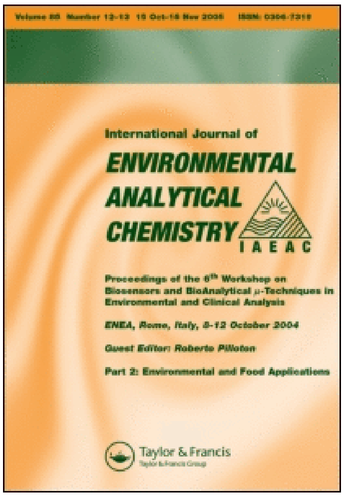

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USE OF CARBON-13 LABELLED DIOXINS MIXTURE FOR ANALYSIS OF POLYCHLORINATED DIBENZO-p-DIOXINS IN ENVIRONMENTAL SAMPLES BY GC-MS

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Positive identification and quantitation of polychlorinated dibenzo-p-dioxins (PCDD) in complicated environmental samples is described using a C-13 labelled dioxin mixture as an internal reference standard. Environmental samples are spiked with the C-13 labelled dioxin mixture and monitored for labelled and unlabelled dioxins using GC-MS in the electron impact selected ion monitoring (EISIM) mode. The C-13 labelled dioxin mixture and a municipal solid waste (MSW) incinerator fly ash extract show the same number of isomers in each tetra to octa-chlorodioxin congener groups. Quantitation of the C-13 labelled dioxin mixture was carried out using a reference standard mixture of unlabelled dioxins consisting of at least one isomer for each congener group. The C-13 labelled dioxin standard is highly useful for the determination of retention windows for tetra- to octa-chlorodioxins, identification of dioxins in each congener group, and calculation of the recovery of dioxins in samples that require extensive sample clean-up prior to GC-MS analysis. Its application for retention time window determination and as an internal reference standard for quantitation of dioxins in MSW incinerator fly ash extract and identification of dioxins in a complex sample from a PCB fire is demonstrated.

KEY WORDS: Dioxins, fly ash, C-13 labelled dioxins standard, quantitation of dioxins.

INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDD) are highly toxic chemical found in the environment. Until recently, the major sources of these pollutants were considered to be specific chemical manufacturing processes, where they occur as trace contaminants in the production of chlorophenoxy compounds used as precursors for certain herbicides, insecticides, bactericides and wood preservatives. The PCDD were first discovered in the municipal solid waste incinerator fly ash by Olie.[1] It is now generally accepted that a common source of dioxins is combustion of municipal and industrial waste,[2–4] sewage sludge incineration,[5] and forest fires and wood burning units.[6] The PCDD and equally toxic polychlorinated dibenzofurans (PCDF) have been detected also in soot samples of accidental fires of electrical transformers filled with polychlorinated biphenyls (PCB).[7]

Various mechanisms have been proposed for the formation of PCDD during incineration.[8] Laboratory experiments show that traces of PCDD can form from pyrolysis of chlorobenzenes, chlorophenols[9] and chlorinated biphenyls.[10] Formation of PCDD by catalytic activity of fly ash and quantitation of PCDD and PCDF in fly ash samples from different MSW incinerators located in different countries has been reported.[11–13]

The analysis of the environmental samples for PCDD requires rigorous methodology for positive identification and quantitation. Environmental samples give several difficulties due to sample complexity and strong interferences of other chlorinated organic compounds. GC-MS in the electron impact selected ion monitoring (EISIM) mode is generally used for analysis of PCDD. Use of open column chromatography and HPLC for sample clean up and then GC-MS analysis of PCDD in fish,[14] sediments,[15] fly ash[16,17] samples has been reported. Extreme care is required in sample preparation and measurement procedures in order to eliminate the possibility of false positive or false negative results due to interferences. This difficulty in PCDD analysis can be eased if samples are spiked by C-13 labelled dioxin mixture containing all the C-13 labelled dioxin isomers. However, this procedure was not possible to date, due to non availability of C-13 labelled dioxin isomers.

This paper shows application of C-13 labelled dioxin mixture containing a full range of isomers for analysis of fly ash extract and complex PCB fire sample for PCDD.

EXPERIMENTAL

The C-13 labelled dioxin mixture used in this study was synthesised in our laboratory and is now available commercially.[18] The Ontario fly ash sample was obtained from R. E. Clement (Ontario Ministry of The Environment, Toronto, Canada). The PCB fire sample was obtained from a previous study.

In a given experiment 40 g fly ash was soxhlet extracted for 48 hours using benzene as the solvent. Benzene extract was concentrated to 1 mL by rotary evaporation, then 20 uL of fly ash extract was spiked by the C-13 labelled dioxin mixture prior to GC-MS/EISIM analysis. PCB fire sample was spiked by C-13 labelled dioxin mixture prior to a normal phase high performance liquid chromatography (HPLC) separation. An HPLC separation of PCDD from PCB was achieved using an alumina column that was packed in our laboratory (19). The HPLC fraction containing the PCDD was further analyzed for PCDD using GC-MS/EISIM technique.

The GC-MSD instrument used was the Hewlett Packard 5890/5970. A Waters LC-510, equipped with NEC data system was used for isolation of dioxins from PCB. The unlabelled dioxin mixture used for quantitation of C-13 labelled dioxin mixture consisted of 1,2,3,4-TCDD, 2,3,7,8-TCDD, 1,2,3,7,8-P5CDD, 1,2,3,4,7,8-H6CDD, 1,2,3,4,6,7,8-H7CDD and OCDD.

