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QUANTITATIVE ASPECTS IN THE DETERMINATION OF POLYCHLORINATED DIBENZO-*p*-DIOXINS BY HIGH-RESOLUTION GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

Polychlorinated dibenzo-*p*-dioxins (PCDDs) are a group of compounds consisting of 75 isomers which are difficult to quantitate because of the lack of sufficient reference standards. A method of quantitation is developed which uses relative response factors for each isomeric group. Variations in gas chromatographic–mass spectrometric parameters do not bias the quantitation method for PCDDs. Testing the method using an environmental sample gives consistent data that can be used to study PCDD content in a number of samples.

INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins (PCDDs) are known toxins and carcinogens. These compounds have been found on fly ash from municipal incinerators worldwide at ppm levels[1–5]. Quantitative measurements of these compounds in a complex mixture of other organic compounds presents difficulties because the availability of only a few reference standards and the effects of changing instrument parameters.

In the past, the determination of PCDDs has been performed using packed-column gas chromatography–mass spectrometry (GC–MS). In that work, published data have indicated that the relative standard deviation for the quantitation of PCDDs in environmental samples can be 30%[6,7,9].

Further studies using packed-column GC–MS have focused on the effect of using a glass-jet or silicone membrane separator on the relative peak-ratio response of the PCDD congeners using a high-resolution mass spectrometer[8]. When total congener concentrations are desired, the summed area of the congener isomeric peaks are used for quantitation. Area response factors of other PCDDs relative to tetrachlorodibenzo-*p*-dioxin (TCDD) were included in this study[8].

Ideally, area response factors of the individual dioxins should be determined using reference standards. However, this is neither possible nor practical in the case of these compounds. The dioxins comprise a group of 75 related compounds in which some isomeric classes, such as tetrachlorinated isomers, contain 22 compounds. Ref-

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erence standards for each isomer are not commercially available because of the difficulty in synthesizing individual compounds and their extreme toxicity. A reasonable calibration procedure must be used in view of this situation. It is assumed that all isomers in one class have close response factors based on peak areas. Response factors relative to the other compounds for which references are available are then determined. A plot of these relative responses from tetra- to octachloro dibenzo-*p*-dioxins follows a curve, from which factors for those compounds, for which no references are available, can be determined. This procedure can be used with any GC–MS instrument system. However, the data will differ and must be determined for each system. The results reported here are a study of this procedure and the effects of such variables as electron-multiplier voltage and chromatographic stationary phase on the relative responses of PCDDs.

EXPERIMENTAL

Sample extraction and concentration

A sample of fly ash was collected from an electrostatic precipitator from an Ontario municipal incinerator. This sample was extracted in triplicate using procedures described in an earlier study[1]. These procedures include: 16-h Soxhlet extraction of 15–20 g sample with 200 ml of distilled-in-glass benzene, cencentration to 100 μl by rotary evaporation under aspirator vacuum and analysis by GC–MS with no additional sample treatment. Each extract was analyzed in duplicate for this study.

GC–MS analysis

A Hewlett-Packard 5992A GC–MS–calculator was used in the selected-ion monitoring (SIM) mode for PCDD determination. This instrument was equipped with a cool on-column injection port, 30 m × 0.32 mm I.D. fused-silica capillary columns with stationary phases, DB-1701 or DB-5, capillary glass restrictor inlet, *x–y* plotter and dual floppy disk system. The He carrier-gas flow-rate was 3 ml/min.

The ions monitored for TCDD were 319.9 and 321.9. The ions selected for penta- (P5CDD), hexa- (H6CDD), hepta- (H7CCD) and octachlorodibenzo-*p*-dioxin (OCDD) were, 355.9, 389.8, 425.8 and 459.7, respectively. A dwell-time of 50 msec was used for each ion.

SIM areas generated by the GC–MS data system were used for quantification. A standard mixture of 1,2,3,4-TCDD, 1,2,3,4,7,8-H6CDD, 1,2,3,4,6,7,8-H7CDD and OCDD was prepared in benzene for quantitation. P5CDD was quantified using a response factor intermediate to that of TCDD and H6CDD standards.

Chromatographic conditions were; initial oven temperature, 180°C, to 280°C or 300°C final temperature at 4°C/min for the DB-1701 or DB-5 coated columns. Injection-port temperature was 70°C.

Before operating in SIM mode, the mass spectrometer was tuned daily by the manufacturer-supplied programme AUTOTUNE using a perfluorotributylamine calibration standard.

