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METHODS OF ANALYSIS FOR POLYCHLORINATED DIBENZO-p-DIOXINS (PCDDs) AND POLYCHLORINATED DIBENZOFURANS (PCDFs) IN BIOLOGICAL MATRICES - LITERATURE REVIEW AND PRELIMINARY RECOMMENDATIONS

METHODS OF ANALYSIS FOR POLYCHLORINATED DIBENZO-p-DIOXINS (PCDDs) AND POLYCHLORINATED DIBENZOFURANS (PCDFs) IN BIOLOGICAL MATRICES - LITERATURE REVIEW AND PRELIMINARY RECOMMENDATIONS

by

John S. Stanley

TASK 6
FINAL REPORT
February 16, 1984

EPA Prime Contract No. 68-01-5915
MRI Project No. 4901-A(6)

For

U.S. Environmental Protection Agency
Office of Toxic Substances
Field Studies Branch, TS-798
Washington, DC 20460

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PREFACE

This report presents a literature review of the analytical methods used for the measurement of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in human adipose tissue. Also included in this report are recommendations from a meeting of scientists recognized for their efforts in PCDD and PCDF analyses held April 27th and 28th at MRI. This work was accomplished on MRI Project No. 4901-A, Task 6, "Planning Survey and Analysis Projects," for the U.S. Environmental Protection Agency (EPA Prime Contract No. 68-01-5915). The review was conducted and the document prepared by Dr. John S. Stanley, with assistance from Jerry Hurt, Barbara Mitchell, Kathy Funk, Lanora Moore, Cindy Melenson, Carol Shaw, Gloria Sultanik, Judy Daniels and Mary Walker. MRI would also like to thank the people listed in Appendix A for their cooperation, as well as David Redford, Madeline O'Neill-Dean and Daniel Heggem of FSB/OTS, EPA.

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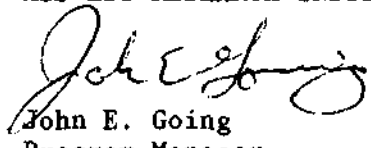

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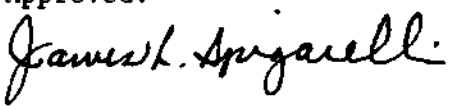

James L. Spigarelli, Director
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LIST OF TERMS, ABBREVIATIONS, AND SYMBOLS

Accuracy	Closeness of analytical result to "true" value.
AOAC	Association of Official Analytical Chemists.
Congener	One of 75 PCDDs or 135 PCDFs, not necessarily the same homolog.
DDE	1,1,-Dichloro-2,2-bis(p-chlorophenyl)-ethylene.
DDT	1,1,1-Trichloro-2,2,-bis(p-chlorophenyl)-ethane.
2,4-D	2,4-Dichlorophenoxyacetic acid.
ECD	Electron capture detector.
EI	Electron impact ionization (mass spectrometry).
EIMS	Electron impact mass spectrometry.
FID	Flame ionization detector.
GC	Gas liquid chromatography (column type unspecified).
GC/MS	Gas liquid chromatography/mass spectrometry (ionization mode unspecified).
HCDD	Hexachlorodibenzo-p-dioxin.
HpCDD	Heptachlorodibenzo-p-dioxin.
Homolog	One of the eight degrees of chlorination of PCDDs and PCDFs.
HPLC	High performance liquid chromatography.
HRGC	High resolution gas chromatography, glass or fused silica.

HRMS	High resolution electron impact mass spectrometry.
Internal standard	Standards used expressly for quantitation added to sample extract immediately prior to the analytical determination. Internal standards are used for PCDD and PCDF analyses to accurately measure recoveries of spiked surrogate compounds.
Isomer	One of up to 22 PCDDs or 38 PCDFs possessing the same degree of chlorination (1,2,3,4-TCDD and 2,3,7,8-TCDD are different isomers).
KOH	Potassium hydroxide.
LOD	Lower limit of detection (see also MDL). Lowest concentration at which an analyte can be identified as present in a sample at a stated statistical confidence level.
LOQ	Lower limit of quantitation. Lowest concentration to which a value can be assigned at a stated statistical confidence level.
LRMS	Low resolution mass spectrometry.
MDL	Method detection limit.
Mean	Arithmetic mean.
MS	Mass spectrometry.
m/z	Mass-to-charge ratio.
NRCC	National Research Council of Canada.
OCDD	Octachlorodibenzo-p-dioxin.
PCB	Polychlorinated biphenyl.
PCDD	Polychlorinated dibenzo-p-dioxin (including monochlorodibenzo-p-dioxins).
PCDF	Polychlorinated dibenzofuran (including monochlorodibenzofuran).
PGC	Packed column gas liquid chromatography.
ppb	Parts per billion (1×10^{-9} g/g, ng/g).
ppm	Parts per million (1×10^{-6} g/g, µg/g).

ppt	Parts per trillion (1×10^{-12} g/g, pg/g).
Precision	Reproducibility of an analysis, measured by standard deviation (SD) of replicates.
QA	Quality assurance. An organization's program for assuring the integrity of data it produces or uses.
QC	Quality control. The specific activities and procedures designed and implemented to measure and control the quality of data being produced.
RP-HPLC	Reverse phase high performance liquid chromatography.
RSD	Percent relative standard deviation (SD/mean x 100).
SD	Standard deviation.
Sensitivity	The slope of instrument response with respect to the amount of analyte. Also used colloquially to refer to lowest detectable amount of analyte.
SIM	Selected ion monitoring (also mid or mass fragmentography).
Surrogate	Standard compounds added to the sample prior to any analytical manipulations for the express purpose of measuring recovery through extraction, cleanup, etc., and to provide true internal standard quantitation.
TCDD	Tetrachlorodibenzo-p-dioxin.
$^{13}C_{12}$-TCDD	Carbon-13 stable isotope labeled TCDD.
$^{37}Cl_4$-TCDD	Chlorine-37 stable isotope labeled TCDD.
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid.

SECTION 1

SUMMARY

The published literature on polychlorinated dibenzo-p-dioxins (PCDDs) analyses for biological matrices is reviewed. The analytical methods are discussed for sample extraction, cleanup, and instrumental analysis.

This report also presents a synopsis of a discussion meeting concerning the analysis of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) held at Midwest Research Institute (MRI) on April 27 and 28, 1983. The primary objective of this meeting was to define the needs of an analytical method for the analysis of PCDDs and PCDFs in human adipose tissue. This method will be used in the future for population studies.

Several major programs were identified as necessary to achieve these goals. These included (a) the need for establishing a repository of PCDD/PCDF standards of known quality; (b) the organization and implementation of a strong quality assurance program; (c) the acquisition of sufficient human adipose tissue to generate a homogeneous sample matrix for the QA program; (d) independent studies of extraction procedures using bioincurred radiolabeled PCDDs; (e) intralaboratory ruggedness testing of a proposed analytical method; and (f) interlaboratory evaluation of the proposed method. Simultaneous activity in several of these areas is necessary in the coming months.

SECTION 2

INTRODUCTION

Polychlorinated dibenzodioxins (PCDDs) are a series of compounds with varying chlorine atom substitution on the dibenzo-p-dioxin parent compound. Table 1 presents the 75 possible positional isomers distributed from monochloro- to octachlorodibenzo-p-dioxin. The dioxin considered to be most toxic is the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

The potential long-term consequences of exposure to PCDDs, particularly 2,3,7,8-TCDD, are an issue of increasing public concern. Highly intense analytical and toxicological investigations have been conducted in recent years as a result of the presence of TCDD as an unexpected contaminant in the defoliant, Agent Orange, which is a formulation of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4-dichlorophenoxyacetic acid (2,4-D), and related ester herbicides. Also, the accidental release of TCDD from a factory near Seveso, Italy, the discovery of TCDD contaminated soil in Missouri, and the indication that PCDDs are emitted from numerous combustion sources have generated a demand for highly sensitive and specific analytical measurements for these contaminants in a wide spectrum of matrices. Table 2 presents some of the highly diverse sample matrices that have been analyzed for PCDDs, particularly for 2,3,7,8-TCDD.

The need to determine PCDDs in these diverse matrices has resulted in the development of a number of well-documented approaches to analysis. Although the exact approaches vary between laboratories, the basic requirements of all methods include quantitative extraction, efficient cleanup and separation from the bulk of the sample matrix and chlorinated compounds that might act as interferences, and sensitive and specific methods of instrumental analysis. The early work of Baughman and Meselson (1973) has been refined and expanded to accommodate complex matrices and to achieve detection at parts per trillion levels in numerous samples.

The overall objective of this review and preliminary method recommendation is to assist the EPA's Office of Toxic Substances (OTS) in proposing an analytical method for PCDDs in human adipose tissue in conjunction with the Veterans Administration's (VA) Agent Orange study. The Field Studies Branch of EPA/OTS has for many years been directly involved with the EPA's National Human Monitoring Network. The Network has adipose specimens archived which may provide evidence of exposure to Agent Orange. Part of the overall plan is (a) the identification of specimens for which exposure can be documented, and (b) the analysis of those specimens for evidence of exposure. The second part of the study is addressed in this document.

TABLE 1. MOLECULAR FORMULA, MOLECULAR WEIGHT, AND NUMBER OF ISOMERS OF PCDD

Chlorinated dibenzo-p-dioxin	Molecular formula	Total number of isomers
Monochloro (MCDD)	$C_{12}H_7ClO_2$	2
Dichloro (DCDD)	$C_{12}H_6Cl_2O_2$	10
Trichloro (T_3CDD)	$C_{12}H_5Cl_3O_2$	14
Tetrachloro (TCDD)	$C_{12}H_4Cl_4O_2$	22
Pentachloro (P_5CDD)	$C_{12}H_3Cl_5O_2$	14
Hexachloro (HCDD)	$C_{12}H_2Cl_6O_2$	10
Heptachloro (HpCDD)	$C_{12}HCl_7O_2$	2
Octachloro (OCDD)	$C_{12}Cl_8O_2$	1

TABLE 2. SAMPLE TYPES ANALYZED FOR TCDD

Human milk	Water, soil and sediment[a]
Human adipose tissue[a]	Workplace air samples
Beef liver[a]	Fly ash samples
Beef adipose tissue[a]	Gasoline and diesel automobile exhaust
Beef blood	Chemical products[a]
Wildlife samples - deer, elk, shrew, etc.[a]	Chemical process streams[a]
Fish[a]	Municipal incinerator[a]

Source: Harless, R. L., and R. G. Lewis, "Quantitative Determination of 2,3,7,8-Tetrachlorodibenzo-p-dioxin Residues by Gas Chromatography/Mass Spectrometry," in Chlorinated Dioxins and Related Compounds. Impact on the Environment, O. Hutzinger, R. W. Frei, E. Merian, and F. Pocchiari (Eds.), Pergamon Press, 1982, pp. 25-36.

a 2,3,7,8-TCDD residues were confirmed and quantified. Presence of other TCDD isomers confirmed in various samples.

This report reviews methods used for analysis of PCDDs in biological matrices. Section 3 describes the literature review procedures. Analytical methods are reviewed in Section 4 in terms of sample preparation, extraction, instrumental analysis, quantitation, and quality assurance. The advantages of specific methodologies, the purpose of specific steps, and the limitations of the particular technology are discussed. Section 5 presents a synopsis of a meeting held at MRI to discuss analytical approaches to the analysis of human adipose for PCDDs and PCDFs. Section 5 also provides recommendations for identifying an analytical method and organization of major program areas for method validation and sample analyses. Appendix A provides a list of persons who provided peer reviews and were invited to attend the meeting held at MRI. Appendix B provides the schedule of discussion topics for that meeting. Appendix C is a bibliography of references compiled and reviewed for this literature review.

SECTION 3

LITERATURE ACQUISITION AND REVIEW PROCEDURE

This section describes how the published literature on analytical techniques for PCDDs in biological matrices was reviewed and presents in tabular form some suggested criteria for rating published methods.

SOURCES OF INFORMATION

Computerized and manual searches and relevant references in recent articles were used. Also, many documents not available in the open literature were obtained from the working files of MRI scientists professionally involved in PCDD research. Recent issues of several key journals (Analytical Chemistry, Journal of Chromatography, Journal of the Association of Official Analytical Chemists, Environmental Science and Technology) were searched manually to pick up any recent references not yet in the computer data bases. In addition, several leading scientists (Appendix A) were called to discuss analytical approaches. In these discussions, they were asked to send copies or give references to any recent publications or preprints.

The computer searches were done using DIALOG. Chemical Abstracts (CA) files were searched back to 1978, printing all references containing "polychlorinated dibenzo-p-dioxin," "PCDD," "TCDD," CAS registry numbers, and synonyms and keywords beginning with the following notations: "anal," "detn," "quant," "measure," "tissue," "milk," "adipose" and "biol." A similar search was performed on the National Technical Information Service data base (including Smithsonian Science Information Exchange) and the Toxline data base.

Once the primary search data had been reviewed, it became apparent that several authors were of primary interest and all of their recent (1980 to 1983) publications were retrieved by a CA name search. These authors included H. Buser, W. Crummet, A. Dupuy, M. Gross, R. Harless, L. Lamparski, T. Nestrick, C. Rappe, D. Stalling, T. Tiernan, H. Tosine, and A. Young.

References contained in primary literature and review articles were also checked to assure that no important articles had been missed by the computer search. Several articles were added to the files by these searches.

REVIEW PROCEDURE

All articles cited in the bibliography of this document were surveyed for relevant analytical details. The salient features of each article were noted and any key subject areas were listed. Each citation was cross filed in applicable key subject areas such as extraction, cleanup, HRGC/MS, method validation, interlaboratory study, etc.

The analytical methodologies for the analysis of PCDDs have previously been reviewed by several authors (Harless, 1977; Firestone, 1978; McKinney, 1978; Hass and Friesen, 1979; Buser, 1980; Cairns et al., 1980; Esposito et al., 1980; Baker, 1981; NRCC, 1981; Fishbein, 1982; Karasek, 1982; Mahle and Shadoff, 1982; Tiernan, 1983). Although these reviews were directed principally toward the final measurements with mass spectrometry, they contained a wealth of information in terms of consolidated analytical results and method performance data.

The National Research Council of Canada (NRCC, 1981) and Mahle and Shadoff (1982) have directed attention to complete analytical methods. The NRCC rated analytical methods current to 1981 by the criteria listed in Table 3. None of the techniques reviewed by NRCC received the highest point rating since no method had been fully evaluated through collaborative testing. Mahle and Shadoff (1982), on the other hand, rated methods from low to high with respect to the technical aspects of extraction and cleanup, separation of isomers, and detection and quantitation. Table 4 is an example of the rating scheme reported by Mahle and Shadoff (1982).

TABLE 3. CRITERIA FOR RATING PUBLISHED PCDD ANALYTICAL METHODS

Point rating	Essential elements
1 (highest)	Complete quality assurance as described by ACS (1980). An ideally developed, evaluated method including collaborative studies.
2	Isomer specific, extensive recovery studies, interferences removed and separation achieved through extensive chemical workup; lacks collaborative evaluation and assumes confirmation.
3	Incompletely isomer specific, some recovery studies, interferences partially removed and partial separation achieved through chemical workup; lacks collaborative evaluation and assumes confirmation.
4	Essentially a screening method for most homologs, interferences partially removed and partial separation through limited chemical workup; lacks collaborative evaluation and assumes confirmation.
5	Same as 4, except inadequately documented for recovery, cleanup, etc.
6	Insufficient for the present state of the art.

Source: National Research Council of Canada, "Polychlorinated Dibenzo-p-dioxins: Limitations to the Current Analytical Techniques," NRCC No. 18576, ISSN 0316-0114 (1981).

TABLE 4. RELATIVE EFFICIENCY OF VARIOUS METHODS USED AT EACH STAGE OF ANALYSIS

Method[a]	Description
	Stage I: sample preparation
L	Chemical treatment and/or extraction without chromatography
M	L + column chromatography
H	M + HPLC
	Stage II: sample introduction
L	No gas chromatography (direct probe)
M	Packed column GC
H	Capillary column GC
	Stage III: mass spectrometry
L	Low resolution (300-2000)
M	Medium resolution (> 2000-9000)
H	High resolution (> 9000)

Source: Mahle, N. H., and L. A. Shadoff, "The Mass Spectrometry of Chlorinated Dibenzo-p-dioxins," Biomedical Mass Spectrometry, 9:45-60 (1982).

a L = Low, M = medium, H = high.

SECTION 4

ANALYTICAL METHODS - A REVIEW

The analytical methods applicable to the measurement of PCDDs in biological matrices are discussed in this section. The quality and limitations of the applicable methods are frequently documented by referral to data published in the literature. Most of the methods reviewed in this section allow the simultaneous analysis of polychlorinated dibenzofurans (PCDFs) and PCDDs in biological matrices.

EXTRACTION

Reliable PCDD analyses begin with the quantitative extraction of the analytes from the sample matrix. In general, the extraction method is dependent on the sample type and the complexity of the matrix. Extraction methods used in preparing biological samples have included neutral extractions, alcoholic potassium hydroxide saponifications, and acidic digestions followed by transfer of the PCDDs into an organic solvent such as hexane, methylene chloride, or petroleum ether.

Neutral extraction of fatty tissues, liver, and milk have been reported in several studies. The procedures begin with homogenization of the tissues with anhydrous sodium sulfate (Na_2SO_4) in ratios of 1 part tissue to 4-10 parts Na_2SO_4. The resulting dry mixture can then be Soxhlet extracted, packed into a chromatography column and eluted, or it can be blended directly with an organic solvent. Ryan et al. (1980), Albro and Corbett (1977), and Hass et al. (1978) have blended liver samples directly with chloroform and methanol, then subsequent back extracted with aqueous solutions. O'Keefe et al. (1978) have used an approach that consists of rendering the fatty sample and dissolving it in hexane. Shadoff (1980) has reported the used of a cellulose gauze to absorb the fat content of human milk samples as the first step in analyzing human milk samples for 2,3,7,8-TCDD. The cellulose gauze with the adsorbed milk sample was extracted with hexane under refluxing conditions. An additional neutral extraction procedure has been described by DeRoos et al. (1982). High pressure liquid carbon dioxide extraction of fish samples proved to be quantitative for samples (5 g) spiked at 20 to 200 parts per trillion of 2,3,7,8-TCDD.

The saponification of fatty tissues with alcoholic KOH preceding the extraction of PCDDs from the matrix with an organic solvent evolved from the early work of Baughman and Meselson (1973). Modifications of this procedure have been used for preparation of most samples for analysis for 2,3,7,8-TCDD under the Dioxin Monitoring Program (DMP). The digestion carried out under the reflux conditions as presented by Baughman and Meselson (1973), however,

may lead to the destruction of the higher chlorinated homologs of the PCDDs. Table 5 presents the estimated half-lives ($t_{\frac{1}{2}}$) of several PCDD compounds including the hexa-, hepta-, and octachloro-homologs, with no sample matrix in refluxing KOH solution. As indicated on Table 5, the concentrations of the octa- and heptachlorinated homologs are significantly reduced during the recommended 1.5- to 2-hr reflux step.

TABLE 5. ESTIMATED HALF-LIVES ($t_{\frac{1}{2}}$) OF SEVERAL DIOXINS IN REFLUXING KOH SOLUTION[a]

Dioxin[b]	$t_{\frac{1}{2}}$
1,2,3,6,7,8- and 1,2,3,7,8,9-HCDD	7 hr
1,2,4,6,7,9- and 1,2,3,4,7,8-HCDD	2 hr
1,2,3,4,6,7,8-HpCDD	23 min
1,2,3,4,6,7,9-HpCDD	16 min
1,2,3,4,6,7,8,9-OCDD	4.5 min

Source: Firestone, D., JAOAC, 60:354-356, 1977. Report on Oils and Fats

a Ten to 40 ng dioxin refluxed gently with 50 ml 32% aqueous KOH solution and 20 ml ethanol.

b HCDD = hexachlorodibenzo-p-dioxin; HpCDD = heptachlorodibenzo-p-dioxin; OCDD = octachlorodibenzo-p-dioxin.

Lamparski et al. (1978) have studied this effect in somewhat greater detail. Table 6 presents data for the decomposition of hexa- (HCDD) and octachlorodibenzo-p-dioxin (OCDD) based on the effects of KOH concentration, time, and digestion temperature. These data were generated during a study of the determination of pentachlorophenol, hexa- and octachlorodibenzo-p-dioxin in bovine milk. As can be seen from these data, lengthy periods of digestion at elevated temperatures will drastically reduce HCDD and OCDD concentrations. To avoid this problem, a less alkaline digestion matrix or shaking at room temperature rather than refluxing has been used to prepare samples for extraction.

TABLE 6. EFFECT OF POTASSIUM HYDROXIDE CONCENTRATION, TIME, AND TEMPERATURES ON POLYCHLORODIBENZO-p-DIOXIN STABILITY

Temperature digestion, °C	Digestion time, h	Percent KOH concentration	HCDD		OCDD	
			Initial concentration, ppb	Percent decomposition	Initial concentration, ppb	Percent decomposition
22	24	20	1	14	1	44
35	24	20	1	54	1	72
60	24	20	1	72	1	> 95
80	24	20	1	> 95	1	> 95
22	1	4	0.1[a]	10[a]	0.1[a]	10[b]
22	2	4	0.1	10[b]	0.1	10[b]

Source: Lamparski, L. L., N. H. Mahle, and L. A. Shadoff, "Determination of Pentachlorophenol, Hexachlorodibenzo-p-dioxin, and Octachlorodibenzo-p-dioxin in Bovine Milk," J. Agric. Food Chem., 26:1113-1116 (1978).

a Lower detection limits are possible because no sample matrix is present.

b These values are reported to one significant figure.

Tiernan and Taylor (1983, personal communication) have provided additional data reflecting that saponification at elevated temperatures also provided degradation of OCDD in beef adipose tissue. Aklaline conditions at room temperature (22°C) with shaking (12 hr) provided complete digestion of liver tissue with quantitative recovery of a chlorine-37 labeled OCDD internal standard. These researchers, however, point out that heating was necessary for complete digestion of the beef adipose tissue.

Langhorst and Shadoff (1980) and Tosine et al. (1982, 1983) have provided the only published reports on the use of acid digestion of a biological sample matrix prior to the determination of PCDDs. Langhorst and Shadoff (1980) reported that 30-g samples of human milk were digested with 200 ml of concentrated HCl prior to solvent extraction. The advantage of the extraction procedure is that it eliminates the caustic digestion that affects the stability of the higher chlorinated dioxins. The reported recoveries of stable isotope-labeled PCDDs from spiked milk homogenates ranged from an average of 36% for the tetra- to 78% for the hexachlorodibenzo-p-dioxin. Validation data for reagent blanks were also presented and recoveries varied from an average of 34% for the tetra-, to 85% for the hexa-, to 31% for the octachlorodibenzo-p-dioxin. However, it is not clear from the data presented what effect the concentrated HCl digestion had on the recovery of these components.

Regardless of the exact extraction procedure employed, the reliability of the data in most of the studies has been supplemented by the repeated recovery of surrogate compounts spiked into the sample prior to extraction. Typically, carbon-13 or chlorine-37 stable labeled PCDDs were added at concentration levels 10 to 100 times higher than the analytical method limit of detection.

In summary, three methods for the extraction of PCDDs from biological matrices have been reported, although there has been no study intended to address the advantages of one procedure over another. Brumley et al. (1981) have reported on six different extraction and cleanup procedures with one common instrument analysis approach for final analysis. However, this study lacks the specificity to identify differences arising from the various extraction techniques since all sample preparations were completed with different cleanup steps. Thus, the need remains to evaluate the three extraction procedures with a common sample source followed by a consistent cleanup procedure and final analysis.

One possibility for determining the true extraction efficiency of PCDDs and PCDFs in adipose tissue with any of the three procedures will require the use of bioincurred radiolabeled compounds. Radiolabeled PCDD and PCDF compounds are used to provide a measurement independent from GC/MS techniques. This approach to study the extraction mechanism was proposed recently at MRI during a meeting to discuss approaches to the analysis of human adipose differences for PCDDs and PCDFs.

CLEANUP

The effective separation of PCDDs from materials coextracted from the sample matrix has required a combination of efficient cleanup techniques. The cleanup methods used for isolating PCDDs have been developed by several analysts. Table 7 is a summary of cleanup procedures used for biological matrices. The cleanup procedures reviewed include acid and base washes, liquid-liquid partition, column chromatography with alumina, florisil, silica gel, chemically modified silica, and carbon impregnated foam. Reverse phase (RP) and normal high performance liquid chromatography (HPLC) have been used to remove interferences that are chemically similar to the PCDDs and to improve isomer specificity with the final instrument determination.

A large percentage of the lipid materials in tissue extracts are presumably sulfonated or saponified with the concentrated sulfuric acid or strong base washes. These procedures promote the degradation and hydrolysis of complex molecules including some pesticide residues. Many of the procedures listed in Table 7 used a concentrated sulfuric acid wash. Several of the methods followed the acid wash with a saponification step using a basic solution, typically 1N KOH. As can be seen from the data presented in Table 6, there should be little or no adverse effect of the base at this concentration on the stability of the hexa- through octachlorinated PCDD homologs. Some samples however have a tendency to form emulsions with a wash procedure.

