GENERAL ELECTRIC
DIOXIN AND FURAN

6-3-93

PRODUCTION

NPC # 783596-784088
GENP# 010785-011277

GENERAL ELECTRIC

CORPORATE ENVIRONMENTAL PROGRAMS
GENERAL ELECTRIC COMPANY • FAIRFIELD, CONNECTICUT 06431

July 22, 1985

Dr. Daniele DuPont
Centre de Sante
1 REQ
1800 Montee Ste-Julie
Varennes, PQ
JOL 2 PO
CANADA

Dear Dr. DuPont:

As we discussed by phone, I am sending you a copy of the program for the dioxin/furan meeting in September. It appears to me to be a very comprehensive program and should be useful for someone making decisions about such chemicals.

Very truly yours,

Steve Hamilton

S. B. Hamilton, Manager-
Environmental Science & Technology

SBH:cas
Enclosure

783596

GENP 010785

RECEIVED
JUL 8 1985
S. B. HAMILTON

PRELIMINARY PROGRAMME

DIOXIN 85

5 TH INTERNATIONAL SYMPOSIUM ON CHLORINATED DIOXINS AND RELATED COMPOUNDS

SEPTEMBER 16 - 19, BAYREUTH (FRG)

783597

GENP 010786

GENERAL ELECTRIC
GENERAL ELECTRIC COMPANY
FAIRFIELD, CONNECTICUT 06431

STEPHEN B. HAMILTON, JR.
MANAGER - ENVIRONMENTAL SCIENCE AND TECHNOLOGY
(203) 373-3316

August 1, 1985

Ms. Suzanne Rudzinski, Chief
Chemical Regulation Branch
U.S. Environmental Protection Agency
Washington, DC 20460

Dear Ms. Rudzinski:

Thank you for the opportunity to review the package of documents relating to the control of halogenated dioxins and furans that may be produced inadvertently in the manufacture of chemicals. I will address the questions as you have listed them, below.

1. The rationale for developing the list of chemicals seems to be reasonable. The application of the rationale in the list of chemicals has led to some apparent inconsistencies, for example: tetrabromophthalic anhydride (632-79-1) is classified under 2a (polybrominated phenols and derivatives) which does not seem appropriate. Tetrachlorophthalic anhydride (117-08-8) appears to be correctly classified as "3." Does the Group # defined in the document, "Development of List...," correspond to the "Priority" classification in Table I? If so, the assignment of Priority number sometimes disagrees with the Group #. For example, there are no chemicals given Priority 2d, but 344-07-0, 392-56-3 (both assigned 2a) fit the description. Finally, unless data exists to the contrary, the inclusion of halogenated aliphatics and titanium dioxide in Group 3 seems inappropriate. On the other hand, mono-halogenated phenols should perhaps be elevated to the list for testing because of the possibility that they may be contaminated with higher halogenated phenols.

2. I can think of no other chemicals that should be tested.

3. I do not see major problems per se in providing only a skeleton of an analytical framework and relying on industry to fill the gap. However, certain necessary guidance is absent from the skeleton. There is no direction as to whether the analytical scheme be devised to determine all PCDFs and PCDDS, only tetra through octa, or only 2,3,7,8- substituted congeners, which are considered to be the structures having the greatest biological activity.

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Is the analysis supposed to be quantitative for unique positional isomers, or only for total tetra, penta, etc., substituted dioxins and furans? The more specific and the more comprehensive the analysis must be, the more development work, especially in terms of labeled and unlabeled standards, the more time-consuming and costly the analyses must be, and greater the probability that incompatible and unusable information will be generated.

- Re the development of analytical standards:
  This is a mammoth job since every individual structure quantitatively analyzed should have both a labeled and unlabeled standard for determination of response factors and recoveries. The introduction of the concern about mixed bromo and chloro structures greatly enlarges the problem. I recommend that this latter concern be dropped, except for the testing of mixed bromo and chloro chemicals. I further recommend that EPA take the lead in synthesizing and making available the required standards.

- Re the development of cleanup methods.
  Each chemical represents an individual case and will require development work. Most will probably be solvable - some may be quite recalcitrant and require special considerations.