Retention windows for tetra- to Octa-chlorodibenzodioxins were determined using the Ontario flyash extract and C-13 labelled dioxin mixture. A total of 20 ions were monitored as a single group using GC-MS/EISIM technique. Redrawing of individual mass chromatograms of ions monitored for a congener group and calculation of intensities of the ions provided the information of an elution time retention window of all isomers in a congener group. Retention windows determined and used for analysis of PCDD were 17 to 20.2, 24.8, 28, 34 and 40 minutes for tetra- to octa-chlorodibenzodioxin congeners, respectively.

RESULTS AND DISCUSSION

Total ion current (TIC) traces for Ontario flyash extract and C-13 labelled dioxin mixture obtained by using GC-MSD in EISIM mode are shown in Figure 1. Thirty ions were monitored such as M, M+2 and M-COCl for both labelled and unlabelled tetra to octa chlorodibenzodioxins (Table 1). For the positive identification of PCDD in complex environmental samples several criteria has to be satisfied.[19,20] One of the important criteria is elution of dioxin congener groups in definite retention windows. To fulfil the criteria of the elution of dioxin congener groups in definite retention windows one has to determine the retention windows prior to analysis of any sample for PCDD. Retention window determination can be carried out using two methods. In the first method a mixture of dioxin isomers, first eluting and last eluting in each congener group can be used to detect the retention window for a congener group. For this method it is required to have nine isomers for determination of tetra to octa chlorodibenzo-p-dioxin retention windows. In a second method an environmental sample having mixture of all tetra- to octa-chlorodibenzo-p-dioxins can be used. To apply the first method one should have nine pure dioxin isomers. To date such specific isomers are not available from commercial sources. By using an environmental sample containing all dioxin isomers, it is possible to determine the retention windows, however, environmental samples usually contain a number of other polar compounds,[17] that can deteriorate the GC capillary column. It has been reported that a flyash extract containing high levels of dioxins also contains all isomers in each congener group.[7,20] Ontario flyash has been studied extensively in our laboratory.[12,16,17] Based on the reported number of isomers in a flyash sample, it can be expected that the Ontario flyash contains all isomers in the tetra- to octa-chlorodioxin congener groups. Since the C-13 labelled dioxin mixture and Ontario flyash extract show the same number of peaks (Fig. 1) separated on a DB-5 column, it should be possible to use the C-13 labelled dioxin mixture for retention window determination.

The purity of the C-13 labelled dioxin mixture was checked by using another criterion for positive identification of dioxins. The three ions M, M+2 and M-COCl for each C-13 labelled congener group were re-plotted and their intensities were measured as area counts. The intensities observed were within ±5% of the theoretical values. For clarity, only the most intense ion for each congener group is shown in Figure 2, which shows that the peaks in the mass chromatograms of tetra- to octa-chlorodioxin congeners, and in the TIC of C-13 dioxin mixture in specified retention windows are the same. Mass chromatograms of the M, M+2 and M-COCl ions for unlabelled dioxins, labelled and unlabelled PCDF, in the C-13 labelled dioxin mixture show no peaks, indicating that the C-13 labelled dioxin mixture is free from native PCDD and PCDF isomers.

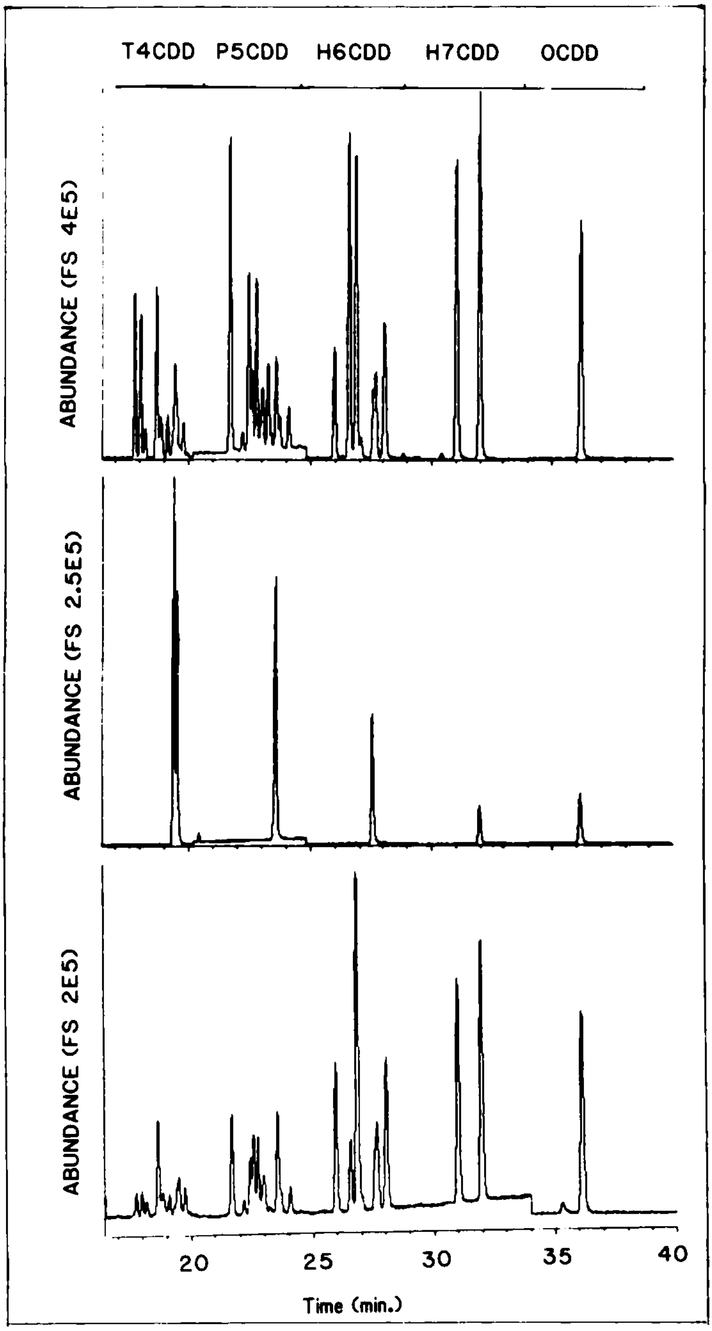

Figure 1 Total ion current (TIC) traces of Ontario flyash extract (upper), mixture of unlabelled dioxin standard (middle) and C-13 labelled dioxin mixture (lower). Chromatographic conditions: 30 m × 0.32 mm I.D. DB-5 fused silica Capillary column, temperature 80 °C for 1 minute, programmed to 230 °C at 15 °C/min., then programmed to 300 °C at 3 °C/min., final time 20 minutes.