The decision to use a chemically modified acid or base silica column is based on the analyst's experience. The advantages of using the impregnated column materials include less manipulation of samples, reduced exposure to active glass surface, and greater rate of sample turnover. The emulsion problem is not encountered with treated columns. However, the eluent flow from concentrated acid columns may become restricted due to impaction from precipitated or charred coextractives in samples with high concentrations of lipid and other oxidizable compounds. Langhorst and Shadoff (1980) have overcome this problem in human milk analyses by using a precolumn with a lower acid (22%) loading prior to the more concentrated acid (44%) column. The 22% acid column is a less effective reagent than the 44% acid column but is also less prone to plugging or reduced flow. The combination of these reagents was reported as quite successful.

Column chromatography following the acid/base extract treatment is used to separate PCDDs from chlorinated residues such as the organochlorine pesticides and polychlorinated biphenyls (PCBs). Alumina is the most widely used adsorbent material, as indicated in Table 7. Florisil and silica have been used in a few specific procedures as a means of separating bulk interferences preceding final separation with alumina columns. The final column chromatography step in many instances was accomplished using micro-columns of alumina (1.0 g) in disposable Pasteur pipettes. Harless et al. (1980) have used two such columns in sequence as the final cleanup step.

A 10% silver nitrate impregnated silica column has been used by Lamparski et al. (1979) preceding the final column for the analysis of fish. The silver nitrate column is effective for the removal of DDE, chlorinated aliphatic hydrocarbons, and sulfides. The basic alumina column in this sequence is used primarily to separate PCBs from the PCDD-containing fraction.

TABLE 7. A LISTING OF SOME CLEANUP PROCEDURES

Wash		Column chromatography								
Acid	Base	Acid	Base	Alumina	Florisil	Silica gel	Foam charcoal	RP HPLC	HPLC	Reference
+[a]				++[b]						Harless et al. (1980)
+				+	+					Harless et al. (1980)
							+	+	+	Mitchum et al. (1980)
+		+		+		+				Lamparski et al. (1978)
		+		++		$AgNO_3$		+		Lamparski et al. (1979)
		+		++	+					O'Keefe et al. (1978)
+	+			+	+					Firestone et al. (1979)
+				+		+				Mahle et al. (1977)
+	+			+						Baughman and Meselson (1973)
+				+	+					Phillipson and Puma (1980)
		+	+							Fanelli et al. (1980)
+		+	+	+		$AgNO_3$		+	+	Langhorst and Shadoff (1980)
		+		++		$AgNO_3$		+		Tosine (1981)
		+		++	+					Norstrom et al. (1981)
+				+		+				Hummel (1977)
+	+			+						Chess and Gross (1980)
	+	+		+		+				Buser (1978)
+										Baughman and Meselson (1971)
	+					+				Hummel (1977)
+								+		Ryan and Pilon (1980)
+				+						Haas et al. (1978)
				+						Haas et al. (1978)
+	+			+						Tiernan et al. (1980)
+		+								DiDomenico et al. (1979)
		+								DiDomenico et al. (1979)
						TLC				Levin and Nilsson (1977)
+				+						Albro and Corbett (1977)

Source: National Research Council of Canada (NRCC), "Polychlorinated Dibenzo-p-dioxins: Limitations to the Current Analytical Techniques," NRCC No. 18576, 1981, 172 pp.

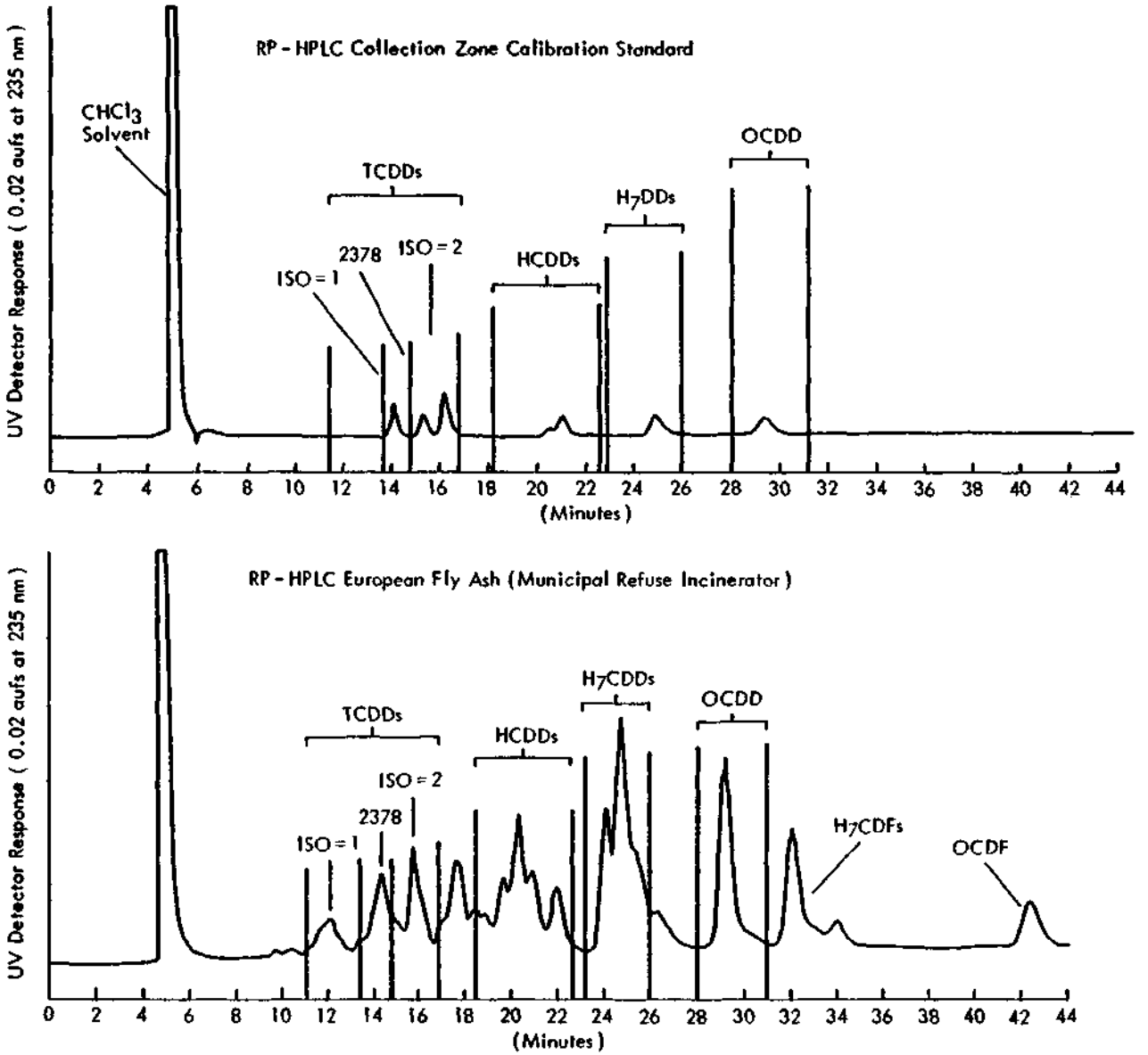
a + indicates used only as one step of the procedure.

b ++ indicates two separate columns were used.

The separation of PCDDs into fractions containing combinations of the various isomers prior to final instrumental analyses has been accomplished using reverse phase (RP) and/or normal HPLC technique. Lamparski et al. (1979) and Langhorst and Shadoff (1980) have used RP-HPLC cleanup to provide additional removal of contaminants (e.g., PCBs, DDE, phthalates) and to remove components that are very similar to dioxins, such as chlorinated benzylphenyl ethers. Specific fractions of the eluent from the RP-HPLC are collected for analysis of PCDDs by homolog. This approach is especially significant for studies that require data on low parts per trillion concentration levels for tetra- to octa-PCDD homologs. Typically, the low parts per trillion measurements require final concentration of sample extracts to 10-20 μl. Instrumental analysis for a specific PCDD homolog may consume a major portion of the extract, presenting difficulties if the need exists to include other PCDD homologs. The RP-HPLC separation of the sample extract as shown in Figure 1 allows collection of PCDDs by homolog, enabling the measurement of all PCDDs at low parts per trillion levels. This approach has been demonstrated by Langhorst and Shadoff (1980) for the analysis of tetra-, hexa-, hepta-, and octachlorodibenzo-p-dioxins in human milk. Langhorst and Shadoff (1980) have also used RP and normal silica HPLC for separation and identification of 2,3,7,8-TCDD from the other TCDD isomers in extracts from human milk.

Regardless of the specific cleanup procedure, the analyst must take precautions to ensure that adsorbents are fully activated and method blanks do not yield extracts with high backgrounds. Huckins et al. (1976) have reported on some contaminants and limitations of silica gel for the chromatographic separation of polychlorinated aromatics and pesticides. The data presented in this paper implicated the presence of sulfuric acid in silica gel as responsible for producing contaminants that interfered with the analysis. It is our experience that sulfuric acid modified silica gels and batch extractions with concentrated sulfuric acid generate contaminants that appear to be oxygenated compounds with aliphatic moieties. These artifacts can be removed by base modified silica gel, batch extraction with a base and/or the use of fully activated basic alumina.

As mentioned earlier, the approach to the determination of PCDDs in biological matrices is dependent on the experience of the analyst and the associated laboratory. The actual extraction and cleanup procedures practiced may differ markedly from one laboratory to another. In view of the variety of methods in use, a comparison of six different extraction and cleanup procedures was conducted by Brumley et al. (1981) with respect to the analysis of 2,3,7,8-TCDD in fish. The relative efficiency of the different methods was determined based on two criteria: (1) the relative number and amounts of undesired components present in the final extracts, and (2) the extent to which these components interfered with TCDD analysis. The objective of the study was to compare the overall efficiency of the six available analytical cleanup procedures using a common GC/MS (low resolution) analysis approach. Six fish samples were submitted to six participating laboratories including the Bureau of Foods, Food and Drug Administration (BF/FDA), Detroit District/FDA, Dow Chemical Company, the Environmental Protection Agency (EPA), Fish and Wildlife Service (FWS), and the New York State (NYS) Department of Health. The samples were prepared for TCDD analysis according to the procedure routinely



Source: Lamparski, L. L., and T. J. Nestrick, "Determination of Tetra-, Hexa-, Hepta-, and Octachlorodibenzo-p-dioxin Isomers in Particulate Samples at Parts per Trillion Levels," Anal. Chem., 52, 2045-2054 (1980).

Figure 1. RP-HPLC fractionation chromatograms of (a) calibration standard and (b) European refuse incineration fly ash demonstrating the application for collection of PCDDs by homolog.

used by each laboratory. All extracts were then analyzed by one laboratory using gas chromatography/mass spectrometry by selected ion monitoring (GC/MS-SIM), scanning GC/MS, and GC/ECD (electron capture detector).

This study did not evaluate the overall analytical method used by any of the participating laboratories. The results of the evaluation of the cleanup efficiency did not necessarily reflect upon the validity of TCDD analyses performed by the participating laboratories using these combined cleanup and MS procedures. Figures 2 to 6 are schematic representations of the extraction and cleanup procedures used by the different participating laboratories.

As part of this study, each laboratory was asked to specify the time and personnel requirements necessary for sample preparations. Table 8 is a summary of the resources required to extract and clean up fish samples. As indicated on Table 8, the EPA-neutral procedure and FWS carbon/dual column procedure provided the most rapid turnaround time. The methods that require the use of HPLC equipment were more labor intensive.

Figure 7 presents data from representative sample extracts prepared by the six laboratories, as determined by GC/ECD at the BF/FDA laboratory. The results indicate that the BF/FDA, Detroit District/FDA, Dow, and FWS sample preparations provide extracts that are significantly less complex than the other approaches. Further analysis by BF/FDA of the sample extracts using low resolution GC/MS-SIM yielded the data presented in Table 9. Twelve ions were monitored, including eight ions representative of the molecular ion cluster and the loss of COCl, two ions representative of the internal standard [$^{13}C_{12}$] TCDD, and two ions representative of possible interferences arising from tetrachloromethoxybiphenyl. Analysis of all 12 ion chromatograms for all six of the participating laboratories indicated that only the NYS, Dow, and FWS cleanup procedures provided sample extracts with no interference at the retention time of TCDD. The summary of results (Table 9) obtained by GC/MS-SIM indicate whether TCDD was confirmed in the sample and quantitation of observed responses for the appropriate ions.

Based on their findings, Brumley et al. (1981) placed the six extraction-cleanup procedures into four categories. The Dow and FWS procedures were in the first category because TCDD was confirmed and quantitated and the ion currents for the 12 ions monitored indicated that the extracts were free of interferences. The NYS procedure was placed in a second category since the overall levels of coextractants appeared to be significant. The FDA and EPA procedures comprised the third and fourth categories, respectively, because of excessive amounts of coextractive, greater than 100% recovery of the surrogates, and interferences appearing for the monitored ions.

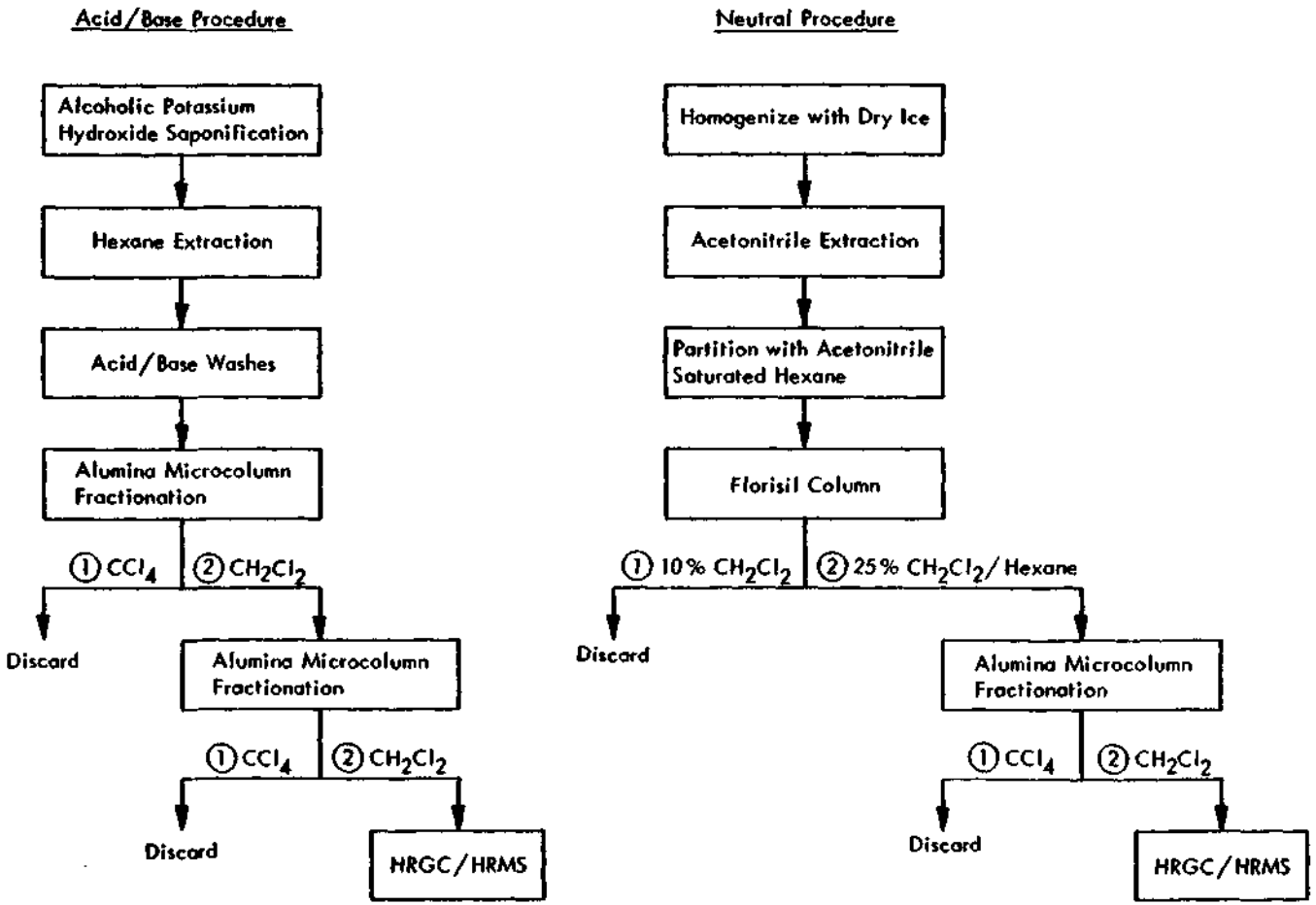


Figure 2. Schematic of EPA sample preparation procedures for preparation of biological matrices for TCDD analyses.

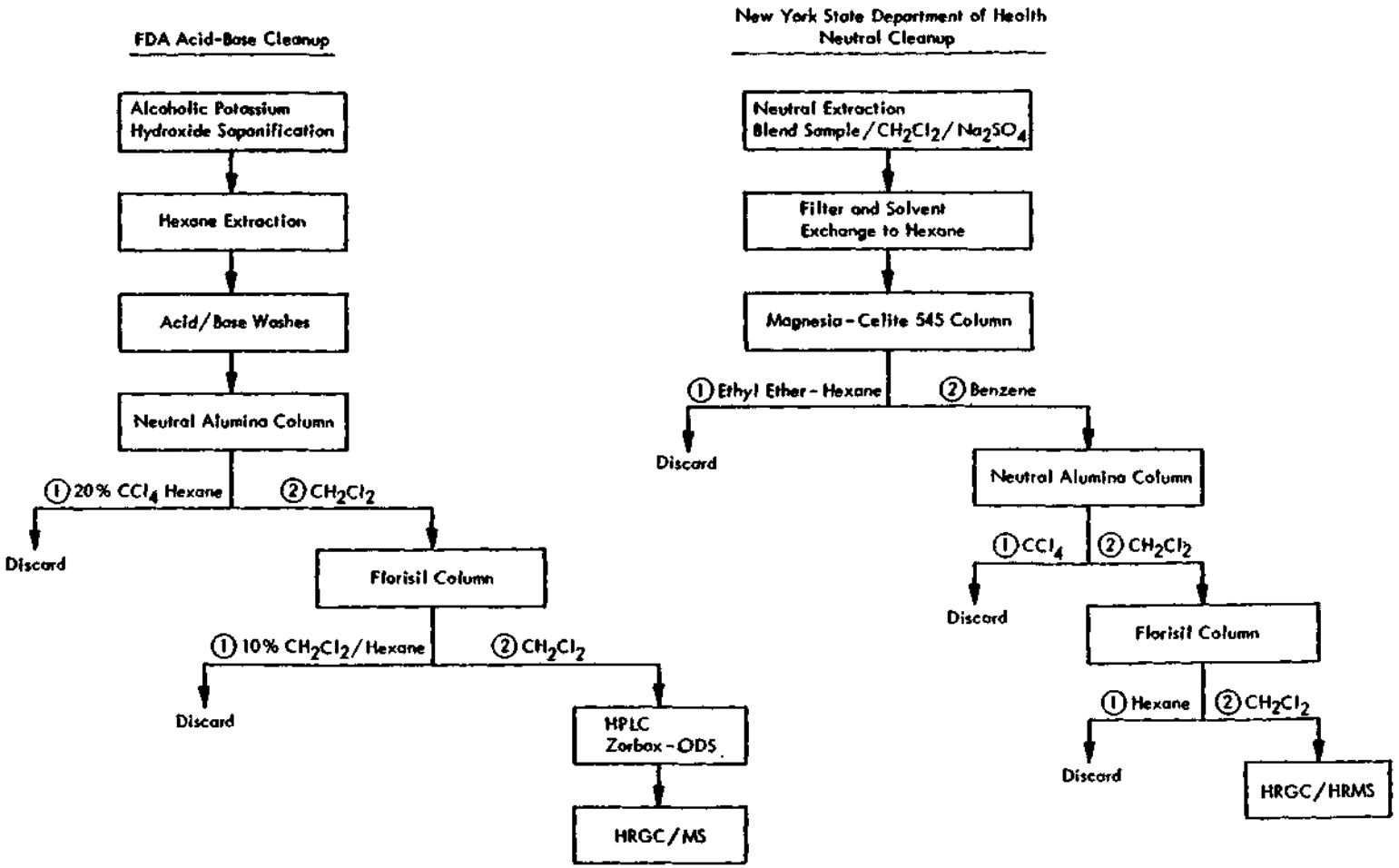
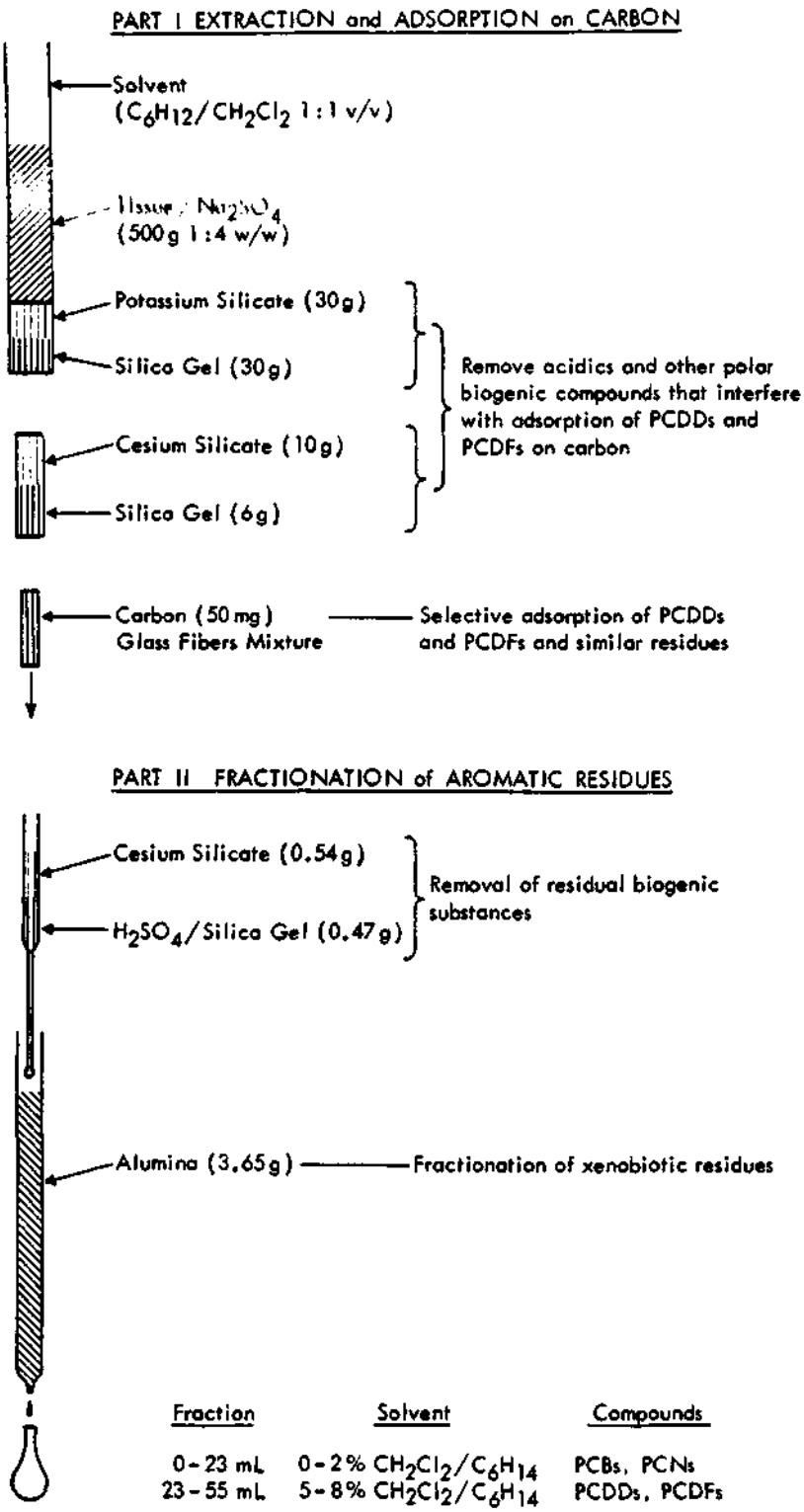
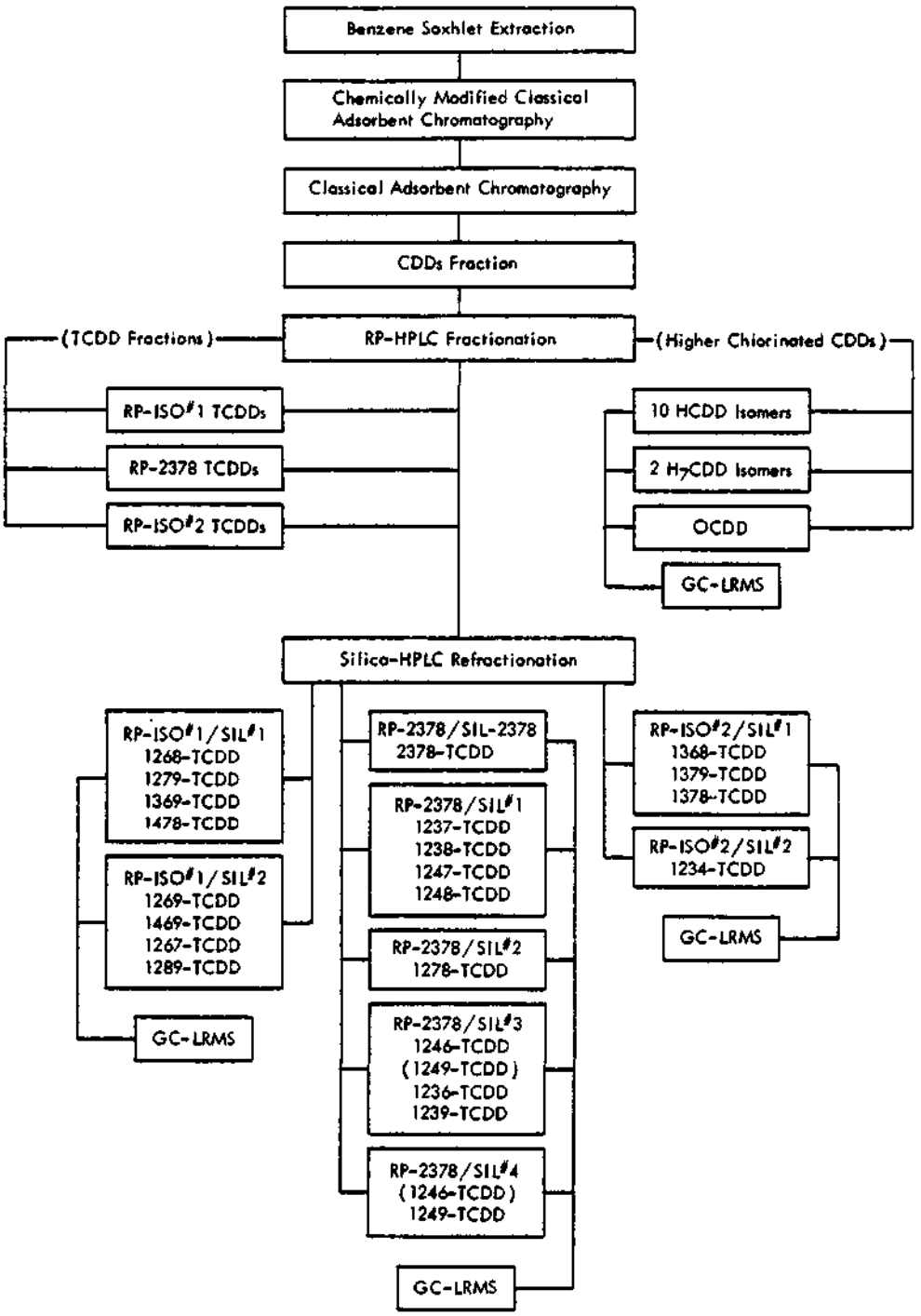


Figure 3. Schematic presentation of the sample preparation schemes used by FDA and laboratories and the New York State Department of Health in collaborative study of fish sample preparation and analysis.