- Re reliable data and comparable results.
  This answer depends to a large extent on the availability of analytical standards and on EPA's simplifying and prioritizing the analytical task. Such a prioritization scheme might be to require the quantitative analysis of the most probable furans and dioxins and a sample representative of the biologically active congeners, such as the 2,3,7,8-tetra substituted dioxins and furans. This would greatly simplify the task and improve chances of getting reliable and comparable data.

4. The sensitivity of the mass spectrometer is limited to one picogram ($1x10^{-12}$ gram) for each individual structure to be analyzed. At one ppb ($1x10^{-9}$), the sample could be analyzed by injecting one mg ($1x10^{-3}$), the maximum sample size that can be injected into a GC, into a GC/MS system. At 0.1 ppb, the sample would have to be concentrated tenfold. This question boils down to one of cleanup. It should also be recognized that at the ppt level, background contamination could also be a factor.

5. There are probably 4 or 5 laboratories worldwide that could perform these tests on the simple cases now. The limiting factor is availability of standards.

6. The sampling and QA plans seem adequate.

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7. General Electric has developed methodologies not referenced in the Draft Final Report dated June 3, 1985, by D. Steele and J. Stanley. The objective of our work was to develop quantitative analyses of PCB fluids for the most biologically active tetra, penta, and hexa chlorinated PCDF structures having the 2,3,7,8-substitution backbone. The work involved the synthesis of all tetra through octa unlabeled PCDFs and C-14 labeled samples of those structures to be quantitatively analyzed. The analytical scheme involved GC-GC-MS instrumental analysis, in some cases preceded by cleanup procedures to extract the furans from the PCBs. This work is described in two papers enclosed for your interest (Target Compound Analysis by Two-Dimensional Gas Chromatography-Mass Spectrometry and Isomer Specific Analysis of Selected Chlorodibenzofurans), both by W.V. Ligon and R.J. May. This analytical approach was also applied to 2,3,7,8-TCDD in hexachlorophene (7-30-4; 2,2'-Methylenebis (3,4,6-trichlorophenol), which is on your list, and provided a quick, quantitative result (see also Determination of Chlorodibenzofurans and Chlorodibenzodioxins using Two Dimensional Gas Chromatography-Mass Spectrometry, by the same authors, enclosed).

8. I have no additional information to provide regarding biological testing systems. I am familiar with the work of Gierthy, et al, at New York State and believe that this work shows real promise since it detects the strongest biological activity of these chemicals, i.e., keratinization. I believe that EPA should devote research funds to the area of biological testing since it could lead to more meaningful data about the toxic potency represented by the impurities in the tested chemical.

I hope these comments will prove useful, and I stand ready to assist further if desired.

Very truly yours,

S. B. Hamilton, Manager-
Environmental Science & Technology

SBH:cas
Enclosures

783600

Journal of Chromatography, 294 (1984) 87–98
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CHROM. 16,672

ISOMER SPECIFIC ANALYSIS OF SELECTED CHLORODIBENZOFURANS

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(First received December 13th, 1983; revised manuscript received February 16th, 1984)

SUMMARY

Two-dimensional gas chromatography has been used to provide full chromatographic resolution of three chlorodibenzofuran isomers. The materials studied were: 2,3,7,8-tetrachlorodibenzofuran, 2,3,4,7,8-pentachlorodibenzofuran and 1,2,3,7,8,9-hexachlorodibenzofuran. The materials were detected using medium resolution mass spectrometry.

INTRODUCTION

Chlorination of dibenzofuran can potentially yield a total of 135 unique products. These products vary both in degree of chlorination and in the positional substitution of chlorine atoms on the basic ring structure. Certain members of this group which have a 2,3,7,8 substitution pattern are known to have measurable physiological effects in a number of animal species. Other members of the group show little biological activity. For this reason, any attempted evaluation of the potential physiological activity of a given chlorination mixture which is based solely on chemical analysis must utilize techniques which allow quantification of the active components.

Analyses of these mixtures by gas chromatography–mass spectrometry (GC–MS) is the method of choice because of the ability of this technique to deal with complex mixtures and small amounts. Such analyses have been reported by a number of workers[1,2]. Such work has failed, however, to provide unambiguous chromatographic resolution of the most interesting species. Without effective separations, it is not possible to ensure that the observed signals accurately reflect the amounts present. If reliable quantification cannot be ensured correlations of analytical data with clinical or epidemiological data are meaningless.

