and false negatives were 27 and 1 for GC/MS/MS, 6 and 10 for GC/EIMS, and 25 and 9 for the CLP data. Causes of erroneous results are discussed.

Introduction

The United States Environmental Protection Agency's (EPA) Contract Laboratory Program (CLP) has used dual-column gas chromatography (GC) with electron capture detection (ECD) methodology (*1*) for the analysis of organochlorine pesticides for many years. Briefly, a soil sample extract is injected into a single injector that splits the sample between two GC columns of different liquid phases for separation and subsequent ECD detection. Each organochlorine pesticide is identified on the basis of its occurrence in each of two specified retention time windows. The ECD detector is used for quantitation since it is both sensitive and specific to halogenated compounds. This is an excellent technique for a skilled analyst to ensure the identity of the detected peaks. Concerns arise, however, when analyses are performed according to contractually specified methods, and the commercial laboratory has no background sample information to predict potential interferences. It is conceivable that false positive identifications may occur when interference peaks are within the retention time window limits of target analytes. To avoid this problem, CLP methodology allows for GC/MS analysis for confirmation of positive ECD pesticide identifications (*1*).

The development of highly sensitive gas chromatography/mass spectrometry (GC/MS) instruments has provided a solution to questionable identifications without sacrificing sensitivity. The ion trap mass spectrometer, a recently commercialized innovation, can identify analytes at the picogram level in the full-scan mode. This translates to method detection limits (MDLs) in the parts per billion (ppb) range. As a high-sensitivity detector, the ion trap mass spectrometer has the potential to perform sensitive and specific analyses with greater certainty of correct identification than the current ECD-dual-column pesticide method. In fact, ion trap data has been used in EPA Method 525.2 for analysis of drinking water (*2*).

In addition, ion trap detectors have recently been modified to perform tandem mass spectrometry (MS/MS). This capability enables the ion trap to isolate an ion of interest and then produce characteristic progeny ions by collision-induced dissociation (CID). This approach can unambiguously distinguish the compound of interest from other compounds that have parent ions of the same mass-to-charge ratio (m/z). The ability to trap an ion of interest (MS/MS in time) and then selectively remove the matrix ions from the manifold makes it possible to directly analyze for specific compounds at very low levels in complex matrixes or sample extracts (*3, 4*).

Ion trap GC/EIMS has developed as a useful tool in the analysis of pesticides in a variety of matrices in recent years (*5*−*10*). One application that has not been adequately evaluated is the analysis of pesticides in the environmental matrices of soil and water at hazardous waste sites. A potential advantage of ion trap GC/MS analysis is a more positive identification of the pesticide if existing detection limit and quality control criteria can be met.

In accordance with the EPA policy of evaluating new analytical techniques for possible use under Superfund contracts, the CLP provided residual sample extracts for this study after the routine CLP analyses had been completed. Extracts with a variety of pesticides at several concentration levels were used in the study. These extracts were analyzed as blinds; the analyst was not provided the CLP reported concentrations until after the results were compiled.

This study was designed to evaluate the performance of the ion trap GC/MS in a variety of operational modes for the analysis of pesticides using authentic sample extracts. The ion trap GC/MS data was compared with the dual-column GC/ECD analytical data provided by the CLP contract laboratories. The comparisons were made on a qualitative as well as a quantitative basis.

Representative samples with detected pesticides from EPA Region 1 (New England) were identified from the CLP Analytical Results Database. In support of Regional needs, the CLP laboratories were requested by EPA Region 1 to provide the investigators with the residual sample extracts.

Experimental Section

Mass Spectrometer. GC/MS/MS and GC/EIMS were performed on a Varian (Walnut Creek, CA) Saturn III ion trap mass spectrometer equipped with a waveboard and MS/MS software. Both EI and MS/MS were run with emission current

* Corresponding author e-mail: Pyle.Steven@epamail.epa.gov; phone: (702)798-2529; fax: (702)798-2142.
† Senior Environmental Employment Program Enrollee.