RESULTS AND DISCUSSION

An investigation into the possibility of mass discrimination by the injection

technique was carried out. This was determined by injecting the standard PCDD solution into the GC–MS instrument with the injection port and oven temperature 40°C below the benzene-solvent boiling point. The previously injected amount was then bracketed by two injections of higher and lower amounts of the PCDD standard solution under the conditions described in the Experimental section. These results are shown in Fig. 1.

The injection volume error was included in Fig. 1 as dashed lines and shows that under either chromatographic conditions the response per amount injected is within the experimental error for that compound. Therefore, mass discrimination is considered to be minimal for PCDDs in this system using this injection procedure.

The injection volume error is the result of volume measurement uncertainty. Typically, 1.0 μl is injected into the GC MS system with a 10-μl Hamilton syringe using the solvent-plug technique. This requires that a volumetric reading be made on both sides of the sample plug in the syringe barrel, which leads to a total volumetric error of 0.1 μl per sample (2 × 0.05 μl) since the smallest graduation is 0.1 μl. Thus a variation of 0.1 μl in 1.0 μl results in a 10% error as shown in Fig. 1.

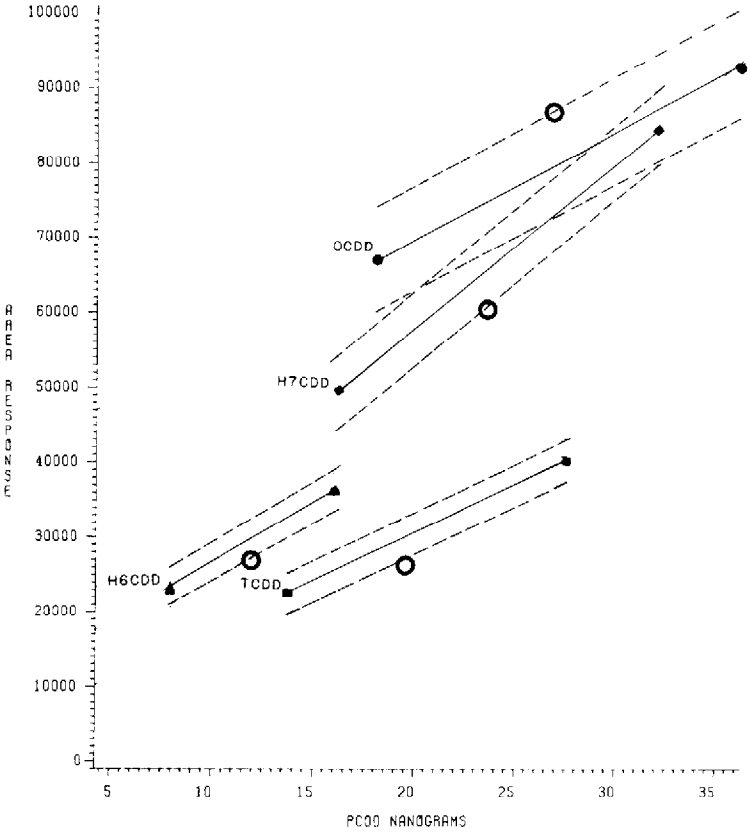

Fig. 1. Graph showing that mass discrimination of PCDD is within the 10% range of error expected from injection volume measurement uncertainty. The circles represent injection of the standard at 40°C below the solvent boiling temperature. The two points forming the line are from injection of the standard under experimental conditions.

TABLE I

RELATIVE AREA RESPONSE FACTORS (± S.D.) FOR PCDD CONGENERS FOR DIFFERENT CHROMATOGRAPHIC SYSTEMS

	DB-5	*DB-1701*	*Ref. 8*
TCDD	1.0	1.0	1.0*
P5CDD	**	**	0.52 ± 0.02
H6CDD	0.57 ± 0.08	0.71 ± 0.08	0.44 ± 0.02
H7CDD	0.50 ± 0.04	0.61 ± 0.08	0.46 ± 0.02
OCDD	0.46 ± 0.07	0.56 ± 0.06	0.32 ± 0.01

* 2,3,7,8-TCDD was reported as 0.89.
** Standard was not available.