In this paper, we report the chromatographic resolution of three important components of the set: 2,3,7,8-tetrachlorodibenzofuran, (2378-TCDF); 2,3,4,7,8-pentachlorodibenzofuran, (23478-PenCDF); and 1,2,3,7,8,9-hexachlorodibenzofuran, (123789-HexCDF). Two-dimensional GC methods were used to obtain the separations. Analysis was by mass spectrometry.

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CHROM. 16,671

TARGET COMPOUND ANALYSIS BY TWO-DIMENSIONAL GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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(First received December 13th, 1983; revised manuscript received February 16th, 1984)

SUMMARY

The use of two-dimensional gas chromatography* for the analysis of specific target compounds in complex matrices in combination with mass spectrometry has been investigated. The combination of a high capacity, high polarity packed first gas chromatography column followed with a low capacity, low polarity, high resolution second column has been found useful. A component of interest is switched from the first column into a cold trap and then flash-evaporated into the second column. This combination allows part per billion analyses in complex mixtures such as soil extracts, crude oils, and biological extracts without any prior sample cleanup whatever. Two important advantages arise naturally therefore from this approach: a significant reduction in analysis time and a major improvement in the specificity of the analysis. The method offers a relatively inexpensive yet powerful alternative to mass spectrometry–mass spectrometry.

INTRODUCTION

In recent years considerable interest has been focused on the development of analytical methods for part per billion (10^9) and even part per trillion (10^{12}) analyses of selected organic species. Quantification of such materials in unusually complex matrices such as soil and biological extracts has been especially widely studied. The analyses which have been devised for 2,3,7,8-tetrachlorodibenzodioxin in environmental samples, are representative of the current analytical approach to such problems[1,2]. Typically a method will involve an arduous multistep purification procedure, including, for example, liquid chromatography and gel permeation chromatography. These preliminary isolation steps are then usually followed by mass spectrometry or gas chromatography–mass spectrometry (GC–MS) for detection and quantification. In the present paper, we show that two-dimensional GC can eliminate much and in many cases all requirements for sample cleanup even in very complex matrices.

* Note by the Editor: The authors insisted on calling this "two dimensional" against the recommendations of our referees (see also I. M. Hais, *J. Chromatogr.*, 187 (1980) 466) and against the judgement of the Editor.

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GENP 010791

Determination of Chlorodibenzofurans and Chlorodibenzodioxins Using Two-Dimensional Gas Chromatography-Mass Spectrometry.

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General Electric Company
Corporate Research and Development
Schenectady, New York, 12301

ABSTRACT

Polychlorinated biphenyl fluids, flyash extract, and hexachlorophene have been successfully analyzed for 2,3,7,8-tetrachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzodioxin at part per billion levels by two dimensional gas chromatography-mass spectrometry without resort to preliminary isolation procedures. Detailed experimental procedures are provided. Systematic errors, fundamental limitations and interferences are documented.

INTRODUCTION

Chlorodibenzofurans and chlorodibenzodioxins are known to occur in the environment and in certain industrial materials at low levels. Techniques for the determination of these materials have generally involved multi-step isolation procedures followed by gas chromatography-mass spectrometry (GC-MS). The work of Harless et al is typical (1). A review of various methods has been published (2). We wish to report that for certain classes of sample, important congeners of each of these materials can be determined at part per billion (ppb) levels by two-dimensional-GCMS (GC-GC-MS) without the use of

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PYROLYSIS AND COMBUSTION OF AROCLOR 1254 CONTAMINATED DIELECTRIC FLUIDS

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Electric Power Research Institute

DIOXIN 85-5th International Symposium on Chlorinated Dioxins and Related Compounds

ABSTRACT Pyrolysis and combustion products were determined for several levels of PCB contamination in mineral oil and other dielectric fluids. Yields of PCDFs were roughly proportional to the quantity of PCB in the feed.