Table 1 Ions monitored for C-13 labelled and unlabelled PCDD.

Compound Ions	*T4CDD*	*P5CDD*	*H6CDD*	*H7CDD*	*OCDD*
C-13 Labelled					
M	331.9	365.9	399.9	433.8	–
M+2	333.9	367.9	401.9	435.8	469.7
M+4	–	–	–	–	471.7
MI-COCl	269.9	303.9	337.9	371.8	407.7
Unlabelled					
M	319.9	353.9	387.9	421.8	–
M+2	321.9	355.9	389.9	423.8	457.7
M+4	–	–	–	–	459.7
MI-COCl	258.9	292.9	326.9	360.8	396.7

MI = Most intense ion in M, M+2 and M+4.

A standard practice applied by most laboratories analyzing for PCDD involves the use of a limited C-13 labelled dioxin mixture containing one isomer for each congener group as an internal standard or a mixture of unlabelled dioxins as external standard. Quantitative estimation of dioxins can be carried out by comparing the response for known concentration of dioxins in the standard to that of native dioxins present in the sample. Accuracy of quantitation using an external standard can vary according to the run-to-run GC and GC-MS conditions, and variation in injection techniques. True quantitation of the total amount of dioxins using an external standard cannot be assessed when extensive sample cleanup has been done prior to GC-MS/EISIM analysis. Precision in dioxin analysis can be achieved by spiking the environmental sample with a known amount of a C-13 dioxin mixture before sample preparation and then analyzing the C-13 dioxins along with the unlabelled native dioxins in the environmental sample. The percent recovery of C-13 labelled dioxins thus provides a measure of the efficiency of the analytical procedures. Thus, C-13 labelled dioxin standards have a major advantage over unlabelled dioxin standard, however, since only a few labelled dioxin isomers have been prepared and are generally available, the extent to which these procedures can be applied is limited.

We have developed a method of synthesis for a C-13 labelled dioxin mixture. Quantitation of tetra- to octa-chlorodibenzo-p-dioxins in this mixture was carried out using an unlabelled dioxins standard containing at least one isomer for each congener group for tetra to octa-chlorodioxin isomers (Figure 1, middle trace). The dioxins used for quantitation, their concentration and amount of each congener group in C-13 dioxin mixture are shown in Table 2.

Application of C-13 Labelled Dioxin Mixture for Analyzing PCDD in a Fly Ash Extract

An Ontario MSW incinerator fly ash extract was spiked with the C-13 labelled dioxin mixture and both labelled and unlabelled dioxins were monitored simulta-