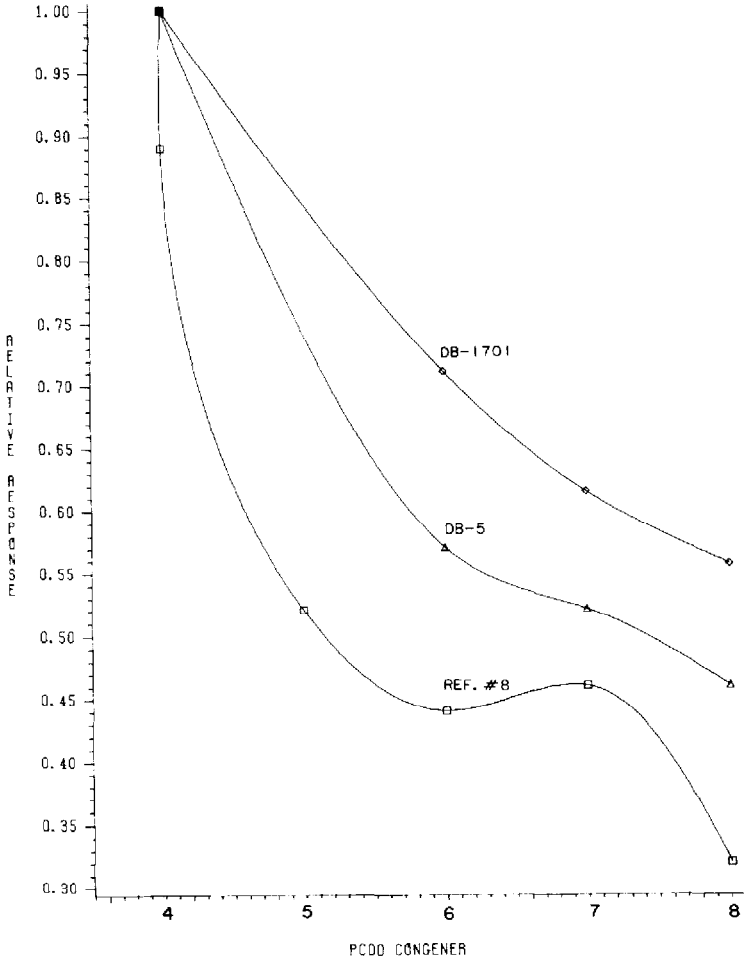

Fig. 2. A plot of the relative area response factors for each PCDD standard compound. Numbers on the *x*-axis represent the number of chlorine substituents in the molecule. Values for DB-5, DB-1701 and ref. 8 are compared using the same isomers.

Relative responses of the PCDDs have been reported using packed-column GC–MS[8]. A comparison of PCDD relative responses using both DB-5 and DB-1701 cross-linked stationary phase wall-coated open tubular (WCOT) fused-silica columns with this data is useful since these columns are becoming more widely used.

Relative response factors were determined by injecting the PCDD standard three times on each column. The mean results and standard deviations are shown in Table I. Both sets of results demonstrate an average relative deviation of 12% attributable to volumetric injection uncertainty. The values obtained are tabulated with those previously published on packed-column GC MS for comparison. It is observed that all the values change with the GC–MS system used for analysis. These data were then plotted as relative response *vs.* PCDD congener in Fig. 2. By smoothly joining the points, the results obtained on this instrument consistently form a hyperbolic-type curve. There is evidence of dependence on stationary phase. This can be expected because different column stationary phases affect peak shape and consequently peak area. The data published using packed-column GC–MS for the same PCDD isomers,

