Guidelines for the Determination of Halogenated Dibenzo-p-Dioxins and Dibenzofurans in Commercial Products (RE-ANNOUNCEMENT of PB88-101050 - see notes field for explanation)

Midwest Research Inst., Kansas City, MO

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16. ABSTRACT

The determination of halogenated dibenzo-p-dioxins (HDDs) and dibenzofurans (HDFs) in commercial products require specific and sensitive analytical procedures. The development of methods for the measurement of HDD and HDF in commercial products is complicated by the diverse range of matrices, the potentially large number of halogenated (specifically chloro, bromo, or bromo/chloro) HDD and HDF congeners and potential interference due to major components of the product matrices.

This report provides (1) a review of the literature pertaining to commercial product analyses for HDDs and HDFs, (2) detail on the total number of bromo, chloro, and bromo/chloro HDDs and HDFs, (3) a tentative scheme for the analysis of HDDs and HDFs in diverse matrices, and (4) guidelines for the high resolution gas chromatography mass spectrometry or electron capture detector analysis of commercial products for HDDs and HDFs. The guidelines specify a rigorous QA/QC program for the analysis of the commercial products.

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GUIDELINES FOR THE DETERMINATION OF HALOGENATED DIBENZO-p-DIOXINS AND DIBENZOFURANS IN COMMERCIAL PRODUCTS

by

David H. Staele
John S. Stanley

FINAL REPORT

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Prepared for:

U.S. Environmental Protection Agency
Office of Pesticides and Toxic Substances
Field Studies Branch
401 M Street, S.W.
Washington, DC 20460

Attn: Ms. Janet C. Remmers, Work Assignment Manager
Mr. Tom Murray, Work Assignment Manager
Dr. Joseph Breen, Program Manager

DISCLAIMER

This document has been reviewed and approved for publication by the Office of Toxic Substances, Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency. The use of trade names or commercial products does not constitute Agency endorsement or recommendation for use.

PREFACE

This document provides guidelines for the determination of halogenated dibenzo-p-dioxins (HDDs) and dibenzofurans (HDFs) in commercial products using instrumental methods of analysis. This report includes a brief review of analytical requirements and existing methods for the analysis of HDDs and HDFs, guidelines for sampling and instrumental analysis for these compounds, and a quality assurance plan.

The analytical guidelines focus on high resolution gas chromatography (HRGC) with either mass spectrometry (MS) or electron capture detectors (ECD) for qualitative identification and quantitation. Bioanalytical techniques may be a more cost-effective alternative, but these procedures are not sufficiently validated at this time to be useful. Therefore, guidelines for bioanalytical methods have not been included in this document.

Preparation of the document was initiated under MRI Project No. 8201 and completed under MRI Project No. 8800 for the U.S. Environmental Protection Agency's (EPA) Office of Toxic Substances, Field Studies Branch, EPA Prime Contract Nos. 68-02-3938 and 68-02-4252, Work Assignments 41 and 33, respectively, Ms. Janet Remmers and Mr. Tom Murray, Work Assignment Managers, and Dr. Joseph Breen, Project Officer. A draft version of this report was submitted for EPA review and approval on June 3, 1985, and a revised draft submitted on June 27, 1985.

The main body and Appendix A (analytical guidelines) of this document were prepared by Mr. David H. Steele and Dr. John S. Stanley, Work Assignment Leader, with input from Dr. Mitchell D. Erickson, Mr. Richard D. Brown, and Ms. LeAnn L. Timmons. Appendices B, C, and D, containing the quality assurance project plan and approaches for sampling commercial products for analysis, were prepared by Dr. John Smith of EPA's Office of Toxic Substances Chemical Regulations Branch.

MIDWEST RESEARCH INSTITUTE

Paul C. Constant
Program Manager

Approved:

John E. Going, Director
Chemical Sciences Department

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I. INTRODUCTION

The Environmental Protection Agency was petitioned (October 22, 1984) by the Environmental Defense Fund (EDF) and the National Wildlife Federation (NWF), pursuant to Section 21 of the Toxic Substances Control Act (TSCA), to issue rules and orders to prevent and reduce environmental contamination by halogenated (specifically brominated and/or chlorinated) dibenzo-p-dioxins and dibenzofurans (HDDs and HDFs). The Agency has reviewed the Petition and has granted certain aspects. In addition, there are a number of relevant Agency activities, planned or currently underway, which address the Petitioners' concerns. The Agency feels that in many cases it is necessary to gather preliminary information before the Agency can make regulatory findings. As part of the preliminary assessments, appropriate analytical methodologies must be identified or developed.

The objective of this work assignment is to identify and/or develop appropriate analytical methodologies to quantitate HDDs and HDFs in commercial products. This report identifies the scope of the problem (Section II) and general considerations for analytical methods (Section III) dealing with the determination of HDDs and HDFs. Existing analytical methods for the determination of chlorinated dioxins and furans are discussed (Section IV), and general guidelines for the sampling and analysis of HDDs and HDFs in commercial products are provided in Section V and Appendix A. A quality assurance project plan and guidance for sampling are contained in Appendices B, C, and D.

II. SCOPE OF PROBLEM

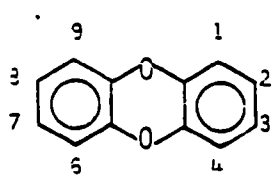
The development of analytical methodologies for the determination of HDDs and HDFs in commercial products is complicated by the diverse range of sample matrices, the potentially large number of brominated, chlorinated, and mixed brominated/chlorinated congeners of the HDDs and HDFs, and by the potential interferences which can be expected to be encountered. The following subsections discuss these considerations individually.

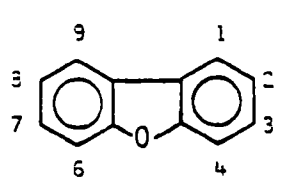
A. Diversity of Sample Matrices

It is anticipated that commercial products which will be analyzed under this program will range in volatility from chlorinated solvents to heavy metal salts and dyes. Acids, bases, and neutral compounds with a wide range of molecular geometries can be expected.

B. Potential Number of HDD and HDF Isomers

Both the dibenzodioxin and the dibenzofuran molecules have eight positions where bromine or chlorine can substitute. These positions are numbered as shown for the dibenzo-p-dioxin and dibenzofuran molecules below.





The total number of unique positional isomers has been calculated (MRI Interim Report, "Analytical Methodologies for Halogenated Dioxins and Dibenzofurans in Commercial Products, May 1, 1985) at 1,700 HDD and 3,320 HDF for bromo, chloro and bromo/chloro-substitution. Within these compounds there are 351 HDDs and 666 HDFs with substitution in the 2,3,7,8-positions. The breakdown of the exact number of positional isomers with degree of halogenation are provided in the general method guidelines (Appendix A, Tables 1 and 2). Although the number of theoretical isomers is high, the actual number of HDDs and HDFs in commercial products may be limited to relatively few. The analyst must first determine the probability of the presence of bromo-, chloro-, or bromo/chloro-HDDs and HDFs in specific commercial products. Clearly, a chlorinated commercial product that has had no contact with bromine needs only to be analyzed for PCDDs and PCDFs. Likewise, a brominated material produced with no intermediate chlorinated materials, needs only to be analyzed for brominated HDDs and HDFs (PBDDs and PBDFs). It is estimated that the bromo/chloro-commercial products requiring analyses of all HDDs and HDFs are very few.

C. Potential Interferences

Interferences are expected to arise from contaminants in solvents, reagents, and glassware, as well as contaminants (halogenated diphenyl ethers, halobenzyl phenyl ethers, bis(chlorophenoxy)methanes, and halogenated phenoxyphenols) which are coextracted with the HDDs and HDFs.

Halogenated diphenyl ethers yield mass spectral fragment ions with exactly the same mass and number of halogen atoms as the corresponding HDFs. Buser (1975) and Mieure et al. (1977) have demonstrated that basic alumina column chromatography will separate the chlorodiphenyl ethers from the PCDDs (polychlorinated dibenzo-p-dioxins) and PCDFs (polychlorinated dibenzofurans). Likewise, Huckins et al. (1978) have demonstrated that a graphitized charcoal column will remove the chlorodiphenyl ether interferences while selectively isolating and concentrating chlorinated dibenzo-p-dioxins.

The halobenzyl phenyl ethers may cause mass spectral interferences to HDDs with mass spectrometry selected ion monitoring (SIM) techniques. Interferences arise with these compounds when using electron capture detectors; however, these compounds are distinguishable from PCDDs by mass spectrometry. These compounds can also be removed from sample extracts by further cleanup on alumina or carbon columns. The bis(chlorophenoxy)methanes have similar gas chromatographic retention characteristics to PCDDs.

The halogenated phenoxyphenols can dehydrohalogenate in the hot inlet of the gas chromatograph to form HDDs. This has been reported for the chlorinated phenoxyphenols (Baker et al. 1981) and for 6-hydroxy-2,2',4,4',5,5'-hexabromobiphenyl (Gardner et al. 1979). It is expected that analogous reactions will occur for the mixed bromo/chloro phenoxyphenols. In order to avoid false positives, these species must be excluded from the final extracts, or the extracts must be methylated to prevent ring closure during injection.

In addition to interferences caused by contaminants in the sample extracts, interferences may be caused by the HDDs and HDFs themselves. This

will occur due to the potentially large number of isomers and to the ion clusters produced by the isotopes of bromine and chlorine. The potential for overlap of the isotope clusters becomes greater as the potential for mixed bromo/chloro-HDDs and HDFs increases. For example, some intereferences might be anticipated for determination of the tetrabromodibenzo-p-dioxin (TBDD) using ions m/z 500 and m/z 502. The interference may result as contribution from dibromopentachlorodibenzofurans or bromoheptachlorodibenzo-p-dioxins which exhibit the same ions in the molecular clusters (Appendix A, Table 5). Again, it should be noted that the probability of this occurring may be small and is undoubtedly a concern only for the mixed bromo/chloro-commercial products. Since little is known regarding the chemical and physical nature of bromo and bromo/chloro-HDDs and HDFs, it is not clear whether these interferences will be resolved by HRGC retention time. The masses and relative abundances of the ions in the molecular ion clusters of the HDDs and HDFs are presented as part of the method guidelines (Appendix A). Possible interferences from the molecular ion clusters of the HDDs and HDFs are also presented in the method guidelines.

III. GENERAL ANALYTICAL METHOD CONSIDERATIONS

The analytical procedures necessary for the quantitative measurement of HDDs and HDFs in commercial products include (1) the quantitative extraction or partitioning of the analytes from the commercial product, (2) separation of the HDDs and HDFs from interferences present in the extract, and (3) separation and quantitation of homologous series or specific congeners by gas chromatography with mass spectral or electron capture detection. Some general considerations concerning these procedures are discussed below.

A. Sample Extraction and Cleanup

The most significant difference in the analysis of HDDs and HDFs in commercial products in comparison with environmental and biological samples will be the extraction and cleanup procedures. The physical and chemical properties of environmental and biological matrices are typically different enough from the properties of the analytes to allow relative ease of separation. In contrast, the commercial products in most cases may be structurally similar to the analytes, complicating the separation and necessitating the complete removal of the matrix to avoid interferences in the final determination.

B. Detection Method

Most of the analyses of commercial products for polychlorinated dioxins (PCDDs), dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and polybrominated biphenyls (PBBs) that have been reported in the literature have employed mass spectrometry (MS) detection. Mass spectrometry will provide both qualitative and quantitative data for HDDs and HDFs. In addition, its primary advantage over other detectors is provided by confirmation of the presence of a specific analyte. Mass spectrometry is a proven tool for providing quantitative information at concentration levels ranging from micrograms per gram to picograms per gram.

Several options are available for mass spectrometry analyses including (1) full mass scan analysis (FMS), which provides identification based on complete mass spectra of individual components; (2) limited mass scan (LMS), which allows the analyst to monitor small mass ranges, possibly full molecular ion clusters; and (3) selected ion monitoring (SIM), which permits the analyst to select specific ions characteristic of the target compounds. The mass spectrometery method sensitivity is increased by decreasing the range of masses or number of ions monitored during an analysis. Thus the SIM method is more sensitive than the LMS or FMS procedures. Full scan mass spectrometry is useful for measurements for the 50 ng/g and higher concentration levels, while the SIM technique will allow measurements as low as 1-10 pg/g. Some caution must be exercised in approaching the final analysis since the lower detection levels require that sample extracts be relatively free of interference.

Another option that is available is high resolution mass spectrometry (HRMS). This approach can be used with any of the techniques discussed above. The advantage of HRMS is that it allows the analyst to lower the contribution of signal due to background and interferences by monitoring the exact masses of the analytes. This usually results in an observed increase of signal to noise and corresponding lower limit of detection (LOD). The major drawbacks of HRMS involve the availability of instrumentation, experienced analysts, cost, and sample turnaround time.

Electron capture detection (ECD) has proven to be effective for monitoring PCDD and PCDF contaminants at the microgram-per-gram (ppm) level in some commercial products (pentachlorophenol, Mieure et al. 1977). It is expected that this technique may be suitable for the analysis of HDDs and HDFs in commercial products. The ECD analysis technique is typically employed on samples on which extensive extraction and cleanup procedures have been performed, producing extracts of acceptable quality for final analysis.

The electron capture detector is highly sensitive to compounds with halogen substitution and provides an attractive, cost-effective procedure compared to mass spectrometry. However, this detector provides qualitative information only, based on a response at a certain retention time. The incidence of false positives will increase as the analyst presses the detection limit from the micrograms-per-gram level to the low nanograms-per-gram level (parts-per-million to parts-per-billion in commercial product). The decision to pursue analysis based on ECD will require that the analyst comply with a well-validated analytical procedure and stringent QA/QC program to minimize the number of false positives during sample analysis.

C. Method Sensitivity

One of the major concerns in using any of the analytical techniques is the ability to achieve the desired limits of detection. Table 1 provides a summary of the reported detection limits for various PCDDs and PCDFs in phenoxyalkanoic acid herbicides (Baker et al. 1981). These detection limits range from 5 to 500 ng/g (ppb). A detection limit of 50 ng/g can be achieved for specific isomers of TCDD and TCDF assuming a conservative instrument sensitivity (quadrupole mass spectrometer) and a 1-μL injection from a 1-mL final extract of a 1-g sample. This detection limit can be lowered by (1) increasing the initial sample size (~ 10 g), (2) decreasing the final extract volume

Table 1. A Summary of the Reported Analytical Limits of Detection for Polychlorodibenzo-p-dioxins (PCDDs) and Polychlorodibenzofurans (PCDFs) in Phenoxyalkanoic Acid Herbicides[a]

Reference	Applicability	Compounds detected	Limit of detection (mg kg^{-1} of sample)
Storherr et al.	2,4,5-T	TCDD	0.05
Brenner et al. (1972)	2,4-D, dichlorprop, mecoprop and 2,4,5-T	TCDD	0.01
Vogel (1976)	2,4,5-T	TCDD	0.03
Ramstad et al. (1977)	Butoxypropyl esters of 2,4,5-T and of fenoprop	TCDD	0.005
Elvidge (1971)	2,4,5-T, ethylhexyl ester of 2,4,5-T and formulations containing the ethylhexyl ester of 2,4,5-T	TCDD	0 05 (on 2,4,5-T content)
Woolson et al. (1972)	2,4-D, 2,4-DB, dichlorprop, 2,4,5-T, fenoprop and dicamba	Di-, tri-, tetra-, penta-, hexa-, hepta, and octa-CDDs	0.5 (for any one of the compounds detected)
Edmunds et al. (1973)	2,4,5-T, the butyl and octyl esters of 2,4,5-T and formulations of the butyl ester of 2,4,5-T (50% in mineral oil)	TCDD	0 05
Crummett and Stehl (1973)	Esters of 2,4,5-T and of fenoprop, Herbicide Orange	TCDD	0.05
Buser et al. (1974)	2,4,5-T, formulations of esters of 2,4,5-T and of amine salts of 2,4,5-T	TCDD	0 001 (on a standard solution)
Huckins et al. (1978)	Herbicide Orange	TCDD and penta-CDD	0.02
Rappe et al. (1978)	Formulations of esters of 2,4,5-T, Herbicide Orange	Di-, tri-, tetra-, penta- and hexa-CDDs, di-, tri-, tetra-, penta- and hexa-CDFs	0.01-0.05
Polzhofer (1979)	2,4,5-T, alkyl esters of 2,4,5-T and formulations	TCDD	0.005 (on 2,4,5-T content)
Carello et al	Fenoprop	TCDD	0 01

[a]Baker PG, Hoodless RH, Tyler JC. 1981. Pestic Sci 12:297-304.

(∿ 20 μL), or (3) use of a more sensitive (∿ 5 pg/μL) mass spectrometer (magnetic sector double focusing instrument). By adjusting these options, the method limit can be lowered to 0.01 to 1 ng/g.

It should be noted that these detection limits are determined by the sensitivity of the instrument, and that sample interferences may significantly increase the detection limit for a given matrix. The working definition of limit of detection, as defined in EPA methods for the determination of 2,3,7,8-TCDD in soil/sediment and water, requires that the signal for the compound is at a minimum three times the background (signal to noise).

D. Analytical Standards

Present commercial sources of HDD and HDF analytical standards include Cambridge Isotope Laboratories (Woburn, Massachusetts), KOR Isotopes (Cambridge, Massachusetts), Ultra Scientific (Hope, Rhode Island), and Wellington Laboratories (Guelph, Ontario, Canada). Table 2 provides a summary of the current availability of specific substituted unlabeled and mass-labeled HDDs and HDFs. Table 2 is intended as a guide only, as suppliers may add or remove reference materials from their offerings at any time. Availability should be confirmed with the suppliers.

A limited number of the mixed bromo/chloro analogs of these compounds are currently available (see Table 2). If analyses for other congeners are to be conducted, standards will have to be custom synthesized and characterized as to identity and purity. Buser (1987) has demonstrated the microsyntheses of several of the mixed congeners by gamma irradiation of PCDDs and PCDFs in dibromoethane. While standards of known purity were not produced, this technique may prove useful in the generation of standards for qualitative use, for example, in the determination of gas chromatographic retention times.

E. Quality Assurance/Quality Control (QA/QC)

A strong QA/QC program is necessary to support the method guideline approach which has been developed for this program. To ensure that the data obtained are of known quality, the QA/QC program detailed in Appendix A (Section 15) includes provisions for the use of carbon-13 labeled surrogates where possible. In addition, replicate sample analysis, the routine analysis of spiked samples, and method blanks have been incorporated. Calibration procedures for instrumentation are also clearly defined for implementation by the analyst.

IV. EXISTING METHODS

A computer search, conducted on the CAS database (1967-1984) yielded no references concerning the analysis of HDDs and HDFs in commercial products. However, analytical methods for the determination of polychlorinated dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated and polybrominated biphenyls (PCBs and PBBs) in various matrices have been reported. It is anticipated that the analytical procedures for PCDDs and PCDFs, summarized below, may be modified for the analysis of bromo- and bromo/chloro-HDDs and HDFs.

Table 2. Currently Available HDD and HDF Standards

Unlabeled		Stable isotope labeled
Chlorinated dioxins:		
Mono:	2	
Di:	2,3	2,7/2,8 (u-$^{13}C_{12}$, 99%)[b]
	2,8	
	2,7/2,8[a]	
Tri:	1,2,3	
	1,2,4	
	2,3,7	
Tetra:	1,2,3,4	1,2,3,4 ($^{13}C_6$, 99%)
	1,2,3,7/1,2,3,8[a]	2,3,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,3,7/1,2,8,9[a]	2,3,7,8 (U-$^{37}Cl_4$, 96%)[c]
	1,2,4,7/1,2,4,8[a]	1,2,3,4 (U-$^{13}C_{12}$, 99%)
	1,2,6,7/1,2,8,9[a]	
	1,2,7,8	
	1,2,8,9	
	1,3,6,8	
	1,3,6,8/1,3,7,9[a]	
	1,3,7,8	
	2,3,7,8	
Penta:	1,2,3,4,7	
	1,2,3,7,8	1,2,3,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,4,7,8	
Hexa:	1,2,3,4,7,8	1,2,3,6,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,3,6,7,8	1,2,3,7,8,9 (U-$^{13}C_{12}$, 99%)
	1,2,3,7,8,9	
Hepta:	1,2,3,4,6,7,8	1,2,3,4,6,7,8 (U-$^{13}C_{12}$, 99%)
Octa:	1,2,3,4,6,7,8,9	1,2,3,4,6,7,8 (U-$^{13}C_{12}$, 99%)
Brominated dioxins:		
Mono:	1	
Di:	2,7	
	2,8	
Tri:	2,3,7	
Tetra:	1,2,3,4	2,3,7,8 (U-$^{13}C_{12}$, 99%)
	1,3,6,8/1,3,7,9[a]	
	1,3,7,8	
	2,3,7,8	
Penta:	1,2,3,7,8	1,2,3,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,4,7,8	
Hexa:	1,2,3,4,7,8	1,2,3,4,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,3,6,7,8/1,2,3,7,8,9[a]	

Table 2 (continued)

Unlabeled		Stable isotope labeled
Hepta:	1,2,3,4,6,7,8	
Octa:	1,2,3,4,6,7,8,9	
Mixed Br/Cl dioxins:		
Tri:	7-Bromo-2,3-dichloro	
Tetra:	2,3-Dibromo-7,8-dichloro	
	2,8-Dibromo-3,7-dichloro	
	2-Bromo-3,7,8-dichloro	
Penta:	2-Bromo-1,3,7,8-tetrachloro	
Hexa:	3-Bromo-1,2,4,7,8-pentachloro	
Chlorinated dibenzofurans:		
Mono:	2	
	3	
	4	
Di:	2,3	
	2,6	
	2,7	
	2,8	
	4,6	
Tri:	1,2,3	
	1,2,4	
	1,3,6	
	1,3,7	
	1,6,7	
	2,3,4	
	2,3,6	
	2,3,8	
	2,4,6	
	2,4,7	
	2,4,8	
	2,6,7	
Tetra:	1,2,3,4	2,3,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,3,6	
	1,2,3,7	
	1,2,3,8	
	1,2,3,9	
	1,2,4,6	
	1,2,4,8	
	1,2,4,9	
	1,2,7,8	
	1,3,4,6	
	1,3,4,7	

Table 2 (continued)

	Unlabeled	Stable isotope labeled
Tetra: (cont.)	1,3,4,8	
	1,3,4,9	
	1,3,6,7	
	1,3,6,8	
	1,3,6,9	
	1,3,7,8	
	1,3,7,9	
	1,4,6,7	
	1,4,7,8	
	2,3,4,6	
	2,3,4,7	
	2,3,4,8	
	2,3,4,9	
	2,3,6,7	
	2,3,6,8	
	2,3,7,8	
	2,4,6,7	
	2,4,6,8	
	3,4,6,7	
Penta:	1,2,3,4,6	1,2,3,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,3,4,7	
	1,2,3,4,8	
	1,2,3,4,9	
	1,2,3,6,7	
	1,2,3,7,8	
	1,2,3,7,9	
	1,2,3,8,9	
	1,2,4,6,7	
	1,2,4,7,8	
	1,3,4,6,7	
	1,3,4,7,8	
	2,3,4,6,7	
	2,3,4,6,8	
	2,3,4,6,9	
	2,3,4,7,8	
	2,3,4,7,9	
Hexa:	1,2,3,4,7,8	1,2,3,4,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,3,4,7,9	
	1,2,3,4,8,9	
	1,2,3,6,7,8	
	1,2,3,6,8,9	
	1,2,3,7,8,9	
	1,2,4,6,7,8	
	1,2,4,6,8,9	
	1,3,4,6,7,8	
	1,3,4,6,7,9	
	2,3,4,6,7,8	

Table 2 (concluded)

Unlabeled		Stable isotope labeled
Hepta:	1,2,3,4,6,7,8 1,2,3,4,6,8,9 1,2,3,4,7,8,9	1,2,3,4,6,7,8 (U-$^{13}C_{12}$, 99%)
Octa:	1,2,3,4,6,7,8,9	1,2,3,4,6,7,8,9 (U-$^{13}C_{12}$, 99%)
Brominated dibenzofurans:		
Di:	2,7 2,8	
Tri:	1,3,8	
Tetra:	2,3,7,8	2,3,7,8 (U-$^{13}C_{12}$, 99%)
Penta:	1,2,3,7,8	1,2,3,7,8 (U-$^{13}C_{12}$, 99%)
Hexa:	1,2,3,4,7,8	1,2,3,4,7,8 (U-$^{13}C_{12}$, 99%)
Mixed Br/Cl furans:		
Tri:	8-Bromo-2,3-dichloro	
Tetra:	6,8-Dibromo-2,3-dichloro 8-Bromo-2,3,4-trichloro	
Penta:	6,8-Dibromo-2,3,4-trichloro	

[a]Mixture of indicated isomers. The relative amounts are unknown; therefore, these standards are not useful for quantitation calibration.
[b]U-$^{13}C_{12}$ indicates that the compound is universally labeled with carbon-13.
[c]U-$^{37}Cl_4$ indicates that the compound is universally labeled with chlorine-37.