S0013-936X(98)00219-3 Not subject to U.S. Copyright. Publ. 1998 Am. Chem. Soc.
Published on Web 08/21/1998

TABLE 1. MS/MS Conditions

window no.[a]	segment time (min)	mass range (amu)	MS/MS ion[b] (*m/z*)	MIW[c] (*m/z*)	ex amp[d] (V)	ex time[e] (ms)	ex store[f] (*m/z*)	MS/MS mass[g] (*m/z*)
1	0−13.0							
2	13.0−14.0	100−250	242.9	4	54.5	20	71.7	207 + 209
3	14.0−15.9	70−190	181.9	4	36.5	20	48.0	145 + 146
4	15.9−16.35	70−190	188.1	1	75.5	25	71.7	160
5	16.35−17.0	70−190	181.9	4	36.5	20	48.0	145 + 146
6	17.0−18.5	100−280	272.8	4	54	20	71.7	237 + 239
7	18.5−19.5	100−270	262.8	6	87	30	87.6	191 + 193
8	19.5−20.6	200−360	352.8	6	49	30	79.6	261 + 263
9	20.6−21.2	150−420	373.8	4	42.25	30	71.7	264 + 266 + 301
10	21.2−21.9	100−380	194.9	6	69.5	25	71.7	157 + 159
10	21.2−21.9	100−380	373.8	4	42.25	25	71.7	264 + 266 + 301
11	21.9−22.6	150−270	246.9	4	66	30	71.7	176
11	21.9−22.6	150−270	262.85	6	75	30	79.6	191 + 193
12	22.6−23.1	100−270	262.8	6	86	30	87.6	191 + 193
13	23.1−23.65	100−240	194.9	6	69	20	71.7	157 + 159
13	23.1−23.65	100−240	235.9	4	62	20	71.7	165
14	23.65−24.2	100−250	243.9	4	64	20	71.7	173 + 207 + 209
15	24.2−25.0	150−280	235.9	4	61	30	71.7	165
15	24.2−25.0	150−280	272.8	4	53	30	71.7	237
16	25.0−26.35	150−330	240.1	1	81.5	30	79.6	212
16	25.0−26.35	150−330	316.85	6	47	30	71.7	243 + 245
17	26.35−26.8	100−230	227	1	78	30	79.6	115 + 152
18	26.8−31.3							
19	31.3−31.9	300−510	498.7	4	97	50	123.4	426 + 428
20	31.9−33.0							

[a] See Table 2 for corresponding compound. [b] MS/MS ion, MS/MS precursor ion. [c] MIW, molecular ion window. [d] ex amp, ion collision excitation energy. [e] ex time, ion collision excitation time. [f] ex store, excitation storage level. [g] MS/MS mass, MS/MS quantitation ion or ions.

TABLE 2. Method Detection Limits ($n = 7$)

				MS/MS MDL[c]		EI MDL	
window no.	compound	*R* time[a] (min)	EI quan mass[b] (*m/z*)	ext std	int std	ext std	int std
4	phenanthrene-d_{10} (IS)	16.06	188				
16	benz[*a*]anthracene-d_{12} (IS)	26.12	240				
2	tetrachloro-*m*-xylene	13.47	242 + 244	0.67	0.65	1.32	1.26
3	α-BHC	14.70	181 + 183	0.43	0.30	1.07	0.97
3	β-BHC	15.43	181 + 183	0.48	0.45	1.43	1.18
3	γ-BHC	15.68	181 + 183	0.26	0.33	0.81	0.82
5	δ-BHC	16.53	181 + 183	0.36	0.30	0.81	0.86
6	heptachlor	17.90	270 + 272 + 274	0.74	0.61	0.73	0.73
7	aldrin	19.00	261 + 263 + 265	0.67	0.78	1.14	1.11
8	heptachlor epoxide	20.22	353	0.86	0.88	0.87	1.04
9	γ-chlordane	20.97	373 + 375	0.78	0.57	0.32	0.35
10	α-chlordane	21.37	373 + 375	0.71	0.64	0.51	0.53
10	endosulfan I	21.38	239 + 241 + 243	1.05	1.08	1.84	1.68
11	*p,p′*-DDE	22.12	316 + 318 + 320	0.80	0.51	0.55	0.80
11	dieldrin	22.23	261 + 263 + 265	1.53	1.31	1.49	1.43
12	endrin	22.88	243 + 245	1.11	1.25	1.10	1.12
13	endosulfan II	23.23	239 + 241 + 243	1.15	1.17	2.29	2.51
13	*p,p′*-DDD	23.44	235 + 237	0.79	0.71	0.40	0.67
14	endrin aldehyde	23.69	243 + 245	0.60	0.89	1.93	1.90
15	endosulfan sulfate	24.45	270 + 272 + 274	0.46	0.74	1.57	1.67
15	*p,p′*-DDT	24.62	235 + 237	0.95	0.90	0.97	0.82
16	endrin ketone	25.91	315 + 317 + 319	0.71	0.93	1.48	1.25
17	methoxychlor	26.41	227	0.80	0.68	1.27	1.29
19	decachlorobiphenyl	31.49	498 + 500	0.57	0.52	0.82	0.56
	average			0.76	0.75	1.12	1.12