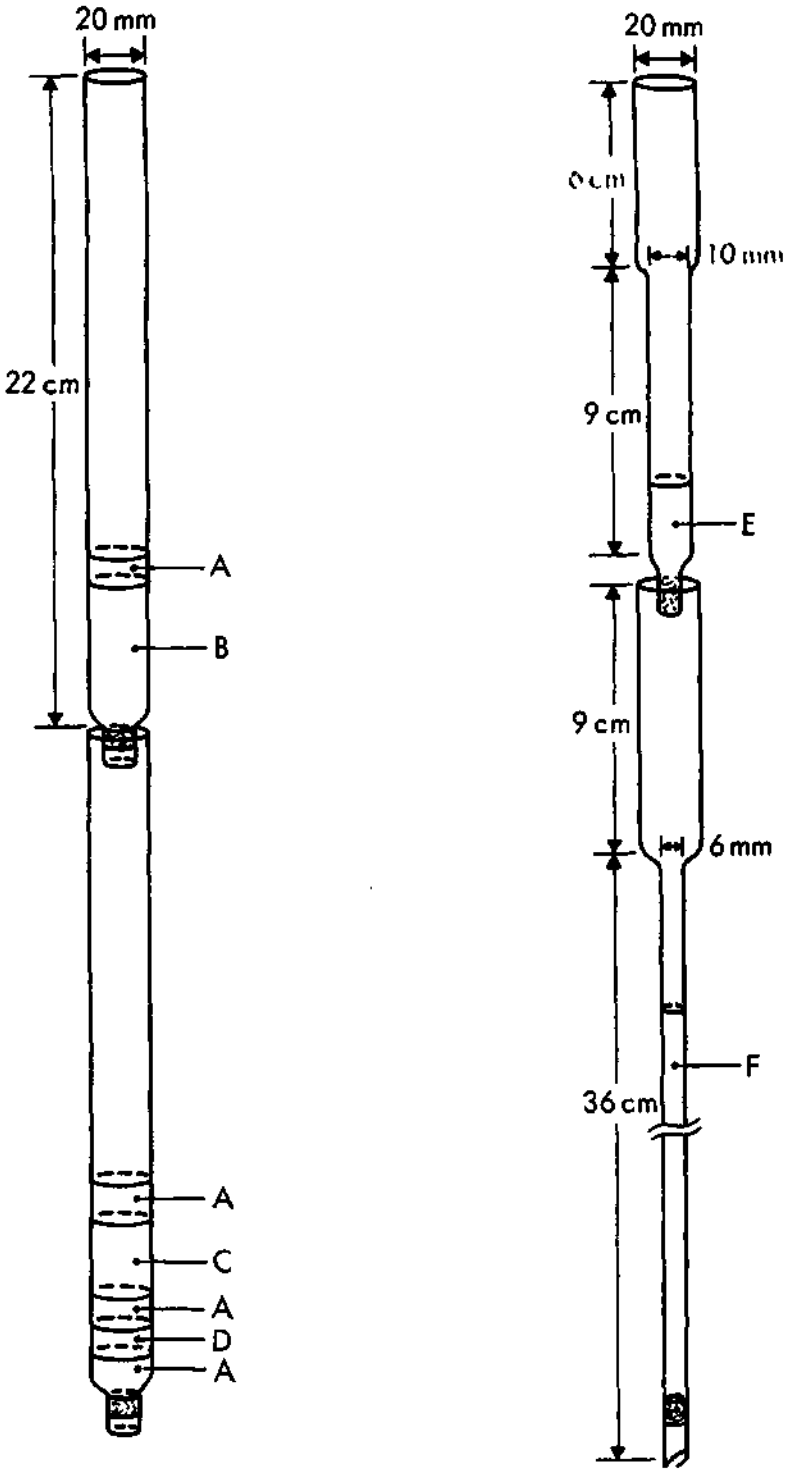
Source: Stalling, D. L., J. D. Petty, L. M. Smith, C. Rappe, and H. R. Buser, "Isolation and analysis or Polychlorinated Furans in Aquatic Samples," in Chlorinated Dioxins and Related Compounds: Impact on the Environment, O. Hutzinger, R. W. Frei, E. Merian, F. Peschari, Eds., Pergamon Press, 1982.

Figure 4. Flow diagram for enrichment and fractionation of PCDDs and PCDFs from tissue samples (FWS procedure).



Source: Lamparski, L. L., and T. J. Nestrick, "Determination of Tetra-, Hexa-, Hepta-, and Octachlorodibenzo-p-dioxin Isomers in Particulate Samples at Parts per Trillion Levels," Anal. Chem., 52, 2045-2054 (1980).

Figure 5. Schematic for sample preparation for PCDD analysis by the Dow analytical approach.



Source: Lamparski, L. L., and T. J. Nestrick, "Determination of Tetra-, Hexa-, Hepta-, and Octachlorodibenzo-p-dioxin Isomers in Particulate Samples at Parts per Trillion Levels," Anal. Chem., 52, 2045-2054 (1980).

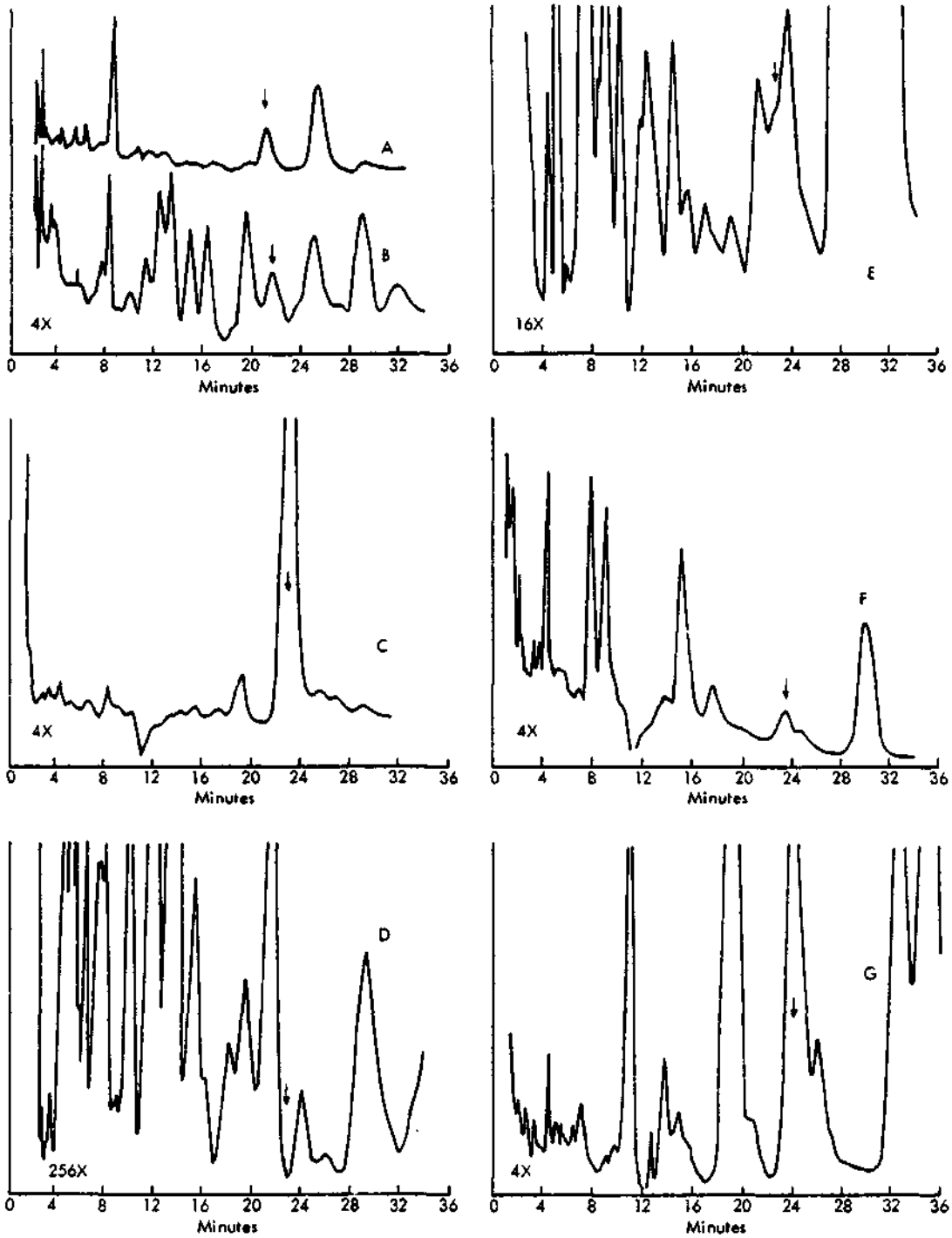
Figure 6. Glass chromatography columns for sample cleanups: A = silica, B = 22% sulfuric acid on silica, C = 44% sulfuric acid on silica, D = 33% 1 M sodium hydroxide on silica, E = 10% $AgNO_3$ on silica, and F = basic alumina.

TABLE 8. RESOURCES REQUIRED TO EXTRACT AND CLEAN UP FISH SAMPLES

Cleanup method	Analyst's per set	Number of samples per set	Extraction-cleanup time, h, per set[a]	Extraction-cleanup time, h, per sample per analyst
FDA acid/base HPLC	1	6	24	4
Dow dual-column/HPLC	2	4	16	8
EPA-A/B	2	4	8	4
EPA-Neutral	1	4	8	2
FWS carbon/dual column	1	6	20	3.3
NYS multicolumn	1	2	16	8

Source: Brumley, W. C., J. A. Roach, J. A. Sphon, P. A. Dreifuss, D. Andrzejewski, R. A. Niemann, and D. Firestone, "Low-Resolution Multiple Ion Detection Gas Chromatographic-Mass Spectrometric Comparison of Six Extraction-Cleanup Methods for Determining 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Fish," J. Agric. Food Chem., 29:1040-1096 (1981).

a Time required for one or two analysts (see second column) to extract and clean up a set of samples.



Source: Brumley, W. CL., J. A. Roach, J. A. Sphon, P. S. Dreifuss, D. Andrzejewski, R. A. Niemann, and D. Firestone, "Low-Resolution Multiple Ion Detection Gas Chromatographi-Mass Spectrometric Comparison of Six Extraction-Cleanup Methods for Determining 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Fish," J. Agric. Food Chem., 29, 1040-1046 (1981).

Figure 7. GC/ECD chromatograms of extracts from unfortified catfish (1/20 of the sample extract). (A) BF/FDA; (B) Det/FDA; (C) Dow; (D) EPA-A/B; (E) EPA-Neut; (F) FWS; (G) NYS. The arrows indicate the retention time of 2,3,7,8-TCDD, as determined by GC of a 2,3,7,8-TCDD standard solution.

TABLE 9. SUMMARY OF GC/MS-SIM RESULTS OF STUDY OF TCDD EXTRACTION-CLEANUP[a]

Sample no.	BF/FDA		DET/FDA		NYS		EPA-A/B		EPA-neut		Dow[b]		FWS	
	conf.	quant.	conf.	quant.	conf.	quant.	conf.	quant.	conf.	quant.	conf.	quant.	conf.	quant.
1	no	5	no	6	no	c	no	c	no	c	no	c	no	9
2	no	67	no	89	yes	77	no	c	no	c	yes	67	yes	47
3	no	34	no	42	yes	57	no	c	no	c	yes	25	yes	22
4	no	188	no	99	yes	128	d	d	no	c	yes	113	yes	117
5	e	e	no	53	yes	38	d	d	d	d	yes	45	yes	56
6	no	178	no	199	yes	107	d	d	d	d	yes	100	yes	96

Source: Brumley, W. C., J. A. Roach, J. A. Sphon, P. A. Dreifuss, D. Andrzejewski, R. A. Niemann, and D. Firestone, "Low-Resolution Multiple Ion Detection Gas Chromatographic-Mass Spectrometric Comparison of Six Extraction-Cleanup Methods for Determining 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Fish," J. Agric. Food Chem., 29:1040-1046 (1981).

a Confirmation of the identity of TCDD was obtained if the responses of the 12 monitored ions for the sample extract were consistent with the responses of the 12 monitored ions of the TCDD standard. Quantitation was based on the observed responses at m/z 322 and 334. Quantitation in nanograms per kilogram.

b Quantitation by the external standard because of the [^{13}C]TCDD carrier.

c No entry in original data presentation.

d Samples were not analyzed due to large amounts of coextractives.

e Some or all of the sample was lost.

INSTRUMENTAL ANALYSIS

The selection of the analytical methodology must take into account a wide concentration range of PCDDs and the possible interferences in different sample matrices. Figure 8 illustrates the detection ranges of analytical techniques that have been used for measurement of PCDDs. This figure presents techniques used in industrial quality control for relatively simple samples at the higher concentration range. Environmental and biological matrices require instrumental methods that have lower limits of detection to achieve parts per billion (nanograms/gram) and parts per trillion (picograms/gram) measurements. Although gas chromatography with electron capture detection (GC/ECD) is capable of low level measurements, the technique lacks the necessary specificity to positively identify PCDDs in a sample extract that contains other halogenated hydrocarbons, pesticides, PCBs, phthalates, etc.

Radioimmunoassay (Luster et al., 1980, 1981) and GC/MS-SIM are comparable with respect to achievable limits of detection. However, radioimmunoassay does not yield the identification of individual dioxins and has been used primarily for the screening of a large number of samples for the presence or absence of PCDDs. Two alternate screening techniques for the presence of PCDDs based on biological or biochemical properties are the hydrocarbon hydroxylase induction assay (Bradlaw and Casterline, 1979) and the cytosol receptor assay (Hutzinger et al., 1981). Since the bioanalytical methods do not provide the specificity necessary for identification of PCDDs, these techniques are not discussed in detail below. For a thorough discussion, see National Research Council of Canada (1981).

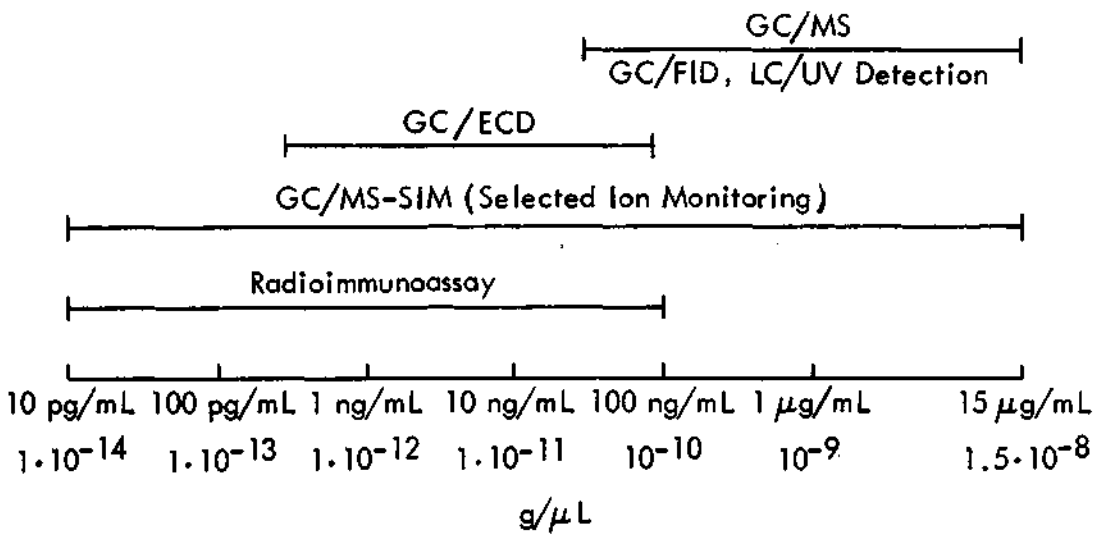
The analytical detection method most frequently reported for the measurement of PCDDs by homolog or by specific isomer in all sample types is gas chromatography combined with mass spectrometry (GC/MS).

Gas Chromatography

The final separation of PCDDs from interferences in the sample extract requires gas chromatography with either packed or capillary columns (HRGC). The NRCC (1981) has compiled a listing of column lengths and liquid phases used for specific and general PCDD analyses.

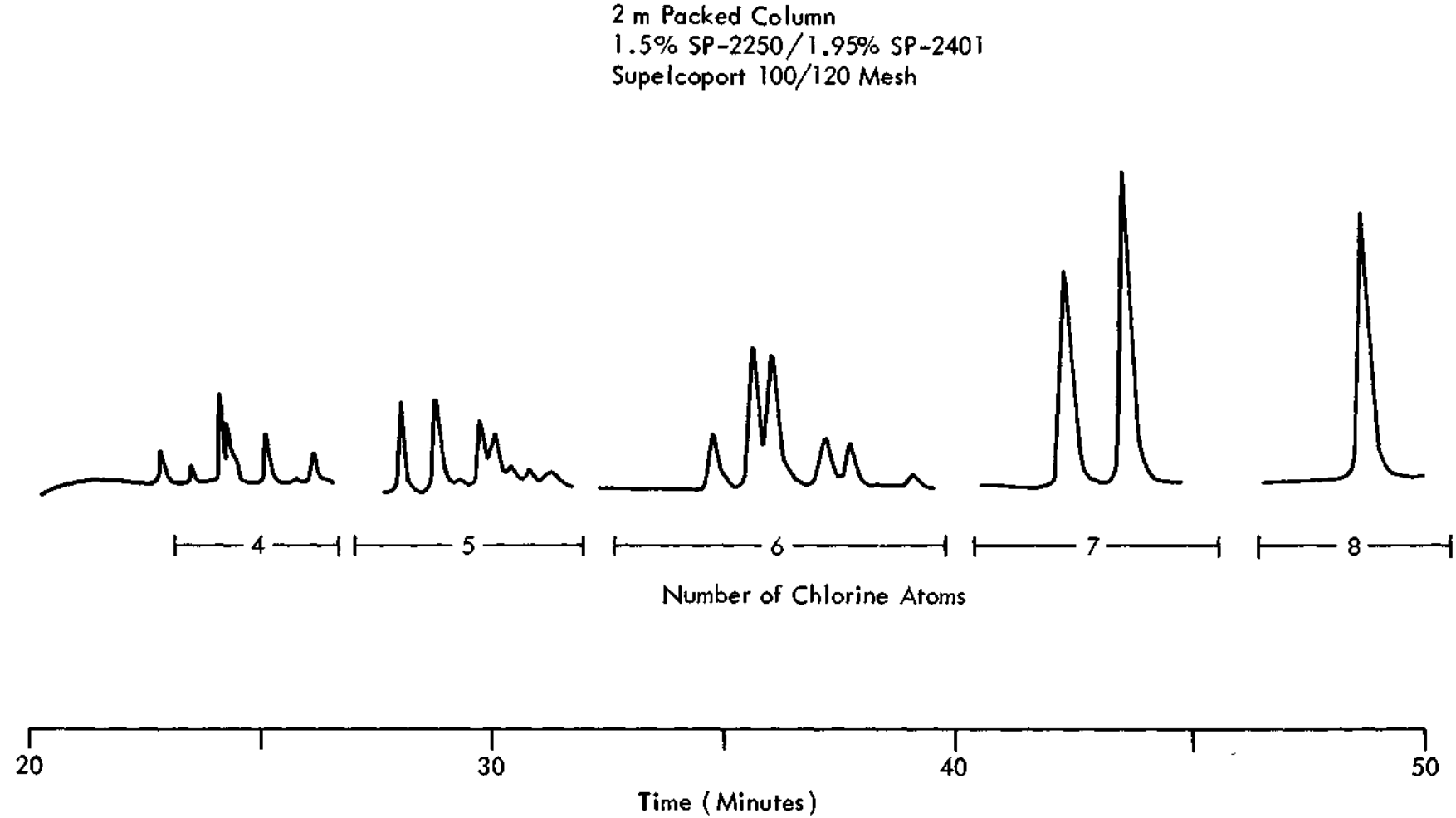
Packed Column Gas Chromatography--

Packed column gas chromatography (PGC) has been used primarily for screening applications to determine the presence of PCDDs and the range of occurring homologs. Figure 9 is an example of packed column gas chromatographic separation of PCDD homologs in an extract from an incinerator fly ash sample (Liberti et al., 1982). The packed column was a 2 m x 1.5 mm ID glass column packed with Supelcoport (100/120 mesh) coated with 1.5% SP-2250 and 1.95% SP-2401. The PCDDs in the sample extract were identified by high resolution mass spectrometry. The packed column chromatogram shown in Figure 9 indicates that tetra- through octachlorodibenzo-p-dioxins were identified in the sample.



Source: Karasek, F. W., and I. Onuska, "Trace Analysis of the Dioxins," Anal. Chem., 54, 309A-324A (1982).

Figure 8. Range of application of some analytical techniques for dioxins. The selection-ion monitoring (SIM) mode of GC/MS is the most applicable.



Source: Liberti, A., P. Ciccioli, E. Brancaleoni, and A. Cecinato, "Determination of Polychlorodibenzo-p-dioxins and Polychlorodibenzofurans in Environmental Samples by Gas Chromatography-Mass Spectrometry," J. Chrom., 242, 111-118 (1982).

Figure 9. PGC/MS chromatogram of PCDD homologs extracted from an incinerator fly ash sample.