A. Instrumental Methods

Standard analytical methods are available for the analysis of 2,3,7,8-TCDD in soils and sediments and water/wastewater (EPA Method 613, RCRA Method 8280, other methods under the EPA Contract Laboratory Program; Harless et al. 1980; McMillin et al. 1983). These methods have been validated for the measurement of 2,3,7,8-TCDD to detection levels of 1 ng/g (ppb) for soils and sediments and 1 ng/L (ppt) for water/wastewater. The soil and sediment protocol has been used for the analysis of over 11,000 samples for 2,3,7,8-TCDD in EPA Region VII through the Contract Laboratory Program (CLP). These methods have not been validated for the analysis of total PCDDs and PCDFs. However, several laboratories have provided sufficient documentation to suggest that modification of these procedures can extend the analysis for total tetrachloro- through octachloro-PCDDs and PCDFs.

The extraction and cleanup procedures for the determination of PCDDs and PCDFs in phenoxyalkanoic herbicides has been reviewed by Baker et al. (1981). Extraction techniques have included steam distillation of TCDD from an alkaline solution of technical 2,4,5-T (Brenner et al. 1972, 1974), hexane extraction from an alkaline solution of 2,4,5-T, liquid-liquid partitioning of TCDD into hexane from a solution of technical 2,4,5-T in dimethylformamide/acetonitrile/water (Vogel 1976), and separation of TCDD from isobutoxy methyl esters of 2,4,5-T and fenoprop using a silica gel column (Ramsted et al. 1977).

Woolson et al. (1972) evaluated 17 different pesticides containing the polychlorophenoxy group for the presence of PCDDs. Extraction was accomplished using hexane extraction from methanolic potassium hydroxide solution. Cochrane et al. (1981) described the analysis of technical and formulated products of 2,4-D as the ester, free acid, and the amine. The 2,4-D esters were transferred directly onto a silica column and eluted with 30% dichloromethane in hexane. The 2,4-D acids were dissolved in acetonitrile/water, and the PCDDs were partitioned into hexane. The 2,4-D amines were dissolved in water and partitioned into hexane.

Analysis of pentachlorophenol (PCP) for PCDDs has been reported by several researchers (Buser 1975, Buser and Bosshardt 1976, Mieure et al. 1977, Blaser et al. 1976, Firestone et al. 1972, Plimmer et al. 1973, Lamberton et al. 1979, Nelsson and Renberg 1974, Pfeiffer et al. 1978, and Miles et al. 1984). The analytical methods included dissolution in a polar solvent and partitioning the PCDDs into a nonpolar solvent, separation and collection of PCDDs by high performance liquid chromatography (Miles et al. 1984), and separation from the commercial product using a macro silica adsorption column (Mieure et al. 1977). Yamagishi et al. (1981) reported a similar procedure for the determination of PCDDs and PCDFs in commercial diphenyl ether herbicides such as nitrophen and chloromethoxynil.

B. Bioanalytical Methods

A possible alternative to instrumental analysis for HDDs and HDFs may arise through the further development of existing bioanalytical or bioassay techniques. These bioanalytical techniques include (1) radioimmunoassay (Albro et al. 1979, McKinney et al. 1981), (2) aryl hydrocarbon hydroxylase

(AHH) induction assay (Bradlaw and Casterline 1979), (3) cytosol receptor assay (Hutzinger 1979), (4) early life stage (E.L.S.) bioassay (Helder and Seinen 1985), and (5) in vitro keratinization assay (Knutson and Poland 1980, Gierthy et al. 1985). Each of these techniques has been evaluated as an alternative technique for screening for the presence of PCDDs and/or PCDFs based on biological/biochemical properties.

The radioimmunoassay, AHH, and the cytosol receptor assay have previously been reviewed (NRCC 1981; NRCC 1984). The disadvantages of these techniques in general are that they do not necessarily respond specifically to PCDDs and PCDFs, they respond to other compounds such as halogenated biphenyls, azoxybenzenes, and nonhalogenated polynuclear aromatic hydrocarbons, and each technique is less sensitive than available analytical methods. The primary advantages of these techniques are lower cost and rapidity of test results.

The in vitro keratinization and E.L.S. bioassays more recently have provided additional options and possibly more specificity for determining the presence of PCDDs and PCDFs in general. Both techniques have been demonstrated to give roughly comparable results with HRGC/MS analysis of total PCDDs and PCDFs in a PCB fire soot (Gierthy et al. 1985) and fly ash from a municipal incinerator (Helder and Seinen 1985).

It is important to note that each of the bioassay techniques is most sensitive to the presence of 2,3,7,8-TCDD. It is speculated that the relative response to other HDDs and HDFs might be dependent on halogen substitution in the 2,3,7,8-positions. It is also important to note that the range of compounds evaluated with each of these bioassay techniques is limited. Evaluation of commercial products for the presence of HDDs and HDFs with any of these bioassay techniques should be completed using a strong QA/QC program (Appendix A, Section 15). It is suggested that the HDDs and HDFs be isolated from the commercial product matrix and other potential contaminants using the methods specified in the guidelines (Appendix) or equivalent techniques. If bioassay techniques are used, it is advised that the analyst follow the recommended QA/QC program (blanks, spikes, replicates, etc.) to avoid false negatives. It may also be necessary to use an alternate technique, such as HRGC/MS, to avoid false positives from the bioassay techniques.

V. PROPOSED METHOD

A flowchart for the general guidelines for the analysis of HDDs and HDFs in commercial products is shown in Figure 1. This scheme, adapted from general separation principles and techniques reported in the literature, is based on the physical/chemical properties of the commercial product; therefore, the exact extraction and cleanup procedure is dependent upon the nature of the product or formulation.

The properties utilized in this scheme are volatility, solubility, acidity, polarity, and molecular geometry. For example, if the product is sufficiently volatile, evaporation may be employed. Differential solubility in immiscible solvents may also be the basis for a liquid-liquid extraction.

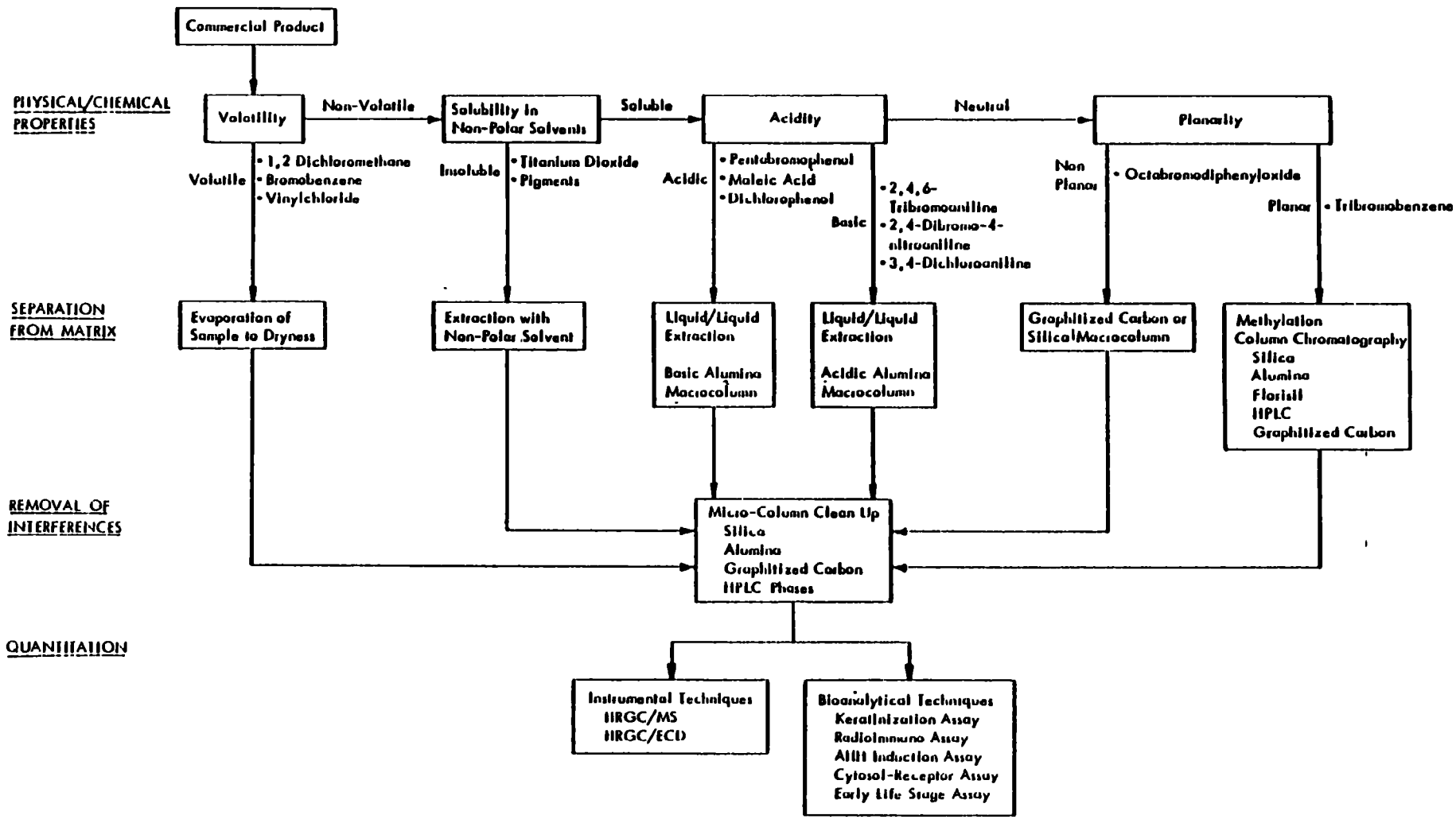


Figure 1. Tentative scheme for the analysis of HDDs and HDFs in commercial products.

For example, a salt may be dissolved in aqueous solution and the HDDs and HDFs extracted with an immiscible nonpolar solvent such as hexane. The solvent, number of extractions, solvent-to-sample ratio, and other parameters are chosen at the analyst's discretion. The planarity of the HDDs and HDFs may be exploited to separate them from a nonplanar matrix using graphitized carbon column chromatography. Column chromatography with alumina, silica, or ion exchange resins may also be applicable for use with some matrices. Other separation techniques may also be used if validated prior to sample analysis. Guidelines for the analysis of HDDs and HDFs in commercial products are presented in Appendix A.

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APPENDIX A

GUIDELINES FOR THE ANALYSIS OF HALOGENATED DIBENZO-*p*-DIOXINS AND DIBENZOFURANS IN COMMERCIAL PRODUCTS

GUIDELINES FOR THE ANALYSIS OF HALOGENATED DIBENZO-p-DIOXINS AND DIBENZOFURANS IN COMMERCIAL PRODUCTS

1.0 Scope and Application

1.1 These guidelines are applicable for the determination of halogenated dioxins (HDDs) and halogenated dibenzofurans (HDFs) in commercial products.

1.2 These guidelines are restricted to use by or under the the supervision of analysts experienced in the use of high resolution gas chromatograpny (HRGC) with mass spectrometric (MS) or electron capture detection (ECD).

1.3 The limit of detection (LOD) and limit of quantitation (LOQ) are dependent upon the specific HDDs and/or HDFs monitored, the mass of sample extracted, the complexity of the sample matrix, the sensitivity of the instrument, and the ability of the analyst to remove interferences and properly maintain the analytical systems. The method accuracy and precision should be determined by each laboratory for each commercial product. Specific LOQs are given in the test rule (EPA 1985).

1.4 The validity of the results depends on equivalent recovery of tne analytes and the surrogate compounds. If the surrogates are not thoroughly incorporated into the matrix, the method is not applicable.

1.5 Certain analytical parameters and equipment designs, which will affect the validity of the analytical results, have been identified. Proper use of the method requires that such parameters or designs should be used as specified. An experienced analyst may make modifications to parameters or equipment identified by the term "recommended." Each time such modifications are made to the method, the analyst should repeat the procedures in Section 15.2.

2.0 Summary

2.1 The product should be sampled such that the specimen collected for analysis is representative of the whole. Statistically designed selection of the sampling position, time, or discrete product units should be employed. The sample should be preserved to prevent HDD and HDF loss prior to analysis. Customary inventory storage may be adequate for commercial products. Guidelines for sample collection, handling, storage, etc., are provided in Appendices C and D.

2.2 The sample is mechanically homogenized and subsampled as necessary The sample is then spiked with surrogate compounds, and the surrogates thoroughly incorporated by further mechanical agitation.

2.3 The surrogate-spiked sample is extracted and cleaned up at the discretion of the analyst. Possible extraction techniques include liquid-liquid partition, column chromatography, etc. The sample volume and solvent composition are then adjusted as necessary for final analysis.

2.4 The HDD and HDF content of the sample extract is determined by an appropriate analytical methodology. Possibilities include high resolution gas chromatography/electron impact mass spectrometry (HRGC/EIMS) operated in the selected ion monitoring mode (SIM), high resolution gas chromatography with electron capture detection (HRGC/ECD), or other analytical procedures which have been validated by the testing laboratory.

2.5 For analysis by HRGC/MS, the HDDs and HDFs are identified by comparison of their retention time and mass spectral abundance ratios or peak areas to those in the calibration standard. Identification by HRGC/ECD is achieved by comparison of retention times.

2.6 HDDs and HDFs are quantitated against the response factors for a mixture of HDD and HDF congeners, using the response of the surrogates to correct for loss of analytes during sample workup and to determine instrument variability.

2.7 HDDs and HDFs identified by the SIM technique may be confirmed by full mass scan HRGC/EIMS, chemical ionization mass spectrometry (CIMS), high resolution mass spectrometry (HRMS), retention on alternate GC columns, or other techniques, provided that the sensitivity and selectivity of the techniques are demonstrated to be adequate.

2.8 The total analysis time is dependent on the sample matrix and the extent of workup employed. The time required for a single HRGC/SIM or HRGC/ECD analysis of a sample is estimated as approximately 1 h.

2.9 Appropriate quality control/quality assurance (QA/QC) procedures are included to estimate the quality of the results. These QA/QC procedures include the use of control charts, and the analysis of blanks, replicates, and standard addition samples. A QA/QC plan should be developed by each laboratory. QA/QC results should be reported with the analytical results on a lot/batch basis.

3.0 Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware, leading to discrete artifacts and/or elevated baseline in chromatograms. All of these materials should be routinely demonstrated to be free from the interferences by the analysis of laboratory reagent blanks as described in Section 16.4. These interferences are especially noted when using electron capture detection.

3.1.1 Glassware should be scrupulously cleaned. All glass should be cleaned as soon as possible after use by rinsing with the last solvent used. This should be followed by detergent washing with hot water and rinses with tap water and reagent water. The glassware should then be drained dry and heated in a muffle furnace at 400°C for 15 to 30 min. Some thermally stable materials may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. National dioxin study procedures snould be used. (Volumetric ware should not be heated in a muffle furnace.) After it is dry and cool, clean glassware should be stored inverted in a contamination-free area or capped with aluminum foil to prevent accumulation of dust or other contaminants.

3.1.2 The use of high purity reagents and solvents will help to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required. All solvent lots should be checked for purity prior to use.

3.2 Interferences may also arise from contaminants that are coextracted with the HDDs and HDFs from the sample. The extent of matrix interferences will vary considerably, depending on the nature and diversity of the samples. Examples are provided below.

3.2.1 Halogenated diphenyl ethers yield mass spectral fragment ions with exactly the same mass and number of halogen atoms as the corresponding HDFs. Buser (1975) and Mieure et al. (1977) have demonstrated that basic alumina column chromatography will separate the chlorodiphenyl ethers from the PCDDs and PCDFs. Likewise, Huckins et al. (1978) have demonstrated that a graphitized charcoal column will remove the chlorodiphenyl ether interferences.

3.2.2 The halobenzyl phenyl ethers may yield mass spectral interferences to HDDs with selected ion monitoring (SIM) techniques. These compounds can also be removed from sample extracts by cleanup on alumina or graphitized carbon columns.

3.2.3 The bis(chlorophenoxy)methanes have gas chromatographic retention characteristics similar to PCDDs. Interferences to analysis may arise when using electron capture detection; however, these compounds are distinguishable from PCDDs by mass spectrometry.

3.2.4 The halogenated phenoxyphenols can dehydrohalogenate in the hot inlet of the gas chromatograph to form HDDs. This has been reported for the chlorinated phenoxyphenols (Baker et al. 1981) and for 6-hydroxy-2,2',4,4',5,5'-hexabromobiphenyl (Gardner et al. 1979). It is expected that analogous reactions will occur for the mixed bromo/chloro phenoxyphenols.

In addition, cyclization of diphenyl ethers and hydroxybiphenyls to form furans has been reported (Norstrom 1976a, 1976b). In order to avoid false positives, these species must be excluded from the final extracts, or the extracts must be methylated to prevent ring closure during injection.

3.2.5 Interferences can be caused by the HDDs and HDFs themselves. This occurs due to the potentially large number of congeners (Tables 1 and 2) and to the ion clusters produced by the isotopes of bromine and chlorine. The masses and relative abundances of the ions in the molecular ion clusters of the HDDs and HDFs are presented in Tables 3 and 4. Possible interferences from the molecular ion clusters of the HDDs and HDFs are presented in Table 5. As an example, a signal observed at m/z 460 can arise from tribromochlorodibenzodioxin, octachlorodibenzodioxin, bromohexachlorodibenzofuran, or dibromotetrachlorodibenzofuran.

Table 1. Theoretical Combinations of Bromo-, Chloro-, and Bromo/Chloro Dibenzo-p-dioxins

Degree of halogenation	Halogen substitution Br	Halogen substitution Cl	No. of theoretical positional congeners	No. of theoretical 2,3,7,8 substituted congeners
1	0	1	2	0
	1	0	2	0
			4	0
2	0	2	10	0
	1	1	14	0
	2	0	10	0
			34	0
3	0	3	14	0
	1	2	42	0
	2	1	42	0
	3	0	14	0
			112	0
4	0	4	22	1
	1	3	70	1
	2	2	114	3
	3	1	70	1
	4	0	22	1
			298	7
5	0	5	14	1
	1	4	70	5
	2	3	140	10
	3	2	140	10
	4	1	70	5
	5	0	14	1
			448	32
6	0	6	10	3
	1	5	42	9
	2	4	114	27
	3	3	140	30
	4	2	114	27
	5	1	42	9
	6	0	10	3
			472	108

Table 1. (concluded)

Degree of halogenation	Halogen substitution Br	Halogen substitution Cl	No. of theoretical positional congeners	No. of theoretical 2,3,7,8 substituted congeners
7	0	7	2	1
	1	6	14	7
	2	5	42	21
	3	4	70	35
	4	3	70	35
	5	2	42	21
	6	1	14	7
	7	0	2	1
			256	128
8	0	8	1	1
	1	7	2	2
	2	6	10	10
	3	5	14	14
	4	4	22	22
	5	3	14	14
	6	2	10	10
	7	1	2	2
	8	0	1	1
			76	76
			Total 1,700 HDDs	Total 351 2,3,7,8 substituted HDDs

Table 2. Theoretical Combinations of Bromo-, Chloro-, and Bromo/Chloro Dibenzofurans

No. of halogens	Classes Br	Classes Cl	No. of positional congeners	No. of 2,3,7,8 substituted congeners
1	0	1	4	0
	1	0	4	0
			8	0
2	0	2	16	0
	1	1	28	0
	2	0	16	0
			60	0
3	0	3	28	0
	1	2	84	0
	2	1	84	0
	3	0	28	0
			224	0
4	0	4	38	1
	1	3	140	2
	2	2	216	4
	3	1	140	2
	4	0	38	1
			572	10
5	0	5	28	2
	1	4	140	10
	2	3	280	20
	3	2	280	20
	4	1	140	10
	5	0	28	2
			896	64
6	0	6	16	4
	1	5	84	18
	2	4	216	48
	3	3	280	60
	4	2	216	48
	5	1	84	18
	6	0	16	4
			912	200

Table 2. (concluded)

No. of halogens	Classes Br	Classes Cl	No. of positional congeners	No. of 2,3,7,8 substituted congeners
7	0	7	4	2
	1	6	28	17
	2	5	84	40
	3	4	140	69
	4	3	140	69
	5	2	84	40
	6	1	28	17
	7	0	4	2
			512	256
8	0	8	1	1
	1	7	4	4
	2	6	16	16
	3	5	28	28
	4	4	38	38
	5	3	28	28
	6	2	16	16
	7	1	4	4
	8	0	1	1
			136	136
		Total HDFs	3,320	Total 2,3,7,8 substituted HDFs 666

Table 3. Characteristic Ions and Their Relative Isotope Abundances in the Molecular Ion Clusters of HBDs

Degree of Halogenation Cl+Br	0	1	2	3	4	5	6	7	8
0	184: 100 185: 13.2 186. 1 2	262: 100 263: 13 2 264. 98.9 265: 12.9 266: 1.2	340: 50.8 341: 6.7 342: 100 343. 13.1 344: 49.8 345. 6 5	418: 34 0 419. 4 4 420: 100 421: 13.1 422: 98 5 423: 12.8 424: 32.9 425: 4.2	496: 17.3 497: 2.3 498: 67.9 499: 8.9 500: 100 501: 13.0 502: 65.8 503: 8 6 504: 16 6 505: 2.1	574. 10 4 575 1.4 576. 51 0 577: 6 6 578: 100 579: 13.0 580. 98 3 581: 12 8 582. 48 6 583: 6 3 584. 9 8 585. 1 2	652: 5.3 653: < 1 654: 31.2 655: 4.1 656: 76.4 657: 9.9 658: 100 659: 13 0 660: 73 8 661: 9 6 662: 29 3 663: 3.8 664: 5 0	730. 3 0 731. < 1 732: 20.8 733: 2 7 734: 61.2 735: 8 0 736. 100 737: 13.0 738: 98 2 739. 12.8 740: 58.1 741: 7 5 742. 19.2 743: 2.5 744: 2.8	808: 1.5 809: < 1 810. 12.1 811: 1.6 812: 41 6 813: 5.4 814: 81 6 815: 10.6 816: 100 817. 13.0 818: 78 6 819: 10 2 820: 38 8 821: 5 0 822. 11.0 823. 1 4 824: 1.4
1	218. 100 219. 13 2 220. 33.8 221: 4 4	296: 76 0 297: 10 0 298: 100 299: 13.1 300: 25 4 301: 3.3	374: 43.6 375. 5 7 376: 100 377. 13.1 378: 70.7 379. 9.2 380: 14.4 381. 1.8	452: 25.9 453: 3.4 454: 84.7 455: 11.1 456: 100 457: 13.0 458: 49.6 459: 6 4 460: 8 5 461: 1 0	530: 14.2 531: 1.8 532: 60.2 533: 7.8 534. 100 535: 13.0 536: 80.6 537: 10 5 548: 31 1 549: 4 0 540: 4 6	608: 7 9 609. 1 0 610. 41 5 611. 5 4 612: 89 0 613: 11 6 614: 100 615: 13.0 616: 61.6 617. 8 0 618: 19 6 619: 2 5 620: 2.5	686: 4 2 687: < 1 688: 26 3 689: 3 4 690: 69 3 691: 9 0 692: 100 693: 13.0 694: 85 2 695. 11 0 696: 42 7 697: 5.5 698: 11.6 699: 1 5 700: 1.3	764: 2.3 765. < 1 766. 16.6 767: 2 2 768: 51 9 769. 6.8 770. 91 7 771: 11.9 772. 100 773 13 0 774. 68.9 775. 89 1 776. 29.2 777: 3.8 778: 6.9	
2	252: 100 253: 13 1 254. 66 4 255. 8 6 256: 11 4 257. 1 4	330: 60.9 331: 8 0 332. 100 333 13.1 334. 46 5 335. 6 0 336. 6 9	408: 38.2 409 5.0 410: 100 411. 13.1 412: 90.4 413: 11.8 414. 32.8 415. 4 2 416 4 3	486. 20.3 487: 2.6 488: 73 0 489: 9.5 490: 100 491: 13 0 492: 64.4 493: 8 4 494: 19.3 495: 2 4 496: 2 2	564: 11.8 565: 1 5 566. 54 2 567: 7 1 568. 100 569: 13.0 570. 94 6 571: 12 3 572: 48 0 573. 6 2 574· 12 3 575. 1.6 576: 1.3	642. 6 2 643: < 1 644. 34 2 645. 4.4 646. 79 5 647 10 4 648. 100 649. 13 0 650 73 0 651 9.5 652 30 8 653. 4 0 654· 6 9	720. 3.5 721: < 1 722: 22 6 723: 2.9 724: 6 4 725: 8 2 726: 100 727: 13 0 728: 96 1 729. 12 5 730: 57 5 731. 7 4 732: 20 8 733. 2 7 734. 4 1		

Table 3 (continued)