[a] *R* time, retention time. [b] EI quan mass, electron impact quantitation mass. [c] MDL, method detection limit (pg/μL).

at 80 μA, automatic gain control (AGC) prescan ionization time at 1500 μs, and manifold temperature at 260 °C. The multiplier voltage for EI was 1850 V (10^5 gain), and the AGC target was 30 000 counts. For MS/MS (also known as nonresident CID), the settings were 2000 V and 15 000 counts. For EI analysis, the mass spectrometer was scanned from 50 to 450 amu in 0.6 s with a mass defect of −50 millimass units per 100 mass units except for the final 4 min, which were scanned from 100 to 550 amu with a mass defect of 0. In each case, the background mass was 49 *m/z*. The MS/MS conditions (excitation time, excitation voltage, segment times, etc.) for each of the 20 windows are given in Table 1.

Gas Chromatograph. A 30 m × 0.25 mm i.d. J&W Scientific (Folsom, CA) fused-silica capillary column with 0.25-μm film thickness of bonded 5% phenyl/95% dimethyl polysiloxane was used to separate the pesticides for subsequent mass spectral detection. The Varian model 3400 gas chromatograph equipped with the model 8200 autosampler

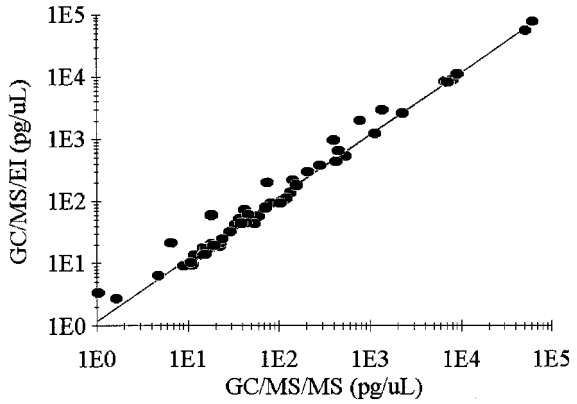

FIGURE 1. Log/log plot of GC/EIMS results versus GC/MS/MS results.

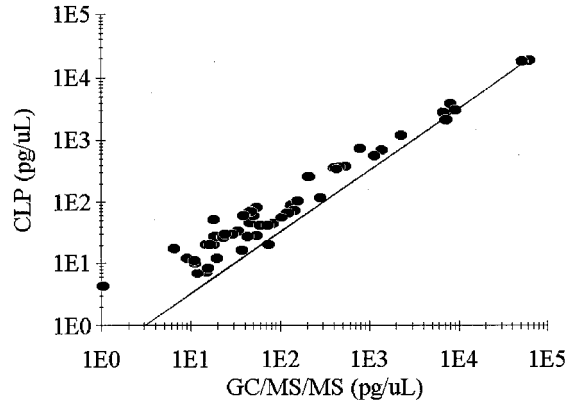

FIGURE 2. Log/log plot of CLP results versus GC/MS/MS results.

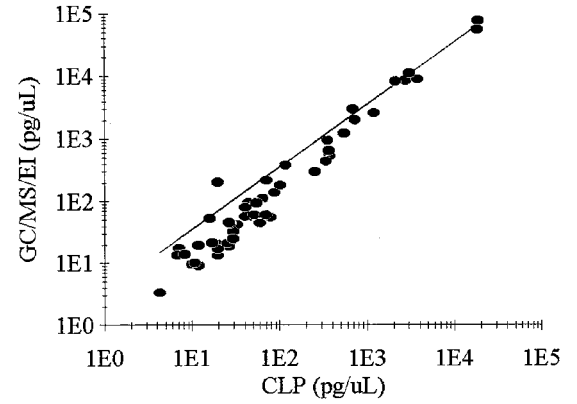

FIGURE 3. Log/log plot of GC/EIMS results versus CLP results.

was held at 60 °C for 2 min, temperature programmed at 12 °C/min to 156 °C, temperature programmed to 276 °C at 6 °C/min, and held at 276 °C for 3 min (total of 33 min). A 1.0-μL portion of a standard solution or sample was injected into a septum-programmable injector (SPI) that was initially held for 30 s at 60 °C and then ramped at 240 °C/min to 300 °C and held for 30 min.