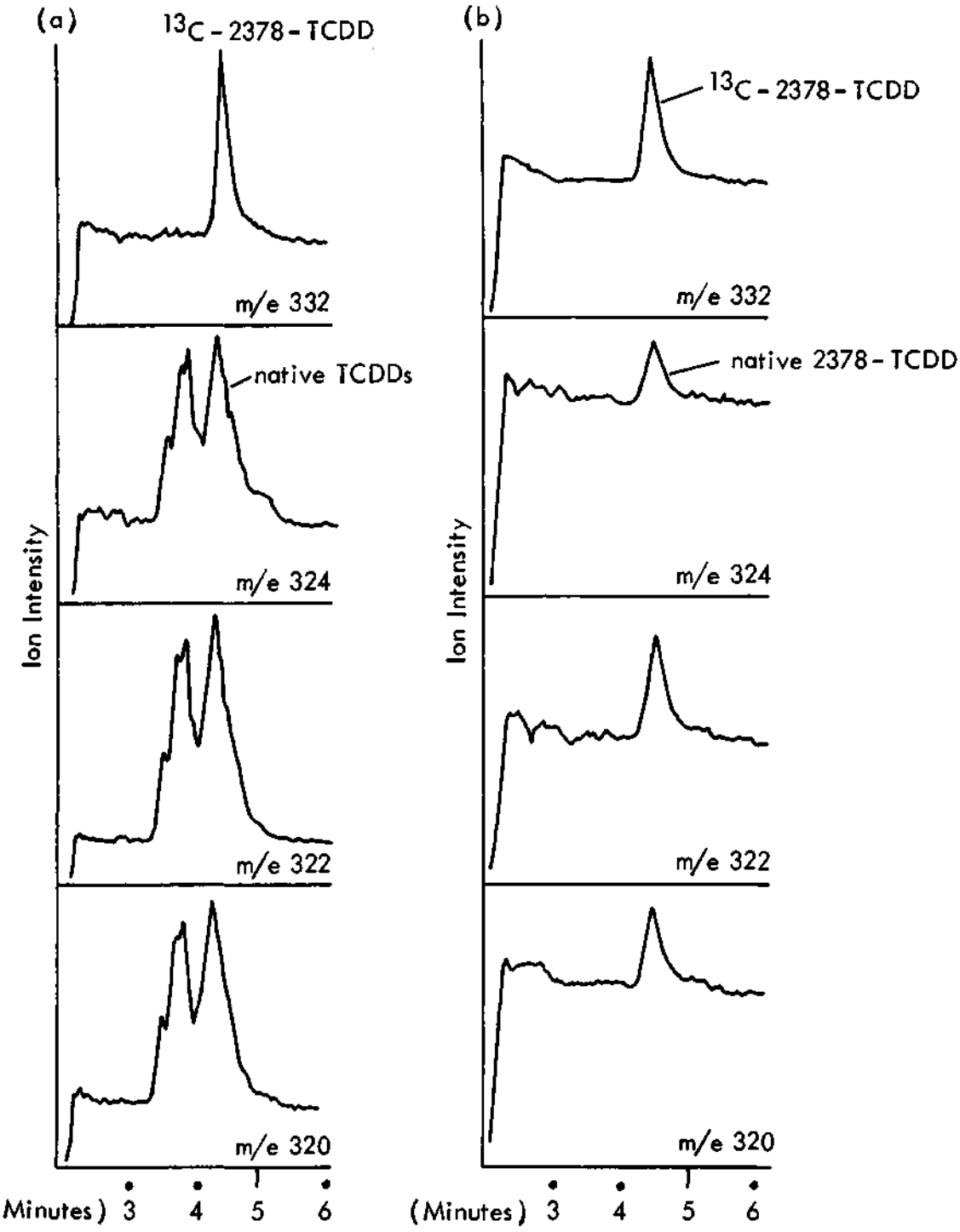
Packed column gas chromatography columns lack the necessary resolution for isomer specific separation of PCDDs other than the hepta- and octachloro-compounds, as indicated in Figure 9. However, Nestrick et al. (1979) have demonstrated the isomer specific determination of 2,3,7,8-TCDD using a packed column following the fractionation of a mixture of the 22 possible TCDD isomers by RP-HPLC and normal HPLC, as discussed in the section on cleanup procedures. The packed GC column used for the specific analysis was a 210 cm x 2 mm ID glass column packed with a 0.6% OV-17/0.4% Poly S-179 on a specially deactivated Chromosorb W-AW (80/100) support. Lamparski et al. (1979), Langhorst and Shadoff (1980), and Lamparski and Nestrick (1980) have used this procedure for the determination of 2,3,7,8-TCDD at spike levels equivalent to 10 ppt in fish, 1 ppt in human milk, and 10 ppt in particulates (fly ash, industrial dust, urban dust, etc.).

Figure 10 presents packed column gas chromatograms of fractions collected from the RP-HPLC and silica HPLC procedures allowing the isomer specific measurement of 2,3,7,8-TCDD by low resolution mass spectrometry (Nestrick and Lamparski, 1980). Quantitation of the peak corresponding to the RP-HPLC fraction for 2,3,7,8-TCDD yielded a value that was approximately four times the concentration found after the extract had been fractionated further with the silica HPLC system. The value obtained before the silica HPLC fractionation was qualified as being the concentration of 2,3,7,8-TCDD plus possibly four unseparated isomers. This demonstrates that PGC can be used for isomer specific PCDD analysis if extended efforts are made to isolate the desired component prior to gas chromatographic separation.

High Resolution Capillary Chromatography--

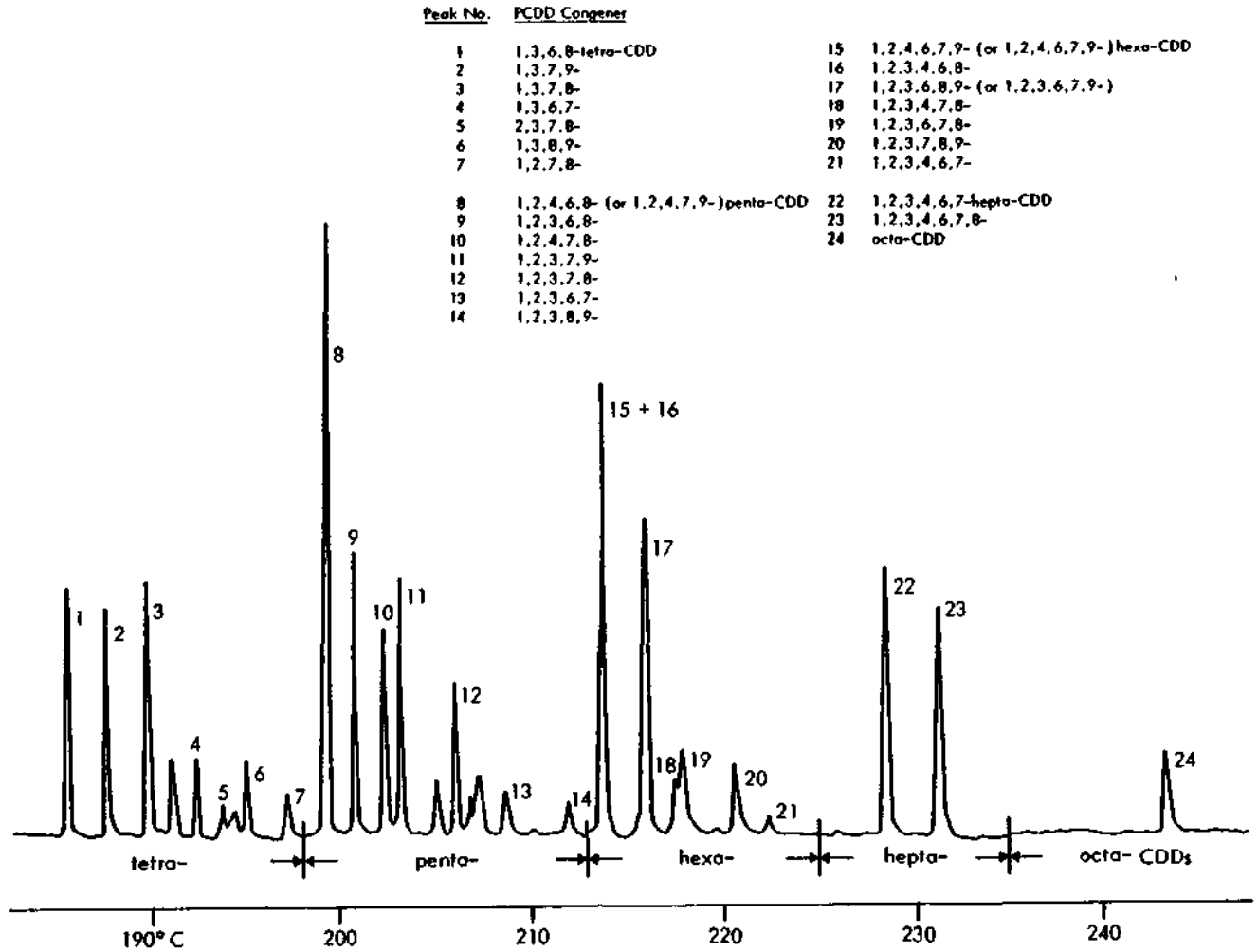
The current approach in many analyses for PCDDs by homolog or for specific isomers is the application of high resolution capillary gas chromatography (HRGC) (either glass or fused silica columns). High resolution glass capillary columns were first used by Buser (1975) for the analysis of PCDDs and PCDFs in chlorinated phenols. Since that time numerous studies have reported qualitative identification and quantitation of PCDDs using HRGC columns for separation. Liquid phases for the HRGC columns have ranged from low (SE-30, OV-17, OV-101) to high polarity phases (Silar 10C, SP-2330, SP-2340), and column lengths have ranged from 18 m for general analysis of PCDD to 60 m for isomer specific measurements. Figure 11 is a chromatogram depicting the elution of tetra- to octa- PCDDs on a HRGC column.

Isomer specific measurements have been of prime importance in most studies (both environmental and biological), particularly for 2,3,7,8-TCDD. Figures 12 and 13 present chromatograms of the mixture of the 22 possible TCDD isomers yielding the isomer specific separation for 2,3,7,8-TCDD. Buser (1980) used the three liquid phases (Figure 12) Silar 10C, OV-17, and OV-101 to determine specific assignments for the 22 isomers. Figure 13 presents the separation of TCDDs on a glass column coated with SP-2330 and a fused silica column coated with SP-2340 that is currently recommended for 2,3,7,8-TCDD specific analyses (EPA, 1982, 1983). In addition to these columns, Harless (1980) has reported isomer specific determination with a 30-m SE-30 column, and the current EPA method for the determination of 2,3,7,8-TCDD in soils and sediments implies that 30-m Durabond DB-5 fused silica columns provide sufficient separation for specific 2,3,7,8-TCDD measurements.



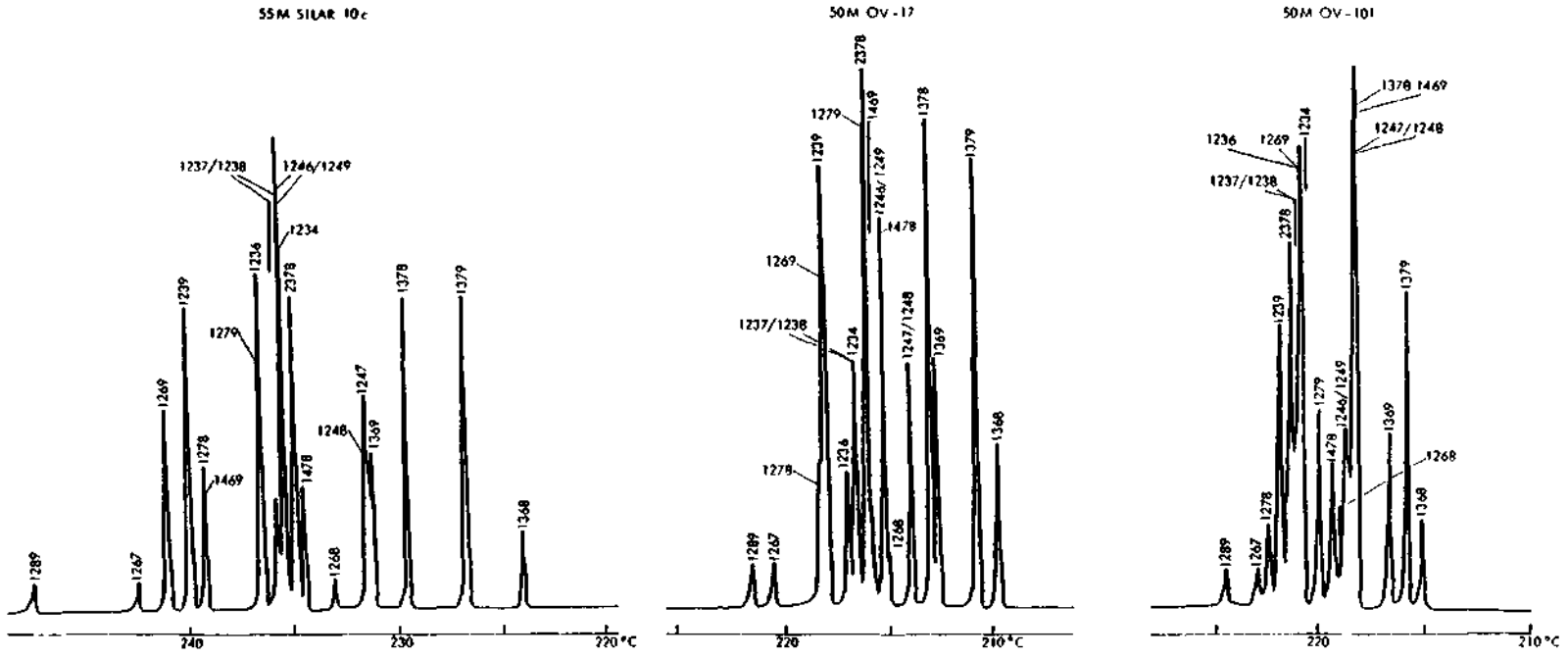
Source: Lamparski, L. L., and T. J. Nestrick, "Determination of Tetra-, Hexa-, Hepta-, and Octachlorodibenzo-p-dioxin Isomers in Particulate Samples at Parts per Trillion Levels," Anal. Chem., 52, 2045-2054 (1980).

Figure 10. Comparative 2,3,7,8-TCDD PGC/MS mass chromatograms for electrostatic fly ash (a) after RP-HPLC and (b) subsequent silica-HPLC.



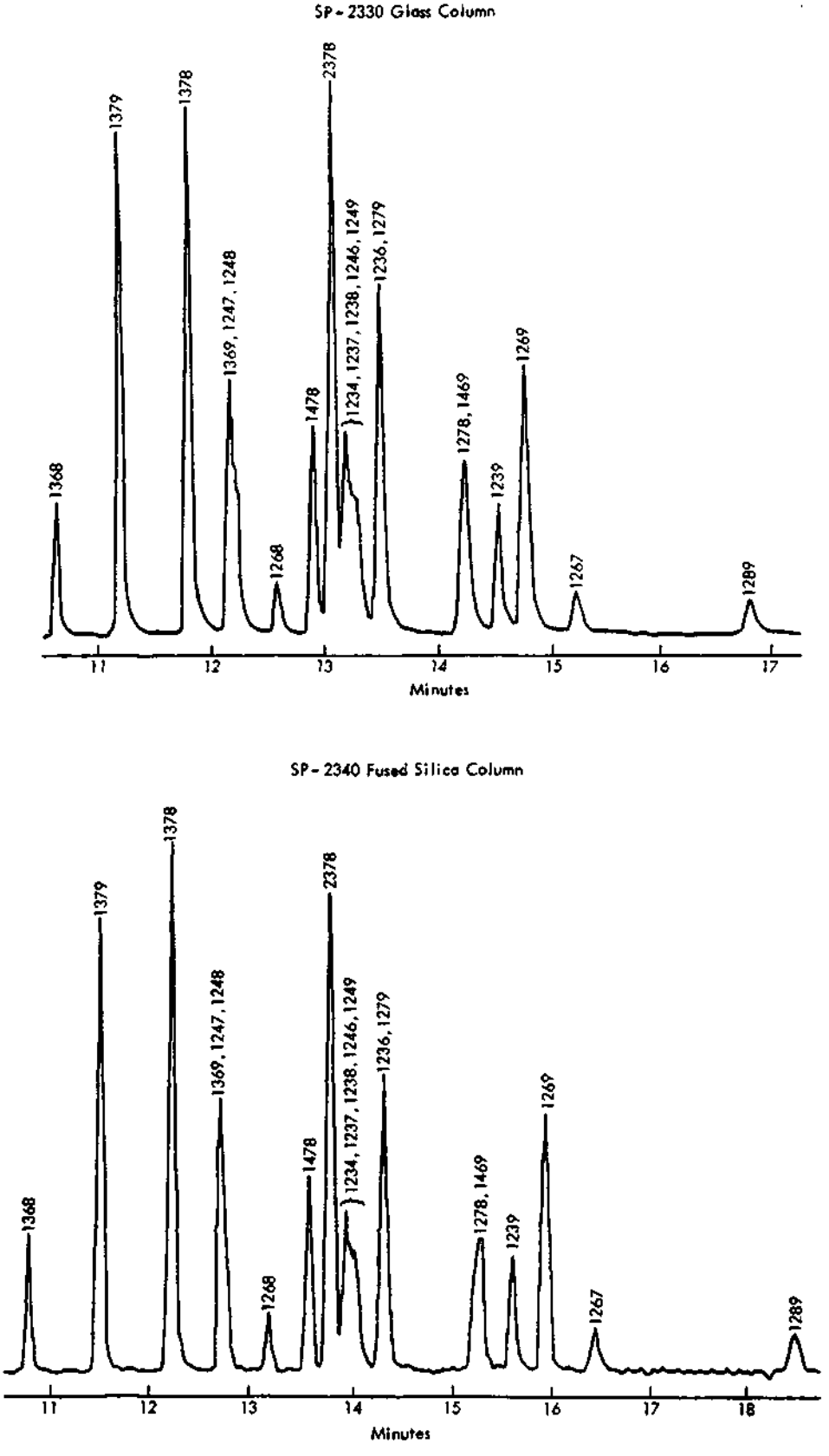
Source: Lustenhower, T. W. A., K. Olie, and O. Hutzinger, "Chlorinated Dibenzo-p-dioxins and Related Compounds in Incinerated Effluents," Chemosphere, 9, 501-522 (1980).

Figure 11. Separation of PCDD-isomers by GC/MS using a high resolution capillary column.



Source: Buser, H. R., and C. Rappe, "High Resolution Chromatography of the 22 Tetrachlorodibenzo-p-dioxin Isomers," Anal. Chem., 52, 2257-2262 (1980).

Figure 12. Mass chromatograms (m/e 320) of a composite pyrolyzate sample showing elution of all 22 TCDD isomers on HRGC columns.



Source: "Rapid Separation of 2,3,7,8-TCDD from Other TCDD Isomers," The Supelco Reporter, 1(4), 1 (1982).

Figure 13. HRGC chromatogram of a mixture of the 22 TCDD isomers on glass and fused silica capillary columns (60 m) coated with SP-2330 and SP-2340, respectively, indicating isomer specific separation for 2,3,7,8-TCDD.

The advantages of using HRGC columns over PGC columns include increased isomer specificity, resolution of interferences from analytes of interest, and increased sensitivity due to less band spreading. Fused silica HRGC columns allow the direct routing of the column into the ion source of the mass spectrometer, a procedure which leads to fewer problems resulting from dead volumes and to greater sensitivity. The major disadvantage of HRGC columns is the ease of overloading by coextractives. This problem has been overcome in most cases, however, by using effective and efficient cleanup procedures prior to HRGC separation of the sample extract.

Gaps in PGC and HRGC Information--

A major deficiency in the area of PGC and HRGC separation is the lack of information regarding the retention times of common interferences with respect to the PCDDs. This information would indicate whether polychlorinated biphenyls (PCBs), the common pesticides (e.g., DDE and DDT), polychloromethoxybiphenyls, or polychlorobenzylphenyl ethers actually elute within the retention windows required for the measurement of the PCDDs. This problem has been partially addressed by Hummel (1977), who considered possible interferences from pesticides and PCBs for the analysis of TCDD. Table 10 provides some of the information for relative retention times and responses for the ions characteristic of 2,3,7,8-TCDD.

Mass Spectrometry

The application of mass spectrometry for the analysis of PCDDs in biological matrices, commercial products, and environmental samples has been reviewed by Hass and Friesen (1979), Cairns et al. (1980), the National Research Council of Canada (1981), Mahle and Shadoff (1982), and Tiernan (1983). Mass spectrometry measurements have been reported for quadrupole (low resolution) and magnetic sector (high resolution) instruments. Electron impact is the most common method of ionization but chemical ionization mass spectrometry techniques have also been reported as a means of confirmation of the identity of PCDDs.

As indicated in Figure 8, MS-SIM techniques are required to obtain the necessary sensitivity for measurement of PCDDs at the parts per trillion concentration range required for biological matrices. The sensitivity of the SIM method is enhanced as a result of making multiple measurements of a few selected ions characteristic of the PCDDs rather than scanning an entire molecular range in the same time frame.

Most of the analytical studies reported in the literature have focused on the measurement of TCDDs. Langhorst and Shadoff (1980) have reported analytical methods for the analysis of tetra-, hexa-, hepta-, and octachlorodibenzo-p-dioxins in human milk based on the RP-HPLC fractionation scheme combined with PGC/MS. The alternative to this approach is computer-sequenced analysis of each PCDD homolog in a single analysis. Tiernan (1983) and Liberti et al. (1982) have emphasized the application of this procedure to provide data at the parts per trillion level for a wide range of PCDD homologs.

TABLE 10. RESPONSE FROM POSSIBLE ENVIRONMENTAL CONTAMINANTS

Compound	Retention time difference from 2,3,7,8-TCDD (sec)[a]	2,3,7,8-TCDD equivalent peak height[b] m/e 320	m/e 322
Chlordane	-251, -194, -184	ND[d]	ND
p,p'-DDE	-158	1	0.2
p,p'-DDD	-83	0.07	0.03
p,p'-DDT	-16	0.01	0.003
Dieldrin	-155	0.005	0.003
Endrin	-120	0.060	0.024
Endosulfan	-96	0.0002	0.0009
Mirex	+257	0.00028	0.00022
PCBs			
Aroclor 1242		ND	ND
Aroclor 1254		ND	ND
Aroclor 1260	-35	0.001	0.020
	+15	0.001	0.015
	+85	ND	0.032
	+187	0.0005	0.008
Toxaphene[c]	-85	0.000002	0.000002
	-38	0.00001	0.00001
	+9	0.00005	0.000005
	+57	0.000005	ND

Source: R. A. Hummel, "Cleanup Techniques for the Determination of Parts per Trillion Residue Levels of 2,3,7,8-TCDD," J. Agric. Food Chem., 25:1049-1053 (1977).

a 2,3,7,8-TCDD retention time 390 sec. Peak width at half-height = 30 sec.

b The ratio of response of the compound at its retention time to the response of an equal weight of 2,3,7,8-TCDD measured at 390 sec.

c Only those peaks near 2,3,7,8-TCDD are listed.

d ND = not detected; no peaks were detected at m/e 320 or 322.

Low Resolution versus High Resolution Mass Spectrometry--

One of the major points of contention in the analyses of low level (ppt) PCDDs is the necessity of low resolution ($M/\Delta M$ = unit) versus high resolution ($M/\Delta M$ = 10,000) mass spectrometry measurements. Many of the methods rely on efficient cleanup steps prior to low resolution mass spectrometry to provide low level backgrounds. Other methods, however, utilize the mass resolving power of single or double focusing mass spectrometers to identify and quantitate low level PCDDs in the presence of other chlorinated compounds (Harless et al., 1980). The need for high resolution mass spectrometry for various extraction and cleanup procedures has been demonstrated by Brumley et al. (1981) via the interference noted for electron capture detector and low resolution mass spectrometry measurements for extracts prepared by six different laboratories.

Hummel and Shadoff (1980) have directed attention to the need for high resolution confirmation of TCDDs in sample extracts, especially when the concentration approaches values of 20 ppt or less as measured by low resolution mass spectrometry. Table 11 provides data presented by Hummel and Shadoff (1980) for the levels of TCDD in beef fat samples analyzed for Phase I of the EPA Dioxin Implementation Plan. The data presented in this table indicate that of 93 total samples analyzed by low resolution mass spectrometry 37 were determined to contain TCDD. Further analyses of these positives by high resolution mass spectrometry yielded that only 20 of the 37 samples contained TCDD. The two control samples identified as positive by high resolution mass spectrometry present the additional problem of false positives for measurements near the detection limit. Additional studies of the extracts after a second cleanup also presented the possibility of false negatives by high resolution mass spectrometry when sample extracts are dirty. Shadoff and Hummel (1980) concluded that analysis by low resolution mass spectrometry is acceptable if suitable control samples demonstrate the absence of interferences. Otherwise, high resolution mass spectrometry should be used for confirming positive results.

Interferences--

Some of the compounds identified as interferences in the analysis of TCDDs by mass spectrometry are presented in Table 12. The alternate methods of resolution are the approaches that have been specifically addressed in the literature. The separation of PCBs, polychlorodiphenyl ethers and polychlorobenzyl phenyl ethers has been reported by Mieure et al. (1977) and Lamparski et al. (1979).

TABLE 11. EPA PHASE I DIOXIN IMPLEMENTATION PLAN
BEEF FAT SAMPLES ANALYZED FOR TCDD

Sample	No. of samples analyzed by PGC/LRMS	No. of apparent positive results by	
		PGC/LRMS	PGC/HRMS[a]
Grazed on treated land	64	19	9
Control	20	9	2
Fortified[b] extracts and solutions	9	9	9

Source: R. A. Hummel and L. A. Shadoff, "Specificity of Low Resolution Gas Chromatography-Low Resolution Mass Spectrometry for the Detection of Tetrachlorodibenzo-p-dioxin in Environmental Samples," Anal. Chem., 52:191-192 (1980).

a In this part of the study, only those extracts showing an apparent positive result or a limit of detection greater than 20 ppt were analyzed by PGC/HRMS.

b The fortification level was 20 to 100 ppt TCDD in the beef fat.