BACKGROUND

The electric utility industry as a major purchaser of PCBs in the past has been left with a legacy of PCB problems of two different dimensions. Although the reality of PCB as a problem is still being debated and evaluated, the industry is retrofilling or replacing its PCB equipment at a steady pace. A short time ago, there were about 40,000 (1) PCB transformers in utility hands with perhaps an equal or larger number owned by nonutility entities. Several well publicized PCB fires (2,3) in the United States have accelerated the effort toward removal of the askarel (generic term for PCB or PCB/tri-/tetrachlorobenzene) equipment.

Two options are open for elimination of PCBs in transformers. The first is retrofilling and the second is replacement of the transformer. Each method has its adherents and its detractors. Retrofilling, in the absence of further improvement of the technology, takes more than a year before a transformer can be reclassified as uncontaminated (in the United States, below 50 ppm PCB). During most of this time, PCB concentration in the transformer fluid is still above 500 ppm because even if one uses the best available technology, between 2 and 5% of the old liquid remains behind after draining a transformer as thoroughly as possible. It is estimated that this residual takes approximately 3 months to come to equilibrium with the replacement fluid. The dilution process must therefore be repeated several times before an appropriately low PCB level is reached. We understand that there is considerable research presently underway to speed up the removal of this trapped material; however, the work has not yet come to fruition.

Where physically possible, complete replacement of the transformer appears to be the easy way out. However, disposal of the transformer requires draining of the PCB followed by flushing with a solvent. The liquids must then be destroyed in a licensed incinerator while the transformer carcass must go to a certified landfill. Certified landfills in the USA are rapidly disappearing and it is also considered possible that these may become the next generation of problem cleanup sites.

A second area of concern is the roughly 2,000,000 (1) mineral oil transformers, which over the years have inadvertently become contaminated with PCBs at the 50 ppm level or higher. About 10% of these are contaminated above 500 ppm and must be treated as PCB transformers.

This pair of problems has fostered a need to learn more about the pyrolysis and combustion of PCB, both in its concentrated state, and at various levels of contamination in retrofill fluids, such as silicone and mineral oil. PCB is also of interest as a contaminant in tetrachloroethylene, because it is a potential retrofill fluid, although it is more likely to be used as a replacement fluid.

WORK PLAN

A project has been sponsored and partially funded by the Electric Power Research Institute (EPRI) for the New York State Department of Health to study pyrolysis and combustion

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products of PCBs, both as a concentrated fluid and as a contaminant in mineral oil and several replacement fluids. In this project we define pyrolysis as the high-temperature decomposition of a fluid in an oxygen-depleted atmosphere. Combustion is defined as oxidation in the presence of an open flame and an excess of air. The work plan includes the investigation of Aroclor 1254, "neat," as well as at a 50, 500, and 5000 ppm impurity level in mineral oil, silicone and tetrachloroethylene. Since a mixture of tri- and tetrachlorobenzene is frequently found as diluent in askarels, these two compounds, both separately and as a blend, also are being investigated.

EQUIPMENT & PROCESS

Pyrolysis

Pyrolysis trials as described by Eadon (4) were conducted using a simple thermostatically controlled apparatus, capable of accommodating a 6-cm diameter metal block within its 9-cm-long heated region. In order to minimize hazards and disposal problems, yet permit sufficient product formation to facilitate detection of PCDDs and PCDFs, pyrolyses were performed on 100 µl samples. In an attempt to differentiate this work from other earlier and ongoing investigations, as well as to simulate more accurately certain catastrophic incidents, pyrolyses were conducted at atmospheric pressure. The necessity of containing the starting materials and nongaseous pyrolysis products in an open system led to the use of an 8 mm ID x 0.5 m glass tube, sealed at one end, and mounted vertically. The material to be pyrolyzed was deposited at the sealed end, then inserted into a tight-fitting hole in the preheated metal block in the heating apparatus. Typically, the liquid refluxed up the inner walls of the tube; the length of the tube and its comparatively large unheated volume kept the reflux level well below the open end in all experiments. To assure containment, the topmost 5 cm of the tube was chilled in dry ice and the end of the tube was connected to a charcoal trap. No visible material was trapped in the chilled region, and excellent mass balances were generally observed.