Degree of Halogenation Cl+ Br+	0	1	2	3	4	5	6	7	8
3	286: 100 287: 13 1 288: 99.1 289: 12 9 290. 33.1 291: 4.3 292: 3.8	364: 50.8 365: 6.7 366: 100 367: 13.1 368: 66.0 369: 8 6 370: 18.4 371: 2.3 372: 1.9	442: 31.0 443: 4.1 444: 91.4 445. 11.9 446: 100 447. 13.0 448: 50.6 449. 6.5 450: 12.1 451. 1.5 452: 1.1	520: 16.4 521: 2.1 522: 6.4 523: 8.4 524: 100 525: 13 0 526: 78 4 527: 10.2 528: 32.6 529: 4.2 530: 6.9	598: 9.3 599: 1.2 600: 45.6 601: 5.9 602: 92.5 603: 12.0 604: 100 605: 13.0 606: 62.0 607: 8 0 608: 22.0 609: 2.8 610: 4 2	676: 4 9 677. < 1 678: 28 7 679. 3 7 680: 71.9 681: 9 4 682: 100 683: 13 0 684: 83.9 685: 10.9 686: 43.4 687. 5.6 688. 13 5 689. 1 7 690: 2.3			
4	320: 75.9 321: 10.0 322: 100 323: 13.0 324: 49.7 325: 6 4 326: 11.1 327: 1 4 328: 1.0	398: 43.6 399: 5.7 400: 100 401: 13.1 402: 84.6 403: 11.0 404: 34.2 405: 4.4 406: 6.8	476: 23.9 477: 3.1 478: 78.2 479. 10.2 480. 100 481: 13.0 482: 64.1 483. 8.3 484: 22.1 485: 2.8 486: 4.0	554: 13.5 555: 1.8 556: 57.6 557: 7.5 558: 100 559: 13.0 560: 91.8 561: 11.9 562: 48.0 563: 61.9 564: 14.5 565: 1 8 566: 2.4	632: 7.1 633: < 1 634: 37.4 635: 4.9 636: 82 5 637: 10.7 638: 100 639: 13.0 640: 72.7 641: 9 4 642: 32.4 643: 4.2 644: 8.7 645: 1.1 646: 1.3				
5	354: 60.8 355. 8 0 356. 100 357: 13 0 358: 66 0 359. 8 6 360. 21 9 361: 2 8 362. 3 7	432: 37.2 433: 4.9 434: 97.4 435: 12.7 436: 100 437. 13 0 438: 52 8 439. 6.8 440: 15 3 441. 2.0 442. 2.4	510: 19.0 511: 2.5 512. 68.5 513· 8.9 514. 100 515. 13 0 516. 77.1 517. 10.0 518: 34.3 519. 44 1 520. 8 9 521. 1.1 522 1.3	588: 10.9 589: 1.4 590: 49 8 591: 6 5 592: 95.5 593: 12.4 594: 100 595: 13 0 596: 62 7 597: 8.1 598: 24 2 599: 3 1 600. 5 7					

Table 3 (concluded)

Degree of Halogenation Cl_4Br_4	0	1	2	3	4	5	6	7	8
6	388: 50.8	466: 28.2	544: 15.6						
	389. 6.6	467: 3 7	545: 2 0						
	390: 100	468: 83.1	546. 61.1						
	391: 13 0	469: 10 8	547: 8.0						
	392: 82.2	470: 100	548. 100						
	393: 10 7	471: 13 0	549: 13 0						
	394: 36.2	472: 64.8	550: 89.7						
	395: 4 7	473: 8.4	551: 11 6						
	396: 9 0	474: 24.7	552: 48 6						
	397. 1 1	475: 3.2	553: 6.3						
	398: 1.2	476: 5.6	554. 16.4						
			555: 2.1						
			556. 3 4						
7	422. 43.6	500. 22 2							
	423: 5.7	501. 2.9							
	424: 100	502: 72 6							
	425. 13.0	503: 9.4							
	426. 98.6	504: 100							
	427: 12.8	505: 13 0							
	428: 54.1	506: 76.6							
	429: 7.0	507: 9.9							
	430: 17.9	508: 36.0							
	431. 2.3	509: 4.6							
	432. 3.6	510: 10.7							
		511: 1 4							
		512: 2.0							
8	456: 33.2								
	457: 4.3								
	458: 87.1								
	459. 11 3								
	460: 100								
	461: 13.0								
	462: 65 8								
	463. 8 5								
	464: 27.1								
	465. 3.3								
	466: 7 2								
	467. < 1								
	468. 1.2								

Table 4 Characteristic Ions and Their Relative Isotope Abundances in the Molecular Ion Clusters of HDFs

Degree of Halogenation Cl+Br	0	1	2	3	4	5	6	7	8
0	168. 100 169: 13 1	246: 100 247: 13.1 248: 98 7 249. 12.9	324: 50.9 325: 6.7 326: 100 327: 13 1 328: 49.6 329· 6 4	402: 34.0 403: 4.4 404: 100 405: 13.0 406: 98.4 407: 12.8 408: 32.7 409. 4.2	480: 17.3 481: 2.3 482: 67.9 483: 8.8 484: 100 485: 13.0 486: 65.7 487: 8 5 488: 16.4 489: 2.1	558: 10 4 559. 1 4 560. 51 0 561: 6.6 562. 100 563. 13.0 564. 98 2 565: 12 7 566: 48 5 567. 6 2 568. 9 8 569 1 2	636: 5.3 637: < 1 638: 31 2 639: 4.1 640: 76 5 641: 19 9 642: 100 643: 13.0 644: 73.7 645: 9.5 646: 29 2 647: 3.7 648. 4.9	714: 3.0 715: < 1 716: 20 8 717. 2.7 718. 61 2 719. 7.9 720. 100 721: 12 9 722: 98.1 723: 12.7 724. 57 9 725. 7.5 726: 19 1 727 2.4 728 2 8	792: 1.6 793: < 1 794. 12.1 795: 1.6 796: 41.6 797. 5.4 798. 81 6 799. 10.6 800. 100 801. 12 9 802: 78 5 803. 10.1 804: 38 7 805. 5.0 806: 11 0 807. 1 4 808: 1 4
1	202: 100 203: 13 1 204: 33 6 205. 4 3	280: 76.1 281. 10.0 282. 100 283. 13 0 284: 25 2 285. 3 2	358: 43.6 359: 5.7 360 100 361. 13.0 362. 70.5 363: 9.2 364: 14.3 365. 1 8	436: 25.9 437: 3.4 438: 84.8 439: 11.0 440: 100 441: 13 0 442: 49 4 443: 6.4 444: 8.4 445: 1.0	514: 14.2 515: 1.8 516: 60.2 517: 7.8 518: 100 519: 13 0 520: 80.5 521. 10.4 522: 31.0 523: 4.0 524: 4.5	592 8.0 593. 1.0 594. 41.6 595. 5 4 596. 89.1 597. 11 6 598. 100 599. 13 0 600. 61 5 601: 8 0 602. 5 4 603: 2 5 604. 2 5	670: 4.2 671: < 1 672: 26 4 673: 3.4 674: 69 3 675: 9.0 676: 100 677: 13 0 678: 85 1 679· 11 0 680: 42 6 681. 5.5 682: 11 5 683: 1 5 684: 1 3	748 2 3 749. < 1 750. 16 7 751. 2 2 752. 52 0 753. 6 7 754. 91 8 755. 11 9 756. 100 757. 12.9 758 68 8 759. 8 9 760. 29 1 761. 3 7 762. 6 9	
2	236: 100 237: 13.1 238: 66 2 239. 8 6 240· 11 3 241: 1 4	314: 61 0 315: 8 0 316: 100 317: 13 0 318. 46 4 319 6 0 320 6 8	392 38.2 393. 5 0 394: 100 395. 13.0 396. 90.3 397 11.8 398 32 6 399 4.2 400 4 2	470: 20.3 471: 2.6 472: 73 0 473: 9.5 474: 100 475: 13 0 476: 64 3 477: 8 3 478. 19 2 479. 2 4 480: 2 2	548: 11.8 549: 1.5 550: 54.2 551: 7.0 552. 100 553. 13.0 554 94.5 555. 12 2 556. 47 9 557· 6.2 558. 12 2 559. 1.6 560. 1.3	626. 6 2 627. < 1 628: 34.2 629: 4 4 630. 79 6 631. 10 3 632 100 633 13 0 634. 73 0 635. 9 4 636. 30 7 637 39 3 638 6 9	704: 3 5 705: < 1 706: 22 6 707: 2 9 708. 63 6 709. 8 2 710. 100 711 13 0 712. 96 0 713 12 4 714. 57 4 715· 7.4 716. 20 7 717 2.6 718 4 1		

Table 4 (continued)

Degree of Halogenation Cl, Br	0	1	2	3	4	5	6	7	8
3	270: 100 271: 13 1 272: 98 9 273. 12 9 274: 32 9 275: 4 2 276. 3.8	348. 50.9 349. 6 6 350: 100 351: 13.0 352: 65.9 353: 8 5 354. 18.3 355. 2.3 356: 1.9	426. 31.1 427. 4.0 428: 91 5 429. 11.9 430. 100 431: 13.0 432: 50.5 433: 6 5 434: 12.1 435: 1 5 436: 1.1	504: 16.4 505: 2.1 506: 64.3 507: 8 4 508: 100 509: 13.0 510: 78.3 511: 10.1 512: 32.4 513: 4.2 514: 6.8	582: 9.3 583: < 1 584: 45.7 585: 5.9 586: 92.5 587: 12 0 588. 100 589: 13.0 590: 61.9 591: 8 0 592: 21.9 593: 2 8 594: 4.1	660: 4 9 661. < 1 662: 28.8 663. 3 7 664: 72 0 665. 9 3 666: 100 667. 12.9 668. 83 8 669: 10 8 670: 43.3 671: 5.6 672: 13 4 673. 17.0 674: 2 3			
4	304: 76.0 305: 9 9 306. 100 307: 13 0 308. 49.5 309: 6.4 310: 11.0 311. 1.4	382: 43.6 383: 5 7 384. 100 385: 13 0 386: 84.5 387. 11 0 388. 34.1 389. 4 4 390. 6.7	460: 23.9 461: 3.1 462. 78.3 463: 10.2 464: 100 465: 13.0 466. 64 0 467. 8 3 468: 22.0 469: 2.8 470: 3.9	538: 13.6 539: 1.8 540: 57.6 541: 7.5 542: 100 543: 13.0 544: 91 6 545: 11.8 546: 47.9 547: 6.1 548: 14.4 549: 1.8 550: 2.3	616: 7.2 617: < 1 618: 37.4 619: 4.9 620: 8 2 621: 10.7 622: 100 623: 12.9 624: 72.6 625: 9.3 626: 32.3 627: 4.1 628: 8.7 629: 1.1 630: 12.9				
5	338. 60.9 339: 8 0 340: 100 341: 13 0 342: 65 8 343: 8 5 344. 21 8 345. 2.8 346. 3 6	416: 37.2 417: 4.8 418: 97.5 419: 12 7 420: 100 421: 13 0 422: 52.7 423: 6 8 424. 15.3 425. 1.9 426: 2.3	494: 19.1 495: 2.5 496: 68.6 497: 8 9 498. 100 499. 13.0 500: 77.0 501. 10.0 502: 34.1 503: 4.4 504. 8.8 505: 1 1 506 1.2	572: 10.9 573: 1.4 574: 49.9 575: 6.5 576: 95 6 577: 12.4 578: 100 579. 12.9 580: 62 6 581: 8.1 582: 24 2 583: 3 1 584: 5.6					

Table 4. (concluded)

Degree of Halogenation Cl_4Br_4	0	1	2	3	4	5	6	7	8
6	372. 50 8	450: 2 8	528: 15.6						
	373: 6 6	451: 3.7	529. 2.0						
	374. 100	452: 83.2	530: 61.1						
	375: 13 0	453: 10 8	531. 7.9						
	376: 82.1	454. 100	532: 100						
	377. 10 6	455: 12 9	533. 12 9						
	378. 36.1	456: 64 7	534. 89 6						
	379. 4 6	457: 8.4	535. 11 6						
	380 9 0	458: 24 6	536. 48 4						
	381: 1.1	459: 3 1	537: 6.2						
	382: 1 2	460. 5 6	538: 16.3						
			539: 2 1						
			540. 3.4						
7	406: 43.6	484: 22.2							
	407: 5.7	485: 2.9							
	408: 100	486: 72.7							
	409. 13.0	487. 9.4							
	410: 98.4	488: 100							
	411: 12.7	489: 12.9							
	412. 53 9	490. 76 5							
	413. 7.0	491: 9 9							
	414. 17.8	492: 36.0							
	415. 2 2	493: 4.6							
	416: 3 6	494: 10.7							
		495: 1.3							
		496: 2 0							
8	440. 33 3								
	441: 4 3								
	442: 87.2								
	443. 11.3								
	444: 100								
	445. 12 9								
	446: 65 7								
	447. 8 5								
	448: 27 0								
	449. 3 4								
	450: 7 1								
	451: < 1								
	452: 1 2								

Table 5. Potential Interferences from Complex Mixtures of HDDs and HDFs

m/z	Ions present
168	F[a]
169	F
184	D[b]
185	D
186	D
202	Cl-F
203	Cl-F
204	Cl-F
205	Cl-F
218	Cl-D
219	Cl-D
220	Cl-D
221	Cl-D
236	Cl_2-F
237	Cl_2-F
238	Cl_2-F
239	Cl_2-F
240	Cl_2-F
241	Cl_2-F
246	Br-F
247	Br-F
248	Br-F
249	Br-F
252	Cl_2-D
253	Cl_2-D
254	Cl_2-D
255	Cl_2-D
256	Cl_2-D
257	Cl_2-D
262	Br_2-D
263	Br_2-D
264	Br_2-D
265	Br_2-D
266	Br_2-D
270	Cl_3-F
271	Cl_3-F
272	Cl_3-F
273	Cl_3-F
274	Cl_3-F
275	Cl_3-F
276	Cl_3-F
280	ClBr-F
281	ClBr-F
282	ClBr-F
283	ClBr-F
284	ClBr-F
285	ClBr-F

Table 5. (continued)

m/z	Ions present	
286	Cl_3-D	
287	Cl_3-D	
288	Cl_3-D	
289	Cl_3-D	
290	Cl_3-D	
291	Cl_3-D	
292	Cl_3-D	
296	ClBr-D	
297	ClBr-D	
298	ClBr-D	
299	ClBr-D	
300	ClBr-D	
301	ClBr-D	
304	Cl_4-F	
305	Cl_4-F	
306	Cl_4-F	
307	Cl_4-F	
308	Cl_4-F	
309	Cl_4-F	
310	Cl_4-F	
311	Cl_4-F	
314	Cl_2Br-F	
315	Cl_2Br-F	
316	Cl_2Br-F	
317	Cl_2Br-F	
318	Cl_2Br-F	
319	Cl_2Br-F	
320	Cl_2Br-F	Cl_4-D
321	Cl_4-D	
322	Cl_4-D	
323	Cl_4-D	
324	Cl_4-D	Br_2-F
325	Cl_4-D	Br_2-F
326	Cl_4-D	Br_2-F
327	Cl_4-D	Br_2-F
328	Cl_4-D	Br_2-F
329	Br_2-F	
330	Cl_2Br-D	
331	Cl_2Br-D	
332	Cl_2Br-D	
333	Cl_2Br-D	
334	Cl_2Br-D	
335	Cl_2Br-D	
336	Cl_2Br-D	
338	Cl_5-F	
339	Cl_5-F	

Table 5. (continued)

m/z	Ions present	
340	Cl_5-F	Br_2-D
341	Cl_5-F	Br_2-D
342	Cl_5-F	Br_2-D
343	Cl_5-F	Br_2-D
344	Cl_5-F	Br_2-D
345	Cl_5-F	Br_2-D
346	Cl_5-F	
348	Cl_3Br-F	
349	Cl_3Br-F	
350	Cl_3Br-F	
351	Cl_3Br-F	
352	Cl_3Br-F	
353	Cl_3Br-F	
354	Cl_3Br-F	Cl_5-D
355	Cl_3Br-F	Cl_5-D
356	Cl_3Br-F	Cl_5-D
357	Cl_5-D	
358	Cl_5-D	$ClBr_2$-F
359	Cl_5-D	$ClBr_2$-F
360	Cl_5-D	$ClBr_2$-F
361	Cl_5-D	$ClBr_2$-F
362	Cl_5-D	$ClBr_2$-F
363	$ClBr_2$-F	
364	$ClBr_2$-F	Cl_3Br-D
365	$ClBr_2$-F	Cl_3Br-D
366	Cl_3Br-D	
367	Cl_3Br-D	
368	Cl_3Br-D	
369	Cl_3Br-D	
370	Cl_3Br-D	
371	Cl_3Br-D	
372	Cl_3Br-D	Cl_6-F
373	Cl_6-F	
374	Cl_6-F	$ClBr_2$-D
375	Cl_6-F	$ClBr_2$-D
376	Cl_6-F	$ClBr_2$-D
377	Cl_6-F	$ClBr_2$-D
378	Cl_6-F	$ClBr_2$-D
379	Cl_6-F	$ClBr_2$-D
380	Cl_6-F	$ClBr_2$-D
381	Cl_6-F	$ClBr_2$-D
382	Cl_6-F	Cl_4Br-F
383	Cl_4Br-F	
384	Cl_4Br-F	
385	Cl_4Br-F	
386	Cl_4Br-F	

Table 5. (continued)

m/z	Ions present			
387	Cl_4Br-F			
388	Cl_4Br-F	Cl_6-D		
389	Cl_4Br-F	Cl_6-D		
390	Cl_4Br-F	Cl_6-D		
391	Cl_6-D			
392	Cl_6-D	Cl_2Br_2-F		
393	Cl_6-D	Cl_2Br_2-F		
394	Cl_6-D	Cl_2Br_2-F		
395	Cl_6-D	Cl_2Br_2-F		
396	Cl_6-D	Cl_2Br_2-F		
397	Cl_6-D	Cl_2Br_2-F		
398	Cl_6-D	Cl_2Br_2-F	Cl_4Br_3-D	
399	Cl_2Br_2-F	Cl_4Br-D		
400	Cl_2Br_2-F	Cl_4Br-D		
401	Cl_4Br-D			
402	Cl_4Br-D	Br_3-F		
403	Cl_4Br-D	Br_3-F		
404	Cl_4Br-D	Br_3-F		
405	Cl_4Br-D	Br_3-F		
406	Cl_4Br-D	Br_3-F	Cl_7-F	
407	Br_3-F	Cl_7-F		
408	Br_3-F	Cl_7-F	Cl_2Br_2-D	
409	Br_3-F	Cl_7-F	Cl_2Br_2-D	
410	Cl_7-F	Cl_2Br_2-D		
411	Cl_7-F	Cl_2Br_2-D		
412	Cl_7-F	Cl_2Br_2-D		
413	Cl_7-F	Cl_2Br_2-D		
414	Cl_7-F	Cl_2Br_2-D		
415	Cl_7-F	Cl_2Br_2-D		
416	Cl_7-F	Cl_2Br_2-D	Cl_5Br-F	
417	Cl_5Br-F			
418	Cl_5Br-F	Br_3-D		
419	Cl_5Br-F	Br_3-D		
420	Cl_5Br-F	Br_3-D		
421	Cl_5Br-F	Br_3-D		
422	Cl_5Br-F	Br_3-D	Cl_7-D	
423	Cl_5Br-F	Br_3-D	Cl_7-D	
424	Cl_5Br-F	Br_3-D	Cl_7-D	
425	Cl_5Br-F	Br_3-D	Cl_7-D	
426	Cl_5Br-F	Br_3-D	Cl_7-D	Cl_3Br_2-F
427	Cl_7-D	Cl_3Br_2-F		
428	Cl_7-D	Cl_3Br_2-F		
429	Cl_7-D	Cl_3Br_2-F		
430	Cl_7-D	Cl_3Br_2-F		
431	Cl_7-D	Cl_3Br_2-F		
432	Cl_7-D	Cl_3Br_2-F	Cl_5Br-D	

Table 5. (continued)

m/z	Ions present			
433	Cl_5Br-D	Cl_3Br_2-F		
434	Cl_5Br-D	Cl_3Br_2-F		
435	Cl_5Br-D	Cl_3Br_2-F		
436	Cl_5Br-D	$ClBr_3$-F	Cl_3Br_2-F	
437	Cl_5Br-D	$ClBr_3$-F		
438	Cl_5Br-D	$ClBr_3$-F		
439	Cl_5Br-D	$ClBr_3$-F		
440	Cl_5Br-D	$ClBr_3$-F	Cl_8-F	
441	Cl_5Br-D	$ClBr_3$-F	Cl_8-F	
442	Cl_5Br-D	$ClBr_3$-F	Cl_8-F	Cl_3Br_2-D
443	$ClBr_3$-F	Cl_8-F	Cl_3Br_2-D	
444	$ClBr_3$-F	Cl_8-F	Cl_3Br_2-D	
445	$ClBr_3$-F	Cl_8-F	Cl_3Br_2-D	
446	Cl_8-F	Cl_3Br_2-D		
447	Cl_8-F	Cl_3Br_2-D		
448	Cl_8-F	Cl_3Br_2-D		
449	Cl_8-F	Cl_3Br_2-D		
450	Cl_8-F	Cl_3Br_2-D	Cl_6Br-F	
451	Cl_8-F	Cl_3Br_2-D	Cl_6Br-F	
452	Cl_8-F	Cl_3Br_2-D	Cl_6Br-F	$ClBr_3$-D
453	Cl_6Br-F	$ClBr_3$-D		
454	Cl_6Br-F	$ClBr_3$-D		
455	Cl_6Br-F	$ClBr_3$-D		
456	Cl_6Br-F	$ClBr_3$-D	Cl_8-D	
457	Cl_6Br-F	$ClBr_3$-D	Cl_8-D	
458	Cl_6Br-F	$ClBr_3$-D	Cl_8-D	
459	Cl_6Br-F	$ClBr_3$-D	Cl_8-D	
460	Cl_6Br-F	$ClBr_3$-D	Cl_8-D	Cl_4Br_2-F
461	$ClBr_3$-D	Cl_8-D		Cl_4Br_2-F
462	Cl_8-D	Cl_4Br_2-F		
463	Cl_8-D	Cl_4Br_2-F		
464	Cl_8-D	Cl_4Br_2-F		
465	Cl_8-D	Cl_4Br_2-F		
466	Cl_8-D	Cl_4Br_2-F	Cl_6Br-D	
467	Cl_8-D	Cl_4Br_2-F	Cl_6Br-D	
468	Cl_8-D	Cl_4Br_2-F	Cl_6Br-D	
469	Cl_4Br_2-F	Cl_6Br-D		
470	Cl_4Br_2-F	Cl_6Br-D	Cl_2Br_3-F	
471	Cl_6Br-D	Cl_2Br_3-F		
472	Cl_6Br-D	Cl_2Br_3-F		
473	Cl_6Br-D	Cl_2Br_3-F		
474	Cl_6Br-D	Cl_2Br_3-F		
475	Cl_6Br-D	Cl_2Br_3-F		
476	Cl_6Br-D	Cl_4Br_2-D	Cl_2Br_3-F	
477	Cl_4Br_2-D	Cl_2Br_3-F		
478	Cl_4Br_2-D	Cl_2Br_3-F		

Table 5. (continued)

m/z	Ions present			
479	Cl_4Br_2-D	Cl_2Br_3-F		
480	Cl_4Br_2-D	Br_4-F	Cl_2Br_3-F	
481	Cl_4Br_2-D	Br_4-F		
482	Cl_4Br_2-D	Br_4-F		
483	Cl_4Br_2-D	Br_4-F		
484	Cl_4Br_2-D	Br_4-F	Cl_7Br-F	
485	Cl_4Br_2-D	Br_4-F	Cl_7Br-F	
486	Cl_4Br_2-D	Br_4-F	Cl_7Br-F	Cl_2Br_3-D
487	Br_4-F	Cl_7Br-F	Cl_2Br_3-D	
488	Br_4-F	Cl_7Br-F	Cl_2Br_3-D	
489	Br_4-F	Cl_7Br-F	Cl_2Br_3-D	
490	Cl_7Br-F	Cl_2Br_3-D		
491	Cl_7Br-F	Cl_2Br_3-D		
492	Cl_7Br-F	Cl_2Br_3-D		
493	Cl_7Br-F	Cl_2Br_3-D		
494	Cl_7Br-F	Cl_2Br_3-D	Cl_5Br_2-F	
495	Cl_2Br_3-D	Cl_5Br_2-F		
496	Cl_2Br_3-D	Br_4-D	Cl_5Br-F	
497	Br_4-D	Cl_5Br_2-F		
498	Br_4-D	Cl_5Br_2-F		
499	Br_4-D	Cl_5Br_2-F		
500	Br_4-D	Cl_5Br_2-F	Cl_7Br-D	
501	Br_4-D	Cl_5Br_2-F	Cl_7Br-D	
502	Br_4-D	Cl_5Br_2-F	Cl_7Br-D	
503	Br_4-D	Cl_5Br_2-F	Cl_7Br-D	
504	Br_4-D	Cl_5Br_2-F	Cl_7Br-D	Cl_3Br_3-F
505	Br_4-D	Cl_5Br_2-F	Cl_7Br-D	Cl_3Br_3-F
506	Cl_5Br_2-F	Cl_7Br-D	Cl_3Br_3-F	
507	Cl_5Br-D	Cl_3Br_3-F		
508	Cl_7Br-D	Cl_3Br_3-F		
509	Cl_7Br-D	Cl_3Br_3-F		
510	Cl_7Br-D	Cl_3Br_3-F	Cl_5Br_2-D	
511	Cl_7Br-D	Cl_3Br_3-F	Cl_5Br_2-D	
512	Cl_7Br-D	Cl_3Br_3-F	Cl_5Br_2-D	
513	Cl_3Br_3-F	Cl_5Br_2-D		
514	Cl_3Br_3-F	Cl_5Br_2-D	$ClBr_4$-F	
515	Cl_5Br_2-D	Br_4-F		
516	Cl_5Br_2-D	Br_4-F		
517	Cl_5Br_2-D	Br_4-F		
518	Cl_5Br_2-D	Br_4-F		
519	Cl_5Br_2-D	Br_4-F		
520	Cl_5Br_2-D	Br_4-F	Cl_3Br_3-D	
521	Cl_5Br_2-D	Br_4-F	Cl_3Br_3-D	
522	Cl_5Br_2-D	Br_4-F	Cl_3Br_3-D	
523	Br_4-F	Cl_3Br_3-D		
524	Br_4-F	Cl_3Br_3-D		