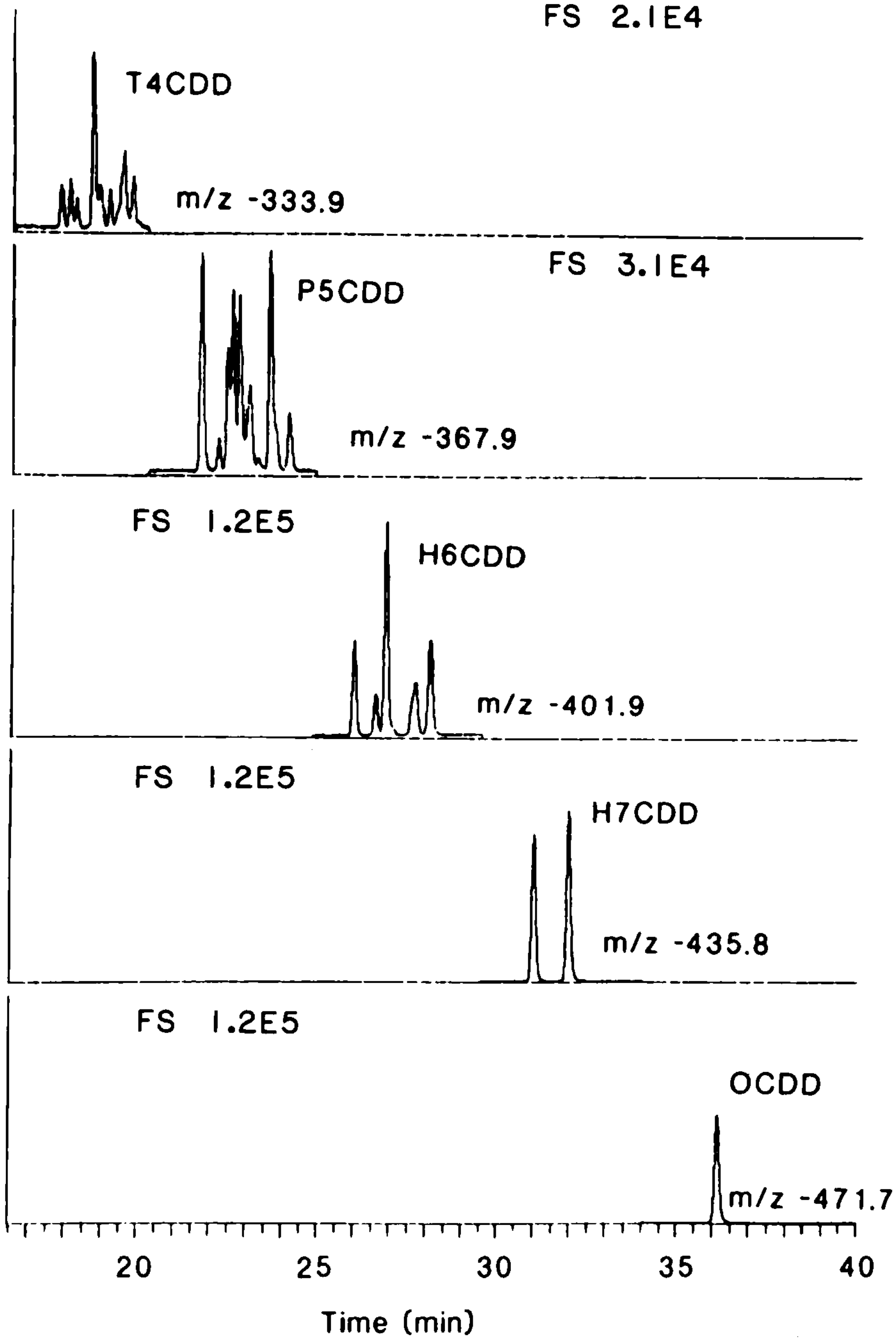

Figure 2 Mass chromatograms for most intense ions of tetra- to octa-chlorodibenzo dioxin congener groups of C-13 labelled dioxin mixture. Chromatographic conditions are given in Figure 1.

Table 2 Quantitation of C-13 labelled dioxin mixture, names and concentration of unlabelled dioxin isomers used for quantitation.

Name of isomer in standard mixture	*Concentration (ng/ul)*	*Concentration of C-13 reference labelled dioxin congener (ng/ul)*
1,2,3,4+2,3,7,8-T4CDD	1.5	1
1,2,3,7,8-P5CDD	1.5	2.8
1,2,3,4,7,8-H6CDD	0.6	4.9
1,2,3,4,6,7,8-H7CDD	0.5	6.6
OCDD	1.3	3.9

neously using the GC-MS/EISIM technique. Added mass chromatograms for tetra- to heptachloro congeners (M and M+2 ions) and for the octa-chloro congener (M+2 and M+4 ions) are shown in Figure 3. The most intense ions for spiked C-13 labelled dioxins are shown in the lower trace of Figure 3. Peaks present in all mass chromatograms were confirmed as dioxins by monitoring three ions for each congener group and comparing the intensities with theoretical values. Since the C-13 labelled dioxin mixture contained all isomers in a congener group, it has a major advantage of identifying retention times of unlabelled dioxins present in the environmental sample. Also, the C-13 labelled dioxin mixture gives one more additional parameter of exact retention time of each peak in a congener group for identification of dioxins. A comparison of quantitative results of the Ontario MSW incinerator fly ash sample for tetra- to octa-chlorodibenzo-p-dioxins using an external standard of unlabelled dioxins and using C-13 labelled dioxin mixture as an internal standard is shown in Table 3.

Application of C-13 Labelled Dioxin Mixture for Analyzing PCDD in a PCB Fire Sample

Sometimes the traditional criteria used for identification of PCDD[20,21] using the GC-MS/EISIM technique are not sufficient for analyzing PCDD in very complicated samples because of interferences. In particular, analysis of a PCB fire sample for PCDD using these criteria can result in false positive identification of interfering compounds as dioxins. PCB accidental fire samples are usually very complicated due to the presence of PCB and formation of traces of a variety of interfering compounds at higher temperatures. The mass chromatograms for M and M+2 (319.9 and 321.9) ions for unlabelled T4CDD in PCB fire sample and M+2 (333.9) ion for C-13 labelled spiked T4CDD reference standard are shown in Figure 4. Applying the two major criteria of retention window and proper intensities, all peaks in the mass chromatogram for native T4CDD can be considered as positively identified tetrachloro-dibenzo-p-dioxins. However, upon comparison of retention times for known peaks in the spiked C-13 dioxin mixture (Figure 4 upper trace), it can be seen that the peaks marked ‘x’ in the mass chromatograms for native T4CDD in PCB sample are the only dioxins; the other peaks, in spite of correct retention window and intensities, are not dioxins.