TABLE 12. SOME COMPOUNDS THAT MAY INTERFERE WITH THE DETERMINATION OF TCDD AT m/z VALUES OF 319.8966 AND 321.8936

Compound	Elemental composition	Ion	Mass lost	m/z	ΔM TCDD	Mass resolution for separation M/ΔM	Alternate means of resolution
Heptachloro-biphenyl	$C_{12}H_3\ ^{35}Cl_7$	M^+	$-2^{35}Cl$	321.8678	0.0258	12476	Alumina micro column, HPLC, HRGC
Nonachloro-biphenyl	$C_{12}H\ ^{35}Cl_9$	M^+	$-4^{35}Cl$	319.8521	0.0445	7189	Alumina micro column, HPLC, HRGC
	$C_{12}H\ ^{35}Cl_8\ ^{37}Cl$	M^+	$-3^{35}Cl\ ^{37}Cl$	321.8491	0.0445	7233	
Tetrachloro-methoxy biphenyl	$C_{13}H_8\ ^{35}Cl_4O$	M^+		319.9329	0.0363	8805	$AgNO_3$ (10%) impregnated silica, alumina, HPLC
	$C_{13}H_8\ ^{35}Cl_3\ ^{37}ClO$	M^+		321.9299	0.0364	8848	
Tetrachloro-benzylphenyl ether	$C_{13}H_8\ ^{35}CL_4O$	M^+		319.9329	0.0363	8813	Alumina micro column, HPLC, HRGC
	$C_{13}H_8\ ^{35}Cl_3\ ^{37}ClO$	M^+		321.9300	0.0364	8843	
Pentachloro-benzylphenyl ether	$C_{13}H_7\ ^{35}Cl_4\ ^{37}ClO$	M^+	$-H^{35}Cl$	319.9143	0.01773	18043	Alumina micro column, HPLC, HRGC
	$C_{13}H_7\ ^{35}Cl_3\ ^{37}Cl_2O$	M^+	$-H^{35}Cl$	321.91138	0.01778	18104	
DDT	$C_{14}H_9\ ^{35}Cl_3\ ^{37}Cl_2$	M^+	$-H^{35}Cl$	319.9321	0.03552	9006	Alcoholic saponification converts DDT to DDE
	$C_{14}H_9\ ^{35}Cl_2\ ^{37}Cl_3$	M^+	$-H^{35}Cl$	321.92917	0.03557	9050	
DDE	$C_{14}H_8\ ^{35}Cl_2\ ^{37}Cl_2$	M^+		319.9321	0.03550	9011	$AgNO_3$ (10%) impregnated silica
	$C_{14}H_8\ ^{35}Cl\ ^{37}Cl_3$	M^+		321.92916	0.03556	9052	

(continued)

TABLE 12 (continued)

Compound	Elemental composition	Ion	Mass lost	m/z	ΔM TCDD	Mass resolution for separation M/ΔM	Alternate means of resolution
Hydroxy-tetrachloro-dibenzofuran	$C_{12}H_4Cl_4O_2$	M^+		319.8966 321.8936	0.00 0.00	a a	NR[b]
Tetrachloro-phenylbenzo-quinone	$C_{12}H_4Cl_4O_2$	M^+		319.8966 321.8936	0.00 0.00	a a	NR
Tetrachloro-xanthene	$C_{13}H_6O\ ^{35}Cl_3\ ^{37}Cl$ $C_{13}H_6O\ ^{35}Cl_2\ ^{37}Cl_2$	M_+^+ M^+		319.9143 321.9114	0.01773 0.01778	18043 18104	NR

Source: Adapted from National Research Council of Canada, "Polychlorinated Dibenzo-p-dioxins: Limitations to the Current Analytical Techniques," NRCC Report No. 18576, ISSN 0316-0114, 1981.

a Cannot be resolved by MS.

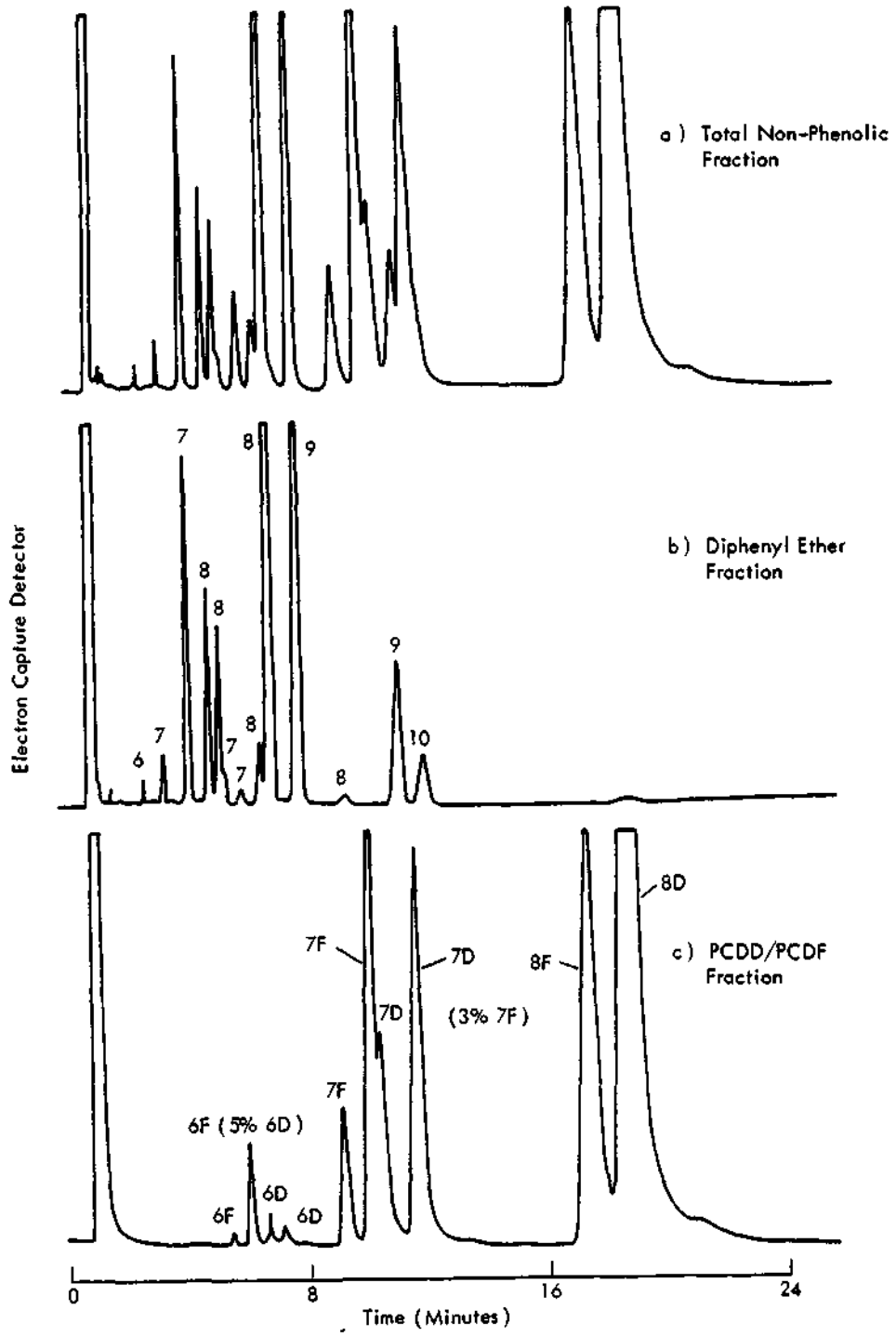
b NR = not reported specifically in the literature.

Mieure et al. (1977) specifically reported that PCBs and polychlorobenzylphenyl ethers are separated from PCDDs on a microalumina column (basic, super grade 1). Figure 14 is an example of separation of chlorinated interferences using the microalumina column. The first chromatogram is representative of the total nonphenolic fraction from technical grade pentachlorophenol obtained after removing the phenolic components with a macroalumina column (Fisher A-540, 5% deactivated). The second chromatogram is the first fraction from the microalumina column and contains the polychlorodiphenyl ethers (hexa- to decachloro), chlorobenzenes, and PCBs. The third chromatogram represents the second fraction collected from the microalumina column that contains PCDDs and PCDFs (hexa- to octachloro). It is concluded from these chromatograms that the octachlorodibenzo-p-dioxin and octachlorodibenzofuran can be measured without possible interferences from the other chlorinated compounds. However, the micro column cleanup is necessary to isolate the lower chlorinated homologs of the PCDDs and PCDFs for specific analysis.

Chlorinated methoxybiphenyls were reported as interferences in the analysis of PCDDs in fish extracts by Phillipson and Puma (1980). These compounds eluted in the retention window for tri- to pentachlorodioxins by PGC/ low resolution mass spectrometry and produced intense molecular ions having the same nominal masses and chlorine isotopic abundances as those observed for the PCDDs. These authors suggested the need for monitoring fragment ions in addition to ions representative of the molecular ion cluster for low resolution mass spectrometry. Alternately, high resolution mass spectrometry may be used as presented in Table 12 to differentiate the PCDDs from interfering chlorinated methoxybiphenyls. This interference might also be removed by the cleanup procedures as described by Nestrick et al. (1980).

Smith and Johnson (in press) have presented detailed information on the potential of interferences to arise from selected congeners of seven families of polychlorinated aromatic compounds with the analytical method for part-per-trillion determinations of PCDFs and PCDDs used by the Fish and Wildlife Service (Stalling et al. 1982). The polychlorinated aromatic compounds evaluated as potential interferences included polychlorinated-biphenyls (PCBs), -naphthalenes (PCNs), -diphenyl ethers (DPEs), methoxy-PCBs (MeO-PCB), -hydroxy-PCBs (HO-PCB), -methoxy-diphenyl ethers (MeO-DPE), -hydroxy-diphenyl ethers (HO-DPE), -benzylphenyl ethers (BzPE) and -biphenylenes. The potential interferences from these compounds arise from the large number of congeners of each chemical family exhibiting chromatographic retention times that are similar to PCDDs and PCDFs and from mass spectral patterns that overlap to varying degrees with PCDDs and PCDFs. In addition, some of these potential interferences have the same nimonal masses and the same number of chlorine substituents as those of PCDDs and PCDFs, making the molecular ions indistinguishable by low resolution mass spectrometry. Also, at least five of the chemical families include compounds that under thermal conversions and/or conversion following ionization produce PCDDs and PCDFs. Table 13 presents the potentially interfereing chemical according to the degree of potential interference that can be encountered.

Smith and Johnson (in press) concluded that the specific polychlorinated aromatic compounds used in this study did not produce a significant number of false positives with the particular analytical procedure. However, these authors do note that only a few of the large number of potential compounds were available for this study.



Source: Mieure, J. P., O. Hicks, R. G. Kaley, and P. R. Michael, "Determination of Trace Amounts of Chlorodibenzo-p-dioxins and Chlorodibenzofurans in Technical Grade Pentachlorophenol," J. Chrom. Sci., 15, 275-277 (1977).

Figure 14. Electron capture chromatograms of (a) entire nonphenolic fraction, (b) first microcolumn fraction containing chlorodiphenyl ethers, (c) second basic alumina microcolumn fraction containing chlorodibenzo-p-dioxins and chlorodibenzofurans.

TABLE 13. INTERFERENCES OF SELECTED CHEMICAL FAMILIES IN MS DETERMINATIONS OF PCDFs AND PCDDs

Family of polychlorinated compounds	Level of interference					
	Overlap of fragmentation patterns		Indistinguishable by LRMS		Indistinguishable by HRMS	
	PCDDs	PCDFs	PCDDs	PCDFs	PCDDs	PCDFs
PCBs	++/+++					
PCNs		+				
DPEs		++/+++		X		X
MeO-PCBs	+++	+++	X	X		X
HO-PCBs		+++		X		X
MeO-DPEs	+++	++	X		X	
HO-DPEs	+++		X		X	
BzPEs	+++		X			
Biphenylenes	++					

Source: L. M. Smith and J. L. Johnson, "Evaluation of Interferences from Seven Series of Polychlorinated Aromatic Compounds in an Analytical Method for Polychlorinated Dibenzofurans and Dioxins in Environmental Samples," in Chlorinated Dioxins and Dibenzofurans in the Total Environment, L. H. Keith, G. Choudry, and C. Rappe (Eds.), Pergamon Press, in press.

NOTE: In the first two columns "+" indicates minor overlap, "++" indicates major overlpa, and "+++" indicates complete overlap.
In the last four columns, an "X" indicates that a particular type of interference is observed.
The abbreviations used for polychlorinated compounds are:
PCBs-biphenyls; DPE-diphenyl ethers; PCNs-naththalenes/ BzPEs-benzylphenyl ethers. The prefixes Meo- designate methoxy and HO- hydroxy.

In summary, a number of chlorinated compounds have been noted to interfere with the mass spectrometry analysis of PCDDs, particularly 2,3,7,8-TCDD. The problems arising from these interferences have been overcome in part by using efficient cleanup procedures or high resolution mass spectrometry, or a combination of the two.

Criteria for Positive Identification of PCDDs--

Positive identification of PCDDs as a particular homolog or specific isomer requires the analyst to ensure that the instrumental response meets specific criteria. Most analysts to date have used the coincident response of a minimum of three different ions from the molecular ion cluster (M^+, $[M-2]^+$, $[M+2]^+$) and from fragment ions (e.g., $[M-COCl]^+$). The retention time of the selected ions must fall within a designated or established retention window. In addition the selected ions must have the correct response ratios. Figure 15 is an example of a HRGC/MS-SIM analysis for 2,3,7,8-TCDD demonstrating these criteria. The ion at m/z 257 is representative of the fragment ion $[M-COCl]^+$, while m/z 320 and 322 are indicative of the molecular ion cluster for TCDD. Isomer specific measurement of the 2,3,7,8-TCDD was accomplished with the SP-2330 glass column discussed earlier. Documentation of the 2,3,7,8-TCDD retention time is represented by m/z 332 for the carbon-13 labeled 2,3,7,8-TCDD.

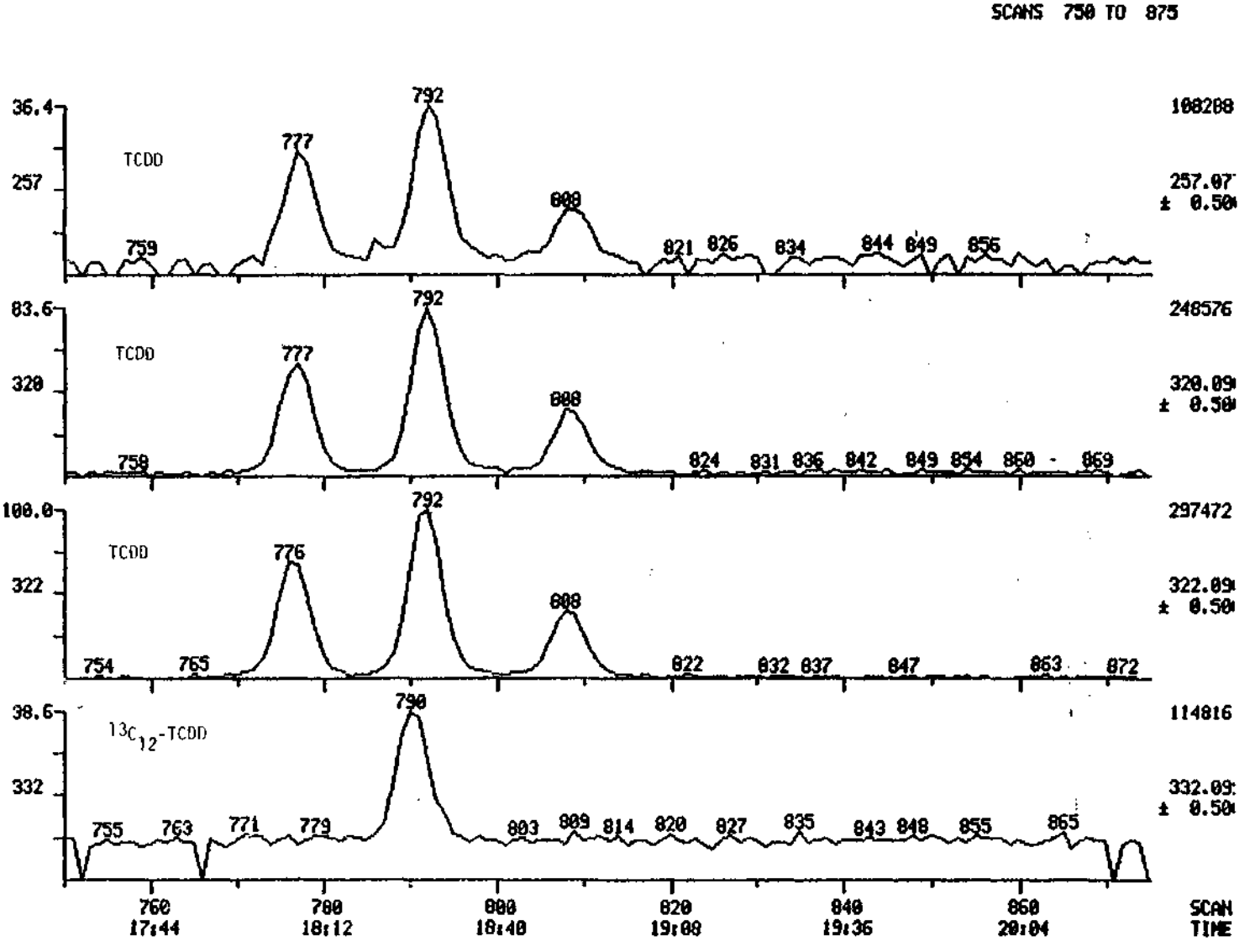
Harless et al. (1980) have specifically designated the following criteria as essential to the final analysis for TCDD by HRGC/HRMS.

1. Correct HRGC-HRMS retention time for 2,3,7,8-TCDD.
2. Correct HRGC-HRMS multiple ion response for ^{37}Cl-TCDD and TCDD masses (simultaneous response for elemental composition of m/z 320, m/z 322, m/z 328).
3. Correct chlorine isotope ratio for the molecular ions (m/z 320 and m/z 322).
4. Correct responses for the co-injection of sample fortified with ^{37}Cl-TCDD and TCDD standard.
5. Response of the m/z 320 and m/z 322 must be greater than 2.5 times the noise level.

Supplemental criteria that Harless et al. (1980) suggested may be applied to highly contaminated sample extracts are:

(A) COCl loss indicative of TCDD structure, and
(B) HRGC-HRMS peak matching analysis of m/z 320 and m/z 322 in real time to confirm the TCDD elemental composition.

Other supplemental information for confirmation of TCDD in particular can be obtained from the partial scan of the TCDD peak (Table 14) when present at parts per billion concentrations (EPA, 1983). Table 15 provides a range of reported relative abundances for the most intense ion in the isotopes clusters.



Source: MRI RC-693-A, "Analytical Chemistry Application of Isotopically Labeled Compounds, 1982.

Figure 15. HRGC/MS-SIM chromatogram of TCDD analysis. Mass charge (m/z) ratios 257, 320, and 322 are representative of natural abundance TCDD isomers, while m/z 322 represents the level of ^{13}C-labeled 2,3,7,8-TCDD internal standard. This chromatogram was obtained on a 60-m SP-2330 glass capillary column. Fifty picograms of the ^{13}C-TCDD were injected.

TABLE 14. PARTIAL SCAN CONFIRMATION FOR TCDD[a]

m/z Ratios	Response ratios
320/324	1.58 ± 0.16
257/259	1.03 ± 0.10
194/196	1.54 ± 0.15

Source: "Determination of 2,3,7,8-TCDD in Soil and Sediment," U.S. Environmental Protection Agency, Region VII, Kansas City, Kansas, February 1983.

a All ions including 160 and 161 must be presented with at least 5% relative abundance to the ion at 322.

TABLE 15. RANGE OF REPORTED PERCENT RELATIVE ABUNDANCES FOR MOST INTENSE ION IN ISOTOPE CLUSTERS FROM ELECTRON IMPACT MASS SPECTRA OF THE CHLORINATED DIBENZO-p-DIOXINS

Ion	Number of chlorines							
	1	2	3	4	5	6	7	8
$[M]^+$	100	100	100	100	100	100	100	100
$[M-Cl]^+$	2	5-7	5	0-10	20	7-10	5-15	3-10
$[M-COCl]^+$	14	20-24	24-36	21-60	40	31-34	28-35	11-35
$[M-2Cl]^+$	0	0-3	1	0-5	15	4-6	0-4	2-5
$[M-C_2O_2Cl]^+$	9	0-3	2	0	5	0	0-3	1-5
$[M-C_2O_2Cl_2]^+$	0	13-18	10-23	13-55	35	17-21	3-25	10-25

Source: Mahle, N. H., and L. A. Shadoff, "The Mass Spectrometry of Chlorinated Dibenzo-p-dioxins," Biomedical Mass Spectrometry, 9:45-60 (1982).

Quantitation

Several variables have been reported for quantitation of PCDDs by mass spectrometry methods. These variables include electron impact versus chemical ionization mass spectrometry, selection and availability of standard compounds, and internal versus external standard calibration.

Electron Impact Versus Chemical Ionization Mass Spectrometry--

Although chemical ionization mass spectrometry, especially the negative chemical ionization mode, has the potential to enhance specificity and sensitivity for individual isomers (Hass et al., 1978; Mitchum et al., 1981; Rappe et al.), electron impact ionization has been used most often for quantitative analysis of PCDDs. The inconsistencies of response factors across a homolog of PCDDs noted with negative chemical ionization (NCI) mass spectrometry (Kuehl and Dougherty, 1980) and the scarcity of all the specific standard PCDDs are disadvantages to its use for routine analysis of PCDDs. Kuehl and Dougherty (1980) have reported that the relative sensitivity for 2,3,7,8-TCDD is roughly a factor of 50 less than that for other TCDD isomers or higher chlorinated dioxins. However, specific analysis for 2,3,7,8-TCDD has been reported for NCI methods (Hass et al., 1978). Hass et al. (1981) have also suggested that both negative chemical ionization and electron impact ionization are necessary to provide reliable measurements for PCDDs and PCDFs in the presence of PCBs and polychlorinated diphenyl ethers.

Selection of Calibration Standards--

There is concern regarding the need for standard compounds representing each homolog to provide appropriate assessment of the possible effects arising from trace levels of PCDDs in biological samples. Nestrick et al. (1982) addressed this problem as a systematic error that affects accuracy and reliability in the analysis of environmental samples for PCDDs. The source of error originated by assuming that the response factors for penta- through octachloro PCDDs were consistent with the response factor for TCDD.

Table 16 provides response factors of several PCDDs relative to 1,2,3,4-TCDD at the molecular ion (m/z) 322. These data illustrate the possible margin of quantitative error that could be introduced by the assumption of a constant response factor for all PCDD homologs. The data from Table 16 indicate differences of approximately 3 to 1 when comparing the response of 1,2,3,4-TCDD to the response for octachlorodibenzo-p-dioxin (OCDD).

Nestrick et al. (1982) also demonstrated the differences in response factors that arise when working with PGC/MS systems that rely on silicone membrane separators and jet separators for introduction of the PCDDs to the ion source of the mass spectrometer. Table 17 summarizes the data and indicates a significant difference for the response factors of hepta- and octa-PCDDs measured with a quadrupole mass spectrometer equipped with silicone membrane or jet separators.

TABLE 16. AREA RESPONSE FACTORS OF PCDDs RELATIVE TO 1,2,3,4-TCDD AT m/z 322[a]

Component	m/z	Rel response (± rel std dev)	No. of replicates
1,2,3,4-TCDD	322	1.00 ± 0.03	5
2,3,7,8-TCDD	322	0.89 ± 0.03	5
1,2,3,7,8-PCDD	356	0.52 ± 0.02	2
HCDD mixture	390	0.44 ± 0.02	4
1,2,3,4,6,7,8-HpCDD	426	0.46 ± 0.01	3
OCDD	460	0.32 ± 0.01	3

Source: Nestrick, T. J., L. L. Lamparski, W. B. Crummett, and L. A. Shadoff, "Comments on Variations in Concentrations of Organic Compounds Including Polychlorinatd Dibenzo-p-dioxins and Polynuclear Aromatic Hydrocarbons in Fly Ash from a Municipal Incinerator," Anal. Chem., 54:824-825 (1982).

a One hundred picograms of each component injected.

TABLE 17. COMPARISON OF RELATIVE PEAK RATIOS OF PCDDs THROUGH A GLASS JET AND SILICONE MEMBRANE SEPARATOR[a]

Component		Rel response (± rel std dev)	No. of replicates
1,2,3,6,7,8-HCDD	membrane	1.00	7
	jet	1.00	4
1,2,3,4,6,7,8-HpCDD	membrane	0.34 ± 0.04	7
	jet	0.63 ± 0.10	4
OCDD	membrane	0.21 ± 0.05	7
	jet	0.38 ± 0.04	4

Source: Nestrick, T. J., L. L. Lamparski, W. B. Crummett, and L. A. Shadoff, "Comments on Variations in Concentrations of Organic Compounds Including Polychlorinatd Dibenzo-p-dioxins and Polynuclear Aromatic Hydrocarbons in Fly Ash From a Municipal Incinerator," Anal. Chem., 54:824-825 (1982).

a All values normalized to HCDD response.

Data summarizing the response factors for PCDDs by homolog or by isomer by electron impact ionization versus chemical ionization mass spectrometry do not appear in the primary literature. There is a need to determine the variability of the response factors for isomers within a homolog in order to evaluate the maximum systematic error that might be encountered in using a response factor for a single isomer within a homolog.

Rappe et al. (in press) have recently reported some response factor data for PCDFs using electron impact and negative chemical ionization mass spectrometry. The data presented indicated the relative response factors for 13 TCDFs varied considerably less with electron impact than with negative chemical ionization. The range of response factors however, was not markedly different for higher chlorinated PCDFs when comparing the two ionization techniques, although the negative chemical ionization absolute response is considerably greater.