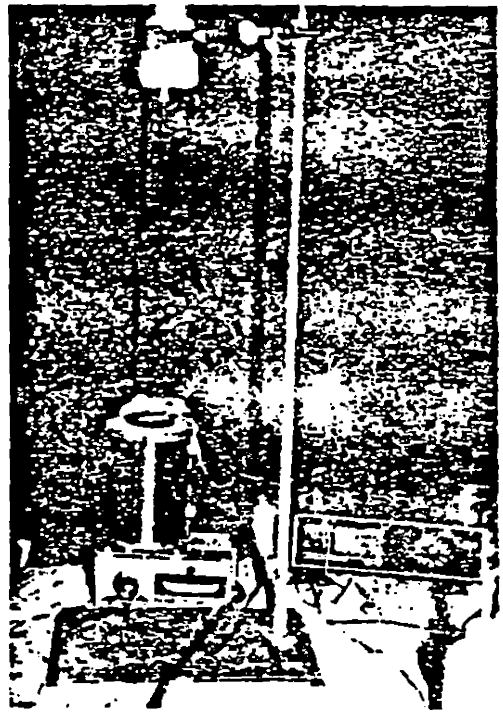

Figure 1: Pyrolysis Equipment

Combustion

The design of the combustion apparatus was also constrained by the necessity of assuring that all discharged gases pass through a trapping system capable of efficient removal of PCBs and any potentially toxic products. The combustion chamber consists of a 1 m quartz tube with a 22-mm ID. One end of the chamber accommodates a modified blast burner and inlet tube through which the end of a 1/16" x 24" (1.6 x 635 mm) syringe needle is introduced via airtight connections. The inlet tube and needle are mounted to allow sample introduction into the flame of the blast burner. Sample addition is accomplished using a 10-ml syringe mounted in a worm syringe drive. The combustion chamber is mounted in a furnace capable of maintaining temperatures of 100-1000°C in three independently controlled zones. The effluent of the combustion chamber is passed through a water filled impinger, then through an XAD-2 packed adsorbent tube which in turn is attached through an orifice to a vacuum line. XAD-2 has been shown here and elsewhere to be an efficient trapping agent for polychlorinated dibenzofurans (PCDFs) and dibenzo-p-dioxins (PCDDs).

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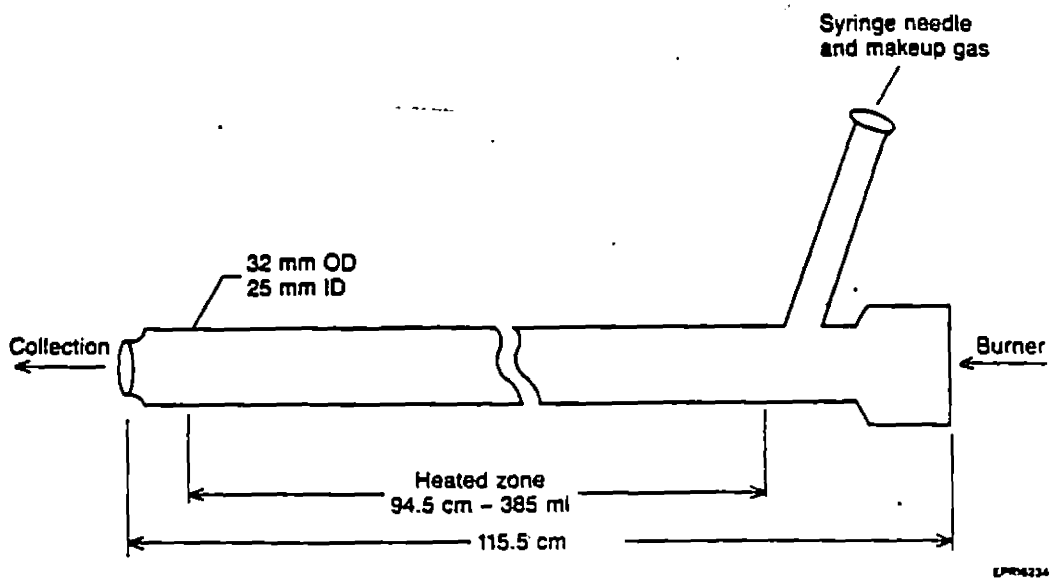