Standards and Samples. A Supelco (Bellefonte, PA) certified stock solution containing 20 pesticides in hexane, each at 2000 μg/mL, was diluted with hexane to appropriate levels in a 2-mL autosampler vial for GC/EIMS and GC/MS/MS analysis. Benz[*a*]anthracene-d_{14} and phenanthrene-d_{10} were purchased as single solutions in methylene chloride from AccuStandard (New Haven, CT). Soil sample extracts were received from CLP laboratories in 2.0-mL sample vials and stored in a refrigerator. A subset of the total available samples was selected for analysis such that each laboratory was represented as well as a range of concentrations.

TABLE 3. Frequency of Pesticide Occurrence in 16 CLP Extracts

compd	MS/MS	EI	CLP	total
p,*p*′-DDT	13	12	13	38
p,*p*′-DDE	14	11	10	35
p,*p*′-DDD	11	10	8	29
dieldrin	6	2	9	17
endosulfan II	5	2	7	14
γ-BHC	4	6	4	14
γ-chlordane	6	5	2	13
heptachlor	4	4	5	13
endosulfan sulfate	7	2	2	11
α-chlordane	6	2	2	10
endrin ketone	5	3	2	10
endrin	2	2	5	9
methoxychlor	4	2	2	8
α-BHC	5	1	2	8
endosulfan I	1	0	7	8
aldrin	2	2	3	7
endrin aldehyde	2	1	4	7
heptachlor epoxide	0	0	1	1
δ-BHC	1	0	0	1
β-BHC	0	0	0	0
total	98	67	88	253

TABLE 4. False Positives and False Negatives vs Concentration

	false positives			false negatives		
level	MS/MS	EI	CLP	MS/MS	EI	CLP
total	27	6	25	1	10	9
<1 pg/μL	14	2	3	0	1	4
>10 pg/μL	7	1	12	0	4	2
>100 pg/μL	0	1	4	0	2	0

Quantitation. All quantitation was performed by the method of internal standardization using either benz[*a*]-anthracene-d_{14} or phenanthrene-d_{10} at the 2 ng/μL level as the internal standard. The quantitation ions for MS/MS and EI are shown in Tables 1 and 2. The EI and MS/MS response curves for quantitation were generated from single hexane standards at five concentration levels ranging from 5 to 1200 pg/μL. This compares to a dynamic range of 5−800 pg/μL for the CLP ECD-dual-column method (*1*). The EI and MS/MS response curves for the pesticides were linear (coefficients of determination were >0.99 for both EI and MS/MS).

Method Detection Limits. Method detection limits (MDLs) were calculated from replicate ($n = 7$) injections at the 5 pg/μL level using the recommended EPA protocol (*11*).

Results and Discussion

After preliminary experimentation, the conditions shown in Tables 1 and 2 were used to analyze 20 pesticides in 16 soil extracts by GC/EIMS and GC/MS/MS for comparison with previously reported CLP results. Relative internal standard precision (%RSD) over the period of data collection was 4.2% and 6.8% for GC/EIMS and GC/MS/MS, respectively. Method detection limits (MDLs) were determined in hexane and are shown in Table 2 with average values of 0.75 and 1.12 pg/μL for GC/MS/MS and GC/EIMS, respectively. MDLs were calculated from replicate injections ($n = 7$) at the 5-pg level using the recommended EPA protocol (*11*).

Figures 1−3 show the correlation between each of two data sets. In each log−log plot, the solid circles represent an XY pair of corresponding pesticide concentrations in a soil or water extract. The solid line in each figure is the least-squares fit to the data set forced through zero. The respective coefficients of determination (R^2) and slopes were