Internal Versus External Standard Quantitation--

Quantitation for PCDDs requires calibration of the instrument with standards bracketing the expected concentration range of any sample extracts. The internal standard quantitation method has been used by most analysts for measurement of the levels of PCDDs in biological and environmental matrices. This method requires response factors be determined for the internal standard versus an authentic analyte. Typically, the stable isotope labeled compounds, such as carbon-13 or chlorine-37 analogs of native PCDDs, are incorporated in calibration solutions and samples as internal standards. The level of the labeled internal standard is usually held constant and the native PCDD is varied for calibration purposes. If response factors are determined to remain constant over the expected concentration range, the true internal standard quantiation method is applicable for calculation of the PCDD concentration.

On the other hand, if the response factor is not consistent across the calibration range, it becomes necessary to use external standards and calibration curves routinely for measurements of PCDD contamination. The EPA methods for analysis of 2,3,7,8-TCDD in water and wastewater (EPA, 1982) and soil and sediment (EPA, 1983) require measurement of the response factors over a designated concentration range at the initiation of any sample analysis event and the daily check of the response factor value. If the response factor does not agree within ± 10% of the value generated for the concentration range, a recalibration is necessary.

True internal standard quantitation provides a correction of the reported value without a true measure of the recovery for each analysis. Recovery can be estimated by comparing the response of the labeled compound in a sample extract versus an external standard. More accurate measurements of method recovery are achieved by using a second internal standard added to the sample extract immediately before GC/MS analyses. For instance, carbon-13 ($^{13}C_{12}$) labeled 2,3,7,8-TCDD can be added as a surrogate prior to sample preparation to provide true internal standard quantitation for native TCDD and chlorine-37 ($^{37}Cl_4$) labeled 2,3,7,8-TCDD can be added to the sample extract prior to GC/MS analyses to provide accurate recovery measurement of the carbon-13 TCDD.

The true internal standard quantitation is accomplished using the following equation:

$$C_x = (As)(I_s)/(A_{IS})(RF)(W)$$

where C_x = concentration of the PCDD in the original sample

As = peak area response for the PCDD quantitation ion

A_{IS} = peak area response for the internal standard quantitation ion

I_S = amount of internal standard added to the sample

W = weight or volume of the sample

RF = response factor

The response factor (RF) is calculated according to the equation

$$RF = (A_{std})(C_{IS})/(A_{IS})(C_{std})$$

where A_{std} = peak area response for the standard PCDD quantitation ion

A_{IS} = peak area response for the internal standard quantitation ion

C_{std} = concentration of the standard PCDD

C_{IS} = concentration of the internal standard

Stable isotope labeled compounds are commercially available for internal standard quantitation of tetra-, hepta-, and octachloro-PCDDs. KOR Isotopes, Division of ICN Pharmaceuticals, and Lamparski and Nestrick (1982) have presented details for the laboratory preparation of carbon-13 ($^{13}C_{12}$) labeled penta- through octachloro-PCDDs from the commercially available carbon-13 ($^{13}C_{12}$) 2,3,7,8-TCDD. Bell (in press) has recently reported on the synthesis of carbon-13 labeled PCDFs.

Regardless of the quantitation technique, the quantitation ion monitored for each PCDD isomer or homolog is selected from the molecular ion cluster. Table 18 provides the exact masses, relative isotope abundances, and the chlorine pattern for the major molecular cluster ions for the mono-through octachlorinated dibenzo-p-dioxins.

Limit of Detection--

The limit of detection is the lowest concentration of an analyte that the analytical method can reliably detect. The limit of detection in most PCDD studies is the concentration of the analyte that gives rise to a response signal that is at least 2.5 times the background noise for the sample matrix.

TABLE 18. EXACT MASSES AND RELATIVE ISOTOPE ABUNDANCES OF MAJOR MOLECULAR CLUSTER IONS FOR PCDDs

No. of chlorines	Exact mass	Relative abundance
0	184.0524	-
1	218.0135	100.00
	220.0105	33.82
2	251.9746	100.00
	253.9716	66.45
	255.9686	11.43
3	285.9356	100.0
	287.9326	99.07
	289.9296	33.10
	291.9266	3.86
4	319.8967	75.93
	321.8937	100.00
	323.8907	49.68
	325.8877	11.13
	327.8847	0.99
5	353.8578	60.86
	355.8546	100.00
	357.8518	65.96
	359.8488	21.91
	361.8458	3.70
6	387.8188	50.78
	389.8158	100.00
	391.8128	82.25
	393.8909	36.23
	395.8068	9.05
7	421.7799	43.56
	423.7769	100.00
	425.7739	98.55
	427.7709	54.10
	429.7679	17.90
8	455.7410	33.21
	457.7380	87.08
	459.7350	100.00
	461.7320	65.76
	463.7290	27.10

Source: Radolovich, G., Midwest Research Institute (personal communication) (1983).

The limit of detection has been found to vary with each sample (Crummett, 1979). The differences in reported limits of detection are dependent on initial sample size, final extract volume, volume of final extract analyzed, residual interferences from the sample matrix, extraction and cleanup procedures, chromatography and instrumental performances, purity of reagents used for preparation of samples, and absolute sensitivity obtainable with any particular mass spectrometer. Figure 16 presents the direct relationships of method limit of detection with respect to initial sample size and final extract volumes. The data generated for Figure 16 were calculated assuming a conservative GC/MS instrumental detection limit of 5 pg per 1.0 µl on-column injection. Based on these data, the instrumental detection limit required for measurement of 1 ppt levels of a PCDD in a 1-g sample concentrated to 10 µl would be 0.1 pg/µl assuming 100% recovery. The only study approaching this level of effort has been presented in part by Harless (1980). Table 19 provides the data presented for the feasibility study regarding the analysis of TCDD at the parts per trillion level in 250-mg samples of human adipose tissue (equivalent to a needle biopsy). These data suggest than an extremely clean and sensitive mass spectrometer was used to measure these levels of TCDD.

Limits of detection have been presented in many of the studies dealing with the analysis of PCDDs in biological matrices. Table 20 is a summary of data presented in a review of TCDD analysis by Shadoff and Hummel (1978). The data presented in Table 20 generated by gas chromatography low resolution mass spectrometry show that the original biological sample matrix has little effect on the average detection limit that is obtainable. The lowest parts per trillion limits of detection were obtained for samples sizes of 10 to 20 g. Final extract volumes were taken to 10 to 20 µl, providing concentration (or enrichment) factors of 1,000 for the larger sample sizes. In comparison, the evidently higher LOD reported for a 1-g blood sample is in part due to the difference in achievable enrichment (100) of TCDD in the final extract. Thus, actual limits of detection vary with the sample extract and instrumental condition. If significant interferences prevent measurement of the desired LOD value with low resolution mass spectrometry, the alternative approach is to use high resolution mass spectrometry.

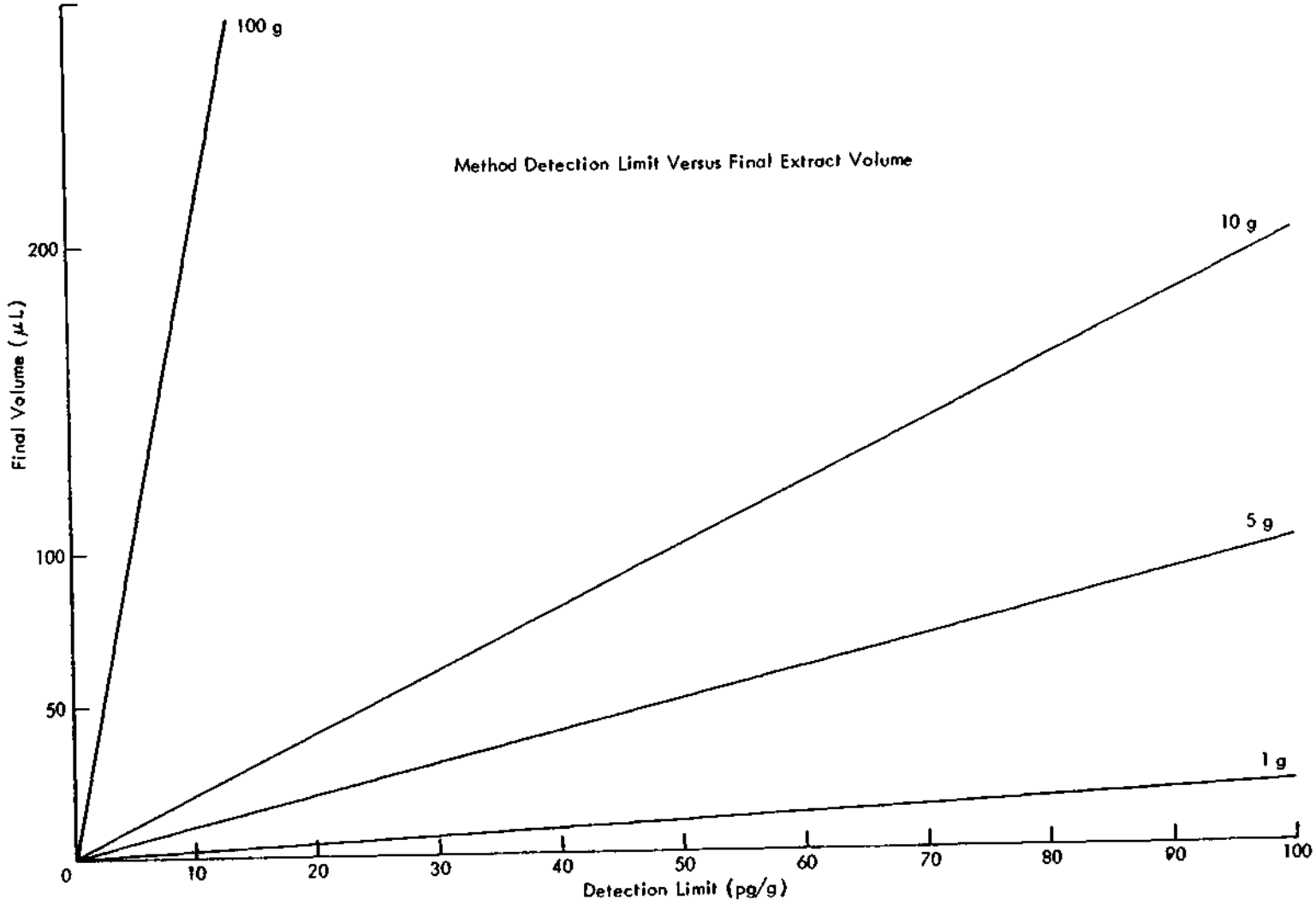


Figure 16. Method detection limit versus final extract volume and initial sample size assuming a GC/MS instrumental detection limit of 5 pg/µl on-column.

TABLE 19. FEASIBILITY STUDY FOR THE QUANTITATIVE DETERMINATION OF TCDD IN QA TISSUE SAMPLES

TCDD detection limit (ppt)[a]	TCDD detected (ppt)[a]	TCDD fortification level[b] (pg)	(ppt)
3	8	1	4
5	16	0	0
1	2	0.5	2[c]
1	3	2	8
1	6	16	6[c]

Source: Harless, R. L., "Analytical Methodology for 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Its Application by the United States Environmental Protection Agency to Human and Environmental Monitoring," presented at the Assistant Administrators Program Review, U.S. EPA, Washington, D.C., April 1980.

a ^{37}Cl-TCDD mean percent recovery - 75%. Values are not corrected for percent recovery losses.

b Each 0.250 g sample was fortified with 0.5 ng ^{37}Cl-TCDD.

c Standard solutions.

TABLE 20. DETECTION LIMITS FOR TCDD IN VARIOUS SAMPLES

	No. of determinations	Limit of detection (ppt) Range	Average
Arkansas and Texas			
Catfish	57	2-22	8
Viscera	2		10
Bass, walleyed pike	52	1-14	7
Sunfish, etc.			
Flesh	11	2-3	3
Viscera	6	2-8	4
Liver	2	5-15	10
Skin	5	2-10	7
Eel			
Flesh	1		7
Viscera	1		5
Skin	1		4
Shark liver	1		11
Sea cucumber			
Flesh	1		1
Viscera	1		1
Crayfish			
Whole	1		4
Muscle	1		4
Viscera	1		7
Tadpole	1		20
Toad	1		3
Rabbit			
Liver	1		8
Pelt	1		2
Beaver liver	11	3-17	9
Opossum			
Liver	1		10
Fat	1		10
Deer			
Liver	3	4-5	4
Fat	1		4
Insects	1		3
Insect larvae	1		8
Diving beetles	1		30
Snails	1		2
Mice			
Liver	1		8
Skin	3	10-40	20
Whole	10	3-8	5
Rat liver	1		20
Shrimp	4	1	1

(continued)

TABLE 20 (continued)

	No. of determinations	Limit of detection (ppt) Range	Average
Beef			
Fat	60	3-10	6
Liver	7	2-7	4
Sheep			
Fat	7	5-15	9
Liver	15	3-10	7
Kidney	12	3-6	5
Muscle	5	2-6	5
Bovine			
Milk (40 g)	28	0.5-1	1
Cream	4	3-5	4
Human milk	6	1-6	3
Rice	21	2-7	4
Rat feed	5	4-6	5
Sheep feed	1		3
Cattle feed	1		6
Grass	2	12-14	13
Seed (grass)	5	2-12	7
Sorghum	2	2-3	3
Leaves	1		4
Roots	2	4-6	5
Soil	100	3-10	6
Water	4	0.1-0.2	0.2
Blood (1 g)	2	40	40

Source: Shadoff, L. A., and R. A. Hummel, "The Determination of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Biological Extracts by Gas Chromatography Mass Spectrometry," Biomed. Mass Spectrom., 5:7-13 (1978).

Quality Assurance

All studies concerning the analysis of biological samples for PCDDs have included some form of a quality assurance (QA) program. The routine use of stable isotope labeled PCDDs as surrogates for internal standard quantitation and method recovery is practiced most frequently as a QA procedure. Studies undertaken by the Dioxin Monitoring Program (Harless et al., 1980) included the use of stable labeled surrogates, submission of blind samples, duplicates, and blanks to the analyst, establishing of criteria for the positive identification of PCDDs, and interlaboratory studies to bolster the significance and validity of TCDD data generated. The methods adopted by EPA for the analysis of TCDD in water, wastewater (EPA, 1982), soil and sediment (EPA, 1983) require a specified number of samples to be analyzed in duplicates or as spiked samples at levels near the detection limit. In addition, these methods specify routine performance evaluations with respect to isomer specificity by HRGC, consistency of response factors, evaluation of method blanks, qualitative criteria, and analysis of blind spiked samples. Other analysts (Gross et al., 1981; Langhorst and Shadoff, 1980; Kocher et al., 1978; Stalling et al., 1982; Tosine, 1981; O'Keefe et al., 1978; Mahle, 1977; Mitchum et al., in press) have also completed method validations as QA procedures with respect to the analysis of PCDDs.

Stable Isotope Labeled Compounds--

Chlorine-37 or carbon-13 labeled 2,3,7,8-TCDD were available for use as surrogate compounds for the analysis of 2,3,7,8-TCDD in most studies. The advantage of using these compounds is that they behave exactly as native TCDDs throughout extraction, cleanup, and gas chromatography separation. The mass spectra of the native and stable isotope labeled compounds vary enough to allow differentiation during the analysis. The surrogate compound added to the sample is used as a true internal standard for quantitation. The concentration calculated by the internal standard method provides a recovery correction. The method recovery can be determined by comparing area response of the quantitation ion for the internal standard in the sample extract versus the area response in an external standard. A more accurate measurement of method recovery can be achieved by adding a second internal standard to the sample extract prior to instrumental analysis. For example, TCDD can be measured in a sample by internal standard quantitation with accurate method recovery determination by combining the use of the carbon-13 and chlorine-37 labeled TCDD compounds. Stable isotope labeled compounds are also commercially available for the hepta- and octa-PCDDs. Nestrick and Lamparski (1982) have described techniques for synthesizing carbon-13 labeled penta- to octa-PCDDs from perchlorination of microgram amounts of carbon-13 labeled 2,3,7,8-TCDD.

Langhorst and Shadoff (1980) have reported the analysis of tetra-, hexa- and octa-PCDDs in human milk samples and have provided recovery data for each homolog determined by comparing external standards to the surrogate compounds. Table 21 is an example of the end use of surrogate recovery and internal standard quantitation as presented by Langhorst and Shadoff (1980). These data were generated while validating an analysis method for human milk. The values for percent recovery are the recoveries of the isotopically labeled surrogates. The percent accountability refers to the amount of observed native dioxin corrected for recovery of internal standard compared to the actual

TABLE 21. PERCENT RECOVERY OF INTERNAL STANDARD AND PERCENT ACCOUNTABILITY FOR NATIVE DIOXINS SPIKED INTO CONTROL MILK HOMOGENATE

	2,3,7,8-TCDD				HCDD				OCDD			
	Concn, ppt				Concn, ppt				Concn, ppt			
No.	Added	Found	% Recovery	% Account-ability	Added	Found	% Recovery	% Account-ability	Added	Found	% Recovery	% Account-ability
1	1.0	0.2	42	20	5.3	4.5	70	84	21	21	52	100
2	1.0	0.2	65	20	5.3	3.7	67	70	21	38	39	180
3	1.3	0.7	56	54	6.6	7.0	64	106	27	23	53	85
4	1.3	0.8	33	62	6.6	7.2	57	109	27	40	31	150
5	1.3	0.8	49	62	6.6	7.2	57	109	27	37	45	140
6	2.0	1.6	38	80	9.9	5.7	70	58	41	49	33	120
7	2.0	2.2	96	110	9.9	8.0	86	81	41	41	110	101
8	2.6	2.0	25	77	13	9.0	76	68	53	41	53	77
9	2.6	2.4	25	92	13	9.9	68	74	53	33	49	62
10	2.6	2.2	32	85	13	8.9	85	67	53	42	55	79
11	2.6	1.9	26	73	13	8.1	74	61	53	38	48	71
12	2.6	1.9	21	73	13	13	47	98	53	38	43	71
13	2.6	2.5	11	96	13	13	38	98	53	40	32	75
14	2.6	1.9	23	73	13	13	53	98	53	45	43	84
15	2.6	1.4	34	54	13	10	52	75	53	57	35	108
16	3.9	2.3	34	59	20	17	59	86	80	51	50	64
17	3.9	3.3	25	85	20	11	61	56	80	98	31	120
18	12	10	33	84	60	42	81	70	240	360	45	150
19	12	12	33	97	60	42	85	70	240	270	58	110
avg			37	77			67	81			48	94
std dev			±19	±16			±11	±17			±17	±43

Precision data for eight replicates samples (no. 8-15)

Compound	Conc added, ppt	% Recovery	% Accountability
2,3,7,8-TCDD	2.6	25 ± 7	78 ± 13
HCDD	13	65 ± 13	80 ± 16
OCDD	53	45 ± 8	78 ± 14

Source: Langhorst, M. L., and L. A Shadoff, "Determination of Parts per Trillion Concentrations of Tetra-, Hexa-, Hepta-, and Octachlorodibenzo-p-dioxins in Human Milk Samples," Anal. Chem., 52:2037-2044 (1980).

a Corrected for internal standard recovery.

amount of the native PCDD that was added. The precision of the analysis was also demonstrated by the results of eight replicates (Table 21). These data show the usefulness and applicability of isotopically labeled compounds for producing analytical results of known quality. The data demonstrate the improvement of precision at concentrations much higher than the detection limit and also enlighten the analyst on the difficulties of measurements near the detection limits.

Intralaboratory Validation of Method--

Methods development for the analysis of any particular compound or compounds requires validation of the partial steps (extraction, cleanup, etc.) as well as the entire method. Table 22 is a summary of some of the published method vaidation data for PCDDs reported in the literature. Many of the methods reported were validated using replicate measurements of samples fortified with native PCDDs and/or the available isotopically labeled PCDDs. The mean percent recovery of the native compounds and the isotopic surrogates vary with respect to the validation experiments near the detection limit. The values summarized in Table 22 are indications of the total method performance.

Intralaboratory validation requires a closer study of the individual method steps or procedures. This subject has already been demonstrated in this review with respect to extraction, cleanup and quantitation procedures in general. Table 23 is a specific example of intralaboratory validation of specific steps for a single method. The data in Table 23 were generated by diDomenico et al. (1979) while developing analytical methods for 2,3,7,8-TCDD in environmental samples near Seveso, Italy. The sample extracts were cleaned using a combination of the four procedures alluded to in Table 23 as cleanup steps A to D. Step A was a wash with concentrated sulfuric acid that did not introduce any appreciable losses. Procedure B involved a chromatographic cleanup with sulfuric acid treated Celite 545. Acetonitrile partitioning (Step C) of the extract from Step B proved to be an alternate to Step A but was also found to be more time consuming. Final cleanup (Step D) was accomplished using a micro alumina chromatography column. The data presented in Table 23 are representative of replicate analyses of spike recovery experiments for the individual steps and combination of procedures without the influence of a sample matrix. The recovery values for the extraction and cleanup of soil, grass, and cotton swabs are also compiled in Table 23 and are indicative of the entire method performance for samples spiked with 5 to 550 μg of 2,3,7,8-TCDD.

Two other studies reported in the literature provided statistical evaluation of the method validation data. Langhorst and Shadoff (1980) and Gross et al. (1981) evaluated data for human milk and bovine fat samples, respectively. The methods of sample preparation and mass spectrometry analysis differed significantly between these two studies.

TABLE 22. SUMMARY OF SOME PUBLISHED METHOD VALIDATION DATA FOR 2,3,7,8-TCDD RECOVERED FROM FORTIFIED BIOLOGICAL MATRICES

Reference	Bovine milk	TCDD Level of fortification: Native ng·kg^{-1}	TCDD Level of fortification: Isotope ng·kg^{-1}, ^{13}C, (^{37}Cl)	Number of replicates	Mean % recovery with s.d.: Native	Mean % recovery with s.d.: Isotopes
Langhorst and Shadoff (1980)	Human milk	2.6	166	8	25 ± 7	37 ± 19
Tosine (1981)	Fish	20		6	-	92 ± 4
Gross et al. (1983)	Human adipose	0		1	ND	40
		6		1	150	40
		16		1	125	45
		38		1	110	40
			(^{37}Cl)			
Harless et al. (1980)	Fish, liver	0-125	1000	17	± 15[b]	86 ± 15
	Human milk	0-5	250	17	± 38[b]	68
O'Keefe et al. (1978)	Bovine milk	-	-	4	ND	ND
		0.7	66	4	86 ± 17	71 ± 12
		13	66	4	100 ± 8	71 ± 12
		65	66	4	85 ± 9	87 ± 21
Mahle et al. (1977)	Bovine milk	2	-	3	83.3	-
		-	625[a]	4	-	64
O'Keefe et al. (1978)	Bovine fat	-	-	4	ND	ND
		13	390	4	100 ± 15	77 ± 18
		25	+[c]	4	80 ± 5	77 ± 18
		100	+[c]	4	85 ± 7	77 ± 18
		200	+[c]	4	88 ± 18	105 ± 9
Baughman and Meselson (1973)	Liver	20	1000	9	34 ± 7	27 ± 5
Kocher et al. (1978)	Bovine fat	10		7	76 ± 10	

Source: Adapted from National Research Council of Canada, "Polychlorinated Dibenzo-p-dioxins: Limitations to Current Analytical Techniques," NRCC No. 18576, ISSN 0316-0114, 1981.

a Indicates publishing author's recovery data were converted from ng to ppt or from ppt to % by the Panel.

b These data indicate the mean % accuracy for TCDD obtained with quality assurance samples.

c Plus indicates fortified with isotope but amount not specified clearly.

TABLE 23. RESULTS OF RECOVERY TESTS PERFORMED ON THE ANALYTICAL PROCEDURE, OR ITS SINGLE PARTS[a]

Operation and number of tests	TCDD % Recovery		
	Minimum	Maximum	Average
Cleanup step B, 12	80	124	101 ± 12[b]
Cleanup step C, 15	73	114	91 ± 13
Cleanup step D, 14	95	120	102 ± 7
Cleanup steps B-D, 18	80	115	96 ± 12
Cleanup steps B-C-D, 8	58	93	76 ± 11
Soil, 28[c]	74	101	86 ± 7
Grass, 12[c]	72	98	85 ± 7
Cotton swabs, 46[c]	68	112	86 ± 10

Source: diDomenico, A., et al., "Analytical Techniques for 2,3,7,8-Tetrachlorodibenzo-p-dioxin Detection in Environmental Samples After the Industrial Accident at Seveso," Anal. Chem., 51:735-740 (1979).

a Cleanup step A did not introduce any appreciable 2,3,7,8-TCDD loss provided the operation was performed with the utmost care. This conclusion was reached after a number of recovery tests had been carried out by applying a sequence of cleanup steps including A. 2,3,7,8-TCDD quantity used: 0.1 and 0.01 µg/test in 1 ml solvent.

b Standard deviation.