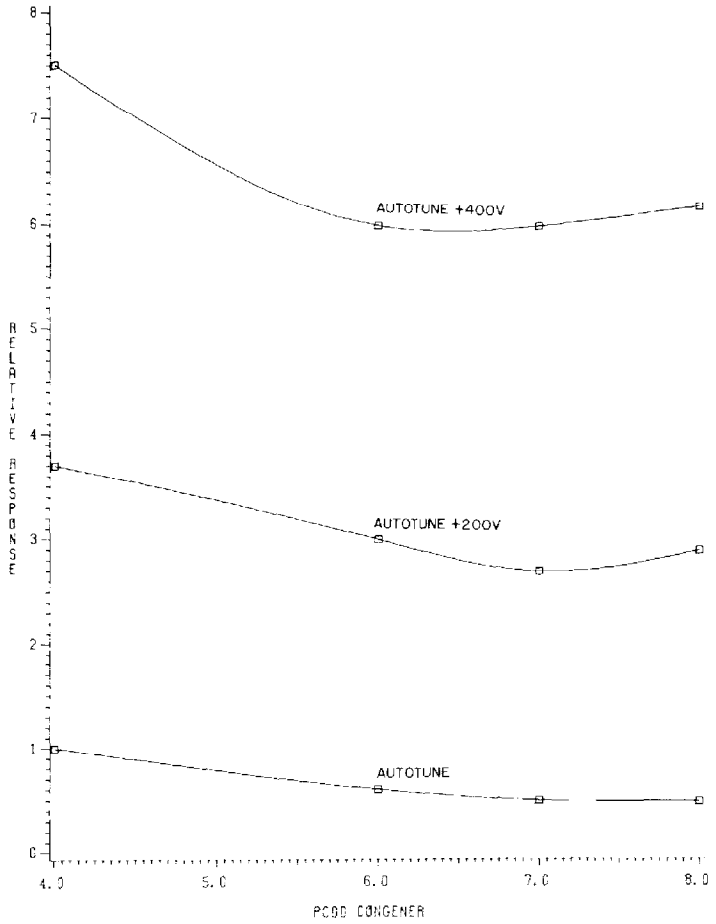

Fig. 3. Comparison of relative area response factors with variation in electron multiplier voltage setting for each PCDD congener. The numbers on the *x*-axis represent the number of chlorine substituents in the molecule.

TABLE II

RELATIVE AREA RESPONSE FACTORS WITH ELECTRON-MULTIPLIER VOLTAGE

All values are relative to TCDD at AUTOTUNE.

	AUTOTUNE	*AUTOTUNE+200V*	*AUTOTUNE+400V*
TCDD	1.0	3.7	7.5
H6CDD	0.6	3.0	6.0
H7CDD	0.5	2.7	6.0
OCDD	0.48	2.9	6.2

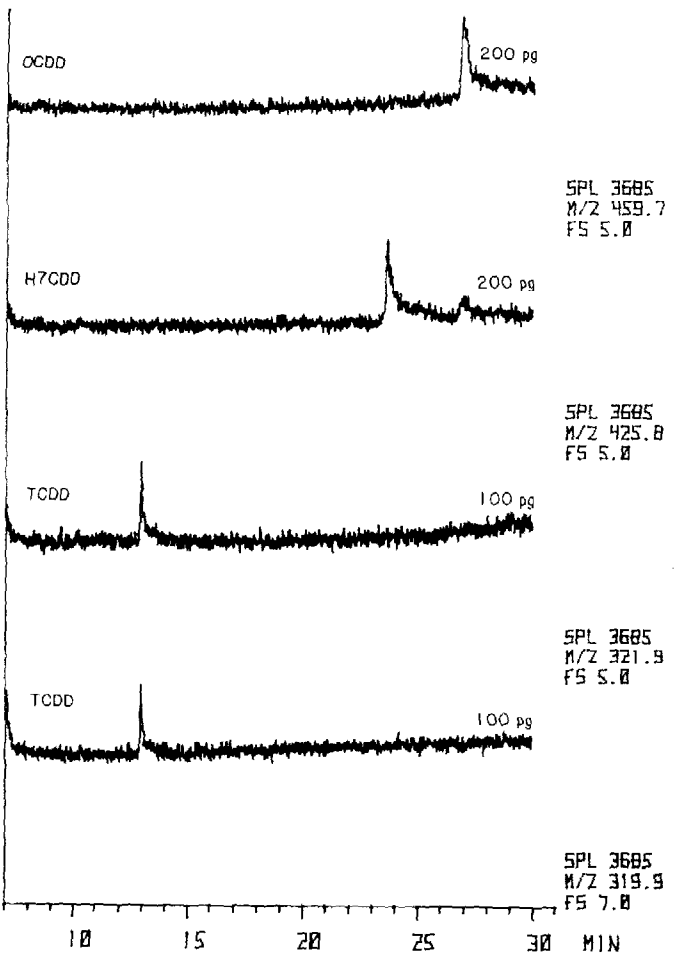

Fig. 4. SIM trace of 100 pg TCDD and 200 pg H7CDD and OCDD injected. This is determined to be the detection limit for these compounds on this system.

TABLE III

RESULTS OF PCDD CONCENTRATION (ng/g) WITH MULTIPLE EXTRACTS AND INJECTIONS WITH DIFFERENT COLUMNS

Values in parentheses represent the coefficients of variation.

	DB-5	*DB-1701*
TCDD	335 (22%)	300 (20%)
P5CDD*	400 (13%)	380 (22%)
H6CDD	690 (18%)	530 (21%)
H7CDD	490 (20%)	430 (17%)
OCDD	470 (21%)	470 (25%)

* Response factor used was interpolated from the curves in Fig. 2.

are shown in Fig. 2, describes a cubic-function curve which is very different from the data obtained using the system under investigation here. This demonstrates the variation in response factors which occurs using different instrumental systems.

From the curves obtained in Fig. 2, it is possible to interpolate a response factor for P5CDD which was unavailable as a pure standard. The response factors for P5CDD relative to TCDD were 0.73 on the DB-5 column and 0.85 on the DB-1701 column. The concentration of P5CDD can then be calculated in a sample using the appropriate factor. This can be done for any of the compounds and illustrates the importance of using a well characterized GC–MS system for accurate PCDD analysis.