Figure 2: Combustion Tube

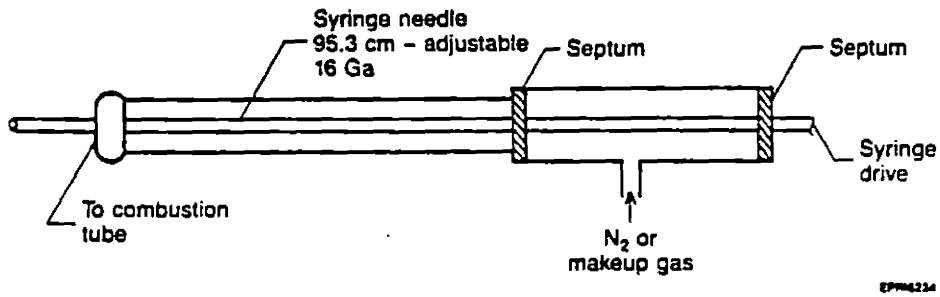

Figure 3: Feed Syringe

ANALYSIS

Because of the hazardous nature of the compounds to be prepared and to check out equipment expeditiously using rapid analytical techniques, the trials were approached stepwise. Initial runs in each case were made with unchlorinated biphenyl to determine the suitability of the equipment and to find a first approximation of proper operating conditions. These runs were followed by trials using individual PCB congeners known to form specific PCDF mixtures. It was anticipated that the relatively simple products formed could be analyzed by capillary GC/EC or GC/FID after chromatographic cleanup. This technique was successful, except in the pyrolysis of contaminated mineral oil. The mineral oil itself produced a complex mixture, and it was necessary, after appropriate up-front cleanup, to resort to the use of GC/MS from the start. Having thus optimized as far as possible operating conditions and analytical procedures, runs were finally made using Aroclor 1254 as the test fluid.

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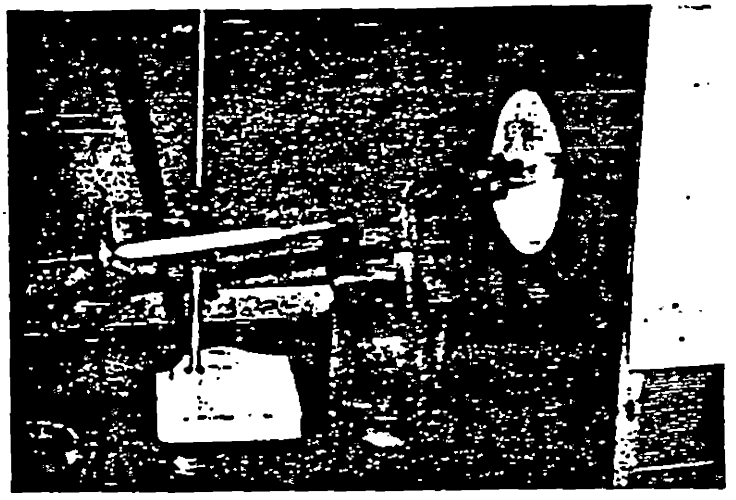

Figure 4: Combustion Product Trap

RESULTS

Pyrolysis of Aroclor 1254 in Mineral Oil, Silicone, and C_2Cl_4

Under the conditions chosen for this project, conversion of PCBs to PCDFs in the trial runs reached an approximate maximum around 550°C. Most of the subsequent runs with Aroclor 1254 were then made at this temperature setting. Where other temperatures were used, they have been noted in the text or tables. All runs were of 15 minutes duration.

To minimize run-to-run experimental variations found in the preliminary work, and to produce larger samples for analysis, a series of six runs was made at each set of conditions. The six runs of each set were always made during a single day and combined randomly (prior to cleanup) into two composites for analysis. Results are tabulated in Tables 1 and 2. Note

TABLE 1: PCDF Formed (ng)/Gram of "Mixture" pyrolyzed: Aroclor 1254 in Insulating Fluids