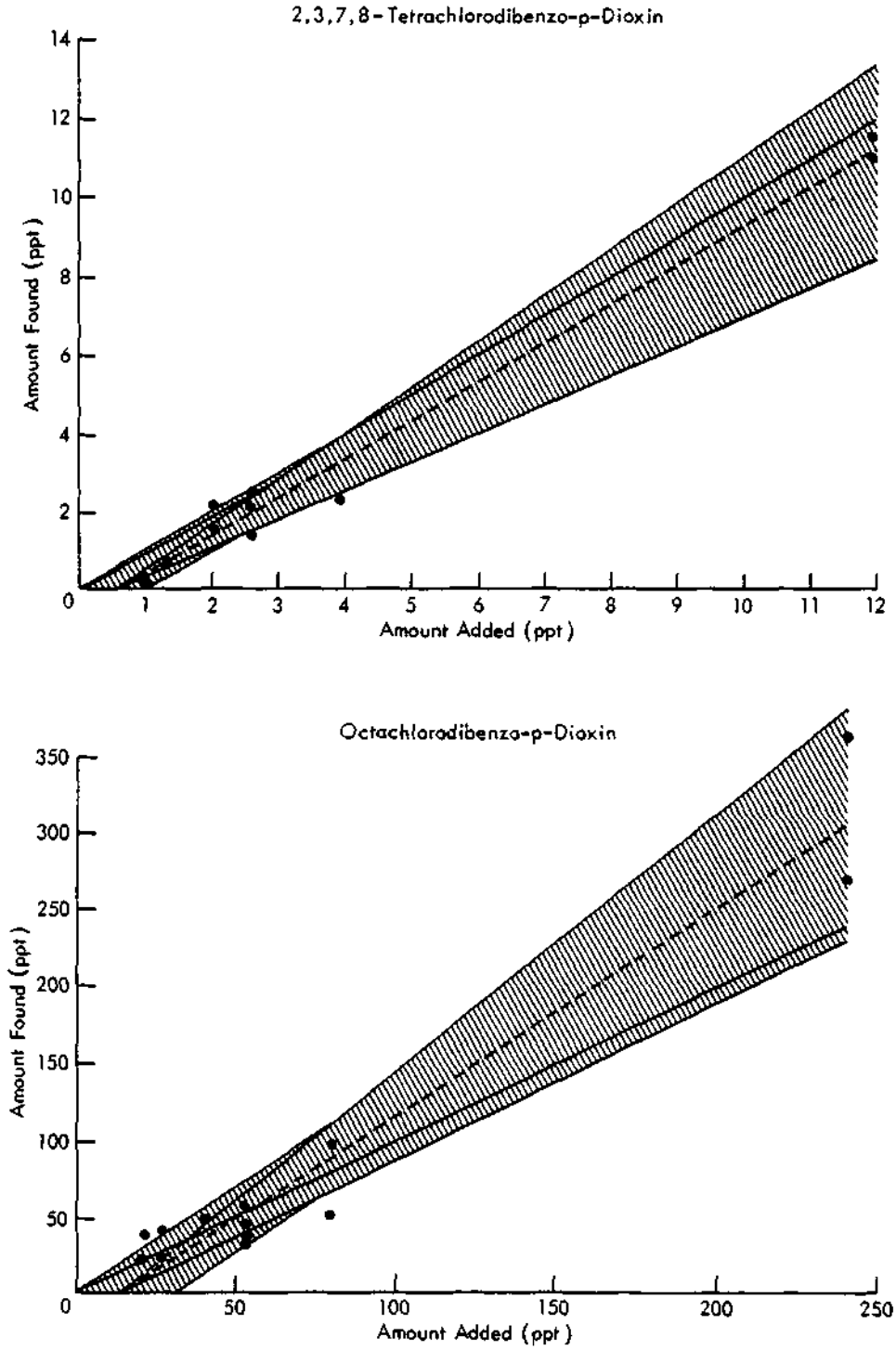
c Values reported take into account 2,3,7,8-TCDD losses due to cleanup steps.

The data generated by Langhorst and Shadoff (1980) for the analysis of tetra-, hexa- and octa-PCDDs for seven controls, seven replicate samples and spiked samples were presented in Table 21. The actual limitations of the method were defined by the dioxin level in the control sample and by statistical treatment of the data. Figure 17 is an example of the statistical data treatment for 2,3,7,8-TCDD and OCDD analysis. The heavy solid line is the actual level of native dioxin spiked. The dashed line represents the least squares fitted line for the dioxin concentration observed. The shaded area represents the total uncertainty of the determination including the error associated with the least squares fitted line and the error associated with the final recovery of dioxin for GC/MS analysis.

The statistical validation of the method practiced by Gross et al. (1981) was generated from analytical results for 26 bovine fat samples and 26 standard solutions spiked at levels ranging from 0 to 81 ppt. The samples and standards were prepared and submitted simultaneously for TCDD analysis by PGC/HRMS with blind sample codes. The sample identifications were decoded when all the analytical results (52 samples) were submitted for statistical analysis. The statistical analysis results for the standard solutions and beef fat samples are illustrated in Figure 18. The theoretical line $y = x$ representing perfect extraction and quantitation was included for comparative purposes. Two sets of upper and lower 95% confidence limits were included for least squares regression of reported values (y) on spiking levels (x). The boundary lines closest to the regression lines represent the upper and lower 95% confidence limits for an infinite number of analyses under the same conditions. The outer boundary lines are indicators of the 95% confidence limits for a single analysis. Based on the results of the statistical analysis of the data, Gross et al. (1981) determined the lower limit of quantitation to fall between 5 and 9 ppt.

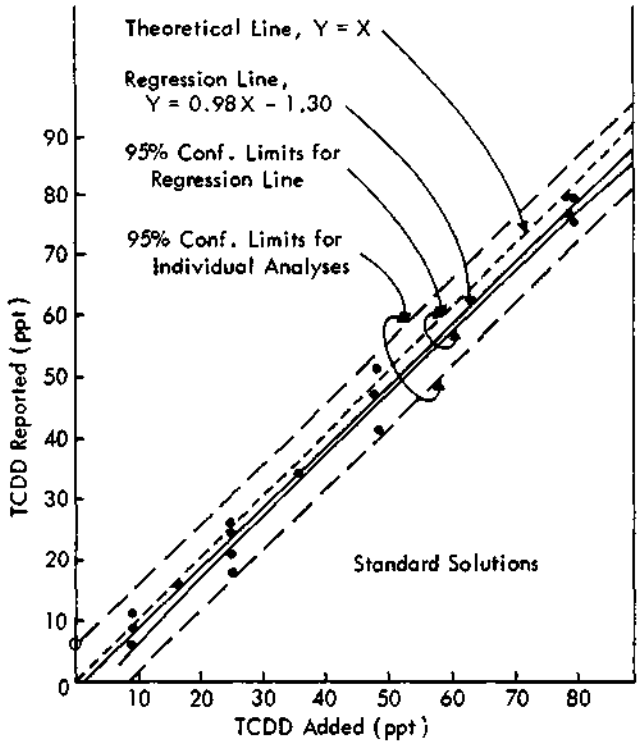
Interlaboratory Studies--

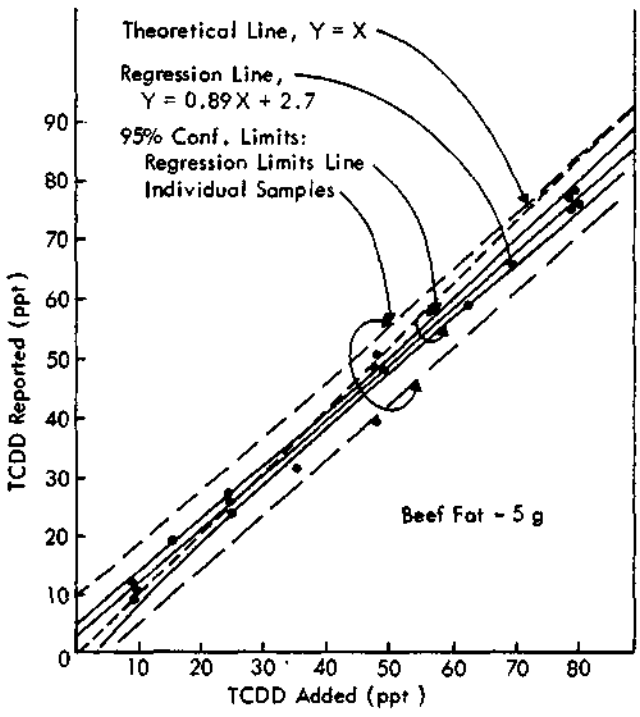
The review of analytical methods for PCDDs presented by the NRCC (1981) included an evaluation of the techniques with respect to applicability to matrix, specificity, method validation, and interlaboratory studies. None of the methods reviewed at the time was given the highest rating because evaluation through a collaborative study was not included. Since that time several interlaboratory studies have been completed or are still in progress. These studies are summarized in Table 24. The only study conducted for biological matrices with directions to follow a specific analytical method is with the pork adipose matrix (EMSL/LV). The participants were instructed to follow the procedures published by Harless et al. (1980) for parts per trillion measurements of 2,3,7,8-TCDD in pork adipose tissues. Samples that were analyzed in other studies were prepared according to Harless et al. (1980), while GC/MS measurements of 2,3,7,8-TCDD were conducted according to the practices of the individual laboratory.



Source: Langhorst, M. L., and L. A. Shadoff, "Determinations or Parts per Trillion Concentrations of Tetra-, Hexa-, Hepta-, and Octachlorodibenzo-p-dioxins in Human Milk Samples," Anal. Chem., 52, 2037-2044 (1980).

Figure 17. Statistical treatment of validation data for 2,3,7,8-TCDD and OCDD in human milk samples.





Source: Gross, M. L., T. Sun, P. A. Lyon, S. F. Wojinski, D. R. Hilker, A. E. Dupuy, Jr., and R. G. Heath, "Method Validation for the Determination of Tetrachlorodibenzodioxin at the Low Parts per Trillion Level," Anal. Chem., 53, 1902-1906 (1981).

Figure 18. Statistical treatment of reported concentrations versus concentrations of TCDD actually added to standard solutions and beef adipose.

TABLE 24. INTERLABORATORY STUDIES AND METHOD VALIDATIONS FOR THE ANALYSIS OF TETRACHLORODIBENZO-p-DIOXINS (TCDD)

Matrix	Method(s)	Number of participating laboratories	TCDD concentration range	Reference
Water Wastewater	EPA Method 613	13	20 - 200 ppt	McMillin et al. (1982)
Soil Sediment	EPA Region VII protocol for soil and sediment	a	1 - 100 ppb	EMSL/LV[b]
Pork adipose	Harless et al. (1980)[c]		1 - 100 ppt	EMSL/LV[b]
Human adipose	Harless et al. (1980)[d]	3	1 - 100 ppt	Dioxin Monitoring Program Gross et al. (1981)
Beef adipose	Harless et al. (1980)[d]	4	1 - 100 ppt	Dioxin Monitoring Program Gross et al. (1981)
Fish	e	6	105 - 121 ppt	Brumley et al. (1983)
Fish	e	13	1 - 100 ppt	Ryan et al. (1983)
Fish	f	8	1 - 200 ppt	O'Keefe et al. (1983)
Beef adipose	Harless et al. (1980)[d]	2	1 - 100 ppt	Dioxin Monitoring Program Gross et al. (1980)
Soil Sediment	EPA Region VII protocol for soil and sediment		1-100 ppt	EMSL/LV[b]
Pottery clay	EPA Region VII protocol for soil and sediment	a	1 - 10 ppb	EMSL/LV[b]

a These samples are used as performance evaluation samples for laboratories involved with analysis of 2,3,7,8-TCDD in soils and sediments.

b Personal communicaton J. Donnelley (1983).

c Participating laboratories were instructed to follow procedure as described by Harless et al. (1980). Some modifications to the method were reported.

d Samples prepared by method described by Harless et al. (1980), but GC/MS conditions varied.

e Each Participting laboratory used current in-house analytical method.

f Sample extracts divided at specific steps of one protocol and submitted to participating laboratories for further analysis.

Some examples of the data generated in these interlaboratory studies are presented in Tables 25 to 28. Table 25 illustrates the results of the analysis of human adipose samples for 2,3,7,8-TCDD. These samples were analyzed initially by PGC/HRMS. A subset of these samples were reextracted and/or reanalyzed at other laboratories to provide interlaboratory validation of reported detections at these low levels. The validation of the analyses was accomplished in two ways. Remaining extracts from PGC/HRMS were reanalyzed using HRGC/HRMS, and portions of the tissues were submitted for reextraction and cleanup followed by HRGC/HRMS. All samples were coded and their identities were not known to the analysts. Based on the interlaboratory validation study, it was confirmed that two of the three samples designated as having heavy exposures contained 2,3,7,8-TCDD at higher levels than those observed for other participants. In addition, 2,3,7,8-TCDD was detected in tissue from other exposed and nonexposed persons designated as controls that were also examined by the interlaboratory studies.

Table 26 provides a comparison of the results obtained by the different methods, PGC/HRMS versus HRGC/HRMS. Gross et al. (1981) have discussed the differences in concentration as reflecting the relatively large uncertainties in quantitation techniques in the parts per trillion range. Some of the variations between sample extracts analyzed by PGC/HRMS and HRGC/HRMS may be due in part to differences in resolution of the 2,3,7,8-TCDD from the other 21 possible isomers. In addition, the results may indicate sample inhomogeneities since different portions of unhomogenized tissue were used in each experiment.

The interlaboratory study reported by Ryan et al. (1983) involved 13 laboratories having experience in determination of low levels (parts per trillion) of 2,3,7,8-TCDD in biological samples. Each laboratory agreed to analyze four fish samples for 2,3,7,8-TCDD using their routine extraction, cleanup and detection procedures. Table 27 presents the data reported by 8 of the 13 laboratories. The relative standard deviation for samples A, C and D is surprisingly low (14.0, 18.4, and 25.3%, respectively) considering the picograms per gram levels in the original sample. This variation is significantly less than that predicted by Horowitz et al. (1980) for low level quantitation.

The recoveries of the internal standard (either carbon-13 or chlorine-37) 2,3,7,8-TCDD are presented in Table 28 for six of the laboratories that used internal standard quantitation. The average recovery of the individual laboratories ranged from 57 to 82% with a relative standard deviation of approximately 25%. The range of all the individual measurements yielded 29 to 109% recovery of the internal standard. This difference in method performance indicated the needs and usefulness of the internal standard quantitation approach.

The results indicated fish samples C and D (Table 27) contained similar levels of 2,3,7,8-TCDD. These data were statistically evaluated according to the methods of Youden to determine variations between laboratories (systematic error) and within laboratories (random error). The results indicated that the difference between laboratories (reproducibility) was somewhat greater than the variance within laboratories (repeatability), although the differences reported were not significant at the 95% confidence level.

TABLE 25. RESULTS OF ANALYSIS OF TCDD IN HUMAN ADIPOSE TISSUE[a]

VA code number	Concentration (ppt)[b]	Detection limit (ppt)	Percent recovery	Ratio[c]
"Heavily Exposed Veterans"				
10	23	4	65	.85
10	35	9	100+	.75
19	ND[b]	3	20	-
26	99	10	90	.77
26	63	6	45	-
"Lightly Exposed Veterans"				
1	ND	5	50	-
13	ND	2	80	-
28	7	4	50	.88
28	8	6	40	.78
34	5	3	100	.85
"Possibly Exposed Veterans"				
6	5	3	65	.90
8	5	3	50	.90
9	ND[a]	3	40	-
11	3	2	55	.77
12	9	3	60	.88
14	4	3	65	.74
16	ND	4	60	-
24	5	3	80	.71
24	5	4	45	-
25	12	4	45	-
25	10	3	100+	.78
27	ND	6	100	-
29	13	5	60	.88
30	ND	3	95	-
"Controls"				
5	4	4	65	1.02
7	3	2	60	.92
17	4,3	3	75	.84
18	ND	4	30	-
20	5	4	50	.86
21	6	3	35	1.07
23	8	2	100	.78
23	6	3	55	-
31	7	4	50	.98
32	4	4	60	.74
33	14	7	100	.94

(continued)

TABLE 25 (continued)

VA code number	Concentration (ppt)[b]	Detection limit (ppt)	Percent recovery	Ratio[c]
"USAF Scientists"				
2	5	2	50	.77
3	4	1	85	.94
4	6	2	50	.76

Source: Gross, M. L., J. O. Lay, P. A. Lyon, D. Lippstred, N. Kangas, R. L. Harless, S. E. Taylor, and A. E. Dupuy, "2,3,7,8-Tetrachlorodibenzo-p-dioxin Levels in Adipose Tissue of Vietnam Veterans" (personal communication).

a Sample sizes ranged from 2.2 to 11.6 g for each extraction. Internal standard amounts used varied from 2.0 - 2.6 ng/extraction.

b ND = not detected.

c Ratio of intensities of m/z 320 and m/z 322. Acceptable values are 0.78 ± 0.10.

TABLE 26. RESULTS OF INTERLABORATORY VALIDATION STUDIES

VA Code	UN-L/UN-L[a]	UNL/RTP[b]	TAC/RTP[c]	TAC/RTP[d]	UN-L/UN-L[e]
"Heavily Exposed Veterans"					
VA-26	63,99	-	173	-	-
VA-10	23,35	36	-	86	-
VA-19	ND(3)[e]	-	-	20	ND(29)
USAF Researchers					
VA-3	4[g]	3	10	-	-
VA-2	5	-	-	24	-
Other Vietnam Veterans					
VA-13	ND(2)	ND(0.2)	ND(7)	-	-
VA-8	5	3	5	-	-
VA-9	ND(3)	3	-	ND(7)	-
VA-15	7	-	-	18	-
VA-34	5	-	-	ND(5)[f]	-
Controls					
VA-17	4,3	-	20	14	-
VA-18	ND(4)[f]	5	8	-	-
VA-21	69	3	12	-	9
VA-31	ND(4)	-	ND(3)	-	-
VA-20	5	-	-	19	20

Source: Gross, M. L., J. O. Lay, P. A. Lyon, D. Lippstred, N. Kangas, R. L. Harless, S. E. Taylor, A. E. Dupuy, "2,3,7,8-Tetrachlorodibenzo-p-dioxin Levels in Adipose Tissue of Vietnam Veterans" (personal communication).

a Extracted at UN-L/analyzed at UN-L (University of Nebraska, Lincoln). The values given in parentheses are the detection limits.

b Portion of the extract from UN-L/analyzed at RTP (Research Triangle Park).

c Extracted at TAC (Toxicant Analysis Center)/analyzed at RTP.

d Another portion of tissue shipped from UN-L, extracted at TAC/analyzed at RTP.

e Extracted at UN-L/analyzed at UN-L. Results obtained with knowledge of the code.

f Poor recovery of internal standard (< 40%).

g Isotope ratio for m/z 320 and n/z 322 not correct.

TABLE 27. CONCENTRATION OF 2,3,7,8-TCDD IN FISH SAMPLES FROM INTERLABORATORY STUDY

Values are single determinations expressed in pg/g (ppt).

Lab No.	Fish sample A	B	C	D
1[a]	104[b]	ND[c](10)	35	45
3	58	ND(1.3)	37	33
4[d]	49	ND(2)	23	19
5[e]	58	ND(1)	34	38
6	ND(5)[f]	ND(5)	51[f]	55
7	72	ND(2.3)	25	32
9	70	ND(5)	33	27
12	60	37[g]	26	32
Av.[h]	61.2	3.6	30.4	32.3
SD	8.5	0	5.6	8.2
CV, %	14.0	-	18.4	25.3
n	6	6	7	7

Source: Ryan, J. J., J. C. Pilon, H. B. S. Conacher, and D. Firestone, "Interlaboratory Study for the Analysis of Fish for 2,3,7,8-Tetrachlorodibenzo-p-dioxin," in press, 1983.

a Also reported GC/ECD values of 103, ND(10), 39, 37 pg/g, respectively.

b Value given judged to be an outlier by Dixon's test; recovery of this sample was judged by the analyst to be high (74%), so an average recovery (51%) was used to calculate value given.

c Not detected followed by bracketed detection limits in pg/g.

d Also reported higher values of 58, ND(2), 37, 38 pg/g for acid-base method; these values are closer to average than neutral method preferred by the analyst.

e Confirmed by atmosphere pressure-negative chemical ionization GC/MS on same extract with values of 54, ND(2.3), 32, and 31 ppt, respectively, for samples A, B, C, D.

f Value given judged to be outlier.

g Value given judged to be outlier; subsequent analysis showed a value of ND(10) pg/g.

h Does not include any outliers or values from laboratory 6.

TABLE 28. PERCENT RECOVERIES OF INTERNAL STANDARD TCDD IN THE INTERLABORATORY STUDY

Lab No.	Av.[a]	SD	CV, %
1[b]	57.0	11.6	20.4
3[c]	69.0	16.6	24.1
4[d]	80.0	12.3	15.4
5[c]	83.1	18.7	22.5
7[d]	35.3	5.2	14.7
9[c]	74.6	18.4	24.7
12[c]	81.8	14.5	17.7
	67.7		
Range	29-109		

Source: Ryan, J. J., J. C. Pilon, H. B. S. Conacher, and D. Firestone, "Interlaboratory Study for the Analysis of Fish for 2,3,7,8-Tetrachlorodibenzo-p-dioxin," in press, 1983.

a Each value represents the average of 4 reported values.

b Fortified duplicate with native 2,3,7,8-TCDD.

c ^{13}C-2,3,7,8-TCDD.

d ^{37}Cl-2,3,7,8-TCDD.

The analytical results for samples C and D, although somewhat limited, were further evaluated to see if there were significant differences for the different analytical methodologies. No differences were determined with this treatment for methods that used digestion or extraction; high or low resolution mass spectrometry; and specific or nonspecific isomer separaton.

Needs for Future Validation Studies--

Although several interlaboratory studies have been conducted, there is need for further validation of specific procedures. The results from such studies presented by Ryan et al. (1983), Brumley et al. (1981), Gross et al. (1980), and O'Keefe et al. (1983) demonstrate that the available methodologies are comparable in performance and provide reasonably valid measurements with respect to other approaches. Critical assessments of specific steps of the methodologies have not been attempted. There is need for a single laboratory to compare the best approach, for example, for initial extraction of PCDDs from the sample matrix (acid digestion, alcoholic saponification, or neutral extraction). Likewise, cleanup procedures should be compared and evaluated to generate information on recovery of analytes and separation from specific contaminants such as PCBs, chlorodiphenylethers, chloromethoxybiphenyls, etc. In order to accomplish this evaluation of methodology, it is important to vary only one parameter at a time. Additional validation of the methods is required if it is necessary to measure other homologs of PCDDs other than TCDD. Another important aspect that must be evaluated when considering interlaboratory validation of a single method is the ease of individual analytical steps. In order to demonstrate and fully evaluate the validity of a method all participating laboratories should be able to manipulate all procedural steps with good precision and accuracy.

SECTION 5

APPLICABLE TECHNIQUES - RECOMMENDATIONS

Following the first submission of the literature review (Sections 1 - 4, this report), MRI was requested to organize a meeting to discuss analytical approaches for the analysis of PCDDs and PCDFs. This Section presents a synopsis of a discussion meeting held at Midwest Research Institute, Kansas City, Missouri, on April 27 and 28, 1983. The specific purpose of this meeting was to discuss analytical methods that are applicable to the analysis of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in human adipose tissues. The discussion meeting was attended by scientists (Appendix A) recognized as experts in the field of PCDD and PCDF analysis. The meeting served as an additional source of information pertaining to specific considerations for low parts-per-trillion measurements of PCDDs and PCDFs in human adipose tissue. The meeting followed the first draft of the written literature review with preliminary method recommendations for analysis of PCDDs in adipose tissue and a peer review (Stanley, 1982) of the initial document.

DISCUSSION MEETING SUMMARY

The meeting was organized to promote open and detailed discussion on the criteria that must be considered for an effective analytical method and study of PCDD levels in human adipose tissue. Scientists recognized as experts in the field of PCDD and PCDF analysis were invited to participate (Appendix A). Most of the participants had previously provided peer review comments to the literature review and preliminary recommendations.

Representatives from EPA/OTS and the VA presented overviews on the design of a general population study to determine PCDD exposure using existing adipose sample repositories and an update of the VA involvement with Agent Orange studies.

A summary of the primary issues identified from the peer reviews of the literature review and preliminary recommendations was presented. These issues included (a) the need for stating the primary objectives of the program, (b) the use of high resolution mass spectrometry (HRMS) versus low resolution mass spectrometry (LRMS), (c) the practical limitations of the proposed extract cleanup procedures, and (d) additional measures for the quality assurance program.

The discussion of methods of analysis were held to four major subject headings. They were: primary objectives of the method, instrumental analysis, sample preparation, and method validation (Appendix B).

Primary Objectives

The primary objective of the method was defined as the need to accurately determine the level of 2,3,7,8-TCDD in human adipose tissue. However, higher chlorinated PCDDs and PCDFs including tetrachlorodibenzofurans are also of interest in the overall program. It was recognized that it may be difficult to achieve this additional data if sufficient sample sizes are not available. It was emphasized that if possible, a method should provide data on PCDDs and PCDFs with chlorine substitution in the 2,3,7,8-positions. The objectives of a method as expressed in the discussion were (a) isomer specific measurement of 2,3,7,8-TCDD, (b) determination of PCDDs and PCDFs with chlorine substitution in the 2,3,7,8-positions, and (c) measurement of total PCDDs and PCDFs by homolog.