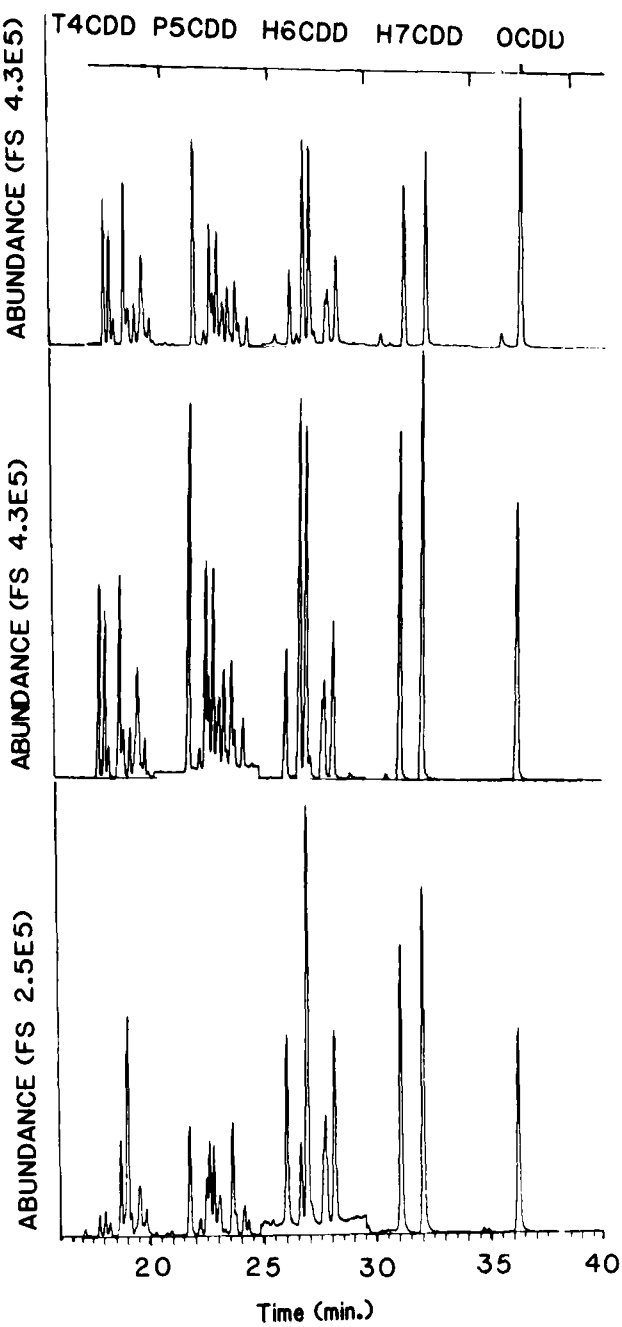

Figure 3 Mass chromatograms for native dioxins in the Ontario flyash extract and spiked C-13 labelled dioxins. Mass chromatogram: (Upper trace) m/z=319.9 (M, T4CDD), m/z=353.9 (M, P5CDD), m/z=387.9 (M, H6CDD), m/z=421.8 (M, H7CDD) and m/z=457.7, (M+2, OCDD); (middle trace) m/z=321.9 (M+2, T4CDD), m/z=355.9 (M+2, P5CDD), m/z=389.9 (M+2, H6CDD), m/z=423.8 (M+2, H7CDD) and m/z=459.7 (M+4, OCDD); (lower trace) m/z=333.9 (M+2, T4CDD), m/z=367.9 (M+2, P5CDD), m/z=401 (M+2, H6CDD), m/z=435.8 (M+2,H7CDD) and m/z=471.7 (M+4, OCDD). Chromatographic conditions are given in Figure 1.

Table 3 Quantitation of Ontario flyash extract for PCDD using unlabelled dioxin standard and C-13 labelled dioxin mixture.

Congener group	*Using unlabelled dioxins as external standard (ng/ul flyash extract)*	*Using C-13 labelled dioxin mixture as internal standard (ng/ul flyash extract)*
T4CDD	13.2	9.2
P5CDD	37.5	32.2
H6CDD	29.4	29.1
H7CDD	50.5	44.1
OCDD	36.5	29.9

Similarly based on the criteria of comparison of retention times of peaks in the retention window, several peaks of interfering compounds were observed in penta-, hexa- and hepta-chlorodioxin windows that do not match the retention times of the peaks in the C-13 dioxin mixture. The peaks marked 'x' in Figures 5, 6, 7 are confirmed as dioxins. It should be emphasized that the analysis of a complicated sample using only the C-13 labelled dioxin isomer for each congener group may also result in false identification of interfering compounds as dioxins. However, using the C-13 dioxin mixture and the additional criteria of comparison of retention times of individual peaks in the C-13 dioxin mixture with that of peaks for native unlabelled dioxins in PCB sample results in a more positive identification of dioxins.

References

1. K. Olie, P. L. Vermeulen and O. Hutzinger, *Chemosphere* **8,** 455 (1977).
2. W. B. Crummett and D. I. Townsend, *Chemosphere* **13,** 777 (1984).
3. C. L. Hail, R. B. Blair, J. S. Stanley, D. P. Redford and R. M. Lucas. In: *Chlorinated Dioxins and Dibenzofurans in the Total Environment* (G. Choudhary, L. H. Keith and C. Rappe, eds) (Butterworth Publishers, 1983), pp. 439.
4. T. Sawyer, S. Dandiera and S. Safe, *Chemosphere* **12,** 529 (1983).
5. A. Cavallaro, *Chemosphere* **9,** 611 (1980).
6. R. C. Lao, C. Chiu, R. S. Thomas, K. Li and J. Lockwood, *Chemosphere* **12,** 607 (1983).
7. C. Rappe, S. Marklund, P. A. Bergqvist and M. Hansson. In: *Chlorinated Dioxins and Dibenzofurans in the Total Environment* (G. Choudhary, H. Keith and C. Rappe, eds) (Butterworth Publishers, 1983), p. 99.
8. G. Choudhary and O. Hutzinger (eds), *Mechanistic Aspects of the Thermal Formation of Halogenated Organic Compounds Including Polychlorinated Dibenzo-p-dioxins* (Gordon and Breach Science Publishers, Inc., New York, 1983).
9. H. R. Buser, *Chemosphere* **8,** 415 (1979).
10. H. R. Buser and C. Rappe, *Chemosphere* **8,** 157 (1979).
11. F. W. Karasek and L. C. Dickson, *Science* **237,** 754 (1986).
12. F. W. Karasek, K. P. Naikwadi and H. Y. Tong, Proc. Machida Workshop, Machida, Japan, 12 and 13 September 1986, pp. 12.
13. K. P. Naikwadi, F. W. Karasek and H. Hatano, Proc. Machida Workshop, Machida, Japan, 12 and 13 September 1986, pp. 1.
14. L. L. Lamparski, T. J. Nestrick and R. H. Stehl, *Anal. Chem.* **51,** 1453 (1979).
15. D. C. G. Muir, B. E. Townsend and W. L. Lockhart, *Environ. Toxicol. Chem.* **2,** 269 (1983).