Table 5. (continued)

m/z	Ions present		
525	Cl_3Br_3-D		
526	Cl_3Br_3-D		
527	Cl_3Br_3-D		
528	Cl_3Br_3-D	Cl_6Br_2-F	
529	Cl_3Br_3-D	Cl_6Br_2-F	
530	Cl_3Br_3-D	Cl_6Br_2-F	Cl_3Br_4-D
531	Cl_6Br_2-F	$ClBr_4$-D	
532	Cl_6Br_2-F	$ClBr_4$-D	
533	Cl_6Br_2-F	$ClBr_4$-D	
534	Cl_6Br_2-F	$ClBr_4$-D	
535	Cl_6Br_2-F	$ClBr_4$-D	
536	Cl_6Br_2-F	$ClBr_4$-D	
537	Cl_6Br_2-F	$ClBr_4$-D	
538	Cl_6Br_2-F	$ClBr_4$-D	Cl_4Br_3-F
539	Cl_6Br_2-F	$ClBr_4$-D	Cl_4Br_3-F
540	Cl_6Br_2-F	$ClBr_4$-D	Cl_4Br_3-F
541	Cl_4Br_3-F		
542	Cl_4Br_3-F		
543	Cl_4Br_3-F		
544	Cl_4Br_3-F	Cl_6Br_2-D	
545	Cl_4Br_3-F	Cl_6Br_2-D	
546	Cl_4Br_3-F	Cl_6Br_2-D	
547	Cl_4Br_3-F	Cl_6Br_2-D	Cl_2Br_4-F
548	Cl_4Br_3-F	Cl_6Br_2-D	Cl_2Br_4-F
549	Cl_4Br_3-F	Cl_6Br_2-D	Cl_2Br_4-F
550	Cl_4Br_3-F	Cl_6Br_2-D	Cl_2Br_4-F
551	Cl_6Br_2-D	Cl_2Br_4-F	
552	Cl_6Br_2-D	Cl_2Br_4-F	
553	Cl_6Br_2-D	Cl_2Br_4-F	
554	Cl_6Br_2-D	Cl_2Br_4-F	Cl_4Br_3-D
555	Cl_6Br_2-D	Cl_2Br_4-F	Cl_4Br_3-D
556	Cl_6Br_2-D	Cl_2Br_4-F	Cl_4Br_3-D
557	Cl_2Br_4-F	Cl_4Br_3-D	
558	Cl_2Br_4-F	Cl_4Br_3-D	Br_5-F
559	Cl_2Br_4-F	Cl_4Br_3-D	Br_5-F
560	Cl_2Br_4-F	Cl_4Br_3-D	Br_5-F
561	Cl_4Br_3-D	Br_5-F	
562	Cl_4Br_3-D	Br_5-F	
563	Cl_4Br_3-D	Br_5-F	Cl_2Br_4-D
564	Cl_4Br_3-D	Br_5-F	Cl_2Br_4-D
565	Cl_4Br_3-D	Br_5-F	Cl_2Br_4-D
566	Cl_4Br_3-D	Br_5-F	Cl_2Br_4-D
567	Br_5-F	Cl_2Br_4-D	
568	Br_5-F	Cl_2Br_4-D	
569	Br_5-F	Cl_2Br_4-D	

Table 5. (continued)

m/z	Ions present		
570	Cl_2Br_4-D		
571	Cl_2Br_4-D		
572	Cl_2Br_4-D	Cl_5Br_3-F	
573	Cl_2Br_4-D	Cl_5Br_3-F	
574	Cl_2Br_4-D	Cl_5Br_3-F	Br_5-D
575	Cl_2Br_4-D	Cl_5Br_3-F	Br_5-D
576	Cl_2Br_4-D	Cl_5Br_3-F	Br_5-D
577	Br_5-D	Cl_5Br_3-F	
578	Br_5-D	Cl_5Br_3-F	
579	Br_5-D	Cl_5Br_3-F	
580	Br_5-D	Cl_5Br_3-F	
581	Br_5-D	Cl_5Br_3-F	
582	Br_5-D	Cl_5Br_3-F	Cl_3Br_4-F
583	Br_5-D	Cl_5Br_3-F	Cl_3Br_4-F
584	Br_5-D	Cl_5Br_3-F	Cl_3Br_4-F
585	Br_5-D	Cl_3Br_4-F	
586	Cl_3Br_4-F		
587	Cl_3Br_4-F		
588	Cl_3Br_4-F	Cl_5Br_3-D	
589	Cl_3Br_4-F	Cl_5Br_3-D	
590	Cl_3Br_4-F	Cl_5Br_3-D	
591	Cl_3Br_4-F	Cl_5Br_3-D	
592	Cl_3Br_4-F	Cl_5Br_3-D	$ClBr_5$-F
593	Cl_3Br_4-F	Cl_5Br_3-D	$ClBr_5$-F
594	Cl_3Br_4-F	Cl_5Br_3-D	$ClBr_5$-F
595	Cl_5Br_3-D	$ClBr_5$-F	
596	Cl_5Br_3-D	$ClBr_5$-F	
597	Cl_5Br_3-D	$ClBr_5$-F	
598	Cl_5Br_3-D	$ClBr_5$-F	Cl_3Br_4-D
599	Cl_5Br_3-D	$ClBr_5$-F	Cl_3Br_4-D
600	Cl_5Br_3-D	$ClBr_5$-F	Cl_3Br_4-D
601	$ClBr_5$-F	Cl_3Br_4-D	
602	$ClBr_5$-F	Cl_3Br_4-D	
603	$ClBr_5$-F	Cl_3Br_4-D	
604	$ClBr_5$-F	Cl_3Br_4-D	
605	Cl_3Br_4-D		
606	Cl_3Br_4-D		
607	Cl_3Br_4-D		
608	Cl_3Br_4-D	$ClBr_5$-D	
609	Cl_3Br_4-D	$ClBr_5$-D	
610	Cl_3Br_4-D	$ClBr_5$-D	
611	$ClBr_5$-D		
612	$ClBr_5$-D		
613	$ClBr_5$-D		
614	$ClBr_5$-D		
615	$ClBr_5$-D		

Table 5. (continued)

m/z	Ions present		
616	$ClBr_5$-D	Cl_4Br_4-F	
617	$ClBr_5$-D	Cl_4Br_4-F	
618	$ClBr_5$-D	Cl_4Br_4-F	
619	$ClBr_5$-D	Cl_4Br_4-F	
620	$ClBr_5$-D	Cl_4Br_4-F	
621	Cl_4Br_4-F		
622	Cl_4Br_4-F		
623	Cl_4Br_4-F		
624	Cl_4Br_4-F		
625	Cl_4Br_4-F		
626	Cl_4Br_4-F	Cl_2Br_5-F	
627	Cl_4Br_4-F	Cl_2Br_5-F	
628	Cl_4Br_4-F	Cl_2Br_5-F	
629	Cl_4Br_4-F	Cl_2Br_5-F	
630	Cl_4Br_4-F	Cl_2Br_5-F	
631	Cl_2Br_5-F		
632	Cl_2Br_5-F	Cl_4Br_4-D	
633	Cl_2Br_5-F	Cl_4Br_4-D	
634	Cl_2Br_5-F	Cl_4Br_4-D	
635	Cl_2Br_5-F	Cl_4Br_4-D	
636	Cl_2Br_5-F	Cl_4Br_4-D	Br_6-F
637	Cl_2Br_5-F	Cl_4Br_4-D	Br_6-F
638	Cl_2Br_5-F	Cl_4Br_4-D	Br_6-F
639	Cl_4Br_4-D	Br_6-F	
640	Cl_4Br_4-D	Br_6-F	
641	Cl_4Br_4-D	Br_6-F	
642	Cl_4Br_4-D	Br_6-F	Cl_2Br_5-D
643	Cl_4Br_4-D	Br_6-F	Cl_2Br_5-D
644	Cl_4Br_4-D	Br_6-F	Cl_2Br_5-D
645	Cl_4Br_4-D	Br_6-F	Cl_2Br_5-D
646	Cl_4Br_4-D	Br_6-F	Cl_2Br_5-D
647	Br_6-F	Cl_2Br_5-D	
648	Br_6-F	Cl_2Br_5-D	
649	Cl_2Br_5-D		
650	Cl_2Br_5-D		
651	Cl_2Br_5-D		
652	Cl_2Br_5-D	Br_6-D	
653	Cl_2Br_5-D	Br_6-D	
654	Cl_2Br_5-D	Br_6-D	
655	Br_6-D		
656	Br_6-D		
657	Br_6-D		
658	Br_6-D		
659	Br_6-D		

Table 5. (continued)

m/z	Ions present	
660	Br_6-D	Cl_3Br_5-F
661	Br_6-D	Cl_3Br_5-F
662	Br_6-D	Cl_3Br_5-F
663	Br_6-D	Cl_3Br_5-F
664	Br_6-D	Cl_3Br_5-F
665	Cl_3Br_5-F	
666	Cl_3Br_5-F	
667	Cl_3Br_5-F	
668	Cl_3Br_5-F	
669	Cl_3Br_5-F	
670	Cl_3Br_5-F	$ClBr_6$-F
671	Cl_3Br_5-F	$ClBr_6$-F
672	Cl_3Br_5-F	$ClBr_6$-F
673	Cl_3Br_5-F	$ClBr_6$-F
674	Cl_3Br_5-F	$ClBr_6$-F
675	$ClBr_6$-F	
676	$ClBr_6$-F	Cl_3Br_5-D
677	$ClBr_6$-F	Cl_3Br_5-D
678	$ClBr_6$-F	Cl_3Br_5-D
679	$ClBr_6$-F	Cl_3Br_5-D
680	$ClBr_6$-F	Cl_3Br_5-D
681	$ClBr_6$-F	Cl_3Br_5-D
682	$ClBr_6$-F	Cl_3Br_5-D
683	$ClBr_6$-F	Cl_3Br_5-D
684	$ClBr_6$-F	Cl_3Br_5-D
685	Cl_3Br_5-D	
686	Cl_3Br_5-D	$ClBr_6$-D
687	Cl_3Br_5-D	$ClBr_6$-D
688	Cl_3Br_5-D	$ClBr_6$-D
689	Cl_3Br_5-D	$ClBr_6$-D
690	Cl_3Br_5-D	$ClBr_6$-D
691	$ClBr_6$-D	
692	$ClBr_6$-D	
693	$ClBr_6$-D	
694	$ClBr_6$-D	
695	$ClBr_6$-D	
696	$ClBr_6$-D	
697	$ClBr_6$-D	
698	$ClBr_6$-D	
699	$ClBr_6$-D	
700	$ClBr_6$-D	
704	Cl_2Br_6-F	
705	Cl_2Br_6-F	
706	Cl_2Br_6-F	
707	Cl_2Br_6-F	
708	Cl_2Br_6-F	

Table 5. (continued)

m/z	Ions present	
709	Cl_2Br_6-F	
710	Cl_2Br_6-F	
711	Cl_2Br_6-F	
712	Cl_2Br_6-F	
713	Cl_2Br_6-F	
714	Cl_2Br_6-F	Br_7-F
715	Cl_2Br_6-F	Br_7-F
716	Cl_2Br_6-F	Br_7-F
717	Cl_2Br_6-F	Br_7-F
718	Cl_2Br_6-F	Br_7-F
719	Br_7-F	
720	Br_7-F	Cl_2Br_6-D
721	Br_7-F	Cl_2Br_6-D
722	Br_7-F	Cl_2Br_6-D
723	Br_7-F	Cl_2Br_6-D
724	Br_7-F	Cl_2Br_6-D
725	Br_7-F	Cl_2Br_6-D
726	Br_7-F	Cl_2Br_6-D
727	Br_7-F	Cl_2Br_6-D
728	Br_7-F	Cl_2Br_6-D
729	Cl_2Br_6-D	
730	Cl_2Br_6-D	Br_7-D
731	Cl_2Br_6-D	Br_7-D
732	Cl_2Br_6-D	Br_7-D
733	Cl_2Br_6-D	Br_7-D
734	Cl_2Br_6-D	Br_7-D
735	Br_7-D	
736	Br_7-D	
737	Br_7-D	
738	Br_7-D	
739	Br_7-D	
740	Br_7-D	
741	Br_7-D	
742	Br_7-D	
743	Br_7-D	
744	Br_7-D	
748	$ClBr_7$-F	
749	$ClBr_7$-F	
750	$ClBr_7$-F	
751	$ClBr_7$-F	
752	$ClBr_7$-F	
753	$ClBr_7$-F	
754	$ClBr_7$-F	
755	$ClBr_7$-F	
756	$ClBr_7$-F	
757	$ClBr_7$-F	

Table 5. (continued)

m/z	Ions present	
758	$ClBr_7$-F	
759	$ClBr_7$-F	
760	$ClBr_7$-F	
761	$ClBr_7$-F	
762	$ClBr_7$-F	
764	$ClBr_7$-D	
765	$ClBr_7$-D	
766	$ClBr_7$-D	
767	$ClBr_7$-D	
768	$ClBr_7$-D	
769	$ClBr_7$-D	
770	$ClBr_7$-D	
771	$ClBr_7$-D	
772	$ClBr_7$-D	
773	$ClBr_7$-D	
774	$ClBr_7$-D	
775	$ClBr_7$-D	
776	$ClBr_7$-D	
777	$ClBr_7$-D	
778	$ClBr_7$-D	
792	Br_8-F	
793	Br_8-F	
794	Br_8-F	
795	Br_8-F	
796	Br_8-F	
797	Br_8-F	
798	Br_8-F	
799	Br_8-F	
800	Br_8-F	
801	Br_8-F	
802	Br_8-F	
803	Br_8-F	
804	Br_8-F	
805	Br_8-F	
806	Br_8-F	
807	Br_8-F	
808	Br_8-F	Br_8-D
809	Br_8-D	
810	Br_8-D	
811	Br_8-D	
812	Br_8-D	
813	Br_8-D	
814	Br_8-D	
815	Br_8-D	
816	Br_8-D	

Table 5. (concluded)

m/z	Ions present
817	Br_8-D
818	Br_8-D
819	Br_8-D
820	Br_8-D
821	Br_8-D
822	Br_8-D
823	Br_8-D
824	Br_8-D

[a]F = dibenzofuran.
[b]D = dibenzo-p-dioxin.

4.0 Safety

Some of the following safety practices are excerpted directly from the EPA Method 613, "Determination of 2,3,7,8-TCDD in Water and Wastewater," Section 4 (October 1984 version). Although these practices were designed for the safe handling of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), they are also applicable for other HDDs and HDFs.

4.1 2,3,7,8-TCDD has been tentatively classified as a known or suspected human or mammalian carcinogen.

Note: Congeners substituted in the 2,3,7,8 positions are reported to be the most toxic. Extreme caution should be taken when handling these compounds as dry powders or in concentrated solutions.

The toxicity or carcinogenicity of other reagents used in these guidelines has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in these guidelines. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis.

4.2 If diethyl ether is used, it should be monitored regularly to determine its peroxide content. Under no circumstances should diethyl ether be used with a peroxide content in excess of 50 ppm, as an explosion could occur. Peroxide test strips manufactured by EM Laboratories (available from Scientific Products Company, Cat. No P1126-8, and other suppliers) are recommended for this test. Procedures for removal of peroxides from diethyl ether are included in the instructions supplied with the test kit.

4.3 Each laboratory must develop a strict safety program for handling 2,3,7,8-TCDD. The following laboratory practices are recommended.

4.3.1 Contamination of the laboratory will be minimized by conducting all manipulations in a hood.

4.3.2 The effluents of sample splitters for the gas chromatograph and roughing pumps on the GC/MS should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohols.

4.3.3 Liquid waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light with a wavelength greater than 290 nm for several days. (Use F 40 BL lamps or equivalent.) Analyze liquid wastes and dispose of the solutions when 2,3,7,8-TCDD can no longer be detected.

4.4 Dow Chemical U.S.A. has issued the following precautions (revised November 1978) for the safe handling of 2,3,7,8-TCDD in the laboratory:

4.4.1 The following statements on safe handling are as complete as possible on the basis of available toxicological information. The precautions for safe handling and use are necessarily general in nature since detailed, specific recommendations can be made only for the particular exposure and circumstances of each individual use. Inquiries about specific operations or uses may be addressed to the Dow Chemical Company. Assistance in evaluating the health hazards of particular plant conditions may be obtained from certain consulting laboratories and from state Departments of Health or of Labor, many of which have an industrial health service. 2,3,7,8-TCDD is extremely toxic to laboratory animals. However, it has been handled for years without known injury in analytical and biological laboratories. Techniques used in handling radioactive and infectious materials are applicable to 2,3,7,8-TCDD.

4.4.1.1 Protective equipment - Throwaway plastic gloves, apron or lab coat, safety glasses, and a lab hood adequate for radioactive work.

4.4.1.2 Training - Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

4.4.1.3 Personal hygiene - Thorough washing of hands and forearms after each manipulation and before breaks (coffee, lunch, and shift).

4.4.1.4 Confinement - Isolated work area, posted with signs, segregated glassware and tools, plastic-backed adsorbent paper on benchtops.

4.4.1.5 Waste - Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors must be trained in the safe handling of waste.

4.4.1.6 Disposal of wastes - 2,3,7,8-TCDD decomposes above 800°C. Low level waste such as absorbent paper, tissues, and plastic gloves may be burned in an incinerator. Gross quantities (milligrams) should be packaged securely and disposed through commercial or governmental channels which are capable of handling extremely toxic wastes. Liquids should be allowed to evaporate in a good hood and in a disposable container.

4.4.1.7 Decontamination - For personal decontamination, use any mild soap with plenty of scrubbing action. For decontamination of glassware, tools, and surfaces, Chlorothene NU Solvent (trademark of the Dow Chemical Company) is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with chlorothene, then washing with any detergent and water. Dishwater may be disposed to the sewer. It is prudent to minimize solvent wastes because they may require special disposal through commercial sources which are expensive.

4.4.1.8 Laundry - Clothing known to be contaminated should be disposed with the precautions described under Section 4.4.1.6. Lab coats or other clothing worn in 2,3,7,8-TCDD work area may be laundered. Clothing should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the launderer knows the problem. The washer should be run through a cycle before being used again for other clothing.

4.4.1.9 Wipe tests - A useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper. Extraction and analysis by gas chromatography can achieve a limit of sensitivity of 0.1 μg per wipe. Less than 1 μg of 2,3,7,8-TCDD per sample indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 μg on a wipe sample constitutes an acute hazard and requires prompt cleaning before further use of the equipment or work space. A high (> 10 μg) 2,3,7,8-TCDD level indicates that unacceptable work practices have been employed in the past.

4.4.1.10 Inhalation - Any procedure that may produce airborne contamination must be done with good ventilation. Gross losses to a ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical work presents no inhalation hazards except in the case of an accident.

4.4.1.11 Accidents - Remove contaminated clothing immediately, taking precautions not to contaminate skin or other articles. Wash exposed skin vigorously and repeatedly until medical attention is obtained.

5.0 Apparatus and Materials

5.1 Sampling containers - Amber glass bottles or jars, 1-L or other appropriate volume, fitted with screw caps lined with Teflon® are suitable for sample containment. Cleaned aluminum foil may be substituted for Teflon® if the sample is not corrosive. If amber containers are not available, samples should be protected from light using foil or an opaque outer container. The containers should be washed, rinsed with acetone or methylene chloride, and dried before use in order to minimize contamination.

5.2 Glassware - Glassware requirements will be dictated by the extraction and cleanup procedures employed by each laboratory. Typical glassware requirements are given below. All specifications are suggestions only. Catalog numbers are included for illustration only.

5.2.1 Volumetric flasks - Assorted sizes.

5.2.2 Pipets - Assorted sizes, Mohr delivery.

5.2.3 Micro syringes - 1.0 µL with fused silica needle for on-column gas chromatographic analysis.

5.2.4 Chromatography columns - Sizes dictated by method employed. A typical column is the Chromaflex column, 400 mm x 19 mm ID (Kontes K-420540-9011) or equivalent.

5.2.5 Kuderna-Danish evaporative concentrator apparatus.

5.2.5.1 Concentrator tube - 10 mL, graduated (Kontes K-570050-1025 or equivalent). Calibration should be checked. Ground glass stopper (size ₮19/22 joint) is used to prevent evaporative losses.

5.2.5.2 Evaporation flask - 500 mL (Kontes K-57001-0050 or equivalent). Attached to concentrator tube with springs (Kontes K-662750-0012 or equivalent).

5.2.5.3 Snyder column - Three-ball macro (Kontes K-503000-0121).

5.3 Balance - Analytical, capable of accurately weighing 0.0001 g.

5.4 Gas chromatographic system.

5.4.1 Gas chromatograph - An analytical system complete with a temperature programmable gas chromatograph and all required accessories including syringes, analytical columns, and gases. The injection port should be designed for on-column injection when using capillary columns. Other capillary injection techniques (split, splitless, etc.) may be used

provided the performance specifications stated in Section 7.1 are met.

5.4.2 Capillary GC column - A 10 to 60 m long x 0.25 mm ID fused silica column with a 0.25 µm thick DB-5 (Supelco Inc., Bellefonte, Pennsylvania) liquid phase is recommended. Alternate liquid phases may include Silar 10-C, SP-2330, SP-2340, DB-225, SE-54, SP-2100, or other liquid phases which meet the performance specifications stated in Section 7.1. The Silar 10-C, SP-2330, and the SP-2340 columns are recommended to achieve isomer specific analyses (e.g., 2,3,7,8-TCDD from the other 21 TCDD isomers).

5.4.3 A data system should be used for manipulation of GC/ECD data. At a minimum, electronic integration should be used. The ideal system will allow for archiving raw chromatographic data on magnetic media.

5.5 Gas chromatograph/mass spectrometer system.

5.5.1 Gas chromatograph - As described in Section 5.4.1.

5.5.2 Capillary GC column - As described in Section 5.4.2.

5.5.3 Mass spectrometer - Should be capable of scanning a range of 400 amu in 1.5 s or less, collecting at least five data points per chromatographic peak, utilizing a 70-eV (nominal) electron energy in the electron impact ionization mode, and producing a mass spectrum which meets all the criteria in Table 6 when 50 ng of decafluorotriphenyl phosphine [DFTPP, bis(perfluorophenyl)phenyl phosphine] is injected through the GC inlet. Any GC to MS interface that gives acceptable calibration points at the working range and achieves all acceptable performance criteria (Section 10) may be used. Direct coupling of the fused silica chromatographic column to the MS is recommended. Glass interfaces, if used, can be deactivated by silylation with dichlorodimethylsilane or an equivalent silylation reagent.

5.5.4 A computer system that allows the continuous acquisition and storage on machine-readable media of all mass spectral data obtained throughout the duration of the chromatographic program should be interfaced to the mass spectrometer. The data system should have the capability of integrating the abundances of the selected ions between specified limits and relating integrated abundances to concentrations using the calibration procedures described in these guidelines. The computer should have software that allows searching any mass spectral data file for ions of a specific mass and plotting such ion abundances versus time or scan number to yield an extracted ion current profile (EICP). The software should also allow integration of ion abundances in any EICP between specified time or scan number limits.

Table 6. DFTPP Key Ions and Ion Abundance Criteria

m/z	Ion abundance criteria
197	Less than 1% of m/z 198
198	100% relative abundance
199	5-9% of m/z 198
275	10-30% of m/z 198
365	Greater than 1% of m/z 198
441	Present but less than m/z 443
442	Greater than 40% of m/z 198
443	17-23% of m/z 442

6.0 Reagents

6.1 Solvents - All solvents should be pesticide residue analysis grade. New lots should be checked for purity by concentration of an aliquot by at least as much as will occur during the sample preparation procedure. The concentrated solvent sample should then be analyzed in the same manner as the samples.

6.2 Standard chlorinated and brominated congeners - Standards of the HDD and HDF congeners, listed in Table 7, are available from Ultra Scientific (Hope, Rhode Island), KOR Isotopes (Cambridge, Massachusetts), and Cambridge Isotope Laboratories (Woburn, Massachusetts).

6.3 Standards containing mixed brominated/chlorinated congeners of the HDDs and HDFs are not commercially available at this time. If analyses for these congeners are to be conducted, standards must be synthesized and characterized as to identity and purity prior to sample analysis. One standard per homolog group may be adequate to conduct the analysis.