Instrumental Analyses

It was a consensus that mass spectrometry is necessary for the identification and quantitation of PCDDs and PCDFs. The criteria for qualitative identification of PCDDs and PCDFs are similar regardless of whether low resolution or high resolution mass spectrometry is used for analysis. These criteria include (1) coincident response of at least two ions characteristic of the molecular ion cluster of a specific homolog, (2) the proper ion response ratio, and (3) the correct retention times. In addition, response of a fragment ion characteristic of the loss of COCl is necessary to confirm the presence of a PCDD congener.

Electron impact ionization mass spectrometry was presented as the most useful for analysis of PCDDs and PCDFs. It was pointed out, however, that other mass spectrometry methods, negative ion chemical ionization in particular, are applicable to the analysis of specific PCDD or PCDF congeners. These alternate mass spectrometry methods also provide additional sensitive confirmatory information.

Method detection limits for analysis of 2,3,7,8-TCDD were estimated at 1 to 5 parts per trillion (ppt), providing that the original sample size is at least 1 to 3 g. It was recognized by the meeting participants that this small sample size may not be sufficient to allow analysis for other PCDDs and PCDFs. The only means of extending a small sample for the analysis of all PCDDs and PCDFs is to isolate the different chlorinated homologs using liquid chromatography techniques. Estimates for method detection limits of octachlorodibenzo-p-dioxins and octachlorodibenzofurans ranged from 20 to 100 ppt.

It was generally recognized that the use of high resolution rather than low resolution is based on the extent that potential interferences are removed from the sample extract. If sufficient extract cleanup is achieved, low resolution mass spectrometry is acceptable for the analysis of PCDDs and PCDFs at low parts per trillion.

Compounds that are known to interfere with the analysis of 2,3,7,8-TCDD were presented in the literature review. A set of compounds that was not considered in the review was chlorinated benzoquinones. The need to study

the potential interferences of these types of compounds was addressed and discussed. The potential interferences to the analysis of higher chlorinated PCDDs and PCDFs have not been identified. It was speculated that compounds similar to the interferences for 2,3,7,8-TCDD analysis, but with greater chlorine substitution, may interfere with the analysis of other PCDDs and PCDFs. These compounds include the polychlorinated biphenyls, benzoquinones, benzylphenyl ethers, and diphenyl ethers.

Sample Preparation

The procedures for sample preparation were discussed with respect to quantitative extractions of PCDDs and PCDFs from sample matrices and the degree of cleanup necessary for instrumental analysis. Several of the participants were asked to describe their analytical preparation schemes and to provide comments as to the advantages or purpose of the particular method steps.

The cleanup procedures discussed were designed with final instrumental technique in mind. The procedure presented in the preliminary recommendation required less stringent cleanup and high resolution gas chromatography/high resolution mass spectrometry. Other procedures require mass extensive cleanup, fractionation of the sample extract with high performance liquid chromatography and analysis by packed column gas chromatography/low resolution mass spectrometry.

Figures 19 and 20 are schematics of the two analytical schemes presented at the meeting following the discussion of sample preparation. These schemes represent routes to final analysis by HRGC/HRMS and HRGC/LRMS. A macro alumina column is recommended to provide additional separation of PCDDs and PCDFs from interferences. If it is necessary to separate PCDDs and PCDFs by homolog, an HPLC step may be necessary. Several of the meeting attendees commented on the advantages of activated charcoal for separating PCDDs and PCDFs from interferences. This step has been proposed as part of the overall scheme for low resolution mass spectrometry.

Considerable discussion centered around the equivalency of extraction procedures. There have been some indirect comparisons of the recovery efficiencies of acidic digestions, basic saponifications, and neutral extractions with fish samples in previous interlaboratory studies. There is a need for a direct comparison of these procedures followed using a common rigorous cleanup procedure to fully evaluate the extraction efficiencies. A more definitive study could be performed by using adipose containing a bioincurred radiolabeled PCDD. Recovery of the radiolabeled PCDD versus recovery of a spiked stable isotope PCDD would provide detailed information on the actual recovery from adipose tissue for each specific technique.

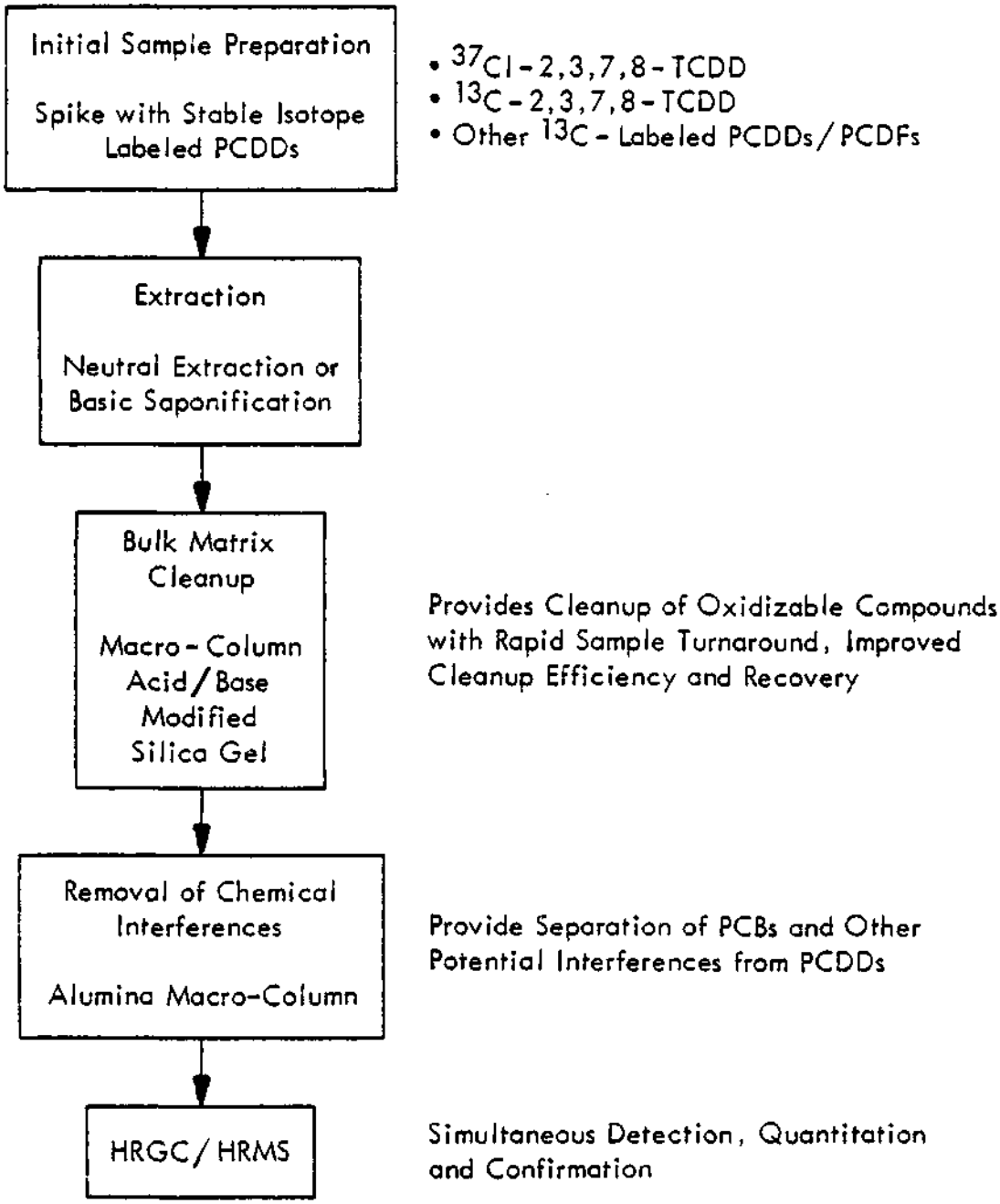


Figure 19. Schematic of proposed analytical method using high resolution mass spectrometry (HRMS).

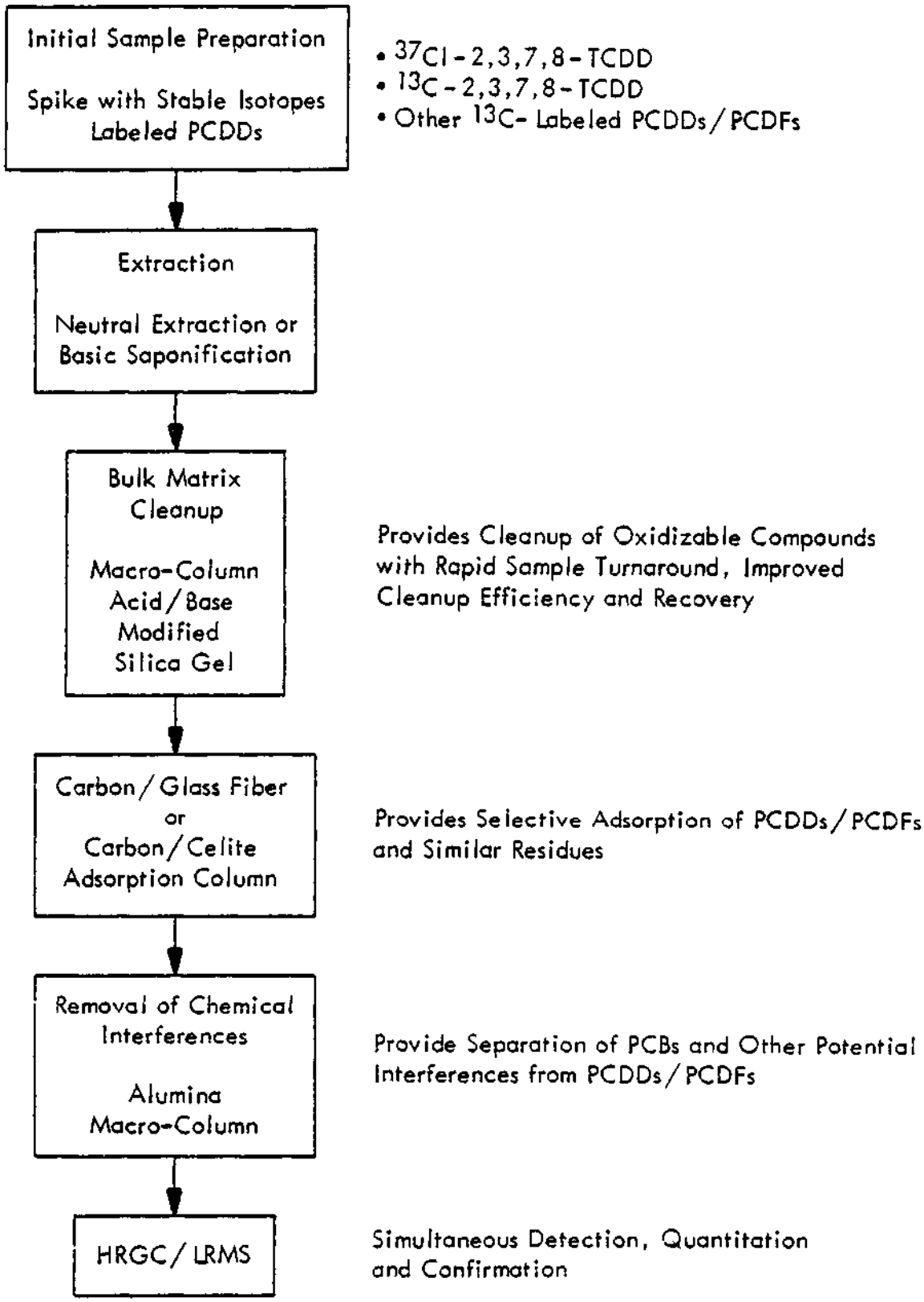


Figure 20. Schematic of proposed analytical method using low resolution mass spectrometry (LRMS).

Method Validation

Validation of a primary analytical method will require participation of at least eight laboratories with a minimum of four samples prepared as Youden pairs at mid-level and lower concentration ranges. A few of the meeting participants felt that a comprehensive evaluation requires the analysis of multiple samples (7-10) at several spiked concentration levels to measure precision of the analytical procedures and to define the actual method detection limit. In addition, an interest was expressed to analyze the same set of samples used for the method validation by alternate analytical methods to independently verify the analysis.

Other points that were presented regarding preparation for a full-scale method validation are presented below. Some samples prepared for the method validation should be spiked with potential interferences to define limitations of the analytical methods. Also, samples of known spiked PCDD concentrations should be provided to a small group of laboratories as a means of identifying potential problems with the written analytical method.

One of the more significant contributions was the suggestion to include actual adipose samples with spiked quality control (QC) samples in the interlaboratory study. Actual adipose samples of sufficient mass would be selected from the repository. These samples would be split and supplied to different laboratories along with the QC samples. The resulting data from the paired laboratories should provide some preliminary information on general population exposure as well as method performance.

Ideally, the method validation study should encompass analyses for tetra- to octachloro-PCDDs and PCDFs. Realistically, this may not be possible because of the significant cost and time required to complete a validation of this magnitude in a single study. It must be kept in mind that the most important issue is analysis for 2,3,7,8-TCDD.

DISCUSSION MEETING RECOMMENDATIONS

The discussion meeting was beneficial in identifying several major programs necessary for the success of the primary analytical method validation and the proposed population studies. These programs include (a) the need for establishing a repository of PCDD/PCDF standards of known quality, (b) the organization and implementation of a strong quality assurance program, (c) the acquisition of sufficient human adipose to generate a homogeneous sample matrix for the QA program, (d) independent studies of extraction procedures using adipose with bioincurred radiolabeled PCDDs, (e) intralaboratory ruggedness testing of a proposed analytical method, and (f) interlaboratory evaluation of the proposed method. Simultaneous activity in several of these areas is necessary in the coming months. The participation of scientists experienced in analysis of PCDDs and PCDFs is needed in many of these programs to aid in designing solid approaches for a successful program. The major action items are discussed in more detail below.

Intralaboratory Testing

A draft of a method will be prepared. The individual steps of the method will be characterized using clean samples or spiked blanks. The total method will be evaluated using adipose tissue spiked with PCDDs and PCDFs. Carbon-14 radiolabeled PCDDs and PCDFs will be used if available to help define critical variables in a more rapid fashion than can be achieved with HRGC/MS. Ruggedness testing of the method will require varying sample sizes, quantities of adsorbent, volumes of solvent, etc., to help define the critical variables and limitations of the method.

The total method including HRGC/MS will be challenged with potential interferences spiked in the sample matrix. A formal method will be written and will undergo peer review to identify uncertainties in the written instructions.

Tissue Program

A large pool of homogeneous adipose tissue is needed to prepare quality control (QC) samples for the overall QA program and interlaboratory validation studies. It is estimated that 40 to 50 kg of adipose tissue are needed to prepare a sufficient number of control samples at known spiked concentration levels with and without the addition of potential interferences. The adipose tissues will be collected through the National Human Monitoring Program network. A repository of the samples will be established. When sufficient samples are collected (40 to 50 kg total), the samples will be pooled and rendered to provide a homogeneous matrix that will be subdivided for spiking procedures. The timing of tissue collection is important since these activities will overlap with the design of the Quality Assurance Program, the Standards Program and needs of the intralaboratory testing and interlaboratory studies.

The following parameters will be considered for collection of the pool of adipose tissues. The adipose tissues will be collected from male trauma victims within 24 hr after death. The specimens will be collected from males born between 1937 and 1952, which is coincident with birthdates for veterans serving in the Vietnam area. All adipose tissues will be frozen until composited for homogenization with other specimen.

A background analysis of the homogenized tissue is necessary to provide information on the levels of PCDDs, PCDFs, and potential interferences. It is recognized that the assistance of laboratories (EPA/RTP; University of Nebraska; Wright State University; Health Protection Branch, Food Division, Canada; Fish and Wildlife Services) with experience in the analysis of PCDDs and PCDFs in adipose tissues will be of benefit in obtaining this information in the most expedient manner. These background analyses must be completed before proceeding with subdividing the homogeneous tissue for spiking purposes as designed under the QA program.

Quality Assurance Program

The Quality Assurance Program will influence the success of the overall program with respect to method validation and performance evaluations for the routine analysis of tissue samples for population studies. The quality assurance program plan will provide details for preparation of fortified tissue samples containing PCDDs and potential interferences. The tissue samples should be spiked with at least one isomer from each PCDD and PCDF homolog.

A subset of QA tissue samples should be spiked with compounds known to interfere with the analysis of PCDDs and PCDFs. This type of performance evaluation sample will provide information on the potential for false positive results.

The QA program will specify the procedures for sample handling, sample coding, frequency of the spiked QC samples, distribution of samples, data handling, and decoding. The design of the QA program must be initiated immediately to provide support to the intra- and interlaboratory method validations.

Standards Program

Procurement of a sufficient quantity of PCDD and PCDF congeners of known quality is essential to provide consistent results from interlaboratory studies, method validations, and actual analysis programs. There is a critical need to establish a repository of the PCDD and PCDF compounds. Currently, participants from the discussion meeting are being surveyed for inventories of PCDDs and PCDFs in specific laboratories. The information gathered from this survey will be useful in identifying needs for procurement or synthesis of specific congeners for the overall program.

Labeled PCDDs are commercially available as carbon-13, chlorine-37, and carbon-14 labeled TCDDs, and carbon-13 labeled octachlorodibenzo-p-dioxin. These compounds will be used as surrogates or internal standards for sample analyses. Stable isotope labeled compounds are not currently available for penta-, hexa-, and heptachloro-PCDDs or any of the PCDFs. If the overall objective of the analysis program is to include tetra- through octachloro-PCDDs and PCDFs, there is a need to study the most cost-effective means to acquire these compounds.

The standards program will also cover collection of potential interferences. Polychlorinated biphenyls and DDE are readily available for addition to samples as interferences. However, compounds such as the chlorinated diphenyl ethers, chlorinated benzylphenyl ethers, and chlorinated benzoquinones may be more difficult to obtain.

Purity of the standard compounds, stable isotope labeled standards, and potential interferences must be known before these compounds can be used for spiking the homogenized tissues for the QA program. Once the purity of the compounds is documented and the repository established, distribution of the compounds to collaborators may occur. Distribution of the standards will be most effective by supplying solutions of accurately determined concentrations.

Bioincurred Program

The need to investigate the extraction efficiency of PCDDs and PCDFs from adipose tissue was discussed at the meeting. A feasible approach to study the extraction efficiency is through use of tissue with bioincurred compounds. The use of carbon-14 radiolabeled 2,3,7,8-TCDD in feeding studies will provide the necessary bioincurred matrix. The recovery of bioincurred carbon-14 labeled compound compared to recovery of spiked stable isotope labeled or native compounds will indicate the adequacy of sample spiking procedures and provide an absolute extraction efficiency.

The bioincurred program will necessarily require several months for completion of the study. Again, there is need for the overlap of this study with other aspects of the total program.

Interlaboratory Studies

Interlaboratory studies are necessary for primary analytical method validation and background analyses of homogenized tissues. The interlaboratory studies required for method validation include a preliminary study of three to four laboratories followed by a full-scale collaborative study with 10 to 12 participants. The preliminary method evaluation will be conducted with samples of known concentration. The purpose of the preliminary study is to familiarize the participants with the method and identify potential difficulties of the method. The analytical method will be refined if necessary based on the preliminary study.

The full-scale method validation will require a significantly larger number of participants. The samples will include the samples prepared under the QA program and will be submitted to the participants under blind codes. The design of the interlaboratory studies should include adipose samples that are (a) spiked near the method limit of detection, (b) spiked with potential interferences, and (c) Youden pairs to determine accuracy and precision.

Actual samples may possibly be included in the interlaboratory validation. These samples would be selected from the pool of samples identified by EPA/OTS and the VA as representative of the general population and Vietnam veterans. Figure 21 is an example of such a study. The TAC sample numbers are included only for illustration purposes. Each actual sample would be split between two laboratories to provide additional data on the accuracy and precision of interlaboratory measurements.

Organization of the interlaboratory studies must begin several months before the actual study. The efforts for organization of the interlaboratory study will overlap with the quality assurance program, standard program, tissue collection, and intralaboratory studies.

Sample Method Validation	Laboratory							
	A	B	C	D	E	F	G	H
Fat	X	X	X	X	X	X	X	X
Fat	X	X	X	X	X	X	X	X
Fat + Interf.	X	X	X	X	X	X	X	X
Fat + Interf.	X	X	X	X	X	X	X	X
Fat + PCDD	X	X	X	X	X	X	X	X
Fat + PCDD	X	X	X	X	X	X	X	X
Fat + PCDD + Interf.	X	X	X	X	X	X	X	X
Fat + PCDD + Interf.	X	X	X	X	X	X	X	X
TAC								
352	X				X			
353		X				X		
354			X				X	
355				X				X
356	X				X			
357		X				X		
358			X				X	
359				X				X

Figure 21. Example of possible interlaboratory organization.

APPENDIX A

INVITED PARTICIPANTS

"METHODS OF ANALYSIS FOR POLYCHLORINATED DIBENZO-p-DIOXINS (PCDDs) IN BIOLOGICAL MATRICES"

EPA/VA/MRI Meeting
April 27-28, 1983

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APPENDIX B

DISCUSSION MEETING SCHEDULE OF EVENTS

MRI

DISSCUSSION OF

"Methods of Analysis for Polychlorinated Dibenzo-p-Dioxins (PCDD) in Biological Matrices"

at

Midwest Research Institute
Kansas City, Missouri

April 27-28, 1983

SCHEDULE OF EVENTS

8:30 - 9:00 - Registration of Participants - Arthur Mag Conference Center

9:00 - 9:10 - J. S. Stanley (MRI) Opening Remarks and Introductions

9:10 - 9:25 - David P. Redford (EPA/OTS) Primary Objectives of EPA/OTS in Assisting the VA with the Sampling and Analysis Program

9:20 - 9:35 - Dr. M. Flicker (VA) Overview of Veterans Administration Agent Orange Programs

9:35 - 9:55 - J. S. Stanley (MRI) Recommendations for Analytical Method - Identifying the Primary Issues from Peer Reviews

9:55 - 12:00 - Instrumental Analysis - Discussion

- Low Resolution vs. high resolution mass spectrometry
- Definition of high resolution mass spectrometry
- Compromises between low resolution and high resolution mass spectrometry
- Quantitation practices
- Criteria for qualitative identification
  - Low resolution mass spectrometry
  - High resolution mass spectrometry
  - Gas chromatography
- Criteria for quantitation
  - Limits of detection
  - Limits of quantitation
- Isomer specificity
- What degree of confidence necessary with any method
- Possible interferences
- Quality assurance/quality control procedures
- Role of screening techniques

12:00 - 1:15 - Lunch

1:15 - 2:30 - Instrumental Analysis Discussion (Concluded)

2:30 - 3:15 - Sample Preparation - Discussion

- Surrogate spiking
- Approaches to preparing spiked samples with native PCDDs
- Extraction procedures--neutral, acid or base
- Cleanup of extract
  Advantages and disadvantages of the proposed cleanup procedures
- Quality assurance/quality control procedures
- Other cleanup procedures

3:15 - 3:25 - Break

3:25 - 5:00 - Sample preparation - Discussion (Concluded)

5:30 - 7:30 - Social Hour (Hilton Plaza Hotel)

April 28, 1983

8:30 - 8:35 - J. S. Stanley - Opening Remarks

8:35 - 9:00 - A. L. Young - VA Need for Primary Analytical Method

9:00 - 11:00 - Method Validation Studies

- Intralaboratory validation of extraction procedure
- Ruggedness testing of method--intralaboratory approach
- Preliminary interlaboratory studies
- Full-scale collaborative study

  . Number of participating laboratories
  . Number of total samples
  . Preparation of spiked tissue samples
  . Availability of native and isotopically labeled standards
  . Needs for spiking samples with potential interferences

10:15 - 10:25 - Break

10:25 - 12:00 - Summary of Discussions and Recommendations

APPENDIX C

BIBLIOGRAPHY

APPENDIX C

BIBLIOGRAPHY

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16. Abstract (Limit: 200 words)

The overall objective of this review and preliminary method recommendation was to assist the EPA's Office of Toxic Substances (OTS) in proposing an analytical method for PCDDs in human adipose tissue in conjunction with the Veterans Administration's (VA) Agent Orange study.

The published literature on polychlorinated dibenzo-p-dioxins (PCDDs) analyses for biological matrices was reviewed. The analytical methods are discussed for sample extraction, cleanup, and instrumental analysis.

This report also presents a synopsis of a discussion meeting organized at the request of EPA/OTS concerning the analysis of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) held at Midwest Research Institute (MRI) on April 27 and 28, 1983. The primary objective of this meeting was to define the needs of an analytical method for the analysis of PCDDs and PCDFs in human adipose tissue.

Several major programs were identified as necessary to achieve these goals. These included (a) the need for establishing a repository of PCDD/PCDF standards of known quality; (b) the organization and implementation of a strong quality assurance program; (c) the acquisition of sufficient human adipose tissue to generate a homogeneous sample matrix for the QA program; (d) independent studies of extraction procedures using bioincurred radiolabeled PCDDs; (e) intralaboratory ruggedness testing of a proposed analytical method; and (f) interlaboratory evaluation of the proposed method.

17. Document Analysis a. Descriptors

2,3,7,8-Tetrachlorodibenzo-p-dioxin
2,3,7,8-TCDD
Polychlorinated dibenzo-p-dioxins
PCDD
Polychlorinated dibenzofurans
PCDF
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