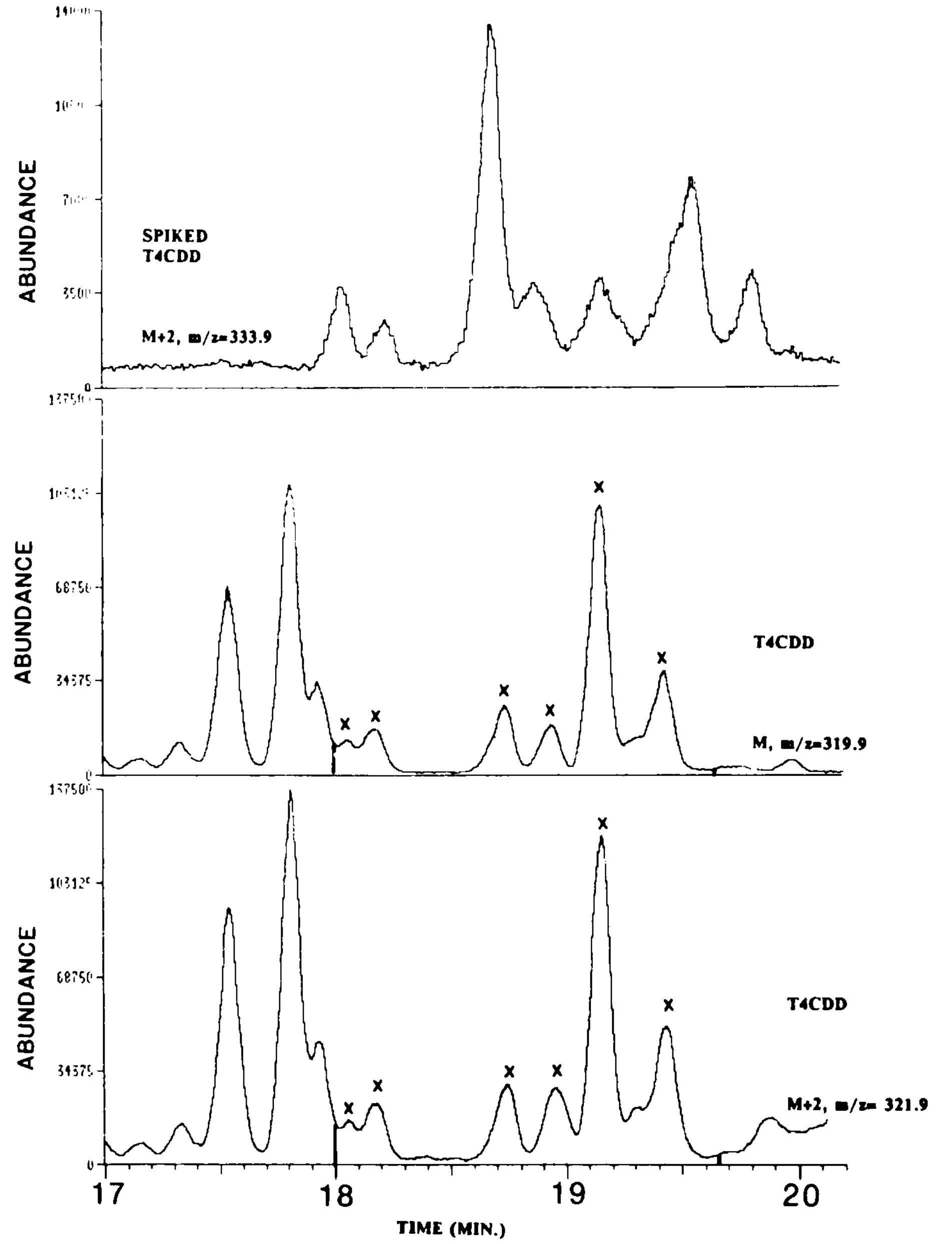

Figure 4 Mass chromatogram for native and spiked C-13 labelled T4CDD in a PCB fire sample. M + 2 (321.9) and M (319.9) ions (lower and middle traces) for native and M2 (333.9) for C-13 labelled T4CDD. Chromatographic conditions are given in Figure 1. Peaks marked "x" are native dioxins.