6.4 Calibration standard stock solution - Primary dilutions of each of the individual HDDs and HDFs which are to be used are prepared by weighing approximately 1 to 10 mg of material with an accuracy of 1% or better. The standard is then diluted to 1.0 mL, or another known volumn, with an appropriate solvent. The concentration is calculated in milligrams per milliliter. The primary dilutions should be stored at 4°C, or lower, in screw-cap vials with Teflon® cap liners. The meniscus is marked on the vial wall to monitor solvent evaporation.

6.5 Working calibration standards - Working calibration standards, similar in HDD or HDF composition and concentration to the samples, are prepared by mixing and diluting the individual standard stock solutions. The mixture is diluted to a known volume with the same solvent that is used for the final sample extract. The concentration is calculated in nanograms per milliliter as the individual components. Dilutions are stored at 4°C, or lower, in narrow-mouth, screw-cap vials with Teflon® cap liners. The meniscus is marked on the vial wall to monitor solvent evaporation. These solutions should be labeled with the analytes and concentration, solvent, initials of the preparer, and the date.

6.6 Alternatively, certified stock solutions, if available from a supplier, may be used in lieu of the procedure described in Section 6.5.

6.7 DFTPP standard - A 50-ng/µL solution of DFTPP is prepared in acetone or another appropriate solvent.

6.8 Surrogate standard solutions - These solutions should be prepared and labeled as described for the calibration standards

Table 7. Currently Available HDD and HDF Standards

Unlabeled		Stable isotope labeled
Chlorinated dioxins:		
Mono:	2	
Di:	2,3	2,7/2,8 (u-$^{13}C_{12}$, 99%)[b]
	2,8	
	2,7/2,8[a]	
Tri:	1,2,3	
	1,2,4	
	2,3,7	
Tetra:	1,2,3,4	1,2,3,4 ($^{13}C_6$, 99%)
	1,2,3,7/1,2,3,8[a]	2,3,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,3,7/1,2,8,9[a]	2,3,7,8 (U-$^{37}Cl_4$, 96%)[c]
	1,2,4,7/1,2,4,8[a]	1,2,3,4 (U-$^{13}C_{12}$, 99%)
	1,2,6,7/1,2,8,9[a]	
	1,2,7,8	
	1,2,8,9	
	1,3,6,8	
	1,3,6,8/1,3,7,9[a]	
	1,3,7,8	
	2,3,7,8	
Penta:	1,2,3,4,7	
	1,2,3,7,8	1,2,3,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,4,7,8	
Hexa:	1,2,3,4,7,8	1,2,3,6,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,3,6,7,8	1,2,3,7,8,9 (U-$^{13}C_{12}$, 99%)
	1,2,3,7,8,9	
Hepta:	1,2,3,4,6,7,8	1,2,3,4,6,7,8 (U-$^{13}C_{12}$, 99%)
Octa:	1,2,3,4,6,7,8,9	1,2,3,4,6,7,8 (U-$^{13}C_{12}$, 99%)
Brominated dioxins:		
Mono:	1	
Di:	2,7	
	2,8	
Tri:	2,3,7	
Tetra:	1,2,3,4	2,3,7,8 (U-$^{13}C_{12}$, 99%)
	1,3,6,8/1,3,7,9[a]	
	1,3,7,8	
	2,3,7,8	
Penta:	1,2,3,7,8	1,2,3,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,4,7,8	
Hexa:	1,2,3,4,7,8	1,2,3,4,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,3,6,7,8/1,2,3,7,8,9[a]	

Table 7 (continued)

Unlabeled		Stable isotope labeled
Hepta:	1,2,3,4,6,7,8	
Octa:	1,2,3,4,6,7,8,9	
Mixed Br/Cl dioxins:		
Tri:	7-Bromo-2,3-dichloro	
Tetra:	2,3-Dibromo-7,8-dichloro	
	2,8-Dibromo-3,7-dichloro	
	2-Bromo-3,7,8-dichloro	
Penta:	2-Bromo-1,3,7,8-tetrachloro	
Hexa:	3-Bromo-1,2,4,7,8-pentachloro	
Chlorinated dibenzofurans:		
Mono:	2	
	3	
	4	
Di:	2,3	
	2,6	
	2,7	
	2,8	
	4,6	
Tri:	1,2,3	
	1,2,4	
	1,3,6	
	1,3,7	
	1,6,7	
	2,3,4	
	2,3,6	
	2,3,8	
	2,4,6	
	2,4,7	
	2,4,8	
	2,6,7	
Tetra:	1,2,3,4	2,3,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,3,6	
	1,2,3,7	
	1,2,3,8	
	1,2,3,9	
	1,2,4,6	
	1,2,4,8	
	1,2,4,9	
	1,2,7,8	
	1,3,4,6	
	1,3,4,7	

Table 7 (continued)

Unlabeled		Stable isotope labeled
Tetra: (cont.)	1,3,4,8	
	1,3,4,9	
	1,3,6,7	
	1,3,6,8	
	1,3,6,9	
	1,3,7,8	
	1,3,7,9	
	1,4,6,7	
	1,4,7,8	
	2,3,4,6	
	2,3,4,7	
	2,3,4,8	
	2,3,4,9	
	2,3,6,7	
	2,3,6,8	
	2,3,7,8	
	2,4,6,7	
	2,4,6,8	
	3,4,6,7	
Penta:	1,2,3,4,6	1,2,3,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,3,4,7	
	1,2,3,4,8	
	1,2,3,4,9	
	1,2,3,6,7	
	1,2,3,7,8	
	1,2,3,7,9	
	1,2,3,8,9	
	1,2,4,6,7	
	1,2,4,7,8	
	1,3,4,6,7	
	1,3,4,7,8	
	2,3,4,6,7	
	2,3,4,6,8	
	2,3,4,6,9	
	2,3,4,7,8	
	2,3,4,7,9	
Hexa:	1,2,3,4,7,8	1,2,3,4,7,8 (U-$^{13}C_{12}$, 99%)
	1,2,3,4,7,9	
	1,2,3,4,8,9	
	1,2,3,6,7,8	
	1,2,3,6,8,9	
	1,2,3,7,8,9	
	1,2,4,6,7,8	
	1,2,4,6,8,9	
	1,3,4,6,7,8	
	1,3,4,6,7,9	
	2,3,4,6,7,8	

Table 7 (concluded)

Unlabeled		Stable isotope labeled
Hepta:	1,2,3,4,6,7,8 1,2,3,4,6,8,9 1,2,3,4,7,8,9	1,2,3,4,6,7,8 (U-$^{13}C_{12}$, 99%)
Octa:	1,2,3,4,6,7,8,9	1,2,3,4,6,7,8,9 (U-$^{13}C_{12}$, 99%)
Brominated dibenzofurans:		
Di:	2,7 2,8	
Tri:	1,3,8	
Tetra:	2,3,7,8	2,3,7,8 (U-$^{13}C_{12}$, 99%)
Penta:	1,2,3,7,8	1,2,3,7,8 (U-$^{13}C_{12}$, 99%)
Hexa:	1,2,3,4,7,8	1,2,3,4,7,8 (U-$^{13}C_{12}$, 99%)
Mixed Br/Cl furans:		
Tri:	8-Bromo-2,3-dichloro	
Tetra:	6,8-Dibromo-2,3-dichloro 8-Bromo-2,3,4-trichloro	
Penta:	6,8-Dibromo-2,3,4-trichloro	

[a]Mixture of indicated isomers. The relative amounts are unknown; therefore, these standards are not useful for quantitation calibration.
[b]U-$^{13}C_{12}$ indicates that the compound is universally labeled with carbon-13.
[c]U-$^{37}Cl_4$ indicates that the compound is universally labeled with chlorine-37.

6.9 Internal standard solution - The chosen internal standard is prepared at a nominal concentration of 1 to 10 mg/mL in an appropriate solvent. The solution is further diluted to give a working standard.

6.10 Solution stability - The calibration standard, surrogate, internal standard, and DFTPP solutions should be checked frequently for stability. These solutions should be replaced after 6 months, or sooner if comparison with quality control check samples indicates compound degradation or concentration change.

7.0 Calibration

The guidelines presented in this section are based on calibration using surrogates and internal standards. Isotope dilution techniques may also be applicable and may be substituted if validated prior to use.

7.1 The gas chromatograph should meet the minimum operating parameters shown in Tables 8 or 9 on a daily basis. If all criteria are not met, the analyst should adjust conditions until all criteria are met.

7.2 The electron capture detector should meet the manufacturer's specifications on a daily basis. The manufacturer's procedure for determining detector performance should be used. If all performance criteria are not met, corrective action, as specified by the manufacturer, should be taken and the procedures repeated until the detector is within specifications.

7.3 The mass spectrometer should meet the minimum operating parameters shown in Tables 10 or 11 on a daily basis. If all criteria are not met, the analyst should retune the spectrometer and repeat the test until all conditions are met.

7.4 If the analytical system (HRGC/MS, HRGC/ECD) has not been demonstrated to yield a linear response or if the analyte concentrations are more than one order of magnitude different from those in the RF solution, a calibration curve should be prepared. If the analyte and RF solution concentration differ by more than two orders of magnitude, a calibration curve must be prepared. A calibration curve should be established with triplicate determinations at three or more concentrations bracketing the analyte levels.

Table 8. Recommended Operating Parameters for High Resolution Gas Chromatographic System for Use with Mass Spectral Detection

Parameter	Recommended	Tolerance
Gas chromatograph	Finnigan 9610	Other[a]
Column	15 m x 0.25 mm ID fused silica	Other
Liquid phase	DB-5 (J&W)	Other nonpolar or semipolar
Liquid phase thickness	0.25 μm	< 1 μm
Carrier gas	Helium	Nitrogen or hydrogen
Carrier gas velocity	30 cm/sec[b]	Optimum performance
Injector	On-column (J&W)[c]	Other
Separator	None[d]	Glass jet or other
Transfer line temperature	320°C	Optimum[e]
Injector temperature	Optimum performance[c]	Optimum performance
Initial column temperature	80°C (2 min)[f]	Other
Column temperature program	80°C to 320°C at 10°C/min	Other
Injection volume	1.0 μL[c]	Other
Tailing factor[g]	0.7 to 1.5	0.4 to 3
Peak width[h]	7 to 10 sec	< 15 sec

[a]Substitutions permitted if performance criteria are met.
[b]Measured by injection of air at 30°C column temperature.
[c]For on-column injection, manufacturer's instructions should be followed regarding injection technique.
[d]Fused silica columns may be routed directly into the ion source to prevent separator discrimination and losses.
[e]High enough to elute all analytes, but not high enough to degrade the column if routed through the transfer line.
[f]With on-column injection, the initial temperature is dependent on the boiling point of the solvent.
[g]Tailing factor is the width of the front half of the peak at 10% height divided by the width of the back half of the peak at 10% height for single analytes in the calibration standard solution.
[h]Peak width at 10% height for single analytes in the calibration standard solution.

Table 9. Recommended Operating Parameters for High Resolution Gas Chromatographic/Electron Capture Detection (HRGC/ECD) System

Parameter	Recommended	Tolerance
Gas chromatograph	Varian 3700	Other[a]
Detector	^{63}Ni; pulsed	$Sc^{3}H$
Column	15 m x 0.25 mm ID fused silica	Other
Liquid phase	DB-5 (J&W)	Other nonpolar or semipolar
Liquid phase thickness	0.25 μm	< 1 μm
Carrier gas	Helium	Nitrogen or hydrogen
Detector make-up gas	Nitrogen	Argon/methane
Carrier gas velocity	30 cm/sec[b]	Optimum performance
Injector	On-column (J&W)	Other
Injector temperature	Optimum performance[c]	Optimum performance
Detector temperature	350°C	Other
Initial column temperature	80°C (2 min)[d]	Other
Column temperature program	80°C to 320°C at 10°C/min	Other
Injection volume	1.0 μL[c]	Other
Tailing factor[e]	0.7 to 1.5	0.4 to 3
Peak width[f]	7 to 10 sec	< 15 sec

[a]Substitutions permitted if performance criteria are met.
[b]Measured by injection of air at 30°C column temperature.
[c]For on-column injection, manufacturer's instructions should be followed regarding injection technique.
[d]With on-column injection, the initial temperature is dependent on the boiling point of the solvent.
[e]Tailing factor is the width of the front half of the peak at 10% height divided by the width of the back half of the peak at 10% height for single analytes in the calibration standard solution.
[f]Peak width at 10% height for single analytes in the calibration standard solution.

Table 10. Recommended Operating Parameters for Quadrupole Mass Spectrometer System

Parameter	Recommended	Tolerance
Mass spectrometer	Finnigan 4043	Other[a]
Data system	Incos 2400	Other
Scan range	240-850 amu	Other
Scan time	1 sec	Other[b]
Resolution	Unit	Optimum performance
Ion source temperature	280°C	250-350°C
Electron energy[c]	70 eV	70 eV
Trap current	0.2 mA	Optimum performance
Electron multiplier voltage	-1,600 V	Optimum
Preamplifier sensitivity	10^{-6} A/V	Set for desired working range

[a]Substitutions permitted if performance criteria are met.
[b]Greater than five data points over a GC peak is a minimum.
[c]Filaments should be shut off during solvent elution to improve instrument stability and prolong filament life, especially if no separator is used.

Table 11. Recommended Operating Parameters for Magnetic Sector Mass Spectrometer System

Parameter	Recommended	Tolerance
Mass spectrometer	Finnigan MAT 311A	Other[a]
Data system	Incos 2400	Other
Scan range	240-850 amu	Other
Scan mode	Exponential (up)	Other
Cycle time	1.2 sec	Other[b]
Resolution	1,000	> 500
Ion source temperature	280°C	250-350°C
Electron energy[c]	70 eV	70 eV
Emission current	1-2 mA	Other
Filament current	Optimum	Optimum
Electron multiplier voltage	-1,600 V	Optimum

[a]Substitutions permitted if performance criteria are met.
[b]Greater than five data points over a GC peak is a minimum.
[c]Filaments should be shut off during solvent elution to improve instrument stability and prolong filament life, especially if no separator is used.

7.5 The HDD or HDF response factors (RF_h) should be determined using Equation 7-1 for the analyte homologs.

$$RF_h = \frac{A_h \times M_{is}}{A_{is} \times M_h} \qquad \text{Eq. 7-1}$$

where:

RF_h = response factor of a given HDD or HDF congener

A_h = area of the HDD or HDF congener peak

M_h = mass of HDD or HDF congener injected (pg)

M_{is} = mass of internal standard injected (pg)

A_{is} = area of internal standard peak

Using the same conditions as for RF_h, the surrogate response factors (RF_s) should be determined using Equation 7-2.

$$RF_s = \frac{A_s \times M_{is}}{A_{is} \times M_s}. \qquad \text{Eq. 7-2}$$

where:

A_s = area of the surrogate peak

M_s = mass of surrogate injected (pg)

Other terms are the same as defined in Equation 7-1.

If specific congeners are known to be present and if standards are available, selected RF values may be employed.

For selected ion monitoring, the primary and secondary ions of the molecular clusters of both the analyte and surrogate are generally used to determine the RF values. If alternate ions are to be used for quantitation, the RF should be determined using those characteristic ions.

The RF value should be determined in a manner which ensures an accuracy and precision of ± 30% or better. The most common procedure is to determine the RF value for at least three different concentration levels to establish a working calibration curve.

Other options include, but are not limited to, triplicate determinations of a single concentration spaced throughout a day or a running mean (RF) based on one value per day for seven consecutive days.

7.6 The relative retention time (RRT) windows for the homologs and surrogates should be determined. The windows should be set wider than observed if all isomers are not available as standards.

8.0 Sample Collection, Handling, and Preservation

8.1 Amber glass sample containers should have Teflon®-lined screw caps. If the sample is noncorrosive, aluminum foil liners, rinsed with methylene chloride, may be substituted. The volume and configuration of the sample containers will be determined by the amount and physical properties of sample to be collected. For dry powders, other containers, such as heavy-walled polyethylene bags, may be appropriate.

8.2 Sample container preparation.

8.2.1 All sample containers and caps should be washed in detergent solution, rinsed with tap water, and then with distilled water. The containers and caps are allowed to drain dry in a contaminant-free area. The caps should be rinsed with pesticide grade hexane and allowed to air dry.

8.2.2 Sample bottles, if used, should be heated to 400°C for 15 to 20 min or rinsed with pesticide grade acetone or hexane and allowed to air dry.

8.2.3 The clean bottles should be stored inverted or sealed until use.

8.3 Sample collection.

8.3.1 The primary consideration in sample collection is that the sample collected is representative of the whole. Therefore, sampling plans or protocols for each individual producer's situation should be developed. The recommendations presented here describe general situations. The number of replicates and sampling frequency should also be planned prior to sampling. Guidelines for the collection of representative samples are provided as Appendices C and D.

8.3.2 Discrete product units - If the product is small enough that one or more discrete units will be used as the analytical sample, a statistically random sampling approach is recommended.

8.3.3 Liquids or free-flowing solids - If possible, the source is mixed thoroughly before collecting the sample. If mixing is impractical, the sample should be collected from a representative area of the source. If the liquid is flowing through an enclosed system, sampling through a valve should be randomly timed.

8.3.4 Solids - Larger bulk solids which should be subsampled to get a reasonably sized analytical sample should be treated on a case-by-case basis. A representative sample should be obtained by designing a sampling location selection scheme such that all parts of the whole have a finite known probability of inclusion.

8.4 Sample preservation - Product samples should be stored as the bulk or packaged product inventory would be stored, or protected from light in a cool, dry area. Intermediates, process samples, or other nonproduct specimens should be stored protected from light at 4°C or lower.

9.0 Sample Preparation

Since a wide variety of matrices may be subjected to analysis by these guidelines, exact extraction/cleanup procedures cannot be specified. Each laboratory should prepare a method document for the specific analysis to be performed.

This section describes general guidelines for subsampling, addition of surrogates, dilution, extraction, cleanup, extract concentration, and other sample preparation considerations. Only those methods that deal directly with commercial products have been presented in detail. Options for analysis are not limited to those presented herein. Additional procedures may be referenced from several analytical protocols dealing with analyses of chlorinated dibenzodioxins and furans in environmental matrices.

Standard analytical methods are available for the analysis of 2,3,7,8-TCDD in soils and sediments and water/wastewater and hazardous wastes (EPA Method 613 1984; RCRA Method 8280, EPA/ERD-L 1984; EPA Region VII 1983; Harless et al. 1980; McMillin et al. 1983). These methods have been validated for the measurement of 2,3,7,8-TCDD to detection levels of 1 ng/g (ppb) for soils and sediments and 1 ng/L (ppt) for water/wastewater. The soil and sediment protocol has been used for the analysis of over 11,000 samples for 2,3,7,8-TCDD in EPA Region VII alone. These methods have not been validated for the analysis of total PCDDs and PCDFs. However, several laboratories have provided sufficient documentation to suggest that modification of these procedures can extend the analysis for total tetrachloro- through octachloro-PCDDs and PCDFs. RCRA Method 8280 is currently being validated for the analysis of tetra- through hexachloro-PCDDs and PCDFs in hazardous wastes.

The extraction and cleanup procedures for the determination of PCDDs and PCDFs in phenoxyalkanoic herbicides has been reviewed by Baker et al. (1981). Extraction techniques have included steam distillation of TCDD from an alkaline solution of technical 2,4,5-T (Brenner et al. 1972, 1974), hexane extraction from an alkaline solution of 2,4,5-T, liquid-liquid partitioning of TCDD into hexane from a solution of technical 2,4,5-T in dimethylformamide/acetonitrile/water (Vogel 1976), and separation of TCDD from isobutoxy methyl esters of 2,4,5-T and fenoprop using a silica gel column (Ramsted et al. 1977).

Woolson et al. (1972) evaluated 17 different pesticides containing the polychlorophenoxy group for the presence of PCDDs. Extraction was accomplished using hexane extraction from methanolic potassium hydroxide solution. Cochrane et al. (1981) described the analysis of technical and formulated products of 2,4-D as the ester, free acid, and the amine. The 2,4-D esters were transferred directly onto a silica column and eluted with 30% dichloromethane in hexane. The 2,4-D acids were dissolved in acetonitrile/water and the PCDDs were partitioned into hexane. The 2,4-D amines were dissolved in water and partitioned into hexane.

Analysis of pentachlorophenol (PCP) for PCDDs has been reported by several researchers (Buser 1975, Buser and Bosshardt 1976, Mieure et al. 1977, Blaser et al. 1976, Firestone et al. 1972, Plimmer et al. 1973, Lamberton et al. 1979, Nelsson and Renberg 1974, and Pfeiffer et al. 1978). The analytical methods range from dissolution in a polar solvent and partitioning the PCDDs into a nonpolar solvent to separation from the commercial product using a macro silica adsorption column (Mieure et al. 1977). Yamagishi et al. (1981) reported a similar procedure for the determination of PCDDs and PCDFs in commercial diphenyl ether herbicides such as nitrophen and chloromethoxynil.

9.1 Sample homogenization and subsampling - The sample is homogenized by shaking, blending, shredding, crushing, or other appropriate mechanical techniques. A representative subsample of an appropriate mass is then taken. The sample size is dependent upon the anticipated HDD or HDF levels and difficulty of the subsequent extraction/cleanup steps.

9.2 Surrogate addition - An appropriate volume of surrogate solution is pipetted into the sample. The final concentration of the surrogates should be in the working range of the calibration curve and well above the matrix background. The surrogates are thoroughly incorporated by further mechanical agitation. For nonviscous liquids, shaking for 30 s should be sufficient. For viscous liquids or free-flowing solids, tumbling for 10 min is recommended. In cases where adequate incorporation may be difficult, such as solids, overnight equilibration with agitation is recommended.

Note: The volume measurement of the spiking solution is critical to the overall method precision. The analyst should exercise caution that the volume is known with an accuracy of 1% or better. Where necessary, calibration of the pipet is recommended.

9.3 Sample preparation (extraction/cleanup) - After addition of the surrogates, the sample is further treated at the discretion of the analyst, provided that the instrumental response of the surrogates meets the criteria listed in Section 7.0. Several possible techniques are presented below for guidance only. The applicability of any of these techniques to a specific sample matrix should be determined by the precision and accuracy of the surrogate recoveries, as discussed in Section 15.2.

9.3.1 Removal of matrix - The techniques used for separation of HDDs and HDFs from the sample matrix will depend on the physical and chemical properties of the commercial product. The properties utilized in this scheme are volatility, solubility, acidity, polarity, and molecular geometry. If the product is sufficiently volatile, evaporation may be employed. Differential solubility in immiscible solvents may be the basis for a liquid-liquid extraction. For example, a salt may be dissolved in aqueous solution and the HDDs and HDFs extracted with an immiscible nonpolar solvent such as hexane. The solvent, number of extractions, solvent-to-sample ratio, and other parameters are chosen at the analyst's discretion. The planarity of the HDDs and HDFs may also be exploited to separate them from a nonplanar matrix using graphitized carbon column chromatography. Column chromatography with alumina, silica, or ion exchange resins may also be applicable for use with some matrices. Other separation techniques may also be used if validated prior to sample analysis. Several illustrative examples, taken from the literature, are presented below.

9.3.1.1 Extraction of chlorophenols with sodium hydroxide (Firestone 1977).

9.3.1.1.1 Weigh 25 g of sample into a 2-L Erlenmeyer flask. Add 1 L of water and 200 mL of 1 N sodium hydroxide.

9.3.1.1.2 If necessary, warm the flask on a steam bath until the sample dissolves

9.3.1.1.3 Add 350 mL of petroleum ether and shake the flask vigorously for about 1 min.

9.3.1.1.4 Drain the lower layer and transfer the upper layer to a second separatory funnel.

9.3.1.1.5 Repeat the extraction with petroleum ether twice more and combine the extracts.

9.3.1.1.6 Wash the combined extracts with 100 mL of water by gently swirling. Repeat the wash twice with 100-mL portions of water. (Wash water should be neutral.)

9.3.1.1.7 Transfer the extract to a 2-L Erlenmeyer flask and dry by adding 200 g of anhydous sodium sulfate. Swirl the flask vigorously and allow it to stand for 30 min.

9.3.1.1.8 Transfer the dry petroleum ether to a second Erlenmeyer flask. Rinse the first Erlenmeyer flask twice with 200-mL portions of petroleum ether and transfer the rinsings to the second Erlenmeyer flask.

9.3.1.1.9 Evaporate the petroleum ether to about 500 mL.

9.3.1.1.10 Transfer to a 500-mL Kuderna-Danish concentrator and evaporate to about 10 mL.

9.3.1.2 Extraction of pentachlorophenol with lithium hydroxide (Buser 1975, 1976).

9.3.1.2.1 Add 4.0 g of sample to 30 mL of methanol in a 250-mL separatory funnel.

9.3.1.2.2 Add 10 mL of 2.5 N lithium hydroxide and 100 mL of water.

9.3.1.2.3 Extract with 40 mL of petroleum ether.

9.3.1.2.4 Drain the lower phase (aqueous) after checking for alkalinity (pH > 12).

9.3.1.2.5 Wash the organic phase with 50 mL of water. Check the last washing for neutrality.

9.3.1.2.6 Dry the organic phase over anhydrous sodium sulfate.

9.3.1.2.7 Concentrate a 20-mL aliquot of the dry organic phase to about 2 mL using a stream of nitrogen.

9.3.1.3 Extraction of 2,4-D amines (Cochrane 1981).

9.3.1.3.1 Transfer 2.0 to 2.5 g of sample to a 250-mL flask.

9.3.1.3.2 Add 100 mL of water and a clean magnetic stirring bar. Mix the solution thoroughly for 10 min on a magnetic stirrer.