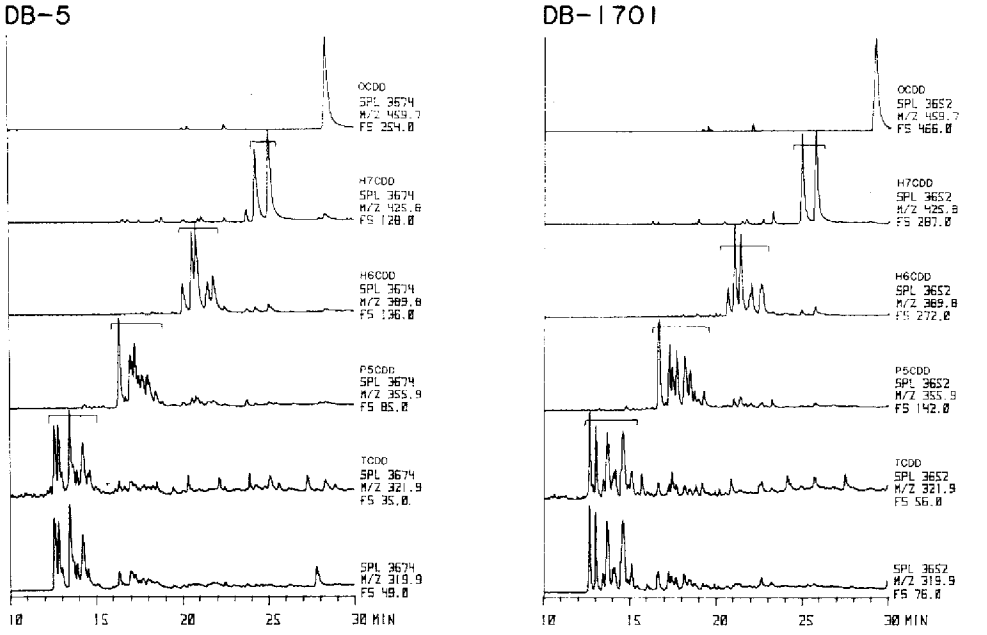

Fig. 5. Comparison of SIM traces for a Canadian fly-ash sample on DB-5 and DB-1701 columns under similar chromatographic conditions.

The relative response data were accumulated with an electron-multiplier voltage set at 200 V above that set by AUTOTUNE. An investigation was undertaken to determine the effect of varying this parameter on the quantitation of PCDDs. The PCDD standard was injected three times at each setting: AUTOTUNE, AUTOTUNE + 200 V and AUTOTUNE + 400 V. The mean values were plotted relative to the congeners at AUTOTUNE in Fig. 3 and listed in Table II. It can be seen that there is not much difference among the curves. This parameter does not affect the relative sensitivity of the instrument to PCDDs. Therefore, quantitation of PCDDs can be deduced to be independent of this variable.

The instrumental detection limit using a criterion of a signal-to-noise ratio of three was then determined for this system using the PCDD standard solution. The data are shown in Fig. 4 with two ions for TCDD and one for H7CDD and OCDD each. It was found that 100 pg/μl of TCDD and 200 pg/μl of H7CDD and OCDD could be distinguished using this criterion.

The total variation involved in extraction, work-up and analysis of a fly-ash sample which contains PCDDs was examined. A homogeneous sample was extracted in triplicate and each concentrated extract was analyzed in duplicate. These extracts were analyzed for PCDDs on both DB-5 and DB-1701 columns. The external standard method was used for quantitation of PCDDs, the reference standards of which were available, and an interpolated response factor was used for P5CDD as described previously. As seen in Table III the data correspond very well for all PCDDs on both columns, within experimental error. The average relative standard deviation is ascertained to be 19%. Since injection volume uncertainty accounts for 10%, then approximately a 9% variation can be attributed to extraction and work-up of one fly-ash sample.

The SIM traces in Fig. 5 are a comparison of the PCDD isomeric patterns obtained using each column. It is observed that although each column had the same efficiency, the DB-1701 column gave better resolution for TCDD and P5CDD isomers than the DB-5 column. This is not surprising because a more polar column has been shown to resolve PCDD isomers better than a non-polar one[10]. This had no apparent effect on the quantitation of the PCDDs as seen in Table II.

The use of relative response factors for determination of PCDD in a complex mixture has been shown to be a reliable method. PCDD quantitation can be independent of some GC–MS parameters with a particular system. It is important that these variables be assessed for each GC–MS system, to avoid analytical errors arising from instrumental parameters.

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