Sample	Description	2378[2] TCDF	Total TCDF	12378[2] PeCDF	Base Peak PeCDF	Total PeCDF	Base Peak HxCDF	Total HxCDF	Total HpCDF	OCDF
42582	100% Aroclor 1254	1,300	8,200	-	-	16,000	8,000	8,000	60	-
42583	100% Aroclor 1254	965	5,500	-	-	10,000	5,100	5,100	-	-
50854	5,000 ppm[1] in mineral oil	15.0	45.0	17.0	62.0	165	87.0	162	24.0	< 2
50855	5,000 ppm in mineral oil	11.8	33.0	17.2	55.0	171	60.0	205	14.0	0.23
50856	500 ppm in mineral oil	1.6	-	1.8	6.7	17.2	6.3	16.8	< 10	-
50857	500 ppm in mineral oil	1.1	3.1	1.9	5.8	18.2	6.8	18.1	2.2	-
50858	50 ppm in mineral oil	-	-	0.4	1.1	3.1	1.5	3.5	-	-
50859	50 ppm in mineral oil	-	-	0.9	1.7	3.4	1.3	-	-	-
45298	5,000 ppm[3,4] in silicone	9.9	107.0	4.5	-	70.0	2.2	6.9	0.15	-
44295	5,000 ppm[5] in tetrachloroethylene	1.2	2.9	4.6	-	28.0	5.5	12.5	0.9	-
42571	Native mix[6] in mineral oil									
	Added	9.3	-	17.2 (23478)	-	32.4 (234678)				56 (OCDD)
	Found	9.1	-	17.5	-	23.0			47	

(1) Each mineral oil sample represents a composite of three separate pyrolyses which were combined prior to analysis to minimize run-to-run variation. All pyrolyses at 550°C for 15 minutes.
(2) Includes coeluters on DB-5 column.
(3) 650°C.
(4) Chlorinated fluorenes(?) at 10x higher level.
(5) 600°C.
(6) Mineral oil spiked with PCDFs to test method.

GENP 010796

TABLE 2: PCDF formed (ng) per Gram of Aroclor 1254 Pyrolyzed in Insulating Fluid

Sample	Description	2378[2] TCDF	Total TCDF	12378[2] PeCDF	Base Peak PeCDF	Total PeCDF	Base Peak HxCDF	Total HxCDF	Total HeCDF	OCDF
42582	100% Aroclor 1254	1,300	8,200	-	-	16,000	8,000	8,000	60	-
42583	100% Aroclor 1254	965	5,500	-	-	10,000	5,100	5,100	-	-
50854	5,000 ppm[1] in mineral oil	3,000	9,000	3,400	12,400	33,000	17,400	32,400	4,800	84
50855	5,000 ppm in mineral oil	2,360	6,600	3,440	11,000	34,200	12,000	41,000	2,800	46
50856	500 ppm in mineral oil	3,200	-	3,600	13,400	34,400	12,600	33,600	-	-
50857	500 ppm in mineral oil	2,200	6,200	3,800	11,800	36,400	13,600	36,200	4,400	-
50858	50 ppm in mineral oil	-	-	8,000	22,000	62,000	30,000	70,000	-	-
50859	50 ppm in mineral oil	-	-	18,000	34,000	68,000	26,000	-	-	-
45298	5,000 ppm[3] in silicone	1,980	21,400	900	-	14,000	440	1,380	30	-
44295	5,000 ppm[4] in tetrachloroethylene	240	580	960	-	5,600	1,100	2,500	180	-

(1) Each mineral oil sample represents a composite of three separate pyrolyses. These were combined prior to analysis to minimize run-to-run variation. All pyrolyses at 550°C for 15 minutes.
(2) Includes coeluters on DB-5 column.
(3) 650°C.
(4) 600°C.

that Tables 1 and 2 report the same data but expressed in different form. Table 1 shows ng PCDF formed per gram of total mixture pyrolyzed, while Table 2 shows ng PCDF per gram of Aroclor 1254.

It may be seen that conversions of PCBs to PCDFs are substantially identical for all congeners and chlorination groups measured for 5000 and 500 ppm, and are less than an order of magnitude different for 100% PCB. Conversion appears to increase slightly at 50 ppm, but this may be the result of measuring error due to analytical difficulties at this level.

Pyrolyses in silicone and tetrachloroethylene show conversion efficiencies qualitatively similar to mineral oil for tetra- and penta-CDF, but are apparently dropping off for higher chlorination levels. Added work must be done to confirm these levels.