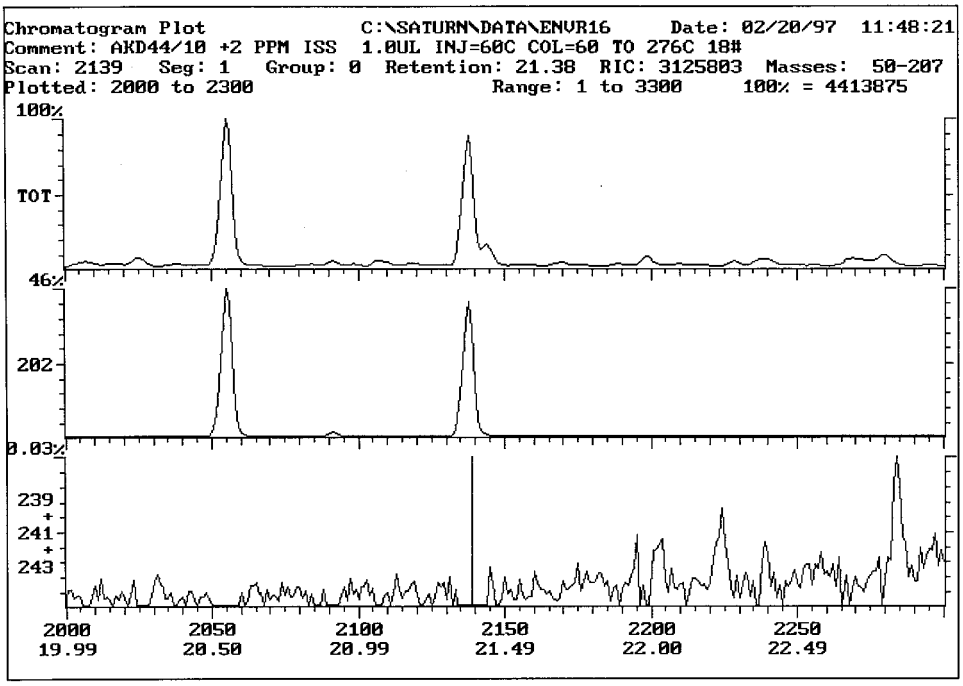

FIGURE 4. GC/EIMS run showing elution of pyrene at endosulfan I retention time.

0.994 and 1.17 (MS/MS vs EI), 0.990 and 0.33 (MS/MS vs CLP), and 0.974 and 3.54 (CLP vs EI). Slopes other than 1 for MS/MS vs CLP and CLP vs EI are due to the CLP reporting the pesticide concentrations in the matrix and not the concentration in the extract. Figure 1 shows minimal deviation from linearity over the entire concentration range, while Figures 2 and 3 show greater deviation from linearity especially at lower concentrations. This is not unexpected since Figure 1 represents single lab data and Figures 2 and 3 compare data from separate labs. Also, the correlation was performed on a nonweighted basis (i.e., regression of the values and not on the log of the values) resulting in a bias at the lower concentrations.

The three data sets (GC/EIMS, GC/MS/MS, and CLP) were compared in order to highlight data inconsistencies. A missing value from one set of data with corresponding values from the other two sets could signify a false negative, and conversely, a lone value in one set with no corresponding values in the other sets could indicate a false positive. In this manner, possibly erroneous results could be detected.

Table 3 shows the frequency of occurrence of the 20 pesticides versus the analytical method. Not unexpectedly, the top three hits were *p*,*p*′-DDD, *p*,*p*′-DDE, and *p*,*p*′-DDT. The method with the most detections was MS/MS (98), followed by CLP (88), and EI (67). The relatively high number of positive identifications for MS/MS are due to its greater sensitivity.

The number of false negatives and false positives, as a function of concentration, is shown in Table 4. Most of the inconsistencies occur at the lower concentration levels (<1 pg/μL), and the instances decrease with increasing concentration. However, there are still seven errors that are >100 pg/μL. Most of these (4) occur in the CLP data as false positive identifications (see Table 3) for endosulfan I (3) or endosulfan II (1). From the EI data of these samples, it was determined that polynuclear aromatic (PNA) compounds were present in these samples and that pyrene (MW = 202) coeluted with endosulfan I (see Figure 4). PNAs give a weak response to an electron capture detector, and it seems reasonable that relatively high PNA concentrations in the sample caused the false positive reported by the CLP laboratory. Inspection of the EI data at the retention time of endosulfan II, however,

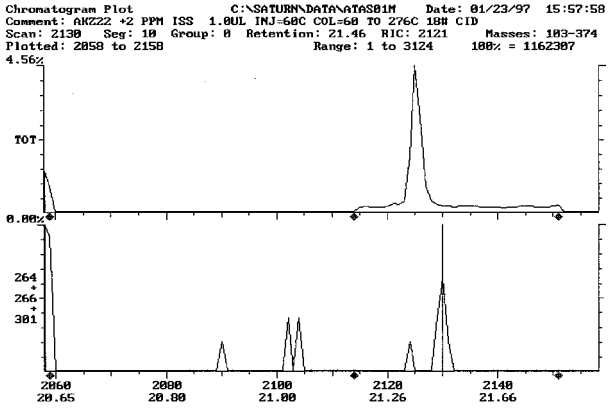

FIGURE 5. GC/MS/MS run showing α-Chlordane at the 110-fg level.