9.3.1.3.3 Transfer the solution to a 250-mL separatory funnel and extract three times with 25-mL portions of hexane. Combine the extracts.

9.3.1.3.4 Wash the hexane extracts twice with 20-mL portions of water.

9.3.1.3.5 Dry the hexane by passage through 15 g of prewashed sodium sulfate.

9.3.1.3.6 Concentrate to 1 to 2 mL.

9.3.1.4 Extraction of 2,4-D acids (Cochrane 1981).

9.3.1.4.1 Dissolve a 10-g sample in 400 mL of acetonitrile-water (1:1) in a 500-mL flask containing 40 mL of methanol.

9.3.1.4.2 Transfer the solution to a 1-L separatory funnel using two rinses of 50 mL of hexane to wash the flask.

9.3.1.4.3 Extract three times with 100-mL portions of hexane. Combine the extracts.

9.3.1.4.4 Wash the combined hexane extracts twice with 50-mL portions of methanol-water (1:1). Discard the rinsings.

9.3.1.4.5 Dry the hexane extract by passage through 30 g of prewashed sodium sulfate in a funnel.

9.3.1.4.6 Reduce the volume to 1 to 2 mL under reduced pressure.

9.3.1.5 Extraction of 2,4-D esters (Cochrane 1981).

9.3.1.5.1 Activate silica gel (M. Kieselgel 60; Merck, Darmstadt, G.F.R.) overnight at 125°C.

9.3.1.5.2 Add 50 g of silica gel to a glass chromatographic column (60 x 2.0 cm ID).

9.3.1.5.3 Transfer 2 g of sample to the head of the column.

9.3.1.5.4 Elute the column with 150 mL of 30% methylene chloride in hexane.

9.3.1.5.5 Discard the first 30 mL of eluent.

9.3.1.5.6 Collect the remaining eluent in a 250-mL round bottomed flask.

9.3.1.5.7 Concentrate to 1 to 2 mL on a rotary evaporator for subsequent cleanup.

9.3.1.6 Removal of an acidic matrix (pentachloropnenol) with alumina (Mieure 1977).

9.3.1.6.1 Heat 190 g of alumina (Fisher Adsorption A-540, 80-200 mesh) at 400°C for 4 h. Cool and pour into a pint bottle. Pipette 10 mL of distilled water into the bottle, cap the bottle, and shake for at least 10 min. The bottle cap should have an aluminum liner. The deactivated alumina has a shelf life of several weeks.

9.3.1.6.2 Place a small amount of glass wool in the bottom of a chromatographic column (30 cm x 1.8 cm ID Pyrex with a Teflon® stopcock). Add benzene to the column until it is half full. Add 40 mL of deactivated alumina. Allow the alumina to settle while tapping the walls of the column. Add 1 in. of sodium sulfate to the top of the alumina. Open the stopcock and adjust the benzene level to the top of the packing.

9.3.1.6.3 Dissolve a 5-g portion of sample in benzene in a 50-mL volumetric flask. Sonicate if necessary to effect solution. Dilute the flask to volume with benzene.

9.3.1.6.4 Pipete a 10-mL aliquot of the sample solution dropwise onto the head of the column.

9.3.1.6.5 Elute the column with benzene, collecting the first 150 mL of eluent.

9.3.1.6.6 Concentrate the collected eluent to an appropriate volume for subsequent cleanup using a Kuderna-Danish concentrator.

9.3.2 Cleanup - Several cleanup techniques are described below as examples of procedures which may be applicable to the analysis of HDDs and HDFs in commercial products. Depending on the complexity of the sample, one or more of the techniques may be required to fractionate the HDDs and HDFs from interferences. For most cleanups a concentrated (1-5 mL) extract should be used.

9.3.2.1 Sulfuric acid cleanup (Firestone 1972).

9.3.2.1.1 Add concentrated extract (in petroleum ether) to a 25-mL graduated cylinder. Stopper and shake the cylinder for about 30 s.

9.3.2.1.2 Let the layers separate and transfer the petroleum ether layer to a 30-mL beaker.

9.3.2.1.3 Rinse the cylinder with 5 mL of petroleum ether and add the rinsings to the beaker.

9.3.2.1.4 Add 1 g of sodium bicarbonate to the petroleum ether solution. Allow to stand for 5 min.

9.3.2.1.5 Transfer the petroleum ether solution to a small vial and evaporate the contents to dryness at room temperature using a gentle stream of nitrogen.

9.3.2.1.6 Dissolve the residue in an appropriate volume of a suitable solvent for further cleanup or for final analysis.

9.3.2.2 Alumina cleanup (Firestone 1972).

9.3.2.2.1 Activate 100-g portions of alumina (Fisher No. A-540) by heating 4 h at 260°C. Transfer alumina, without cooling, to a dry container and close the container tightly. Do not store activated alumina for more than 3 days.

9.3.2.2.2 Quantitatively transfer the concentrated extract (in petroleum ether) to the alumina column.

9.3.2.2.3 Elute the column with 400 mL of petroleum ether (Fraction 1).

9.3.2.2.4 Elute the column with 200 mL of 5% diethyl ether in petroleum ether (Fraction 2).

9.3.2.2.5 Elute the column with 400 mL of 25% diethyl ether in petroleum ether (Fraction 3).

9.3.2.2.6 Elute the column with 400 mL of diethyl ether (Fraction 4).

9.3.2.2.7 Transfer Fractions 3 and 4 to Kuderna-Danish concentrators (keep separate) and evaporate to about 10 mL.

9.3.2.2.8 Remove from steam bath and evaporate just to dryness at room temperature under nitrogen.

9.3.2.2.9 Dissolve the residue in an appropriate volume of a suitable solvent for further cleanup or for final analysis.

9.3.2.3 Alumina cleanup (Buser 1975, 1976).

9.3.2.3.1 Prepare the column by adding 1.0 g of dry alumina to a disposable Pasteur pipete (15 cm x 5 mm ID) containing a plug of glass wool.

9.3.2.3.2 Quantitatively transfer the concentrated extract onto the head of the column.

9.3.2.3.3 Elute the column with 10 mL of 2% methylene chloride in hexane. Discard the eluent.

9.3.2.3.4 Elute the column with 10 mL of 50% methylene chloride in hexane.

9.3.2.3.5 Adjust the sample volume and sample composition as needed for further cleanup or for final analysis.

9.3.2.4 Basic aluminum oxide cleanup (Mieure 1977).

9.3.2.4.1 Add 1.0 g of aluminum oxide (ICN Aluminum Oxide W 200 basic, activity grade Super 1, used as received) to a disposable Pasteur pipet (15 cm x 0.5 cm ID) containing a glass wool plug. Settle the packing by gently tapping 15 to 20 times with a small spatula.

9.3.2.4.2 Quantitatively transfer 0.30 mL of benzene concentrate to the column.

9.3.2.4.3 Elute the column with 10 mL of 2% methylene chloride in hexane. Discard the eluent.

9.3.2.4.4 Elute the column with 10 mL of 50% methylene chloride in hexane.

9.3.2.4.5 Adjust the sample volume and solvent composition as needed for instrumental analysis.

9.3.2.5 Basic alumina cleanup (Cochrane 1981).

9.3.2.5.1 Place a small glass wool plug in the bottom of a glass chromatographic column (25 x 1.5 cm ID). Add 0.5 cm of Ottawa standard sand.

9.3.2.5.2 Add 15 g of basic alumina (Activity I; Woelm, Eschwege, G.F.R., used as received) to the column, then top with 0.5 cm of sodium sulfate.

9.3.2.5.3 Quantitatively transfer 1 to 2 mL of the concentrated sample extract onto the head of the column.

9.3.2.5.4 Elute the column with (1) 100 mL of hexane; (2) 100 mL of 2% methylene chloride in hexane; (3) 100 mL of 5% methylene chloride in hexane; and (4) 100 mL of 10% methylene chloride in hexane.

9.3.2.5.5 Discard Fractions (1) and (2).

9.3.2.5.6 Concentrate Fractions (3) and (4) to 1 to 2 mL, transfer to a 5-mL graduated centrifuge tube with three 1-mL

portions of benzene, then evaporate to dryness.

9.3.2.5.7 Dissolve the residue in an appropriate volume of a suitable solvent for final analysis.

9.3.2.6 Graphitized carbon cleanup (O'Keefe 1978).

9.3.2.6.1 Transfer the extract (15 mL in methylene chloride) to a graphitized carbon column [40 mm x 6 mm ID, packed with Carbopack AHT (Supelco, Inc., Bellefonte, Pennsylvania)].

9.3.2.6.2 Elute the nonplanar aromatic compounds with 50 mL of benzene/diethyl ether (40/60). Discard the eluent.

9.3.2.6.3 Elute the planar aromatic compounds with 70 mL of pyridine.

9.3.2.6.4 Dilute the pyridine fraction with 140 mL of 1% HCl and extract three times with 30-mL portions of hexane.

9.3.2.6.5 Wash the hexane extract with 100 mL of water, then dry over sodium sulfate.

9.3.2.6.6 Adjust the sample volume and solvent composition as needed for instrumental analysis.

9.3.2.7 Graphitized carbon cleanup for TCDD (EPA 1983)

9.3.2.7.1 Prepare 18% Carbopack C on Celite 545® by thoroughly mixing 3.6 g of Carbopack C (80/100 mesh) and 16.4 g of Celite 545® in a 40-mL vial. Activate at 130°C for 6 h. Store in a desiccator.

9.3.2.7.2 Prepare a column using a standard size (5-3/4 in. long by 7.0 mm o.d.) disposable pipet fitted with a small plug of glass wool. Using a vacuum aspirator attached to the pointed end of the pipet, add the Carbopack/Celite mix until a 2-cm column is obtained.

9.3.2.7.3 Preelute the column with 2 mL of toluene followed by 1 mL of 75:20:5 methylene chloride/methanol/benzene, 1 mL of 1:1 cyclohexane in methylene chloride and 2 mL of hexane.

9.3.2.7.4 While the column is still wet with hexane, add 50 µL of sample extract.

9.3.2.7.5 Elute the column sequentially with two 1-mL aliquots of hexane, 1 mL of 1:1 cyclohexane in methylene chloride, and 1 mL of 75:20:5 methylene chloride/methanol/benzene.

9.3.2.7.6 Collect the TCDD fraction by elution with 2 mL of toluene.

9.3.2.7.7 Reduce the volume to near dryness and add isooctane to obtain a final volume of 50 µL.

9.3.2.8 Silica gel cleanup (Pfeiffer 1978).

9.3.2.8.1 This procedure is designed to remove chlorophenols, chlorophenoxy phenols, or other polar impurities extracted from an aqueous solution.

9.3.2.8.2 Dry the silica gel (Bio-Sil A, 100/120 mesh; Bio-Rad Laboratories, Richmond, California) at 180°C in a tube furnace for 1.5 h under a 100 cc/min nitrogen flow. Store the dried silica gel, capped in glass bottles, in a glass desiccator containing phosphorous pentoxide.

9.3.2.8.3 Place a small plug of glass wool in a disposable capillary pipet (150 x 5 mm ID; VWR Scientific, San Francisco, California).

9.3.2.8.4 Add the silica gel to the pipet to produce an adsorbent bed 60 mm in height.

9.3.2.8.5 Transfer 7.5 mL of the hexane extract onto the head of the column.

9.3.2.8.6 Elute the column with 7 mL of hexane, maintaining the flow through the column at 2 to 3 drops per second by applying gentle air pressure at the top of the column using a rubber bulb.

9.3.2.8.7 Collect all of the eluent. Concentrate for final analysis or further cleanup as necessary.

9.3.2.9 HPLC cleanup (Pfeiffer 1978).

9.3.2.9.1 Inject an appropriate aliquot of extract onto a high pressure liquid chromatographic system. An example of an appropriate system is given in Table 12.

9.3.2.9.2 Collect the column eluent within established retention windows for the HDD and HDF homologs. Figure 1 shows a chromatogram obtained using the system outlined in Table 12.

9.3.2.9.3 Adjust the sample volume and solvent composition as necessary for instrumental analysis.

9.3.2.10 HPLC cleanup (RCRA Method 8280)

9.3.2.10.1 Operating parameters: Column temperature, 30°C; column pressure, 172 atm.; flow rate, 1.0 mL/min; UV detector, 235 nm; detector sensitivity, 0.02 UFS; isocratic operating mode, injection via 100 µL sample loop.

9.3.2.10.2 Elution profile for 2,3,7,8-TCDD: An authentic standard of 2,3,7,8-TCDD demonstrated a retention time of 16.0 min. Under the above conditions the retention time of this standard should be verified before proceeding. Typically, collect the entire column effluent, eluting between 16.0 and 22.0 min following introduction of the sample extract onto the HPLC columns, into a 12.0-mL concentrator tube.

Table 12. Example HPLC Parameters for Sample Cleanup (Pfeiffer 1978)

Pump	Waters M-6000A
Injector	Rheodyne Model 7120
Detector	Perkin-Elmer LC-55 or LC-65T
Analytical wavelength	245 nm
Column	ODS/Zorbax (6 μm) (DuPont)
Column dimensions	4.6 mm x 250 mm
Mobile phase	Methanol (100%)
Flow rate	2.0 mL/min
Column temperature	Ambient
Detector sensitivity	0.02 AUFS
Injection volume	6 μL

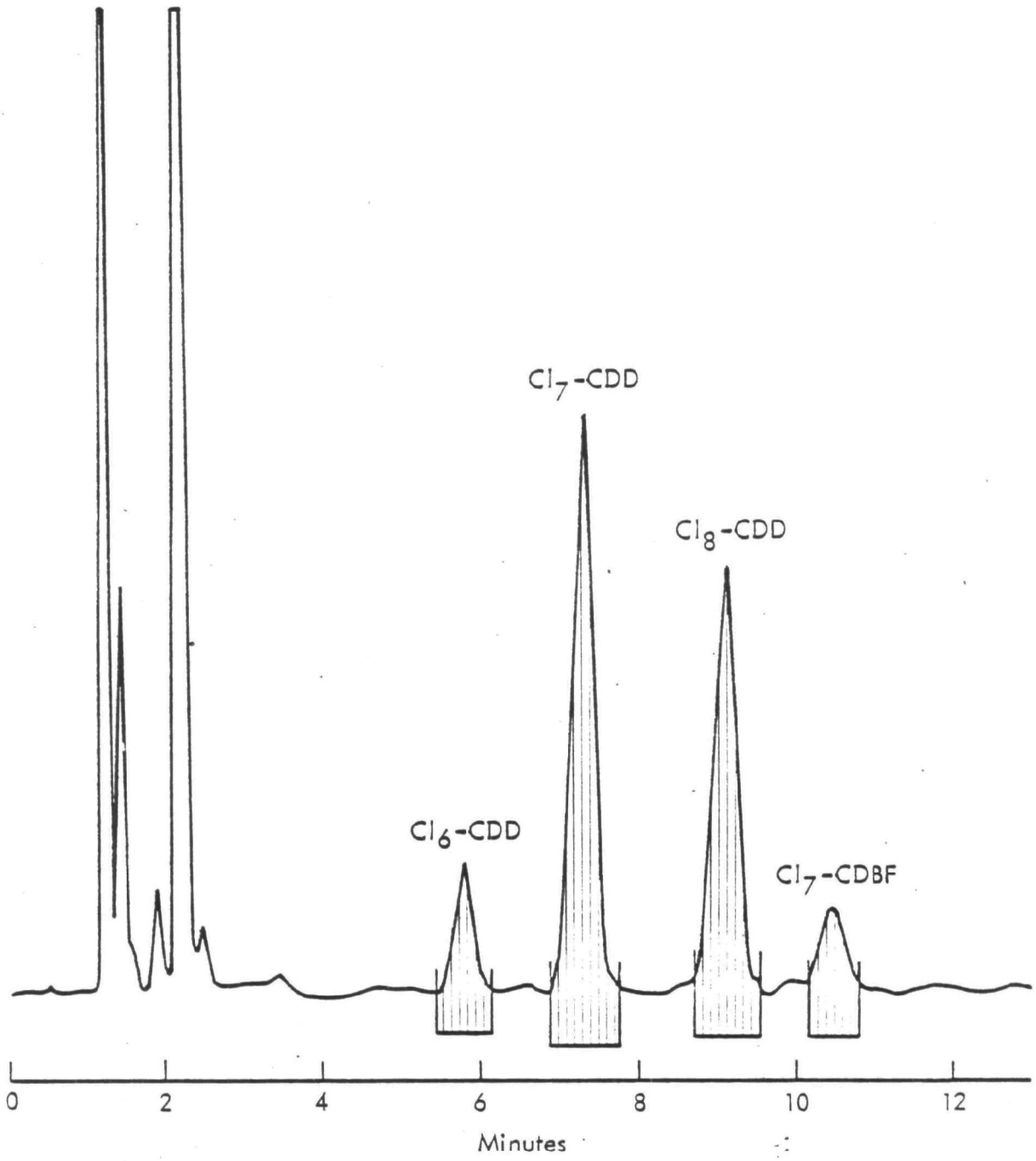


Figure 1. Chromatogram for HPLC cleanup by pentachlorophenol (Pfeiffer 1978).

9.3.2.10.3 Solvent exchange, instrument analysis, and sample concentration: After the methylene chloride/hexane effluent is taken to dryness, add 0.5 mL of methanol to the concentrator tube.

9.3.2.10.4 Using a gentle stream of prepurified nitrogen and a 55°C sand bath evaporate the effluent to 40 µL.

9.3.2.10.5 Using a 100-µL syringe draw the residue solution carefully into the syringe until an air bubble appears in the barrel. Place a second 60-µL aliquot of methanol into the concentrator tube, rotate quickly for rinsing and draw this into the syringe.

9.3.2.10.6 Inject the total volume into the HPLC.

9.3.2.10.7 At the appropriate retention time, position a sample collection bottle to collect the required fraction. Add 5 mL of hexane to the sample bottle.

9.3.2.10.8 Add 2 mL of 5% (w/v) sodium carbonate to the sample fraction collected and shake for 1 min.

9.3.2.10.9 Quantitatively remove the hexane layer (top layer) and transfer to a microreaction vessel. Add an additional 5 mL of hexane, shake 1 min, and transfer the solution to a concentrator tube.

9.3.2.10.10 A Teflon boiling chip is added and the concentrator tube is fitted with a 2-ball Snyder column.

9.3.2.10.11 The sample is concentrated to near dryness in a 90°C water bath.

9.3.2.10.12 After cooling for 10 min, the sample is evaporated just to dryness using a gentle stream of prepurified nitrogen and a 55°C sand bath.

9.3.2.10.13 Transfer the residue to a 1.0-mL microflex vial with a small amount of toluene. Be very careful to transfer the entire rinse solution.

9.3.2.10.14 Again concentrate the residue to 100 µL using a gentle stream of prepurified nitrogen and a 55°C sand bath.

9.3.2.10.15 Store the microflex vial under refrigeration until just prior to GC/MS analyses.

9.4 Miscellaneous cleanup procedures.

Other cleanup procedures may be employed if they are validated prior to use. Possibilities include, but are not limited to, extraction cartridges (e.g., Sep-Pak, Waters Associates), distillation, and thin-layer chromatography.

10.0 Gas Chromatographic/Electron Impact Mass Spectrometric Determination

10.1 Internal standard addition - An appropriate volume of the internal standard solution is pipeted into the sample. The final concentration of the internal standard should be in the working range of the calibration and well above the matrix background. The internal standard is thoroughly incorporated by mechanical agitation.

Note: The volumetric measurement of the internal standard solution is critical to the overall method precision. The analyst should exercise caution that the volume is known with an accuracy of 1% or better. Where necessary, calibration of the pipet is recommended.

10.2 Tables 8, 10, and 11 summarize the recommended operation conditions for analysis. Figures 2 and 3 present, as examples, chromatograms (HRGC/SIM, HRGC/FMS) obtained for an analysis of technical grade pentachlorophenol.

10.3 While the highest available chromatographic resolution is not a necessary objective of this protocol, good chromatographic performance is recommended. With an optimized HRGC system, the probability that the chromatographic peaks consist of single compounds increases, making qualitative and quantitative data reduction more reliable.

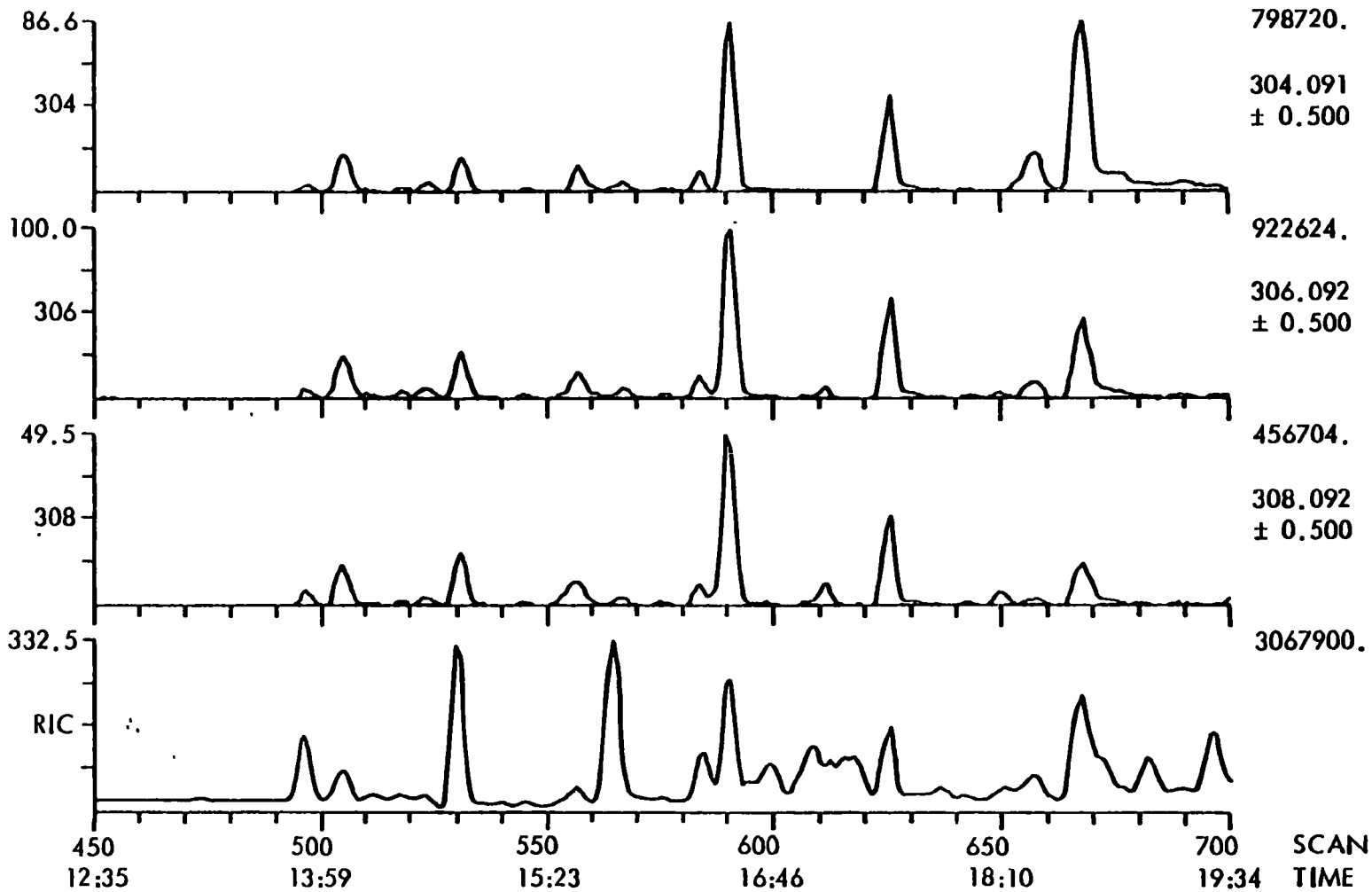
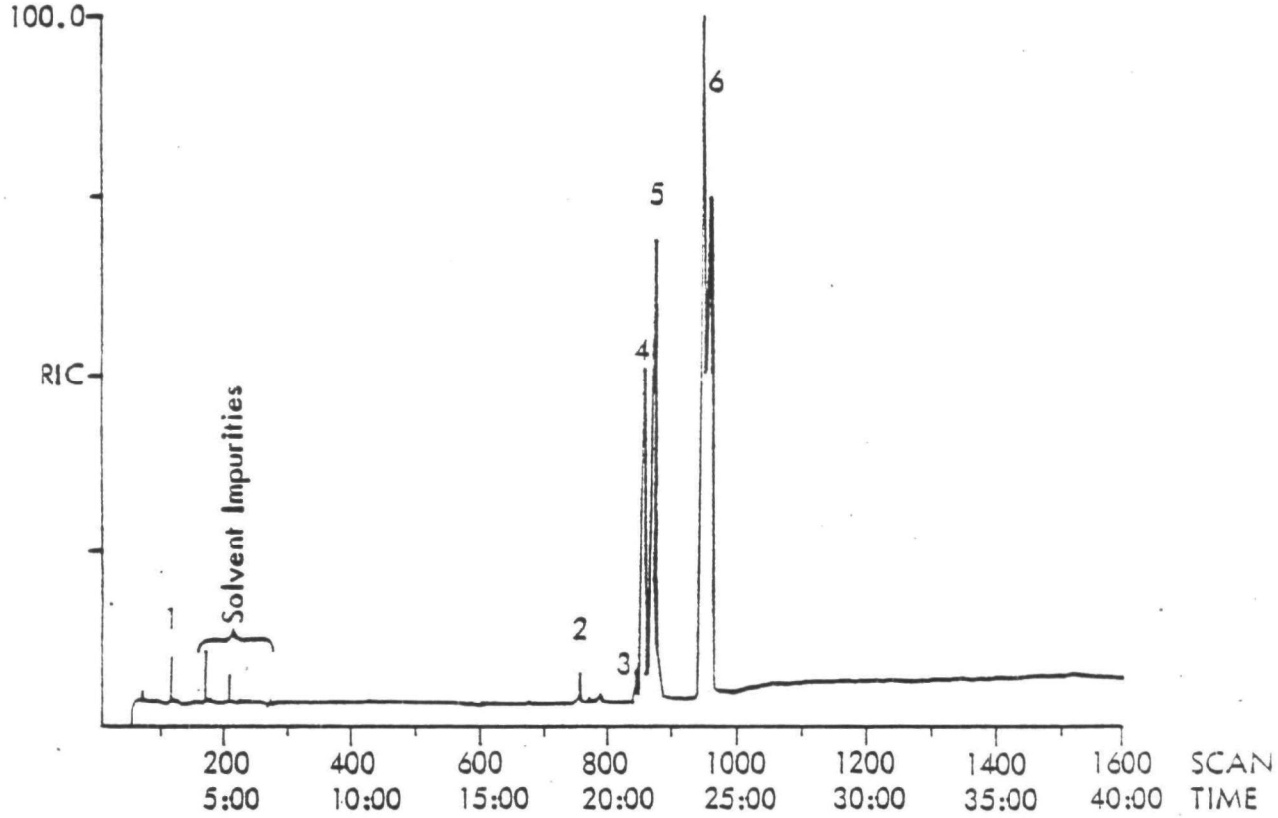


Figure 2. HRGC/SIM determination of tetrachlorodibenzofuran in pentachlorophenol (Midwest Research Institute).