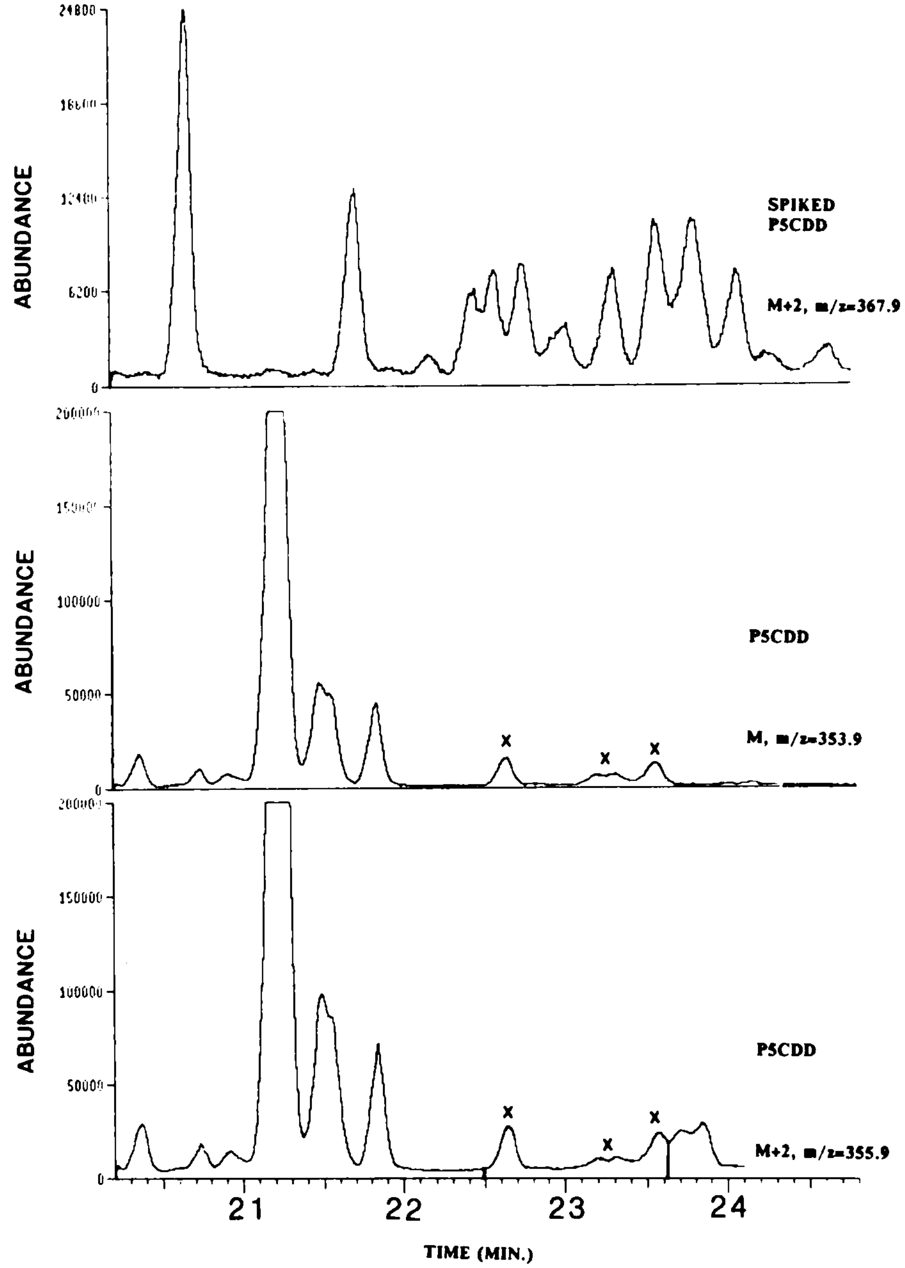

Figure 5 Mass chromatogram for native and spiked C-13 labelled P5CDD in a PCB fire sample. M+2 (355.9) and M (353.9) ions (lower and middle traces) for native and M+2 (367.9) for C-13 labelled P5CDD. Chromatographic conditions are given in Figure 1.