showed no extraneous peak to explain the false positive identification. Another false positive occurred in the EI data as a positive for γ-BHC. A sulfur-containing heterocyclic (MW = 184) eluted here and contained m/z 181 + 183 (γ-BHC quantitation ions) leading to an erroneous identification by the computer software.

The two false negatives in the EI data at the >100 pg/μL level were missing identifications for endrin ketone and endosulfan I. On closer examination of this EI data, two peaks occurred at each of these retention times, a sulfur-containing heterocyclic (MW = 240) and pyrene, but no indication of the two pesticides. However, an examination of the corresponding MS/MS data showed the presence of these two pesticides but not the sulfur-containing heterocycle or pyrene. The absence from the MS/MS data of the two interference peaks is not surprising, because of the intrinsic selectivity of mass spectrometry. An explanation for nondetection of the two pesticides in the EI data may be that this particular sample was diluted 100-fold because of its high concentration. Since the on-column levels were thereby 100 times lower, the pesticides may have been present but below the detection limit of EI analysis. In fact, because of the ability of MS/MS to preferentially isolate the ions of the target compounds, the background noise/ion signal is lower, and

higher sensitivities are possible. This is illustrated in Figure 5. The chromatogram shows α-chlordane, in an undiluted sample, at the 110-fg level.

Conclusions

(a) In general, the ECD-dual-column method results agree well with both the GC/MS/MS and GC/EIMS method results.

(b) In some instances, false positives in the CLP data were caused by extraneous PNA peaks erroneously identified as pesticides.

(c) A greater number of pesticide detections by GC/MS/MS were due to greater sensitivity than GC/EIMS.

Acknowledgments

This research was funded by the U.S. Environmental Protection Agency (EPA) through its Office of Research and Development (ORD), which funded and performed in the research described here. It has been subjected to the Agency's peer review and has been approved as an EPA publication. Neither the EPA nor ORD endorses or recommends any trade name or commercial product mentioned in this paper.

Literature Cited

(1) U.S. EPA Contract Laboratory Program, Statement of Work for Organics Analysis, Multi-media, Multi-concentration. *OLM03.2*; EPA-540/R-94/073; 1994; pp D-1/PEST 1−84.

(2) EPA Method 525.2. Determination of Organic Compounds in Drinking Water by Liquid−Solid Extraction in Capillary Column Gas Chromatography/Mass Spectrometry. EPA-600/R-95/131; 1994.

(3) Pyle, S. M.; Marcus, A. B.; Betowski, L. D.; Winnik, W.; Brittain. R. D. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 183−190.

(4) Pyle, S. M.; Marcus, A. B. *J. Mass Spectrom.* **1997**, *32*, 897−898.

(5) Tadeo, J. L.; Sanchez-Brunete, C.; Garcia-Valcercel, A. I.; Martinez, L.; Perez, R. A. *J. Chromatogr. A* **1996**, *754*, 347−365.

(6) Yang, S. S.; Goldsmith, A. I.; Smetena, I. *J. Chromatogr. A* **1996**, *754*, 3−16.

(7) Lehotay, S. J.; Eller, K. I. *J. AOAC Int.* **1995**, *78*, 821−830.

(8) Papadopoulou-Mourkidou, E.; Patsias, J.; Kotopoulou, A. *J. AOAC Int.* **1997**, *80*, 447−454.

(9) Bao, M. L.; Pantani, F.; Barbieri, K.; Burrini, D.; Griffini, O. *Int. J. Environ. Anal. Chem.* **1996**, *64*, 233−245.

(10) Tuinstra, L. G. M.; Van de Spreng, P.; Gaikhorst, P. *Int. J. Environ. Anal. Chem.* **1993**, *58*, 81−91.

(11) United States Environmental Protection Agency. *Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater*, Appendix A; EPA-600/4-82/057; U.S. EPA: Washington, DC, July 1982.

Received for review March 6, 1998. Revised manuscript received June 29, 1998. Accepted July 2, 1998.

ES9802192