Peak No.	Impurity
1	Hydrocarbon Impurity
2	Hexachlorodibenzofuran
3	Hexachlorodibenzodioxin
4	Heptachlorodibenzofuran
5	Heptachlorodibenzodioxin
6	Octachlorodibenzofuran Octachlorodibenzodioxin

Figure 3. HRGC/FMS analysis of PCDDs and PCDFs (Cl_6-Cl_8) in pentachlorophenol (Midwest Research Institute).

10.4 After performance of the system has been certified for the day and all instrument conditions set according to Tables 8, 10, and 11, inject an aliquot of the sample onto the GC column. If the response for any ion, including surrogates and internal standards, exceeds the working range of the system, dilute the sample and reanalyze. If the responses of surrogates, analytes, or internal standard are below the working range, recheck the system performance. If necessary, concentrate the sample and reanalyze.

10.5 Record all data on a digital storage device (magnetic disk, tape, etc.) for qualitative and quantitative data reduction as discussed in Sections 12 and 13.

11.0 Gas Chromatographic/Electron Capture Detection Screening

11.1 Internal standard addition - An appropriate volume of the internal standard solution is pipeted into the sample. The final concentration of the internal standard should be in the working range of the calibration and well above the matrix background. The internal standard is thoroughly incorporated by mechanical agitation.

Note: The volumetric measurement of the internal standard solution is critical to the overall method precision. The analyst should exercise caution that the volume is known with an accuracy of 1% or better. Where necessary, calibration of the pipet is recommended.

11.2 Table 9 summarizes the recommended operating conditions for analysis. Figure 4 presents, as an example, a chromatogram obtained for an analysis of PCDDs and PCDFs in a sample of technical grade pentachlorophenol.

11.3 While the highest available chromatographic resolution is not a necessary objective of this protocol, good chromatographic performance is recommended. With an optimized HRGC system, the probability that the chromatographic peaks consist of single compounds increases, making qualitative and quantitative data reduction more reliable.

11.4 After performance of the system has been certified for the day and all instrument conditions are optimized, inject an aliquot of the clean sample extract onto the GC column. If the response for any peak, including surrogates and internal standards, exceeds the working range of the system, dilute the sample and reanalyze. If the responses of surrogates, analytes, or internal standard are below the working range, recheck the system performance. If necessary, concentrate and reanalyze the sample.

11.5 If possible, record all data on a digital storage device (magnetic disk, tape, etc.) for qualitative and quantitative data reduction as discussed in Sections 12 and 13.

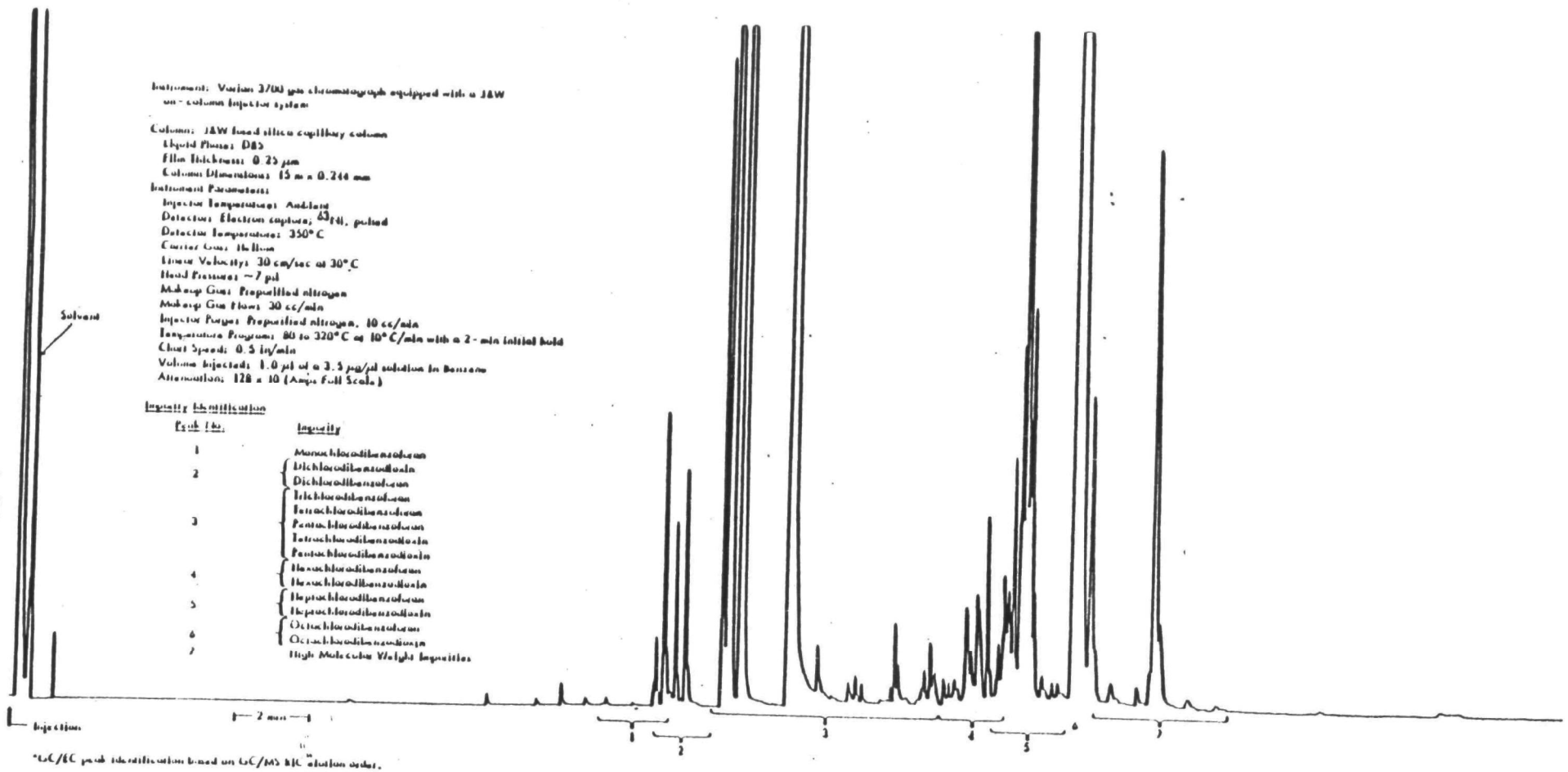


Figure 4. HRGC/ECD profile of PCDDs and PCDFs in pentachlorophenol (Midwest Research Institute).

12.0 Qualitative Identification

12.1 Selected ion monitoring (SIM) or limited mass scan (LMS) data - The identification of a compound as a given HDD or HDF homolog requires that two criteria be met:

12.1.1 (1) The peak should elute within the retention time window set for that homolog; and (2) the ratio of two ions obtained by SIM (Tables 13 and 14) or by LMS (Tables 15 and 16) should match the theoretical ratio within 20%. The analyst should search the higher mass windows to prevent misidentification of a HDD or HDF fragment ion cluster as the molecular ion.

12.1.2 If either of these criteria is not met, interferences may have affected the results and a reanalysis using full scan EIMS conditions is recommended.

12.2 Full mass scan (FMS) data.

12.2.1 The peak should elute within the retention time window set for that homolog.

12.2.2 The unknown spectrum should be compared with an authentic HDD or HDF. The intensity of the three largest ions in the molecular ion cluster should match the theoretical ratio within 20%. Fragment clusters with proper abundance ratios should also be present.

12.2.3 Alternatively, a spectral search may be used to automatically reduce the data. The criteria for acceptable identification should include a high index of similarity.

12.3 Electron capture detection data.

12.3.1 The peak should elute within the retention time window set for that homolog.

12.3.2 Any peak which elutes within the retention time window set for a given homolog should be identified and quantitated as a HDD or HDF.

12.4 Disputes in interpretation - Where there is reasonable doubt as to the identity of a peak as a HDD or HDF, the analyst should either identify the peak as a HDD or HDF or proceed to a confirmation analysis.

Table 13. Characteristic Selected Ion Monitoring (SIM) Ions for HDDs

Homolog	Ion (relative abundance) Primary	Secondary	Tertiary
$C_{12}H_7ClO_2$	218 (100)	220 (34)	-
$C_{12}H_6Cl_2O_2$	252 (100)	254 (66)	256 (11)
$C_{12}H_5Cl_3O_2$	286 (100)	288 (99)	290 (33)
$C_{12}H_4Cl_4O_2$	322 (100)	320 (76)	324 (56)
$C_{12}H_3Cl_5O_2$	356 (100)	358 (66)	354 (61)
$C_{12}H_2Cl_6O_2$	390 (100)	392 (82)	394 (36)
$C_{12}HCl_7O_2$	424 (100)	426 (98)	428 (54)
$C_{12}Cl_8O_2$	460 (100)	458 (87)	462 (66)
$C_{12}H_7BrO_2$	262 (100)	264 (99)	-
$C_{12}H_6Br_2O_2$	342 (100)	340 (51)	344 (50)
$C_{12}H_5Br_3O_2$	420 (100)	422 (98)	418 (34)
$C_{12}H_4Br_4O_2$	500 (100)	498 (68)	502 (66)
$C_{12}H_3Br_5O_2$	578 (100)	580 (98)	582 (49)
$C_{12}H_2Br_6O_2$	658 (100)	660 (76)	660 (74)
$C_{12}HBr_7O_2$	736 (100)	738 (98)	740 (58)
$C_{12}Br_8O_2$	816 (100)	814 (82)	818 (79)
$C_{12}H_6ClBrO_2$	298 (100)	296 (76)	300 (25)
$C_{12}H_5Cl_2BrO_2$	332 (100)	330 (61)	334 (46)
$C_{12}H_4Cl_3BrO_2$	366 (100)	368 (66)	364 (51)
$C_{12}H_3Cl_4BrO_2$	400 (100)	402 (85)	398 (44)
$C_{12}H_2Cl_5BrO_2$	436 (100)	434 (97)	438 (53)
$C_{12}HCl_6BrO_2$	470 (100)	468 (83)	472 (65)
$C_{12}Cl_7BrO_2$	504 (100)	506 (77)	502 (73)
$C_{12}H_5ClBr_2O_2$	376 (100)	378 (71)	374 (44)
$C_{12}H_4Cl_2Br_2O_2$	410 (100)	412 (90)	414 (38)
$C_{12}H_3Cl_3Br_2O_2$	446 (100)	444 (91)	448 (51)
$C_{12}H_2Cl_4Br_2O$	480 (100)	478 (78)	482 (64)
$C_{12}HCl_5Br_2O_2$	514 (100)	516 (77)	512 (68)
$C_{12}Cl_6Br_2O_2$	548 (100)	550 (90)	552 (49)
$C_{12}H_4ClBr_3O_2$	456 (100)	454 (85)	458 (50)
$C_{12}H_3Cl_2Br_3O_2$	490 (100)	488 (73)	492 (64)
$C_{12}H_2Cl_3Br_3O_2$	524 (100)	526 (78)	528 (33)
$C_{12}HCl_4Br_3O_2$	558 (100)	560 (92)	562 (58)
$C_{12}Cl_5Br_3O_2$	594 (100)	592 (96)	596 (63)
$C_{12}H_3ClBr_4O_2$	534 (100)	536 (81)	532 (60)
$C_{12}H_2Cl_2Br_4O_2$	568 (100)	570 (95)	572 (48)
$C_{12}HCl_3Br_4O_2$	604 (100)	602 (93)	606 (62)
$C_{12}Cl_4Br_4O_2$	638 (100)	636 (83)	640 (73)

Table 13 (concluded)

Homolog	Ion (relative abundance)		
	Primary	Secondary	Tertiary
$C_{12}H_2ClBr_5O_2$	614 (100)	612 (89)	616 (62)
$C_{12}HCl_2Br_5O_2$	648 (100)	646 (80)	650 (73)
$C_{12}Cl_3Br_5O_2$	682 (100)	684 (84)	680 (72)
$C_{12}HClBr_6O_2$	692 (100)	694 (85)	690 (69)
$C_{12}Cl_2Br_6O_2$	726 (100)	728 (96)	730 (58)
$C_{12}ClBr_7O_2$	772 (100)	770 (92)	774 (70)

Table 14. Characteristic Selected Ion Monitoring (SIM) Ions for HDFs

Homolog	Ion (relative abundance) Primary	Secondary	Tertiary
$C_{12}H_7ClO$	202 (100)	204 (34)	- -
$C_{12}H_6Cl_2O$	236 (100)	238 (66)	240 (11)
$C_{12}H_5Cl_3O$	270 (100)	272 (99)	274 (33)
$C_{12}H_4Cl_4O$	306 (100)	304 (76)	308 (56)
$C_{12}H_3Cl_5O$	340 (100)	342 (66)	338 (61)
$C_{12}H_2Cl_6O$	374 (100)	376 (82)	372 (51)
$C_{12}HCl_7O$	408 (100)	410 (98)	412 (54)
$C_{12}Cl_8O$	444 (100)	442 (87)	446 (66)
$C_{12}H_7BrO$	246 (100)	248 (99)	-
$C_{12}H_6Br_2O$	326 (100)	324 (51)	328 (50)
$C_{12}H_5Br_3O$	404 (100)	406 (98)	402 (34)
$C_{12}H_4Br_4O$	484 (100)	482 (68)	486 (66)
$C_{12}H_3Br_5O$	562 (100)	564 (98)	560 (51)
$C_{12}H_2Br_6O$	642 (100)	640 (76)	644 (74)
$C_{12}HBr_7O$	720 (100)	722 (98)	718 (61)
$C_{12}Br_8O$	800 (100)	798 (82)	802 (78)
$C_{12}H_6ClBrO$	282 (100)	280 (76)	284 (25)
$C_{12}H_5Cl_2BrO$	316 (100)	314 (61)	318 (46)
$C_{12}H_4Cl_3BrO$	350 (100)	352 (66)	348 (51)
$C_{12}H_3Cl_4BrO$	384 (100)	386 (85)	382 (44)
$C_{12}H_2Cl_5BrO$	420 (100)	418 (98)	422 (53)
$C_{12}HCl_6BrO$	454 (100)	452 (83)	456 (65)
$C_{12}Cl_7BrO$	488 (100)	490 (76)	486 (73)
$C_{12}H_5ClBr_2O$	360 (100)	362 (70)	358 (44)
$C_{12}H_4Cl_2Br_2O$	394 (100)	396 (90)	392 (38)
$C_{12}H_3Cl_3Br_2O$	430 (100)	428 (92)	432 (50)
$C_{12}H_2Cl_4Br_2O$	464 (100)	462 (78)	466 (64)
$C_{12}HCl_5Br_2O$	498 (100)	500 (77)	496 (69)
$C_{12}Cl_6Br_2O$	532 (100)	534 (90)	530 (61)
$C_{12}H_4ClBr_3O$	440 (100)	438 (85)	442 (49)
$C_{12}H_3Cl_2Br_3O$	474 (100)	472 (73)	476 (64)
$C_{12}H_2Cl_3Br_3O$	508 (100)	510 (78)	506 (64)
$C_{12}HCl_4Br_3O$	542 (100)	544 (92)	540 (58)
$C_{12}Cl_5Br_3O$	578 (100)	576 (96)	580 (63)
$C_{12}H_3ClBr_4O$	518 (100)	520 (80)	516 (60)
$C_{12}H_2Cl_2Br_4O$	552 (100)	554 (94)	550 (54)
$C_{12}HCl_3Br_4O$	588 (100)	586 (92)	590 (62)
$C_{12}Cl_4Br_4O$	622 (100)	620 (83)	624 (73)

Table 14 (concluded)

Homolog	Ion (relative abundance)		
	Primary	Secondary	Tertiary
$C_{12}H_2ClBr_5O$	598 (100)	596 (89)	600 (62)
$C_{12}HCl_2Br_5O$	632 (100)	630 (80)	634 (73)
$C_{12}Cl_3Br_5O$	666 (100)	668 (84)	664 (72)
$C_{12}HClBr_6O$	676 (100)	678 (85)	674 (69)
$C_{12}Cl_2Br_6O$	710 (100)	712 (96)	708 (64)
$C_{12}ClBr_7O$	756 (100)	754 (92)	758 (69)

Table 15. Limited Mass Scanning (LMS) Ranges for HDDs

Homolog	Mass range
$C_{12}H_7ClO_2$	218 - 220
$C_{12}H_6Cl_2O_2$	252 - 256
$C_{12}H_5Cl_3O_2$	286 - 290
$C_{12}H_4Cl_4O_2$	320 - 326
$C_{12}H_3Cl_5O_2$	354 - 360
$C_{12}H_2Cl_6O_2$	388 - 394
$C_{12}HCl_7O_2$	422 - 430
$C_{12}Cl_8O_2$	456 - 464
$C_{12}H_7BrO_2$	262 - 266
$C_{12}H_6Br_2O_2$	340 - 344
$C_{12}H_5Br_3O_2$	418 - 424
$C_{12}H_4Br_4O_2$	496 - 504
$C_{12}H_3Br_5O_2$	574 - 584
$C_{12}H_2Br_6O_2$	654 - 662
$C_{12}HBr_7O_2$	732 - 742
$C_{12}Br_8O_2$	810 - 822
$C_{12}H_6ClBrO_2$	296 - 300
$C_{12}H_5Cl_2BrO_2$	330 - 334
$C_{12}H_4Cl_3BrO_2$	364 - 370
$C_{12}H_3Cl_4BrO_2$	398 - 404
$C_{12}H_2Cl_5BrO_2$	432 - 440
$C_{12}HCl_6BrO_2$	466 - 474
$C_{12}Cl_7BrO_2$	500 - 510
$C_{12}H_5ClBr_2O_2$	374 - 380
$C_{12}H_4Cl_2Br_2O_2$	408 - 414
$C_{12}H_3Cl_3Br_2O_2$	442 - 450
$C_{12}H_2Cl_4Br_2O_2$	476 - 484
$C_{12}HCl_5Br_2O_2$	510 - 518
$C_{12}Cl_6Br_2O_2$	544 - 554
$C_{12}H_4ClBr_3O_2$	452 - 458
$C_{12}H_3Cl_2Br_3O_2$	486 - 494
$C_{12}H_2Cl_3Br_3O_2$	520 - 528
$C_{12}HCl_4Br_3O_2$	554 - 562
$C_{12}Cl_5Br_3O_2$	588 - 598
$C_{12}H_3ClBr_4O_2$	530 - 538
$C_{12}H_2Cl_2Br_4O_2$	564 - 574
$C_{12}HCl_3Br_4O_2$	598 - 608
$C_{12}Cl_4Br_4O_2$	634 - 640

Table 15 (concluded)

Homolog	Mass range
$C_{12}H_2ClBr_5O_2$	610 - 618
$C_{12}HCl_2Br_5O_2$	644 - 652
$C_{12}Cl_3Br_5O_2$	678 - 688
$C_{12}HClBr_6O_2$	688 - 698
$C_{12}Cl_2Br_6O_2$	722 - 732
$C_{12}ClBr_7O_2$	764 - 776

Table 16. Limited Mass Scanning (LMS) Ranges for HDFs

Homolog	Mass range
$C_{12}H_7ClO$	202 - 204
$C_{12}H_6Cl_2O$	236 - 240
$C_{12}H_5Cl_3O$	270 - 274
$C_{12}H_4Cl_4O$	304 - 310
$C_{12}H_3Cl_5O$	338 - 344
$C_{12}H_2Cl_6O$	372 - 378
$C_{12}HCl_7O$	406 - 414
$C_{12}Cl_8O$	440 - 448
$C_{12}H_7BrO$	246 - 248
$C_{12}H_6Br_2O$	324 - 328
$C_{12}H_5Br_3O$	402 - 408
$C_{12}H_4Br_4O$	480 - 488
$C_{12}H_3Br_5O$	558 - 566
$C_{12}H_2Br_6O$	638 - 646
$C_{12}HBr_7O$	716 - 726
$C_{12}Br_8O$	796 - 806
$C_{12}H_6ClBrO$	280 - 284
$C_{12}H_5Cl_2BrO$	314 - 318
$C_{12}H_4Cl_3BrO$	348 - 354
$C_{12}H_3Cl_4BrO$	382 - 388
$C_{12}H_2Cl_5BrO$	416 - 424
$C_{12}HCl_6BrO$	452 - 458
$C_{12}Cl_7BrO$	484 - 494
$C_{12}H_5ClBr_2O$	358 - 364
$C_{12}H_4Cl_2Br_2O$	392 - 398
$C_{12}H_3Cl_3Br_2O$	426 - 434
$C_{12}H_2Cl_4Br_2O$	460 - 468
$C_{12}HCl_5Br_2O$	494 - 502
$C_{12}Cl_6Br_2O$	528 - 538
$C_{12}H_4ClBr_3O$	436 - 442
$C_{12}H_3Cl_2Br_3O$	470 - 478
$C_{12}H_2Cl_3Br_3O$	504 - 512
$C_{12}HCl_4Br_3O$	538 - 548
$C_{12}Cl_5Br_3O$	572 - 582
$C_{12}H_3ClBr_4O$	514 - 522
$C_{12}H_2Cl_2Br_4O$	548 - 558
$C_{12}HCl_3Br_4O$	584 - 592
$C_{12}Cl_4Br_4O$	618 - 626

Table 16 (concluded)

Homolog	Mass range
$C_{12}H_2ClBr_5O$	594 - 600
$C_{12}HCl_2Br_5O$	628 - 637
$C_{12}Cl_3Br_5O$	662 - 673
$C_{12}HClBr_6O$	672 - 637
$C_{12}Cl_2Br_6O$	706 - 716
$C_{12}ClBr_7O$	750 - 760

13.0 Quantitative Data Reduction

The guidelines presented in this section are based on quantitation using surrogates and internal standards. Isotope dilution techniques may also be applicable and may be substituted if validated prior to use. The QA record should show in detail the alternative calculation used in arriving at final concentration values.

13.1 Once a chromatographic peak has been identified as a HDD or HDF, the compound is quantitated based either on the integrated abundance of the SIM data or EICP for the primary characteristic ion in Tables 13 and 14. If interferences are observed for the primary ion, use the secondary and then the tertiary ion for quantitation. If interferences in the parent cluster prevent quantitation, an ion from a fragment cluster [e.g., M-63 (M-COCl) for PCDDs or PCDFs] may be used. Whichever ion is used, the RF should be determined using that ion. The same criteria should be applied to the surrogate compounds. If electron capture detection is used, the electronically integrated peak area is used for quantitation.