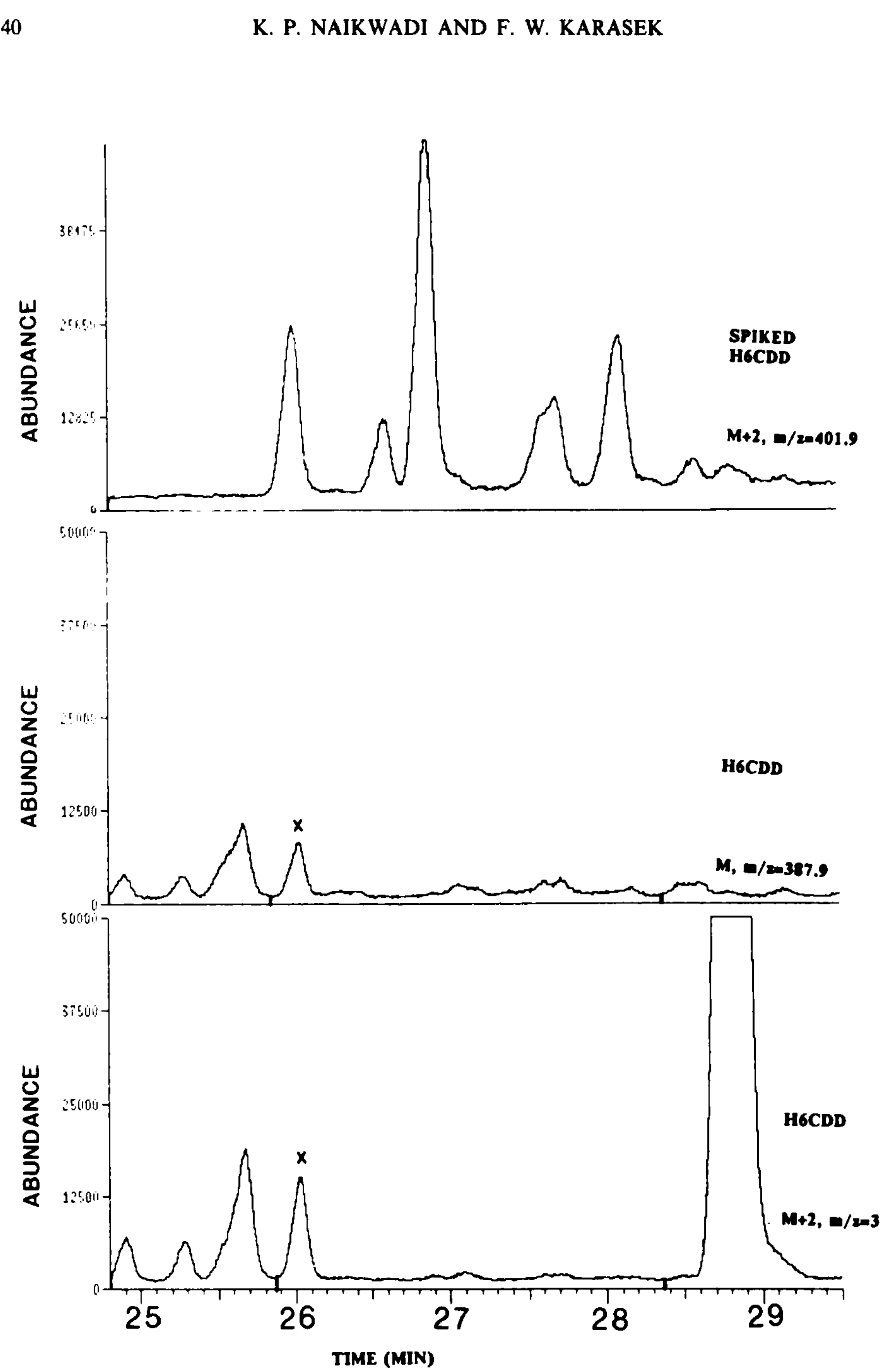

Figure 6 Mass chromatogram for native and spiked C-13 labelled H6CDD in a PCB fire sample. M+2 (389.9) and M (387.9) ions (lower and middle traces) for native and M+2 (401.9) for C-13 labelled H6CDD. Chromatographic conditions are given in Figure 1. Peaks marked "x" are native dioxins.

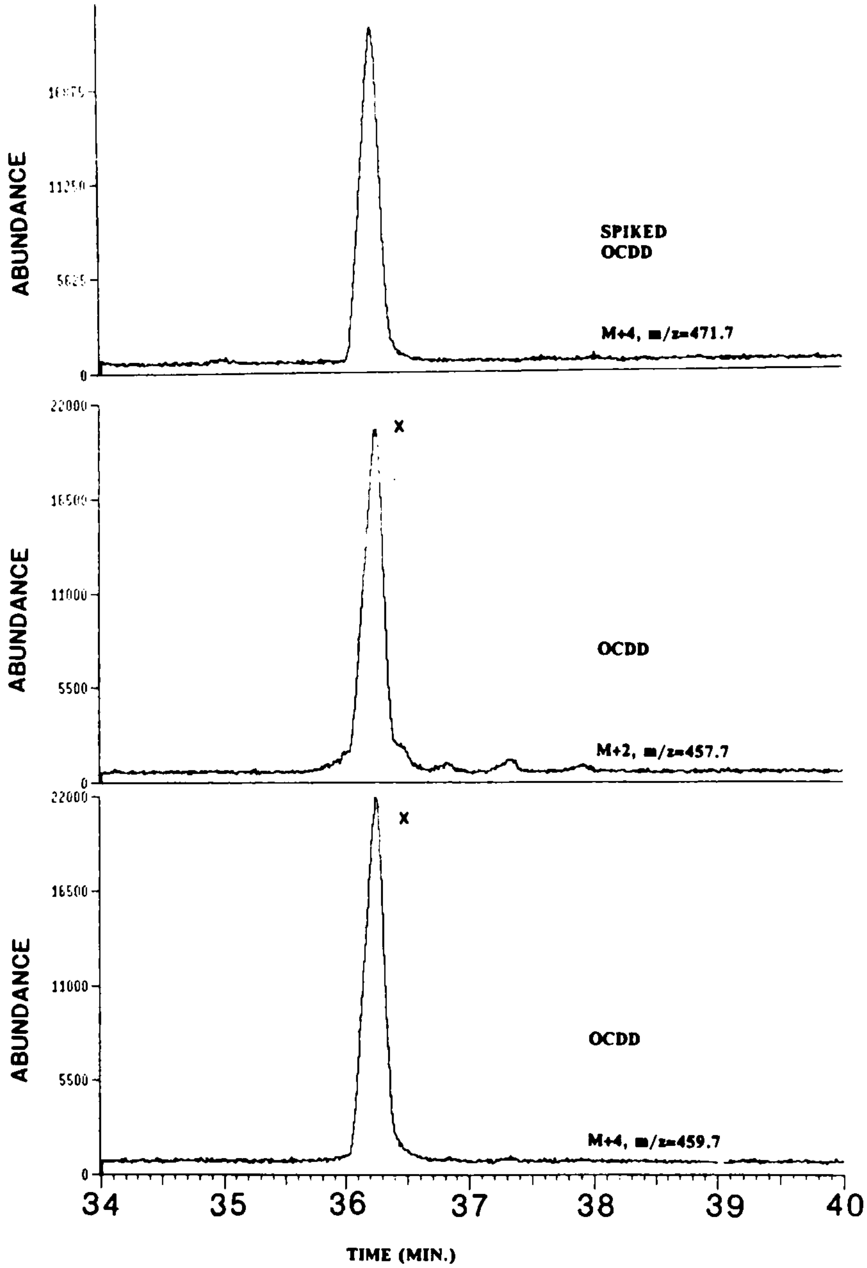

Figure 7 Mass chromatogram for native and spiked C-13 labelled OCDD in a PCB fire sample. M+4 (459.7) and M+2 (457.7) ions (lower and middle traces) for native and M+4 (471.7) for C-13 labelled OCDD. Chromatographic conditions are given in Figure 1. Peaks marked "x" are native dioxins.

16. H. Y. Tong, D. L. Shore and F. W. Karasek, *Anal Chem.* **56,** 2442 (1984).
17. H. Y. Tong, D. L. Shore and F. W. Karasek, *J. Chromatogr.* **285,** 423 (1984).
18. "Femtometric", 17 Wild Goose Court, Newport Beach, California 92663.
19. T. S. Thompson, K. P. Naikwadi and F. W. Karasek, Proc. Technol. Transfer Conference, Toronto (1987), pp. 24.
20. L. H. Keith, C. Rappe and G. Choudhary (eds), *Chlorinated Dioxins and Dibenzofurans in the Total Environment II* (Butterworth Publishers, 1983), pp. 339–375.
21. C. Rappe, *Environ. Sci, Technol.* **18,** 78A (1984).