13.2 Using the appropriate analyte-internal standard pair and response factor (RF_h) as determined in Section 7.3, calculate the concentration of each peak using Equation 13-1.

$$\text{Concentration (ng/g)} = \frac{A_h \times M_{is}}{A_{is} \times M_e \times RF_h} \qquad \text{Eq. 13-1}$$

where:

A_h = area of the characteristic ion (s) for the analyte HDD or HDF peak

A_{is} = area of the characteristic ion (s) for the internal standard peak

RF_h = response factor of a given HDD or HDF congener

M_{is} = mass of internal standard added to extract (ng)

M_e = mass of sample extracted (g)

13.3 If a peak appears to contain non-HDD or non-HDF interferences, which cannot be circumvented by a secondary or tertiary ion, either:

13.3.1 Reanalyze the sample on a different column which separates the interferences; or

13.3.2 Perform additional chemical cleanup and then reanalyze the sample; or

13.3.3 Quantitate the entire peak as HDD or HDF.

13.3.4 Reanalyze the sample using a confirmation technique [i.e., high resolution mass spectrometry (HRMS), NCIMS, etc.].

13.4 Calculate the recovery of the surrogates using the appropriate surrogate-internal standard pair and response factor (RF_s) as determined in Section 7.5 using Equation 13-2.

$$\text{Recovery (\%)} = \frac{A_s \times M_{is} \times 100}{A_{is} \times RF_s \times M_s} \qquad \text{Eq. 13-2}$$

where:

A_s = area of the characteristic ion (s) for the surrogate

A_{is} = area of the characteristic ion (s) for the internal standard

RF_s = response factor for the surrogate compound with respect to the internal standard (Eq. 7-1).

M_{is} = mass of internal standard added to extract (ng)

M_s = mass of surrogate added to original sample (ng)

13.5 Correct the concentration of each peak using Equation 13-3. This is the final reportable concentration.

$$\text{Corrected conc. (ng/g)} = \frac{\text{Conc. (ng/g)} \times 100}{\text{Recovery (\%)}} \qquad \text{Eq. 13-3}$$

13.6 Sum all of the peaks for each homolog, and then sum those to yield the total HDD and HDF concentrations in the sample. Report all numbers in nanograms per gram. The uncorrected concentrations, percent recovery, and corrected concentration should be reported.

13.7 Round off all numbers reported to two significant figures.

14.0 Confirmation

If there is reason to question the qualitative identification (Section 11.0), the analyst may choose to confirm that a peak is not a HDD or a HDF. Any technique may be chosen provided that it is validated as having adequate selectivity and sensitivity. Some candidate techniques include alternate GC column (with EIMS detection), GC/CIMS, GC/NCIMS,

high resolution EIMS, and MS/MS techniques. Each laboratory should validate confirmation techniques to show adequate selectivity between HDDs and HDFs and interferences and adequate sensitivity [limit of quantitation (LOQ)].

If a peak is confirmed as being a non-HDD or a non-HDF, it may be deleted from the calculation (Section 13). If a peak is confirmed as containing both HDD or HDF and interferences, it should be quantitated according to Section 13.3.

15.0 Quality Assurance

Each laboratory must develop a Quality Assurance Project Plan (QAPP) and submit that plan to the Agency for review and approval. The QAPP documents how a laboratory intends to produce data which meet the data quality criteria specified in 40 CFR 707 and 766 (EPA 1987).

A detailed QAPP submitted to the Agency will provide the necessary information required for the Agency to review the specific testing protocols for a commercial product. Figure 5 provides a suggested table of contents for a QAPP as outlined in an EPA Office of Toxic Substances guide for preparation of QAPPs (EPA 1984). The specific quality assurance objectives as stated in the final test rule are summarized in Table 17. Guidance for the development of a QAPP is also given in Appendix B.

16.0 Quality Control

16.1 Each laboratory that uses these guidelines should operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory should maintain performance records to define the quality of data that are generated. After a date specified by the Agency, ongoing performance checks should be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method. QC results should be reported with the analytical results on a lot/batch basis.

16.2 The analysts should certify that the precision and accuracy of the analytical results are acceptable by the following:

16.2.1 The absolute precision of surrogate recovery, measured as the RSD_s of the integrated EIMS or ECD peak area (A_s) for a set of samples, should be ± 30%.

16.2.2 The mean recovery (R_c) of at least four replicates of a quality control check sample should meet Agency-specified accuracy and precision criteria. This forms the initial data base for establishing control limits (see Section 16.3 below).

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Figure 5. Table of contents for a quality assurance project plan (QAPP) as specified by the EPA Office of Toxic Substances (EPA 1984).

Table 17. QC Procedures and Criteria for Analysis of Commercial Products for 2,3,7,8-HDD and 2,3,7,8-HDF Congeners

Analysis event	QC criteria	Corrective action
Instrument mass calibration	Must demonstrate accurate mass calibration daily versus DFTPP or PFK.	Recalibration versus DFTPP or PFK.
Calibration standards	Calibration standards are used to establish the working curve, document response factors and mass calibration. After establishing the working curve, the calibration standards are analyzed to bracket sample analyses. Response factors should vary < 30% from the working curve.	If RF values vary by more than ± 30%, sample analyses must be repeated following recalibration.
Samples	Precision of internal standards must meet 50-150% recovery criteria. Precision of duplicate analyses must meet ± 20% criteria. The limit of quantitation (LOQ) must meet 0.1 ppb for 2,3,7,8-HDD congeners and 1.0 ppb for 2,3,7,8-HDF congeners. LOQ = 10 times background signal-to-noise. Limit of detection (LOD) must be calculated. LOD = 3 times background signal-to-noise.	If accuracy, precision, or sensitivity requirements are not met, the sample must be reanalyzed.
QC samples	Method blanks analyzed with each sample batch. Spiked samples 50-150% recovery.	If method blank gives any positive response, repeat analysis. If recovery results do not meet the criteria, the sample must be reanalyzed.

16.3 Control limits - The laboratory should establish control limits using the following equations:

Upper control limit (UCL) = $R_c = 3\ RSD_c$

Upper warning limit (UWL) = $R_c + 2\ RSD_c$

Lower warning limit (LWL) = $R_c - 2\ RSD_c$

Lower control limit (LCL) = $R_c - 3\ RSD_c$

These may be plotted on control charts. If an analysis of a check sample falls outside the warning limits, the analyst should be alerted that potential problems may exist and corrective action may be indicated. If the results for a check sample fall outside the control limits, the laboratory should take corrective action and recertify the performance of the method (Section 16.2) before proceeding with analyses. The warning and control limits should be continuously updated as check sample replicates are added to the data base.

16.4 Before processing any samples, the analyst should demonstrate through the analysis of a reagent blank that all glassware and reagent interferences are under control. Each time a set of samples is analyzed or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against loss of data caused by contamination.

16.5 Procedural QC - The various steps of the analytical procedure should have quality control measures. These include but are not limited to:

16.5.1 GC performance - See Section 7.1 for performance criteria.

16.5.2 MS performance - See Section 7.2 for performance criteria.

16.5.3 Quantitation - At least 10% of all manual calculations, including peak area calculations, should be checked. After changes in computer quantitation routines, the results should be manually checked.

16.6 A minimum of 10% of all samples, one sample per month or one sample per matrix type, whichever is greater, selected at random, should be run in duplicate to monitor the precision of the method. A difference of 20% or less should be achieved. If the difference is greater than 20%, the analyst should investigate the cause of the imprecision and reanalyze the samples. Failure to satisfy this requirement could invalidate the results obtained for samples analyzed since the last precision determination.

16.7 A minimum of 10% of all samples, one sample per month, or one sample per matrix type, whichever is greater, selected at random, should be analyzed by the standard addition technique. Two aliquots of the sample are analyzed, one "as is" and one spiked with an appropriate amount of HDD and/or HDF congeners. The samples are analyzed together and the quantitative results calculated. The recovery of the spiked compounds (calculated by difference) should be 50-150%. If the sample is known to contain specific HDD or HDF isomers, these isomers should be used for spiking. If the approximate concentrations of analytes are known, the amount added should be such that the spiking level is 1.5 to 4 times the anticipated HDD or HDF levels in the unspiked sample.

16.8 It is recommended that each laboratory adopt additional quality assurance practices for use with these guidelines. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates or triplicates may be analyzed to monitor the precision of the sampling technique. Whenever possible, the laboratory should perform analyses of standard reference materials and participate in relevant performance evaluation studies.

17.0 Method Performance

The method performance has not been evaluated at this time. Limits of detection (LODs), limits of quantitation (LOQ), precision, and accuracy should be documented by each laboratory on a product-by-product basis.

18.0 Documentation

Each laboratory is responsible for maintaining full records of the analyses. Laboratory notebooks should be used for handwritten records. GC/MS data should be archived on magnetic tape, disk, or a similar device. Hard copy printouts may be kept in addition if desired. At a minimum, chromatograms obtained from GC/ECD should be archived with full documentation of all parameters used. If possible, GC/ECD data should be collected and archived on magnetic media (e.g., chromatographic data system with flexible disk capability). QC records should be maintained with the sample analysis records.

The documentation should completely describe how the analysis was performed. Any variances from the protocol should be noted and fully described. Where the protocol lists options (e.g., sample cleanup), the options and specific parameters used (e.g., extraction volumes and solvent compositions) should be stated.

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APPENDIX B

QUALITY ASSURANCE PROJECT PLAN FOR MEASUREMENT OF HALOGENATED DIBENZO-p-DIOXINS (HHDs) AND DIBENZOFURANS (HDFs)

Prepared by

Dr. John Smith
Environmental Protection Agency
Office of Toxic Substances
Chemical Regulation Branch
Washington, DC

QUALITY ASSURANCE PROJECT PLAN FOR MEASUREMENT OF HALOGENATED DIBENZO-p-DIOXINS (HHDs) AND DIBENZOFURANS (HDFs)

For quality assurance (QA) to be an effective method of measurement validation of qualification, a Quality Assurance Project Plan (QAPP) must be prepared and should include the following: history, management, and ultimate location of samples; sampling and sample collection procedures; and extraction, instrumental analysis, and data reduction procedures. The QAPP documents how a laboratory intends to demonstrate its capability to produce data of acceptable quality. The proposed rule is concerned with the following four classes of compounds: brominated dibenzofurans, brominated dibenzodioxins, chlorinated dibenzofurans, and chlorinated dibenzodioxins. Of specific interest within these classes are the compounds substituted with from four to seven chlorine and/or bromine atoms per molecule. Hereafter these compounds will collectively be referred to as halogenated dibenzofurans (HDFs) and halogenated dibenzodioxins (HDDs).

An accurate trace or history of the life of a sample to be chemically analyzed for HDFs and HDDs must be assembled and should contain information beginning with a description of the system, scheme, or survey design for sample collection. The sample collection scheme must be statistically designed to allow measurement of variance of the sampling site. A written record, sometimes called a traceability form, shall follow the sample. Necessary contents of the record are written general descriptions of what happens to the sample, schedules and timetables, disposition, and handling. Following final chemical analysis, the last entry in this trace should be the ultimate location of the sample. Any further use (particularly for another activity), movement, or examination of the sample should be added to the history.

Details of the sampling or sample collection procedures are the second section of the QAPP. Since the history describes handling disposition, schedules, and general descriptions of what happens in sampling, the requirement here is a detailed description of sampling and sample collection. Reasons for using a specific or general sample selection process and reasons for not using other processes must be included here. The rationale for any modifications made to a method must also be given. Estimates of how well the selected samples represent the material to be characterized are an essential part of quality assurance. The greater the variability of composition of a material, the more frequently sampling is required for estimation and characterization purposes. In determining or estimating errors generated in sample collection, control samples or blanks and HDF and HDD reinforced controls or standards (native compounds or, preferably, isotopically labeled compounds) must be sent to the collection site(s) and returned with the samples for identical handling and treatment. Duplicate samples, which are collected, documented, and handled the same as other samples, are necessary for recovery, precision, and accuracy determinations.

The third section of the QAPP is a description of the extraction and chemical analysis or screening test procedures. The QA record should show in detail the mathematical calculations performed in arriving at final concentration values. To determine both extraction efficiency and measurement efficiency, it is necessary to use chemical standards of HDF and HDD mixtures or

individual HDF and HDD compounds in control samples. Once capabilities have been established, the operator must determine precision and accuracy and use those determinations to designate acceptable bounds for analytical performance. The operator must keep control chart records to ensure that instrument readings of standards fall within the range of acceptable performance. The operator must establish and describe the quantitative range of an instrument and analytical procedure. Specific requirements are as follows:

- For chemical analysis, these procedures must be demonstrated capable to reproducibly and repeatedly quantitate HDDs and HDFs at the required limits of quantitation. Quality control check sample analysis begins the determination of system capabilities.

- For HDDs, at least two analyses of the same spiked standard at the required LOQ must be quantifiable to within ± 20% of the true value. The recovery of standard spiked into a product material at the required LOQ and run through the entire chemical analysis must be within 50-150% of the amount spiked, with reference to the instrument standard, 99% of the time.

- For HDFs, at least two analyses of the same spiked standard at the required LOQ must be quantifiable to within ± 20% of the true value. The recovery of standard spiked into a product material at the required LOQ and run through the entire chemical analysis must be within 50-150% of the amount spiked, with reference to the instrument standard, 99% of the time.

Qualitative requirements include: response factors for HDFs and HDDs to be measured, instrument hardware and operating conditions (including type and source of columns, carrier gas and flow rate, operating temperature range, and ion source temperature), and structural assignment and/or library matching of mass spectra. For both qualitative and quantitative measurements, the instrument operator should be blind to the nature or source of samples, particularly to duplicates, blanks, and brominated and spiked samples. The limit of detection (LOD) and limit of quantitation (LOQ) shall be described for each material. Tentative LOD and LOQ definitions are equal to three times and ten times background noise, respectively. Details of quantitative calibration procedures for the known and/or expected range of the HDF and HDD levels in actual samples complete this section of the QAPP.

The last section of the QAPP must include the results of laboratory participation in round robin analytical programs, the results of performance audits, the results of systems audits, analytical results of performance audit samples, persons responsible for all aspects of sampling, chemical analysis, data analysis, corrective actions, and quality assurance/quality control This section also must include a description of how problems are handled and documented, and how corrections in working level notebooks are indicated and explained.

APPENDIX C

GUIDANCE FOR SAMPLING HALOGENATED DIBENZO-p-DIOXINS (HDDs) AND DIBENZOFURANS (HDFs)

Prepared by

Dr. John Smith
Environmental Protection Agency
Office of Toxic Substances
Chemical Regulation Branch
Washington, DC

GUIDANCE FOR SAMPLING HALOGENATED DIBENZO-p-DIOXINS (HDDs) AND DIBENZOFURANS (HDFs)

The following are recommended steps for collecting samples of the following four classes of compounds: brominated dibenzofurans, brominated dibenzodioxins, chlorinated dibenzofurans, and chlorinated dibenzodioxins. Of specific interest within these classes are the compounds substituted with from four to seven chlorine and/or bromine atoms per molecule. Hereafter these compounds will collectively be referred to as halogenated dibenzofurans (HDFs) and halogenated dibenzodioxins (HDDs). Specific subgroups within the collective group addressed by the regulation will be more specifically named. All of the following steps should be carefully recorded.

1. Determine the objectives of the sampling exercise.

In the case of this proposed rule, some objectives might be (1) to measure HDF and HDD levels occurring as by-products or impurities in a product to determine compliance with the rule, and (2) to know the limitations of these measurements.

2. Describe the system or production process from which the sample materials will be collected.

The samples collected can only provide information about the product from which they have been collected, and the completeness of this information depends on how, at what time(s), where, and for what duration the samples are collected. Of particular importance in determining a sampling plan for the process are the continuity, homogeneity, and cyclic characteristics of the production process. Compositing of samples for chemical analysis has benefits in reducing analytical costs but risks in requiring lower instrument quantification levels and reducing the ability to identify whether HDFs and HDDs are from uniform concentrations in all members of a composite or high concentrations in one member and no concentrations in the other members of the composite. Each of the seven samples described in the "Guidance for a Sequential Approach to the Sampling of Dibenzofurans and Dibenzodioxins" may be a randomly selected composite, but the seven may not be composited unless the level of concern is revised to one-seventh of the currently proposed levels.

3. Prepare for sample collection operations.

Professional judgment must be assisted by proven sampling methods. To make a complete analysis, sampling should also determine the presence or absence of HDFs and HDDs in locations or situations not necessarily judged to be most important.

The proposed rule requires random sampling. A random sample selection system includes all possibilities and systematically chooses from among a list or compilation of those possibilities. The way of choosing from among the possibilities is the use of a random number table or through an automated random number generator. A haphazard selection system, the result of selection through convenience and/or purposeful neglect, includes only a portion

of all possibilities, has very little estimating power, and should not be considered a random selection procedure.

For the purpose of incorporating this sampling plan with the EPA "Guidance for a Sequential Approach to the Sampling of Brominated and Chlorinated Dibenzofurans and Dibenzodioxins," the sampling unit for each process will be a cycle. A cycle is defined as the time interval representing a period within which the activity mix of a process is essentially repeated. This cycle may be a batch, a shift, a day, or some other time period.

It is important to use random selection procedures in determining the sampling time and location for each individual cycle (the details for these determinations are given below in parts b and c). Cycles should be sampled, one at a time, until as many as seven cycles have been sampled. If two samples from any of the seven or fewer cycles are analyzed and show measurement levels of HDFs and HDDs above the limit of quantitation, the material is not in compliance with the rule. If none of the seven cycles show HDDs above the required LOQ, no further sampling is necessary until there are changes in the process as described below.

If there are changes known or suspected to change the generation of HDFs and HDDs as by-products or impurities in a process or a cycle, the seven-cycle sampling exercise must be done again. For each defined process and independent cycle:

a. The size of a sample of a material to be analyzed will be determined by the kind of material to be sampled and the sensitivity of the analytical method. If the material is gaseous, a sample size of 10 m^3 is usually sufficient for analysis. If the material is aqueous or liquid, a sample size of 1 L is usually sufficient for analysis. If the material is a solid, a sample size of 100 g is usually sufficient for analysis. Any sample size must have proper documentation demonstrating capability to measure HDDs at the required limits of quantitation.

b. Location(s) of sample collection are defined as a subset of all possible locations of storage of a given material. Physically possible sample collection sites are listed and chosen through random selection procedures.

c. In processes where over seven batches of a material are produced in a year, sample collection times must be selected from a random time period within each individual batch production cycle. The cycle time is completely segmented into intervals long enough to collect a sample, and one interval is randomly selected for the actual collection. For processes where under seven batches of material are produced, random selection of seven samples from a list of production time segments for all batches is employed.

d. Quality assurance of the sampling and actual sample collection exercise includes documentation of: the sampling plan, sample collection procedures, and any traceability records. Special notation should be made of

any deviations from original plans and projections recorded at an earlier stage of the sampling exercise. Especially:

- dates and times of sample collection and chemical analysis of samples;
- exact location and time of sample collection;
- process or product batch or lot identification; and
- traceability records employed in the sample collection and ultimate location of the samples.

APPENDIX D

GUIDANCE FOR A SEQUENTIAL APPROACH TO THE SAMPLING OF HALOGENATED DIBENZO-p-DIOXINS (HDDs) AND DIBENZOFURANS (HDFs)

Prepared by

Dr. John Smith
Environmental Protection Agency
Office of Toxic Substances
Chemical Regulation Branch
Washington, DC

GUIDANCE FOR A SEQUENTIAL APPROACH TO THE SAMPLING OF HALOGENATED DIBENZO-p-DIOXINS (HDDs) AND DIBENZOFURANS (HDFs)

INTRODUCTION

This is the sequential sampling scheme referred to in the Proposed Rule for Processing, Distribution, and Use of Certain Designated Chemical Substances Suspected to Produce and/or Be Contaminated with Dioxins and Furans (49 FR 28172, 28182, July 10, 1984).

For this proposed rule, dioxins and furans are in reality four classes of compounds: brominated dibenzofurans, brominated dibenzodioxins, chlorinated dibenzofurans, and chlorinated dibenzodioxins, and specifically within these classes the compounds substituted with from four to seven chlorine and/or bromine atoms per molecule. Hereafter these particular compounds will collectively be referred to as halogenated dibenzofurans (HDFs) and halogenated dibenzodioxins (HDDs). Specific subgroups within the collective group addressed by the regulation will be more specifically named.

This document contains a statement of the sequential decision scheme, the rationale for its use, the details of sample selection (how much, when, and where), and the statistical properties of the sequential decision scheme. This scheme is part of an overall activity to provide guidance to those who are in a position to respond to the proposed regulations.

PROPOSED TRUNCATED SEQUENTIAL DECISION SCHEME

For a given material at the production/formulation site, select a sample and measure the concentration of HDFs and HDDs. The sampling scheme must be used for all tests and analytical evaluations for determination of HDFs and HDDs. A measurement may be obtained by a screening method which has been demonstrated by the tester to a documented degree of sensitivity and specificity. The confirmatory method for identifying the HDFs and HDDs and the concentrations of these compounds is high resolution gas chromatography/ high resolution mass spectrometry (HRGC/HRMS).

The product sample selection procedure is repeated until either a product sample is found to contain HDFs and/or HDDs or seven samples have been processed, in which case declare the production of the sampled material to be free from HDFs and HDDs. If any of the concentrations of HDDs and/or HDFs in a single product sample exceeds the required LOQ, the product will be deemed to contain HDDs and/or HDFs. In HRGC/HRMS a resolvable chromatographic peak is considered a single brominated dibenzofuran compound, a single chlorinated dibenzofuran compound, a single brominated dibenzodioxin compound, or a single chlorinated dibenzodioxin compound. The number of samples, N, required is not to exceed seven.

REASON FOR NOT USING A SINGLE SAMPLE TO DETERMINE COMPLIANCE

Analysis of a material for HDFs and HDDs being generated as by-products or impurities is subject to two main souces of variation: samples may vary over time and/or location at the site. Secondly, the measured concentration of HDFs and HDDs will vary among repeated measurements on the same sample. Therefore, the measured concentration of a particular single screening test or single gas chromatographic peak will vary among samples selected randomly in time and space so that a single sample would not be representative of the underlying process except for the extreme cases.

In the following paragraphs the variable nature of observations will be expressed by the symbol e, the probability that a sample will have a measured concentration below the required LOQ in a particular gas chromatographic peak.

JUSTIFICATION FOR USING SEQUENTIAL SAMPLING

Given the analytical costs associated with HRGC/HRMS, it is important to utilize statistical procedures which minimize the number of samples which need to be analyzed. Sequential monitoring of a material allows a determination of the presence of HDFs and/or HDDs to be based on fewer samples, on the average, than would be required under an equivalent fixed sample size decision rule because of the large number of samples required to describe a production having undemonstrated variability of HDF and HDD content. For each such screening test or HRGC/HRMS analysis, this efficiency increases with the number of potential HDFs and HDDs in the material and decreases as the magnitude of e increases. For materials containing sufficiently high concentrations of even a single HDF or HDD compound (i.e., e is close to zero for its associated screening test or peak), only one sample will be required to declare the presence of HDFs or HDDs. For most multiple congener materials, the use of the proposed scheme would probably result in the analysis of only four or five samples to identify materials containing HDDs and HDFs above the required LOQ. Even for materials containing a single congener at levels above the required LOQ, the application of the scheme would probably result in the analysis of fewer than seven samples to declare the presence of HDFs and HDDs when e is no larger than 0.7.

TIMES, LOCATIONS, AND QUANTITIES

A material may be produced through a group of activities, within one or more locations at a site. The production of the material is assumed to be cyclic in nature, and within each cycle the distribution and concentration ranges of brominated and chlorinated dibenzofurans and dibenzodioxins are assumed to be consistent. Each cycle, then, is a time interval representing a period within which the activity mix of the production of the material is essentially repeated, such as a batch, shift, or day.

Samples under the proposed sequential sampling plan will be selected from separate cycles of the production process. Further description of sample

selection appears in "Guidance for Sampling Brominated and Chlorinated Dibenzofurans and Dibenzodioxins" (Appendix C). It is important to randomly select the sampling time and location independently for each cycle. Any deviations from this random selection must be documented and justified. As many as seven cycles will be required under the proposed procedure. Changes in the production process, especially changes judged to potentially affect brominated and chlorinated dibenzofuran and dibenzodioxin levels, require that the entire sequential testing effort be redone. If a single production process is the potential source of brominated and chlorinated dibenzofuran and dibenzodioxin exposure through several media, each medium should be separately tested by the sequential sampling.

The size of the sample to be chemically analyzed depends on the medium of the sample and the intended analytical method. Sample sizes are recommended in the "Guidance for Sampling Brominated and Chlorinated Dibenzofurans and Dibenzodioxins."

STATISTICAL PROPERTIES OF THE PROPOSED DECISION SCHEME

In general, two decision errors are possible: (1) declaring materials in compliance to be not in compliance, and (2) declaring processes which are not in compliance to be in compliance. Choice of 0.1 ppb as the test threshold and requiring two samples to exceed it eliminates any significant likelihood of committing an error of the first type. The probability of committing an error of the second type decreases with the number of potential brominated and chlorinated dibenzofuran and dibenzodioxin congeners present in the process and the maximum number of required samples, and increases with the magnitude of e for each such peak. The proposed maximum number of seven samples was chosen because it results in an acceptable probability of the second error without the requirement of an excessive amount of samples to be analyzed to determine the presence of HDFs and HDDs. For the seven samples, the error of the second type will be less than 0.25 for a typical process (i.e., one having five or more peaks) provided the e values for the process are all no larger than 0.92 (assuming unconditional independence or complete gas chromatographic resolution of individual HDF and HDD concentrations).