Analysis of the pyrolysis products of PCBs in both silicone and tetrachloroethylene yielded qualitative indications of several products related to PCDF/PCDD. In silicone, compounds tentatively identified as chlorinated fluorenes were found in the MS scans. Also, in pyrolyzing 1,2,3,4,5 pentachlorobiphenyl in silicone, a series of compounds (which may be methylated chlorinated fluorene) was found. Chlorine atoms plus methyl-groups total four in each case (Figure 5).

Pyrolysis of 2-chlorobiphenyl in silicone has fluorene as a major product. This has been compared with an authentic standard. Other compounds listed above as products in the silicone and discussed in the tetrachloroethylene work below will be compared with authentic standards before their presence is considered confirmed.

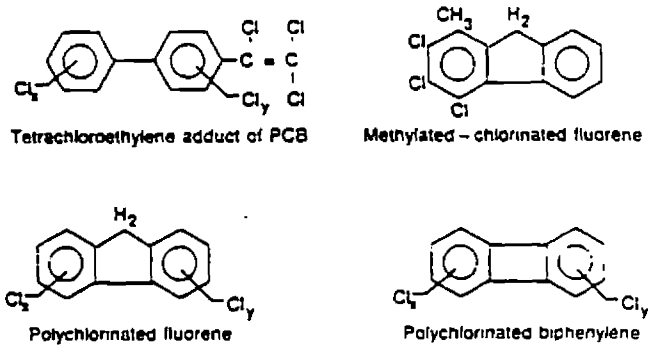

Figure 5: Compounds Related to PCDF/PCDD

GENP 010797

In the combustion and pyrolysis of 1254 in tetrachloroethylene, preliminary work shows that some different tetra- and penta-CDFs are being formed, compared to "neat" 1254 or 1254 in mineral oils. Chlorinated fluorene may also be forming. In addition, a compound that may be a reaction product of PCB and tetrachloroethylene is possible.

Combustion of Aroclor 1254 in Mineral Oil, Silicone and C_2Cl_4

Conversion for the isomer groups studied (Table 3) was near optimum for a combustion wall temperature of 550°C and a 3-second residence time. With feed solutions of mineral oil containing Aroclor 1254 at 50, 500, and 5,000 ppm, tri-, tetra-, and penta-CDF conversion in ng/g PCB fed fell within a narrow range for each of the isomer groups. Under all conditions, hexa- and hepta-CDF formation was not detectable.

Combustion trials with PCBs in silicone were not completed successfully. Large quantities of SiO_2 formed. The finely divided SiO_2 tended to plug the equipment, particularly the feed needle and the sample collection train.

In C_2Cl_4 solution, combustion yields were similar to those found with mineral oil for the $-Cl_3$ isomers. There were substantially greater yields for the more highly chlorinated isomers, reflecting chlorination of lower chlorinated CDFs. Combustion in C_2Cl_4 under oxygen depletion provides a substantial yield of biphenylenes.

TABLE 3: Combustion of Aroclor 1254 in Insulating Fluids at 550°C - 3 Seconds

Solvent	1254 in sol. Combusted ug/ml	PCB's Destroyed %	% PCDFs Formed(1) Cl_3	Cl_4	Cl_5	Cl_6	Cl_7
Mineral Oil	5000	82-88	0.72	0.58	0.17	-(2)	-(2)
Mineral Oil	500	87-92	0.4	0.33	0.084	-(2)	-(2)
Mineral Oil	50	88-90	0.3	0.3	0.056	-(2)	-(2)
C_2Cl_4	5000	68	0.44	1.50	1.35	0.33	0.50
C_2Cl_4	500	92	0.24	0.7	0.66	0.23	0.70
C_2Cl_4	50	72	0.44	1.32	1.0	0.38	0.16
Silicone(3)	-	-	-	-	-	-	-

(1) Based on PCB in feed.
(2) None detected.
(3) Runs unsuccessful. Equipment plugged with SiO_2.

OTHER WORK IN PROGRESS

Pyrolysis and combustion of tri- and tetrachlorobenzene are underway in the laboratory. These results will be of value in assessing the potential fire products from askarels where the PCB is diluted with these solvents.

Preparation of certain synthetic chemicals such as substituted fluorenes is being undertaken. These will be used to confirm the structure of several of the unknowns that appeared during the GC/MS analysis.

DISCUSSION

Part of the original impetus in this project was to determine whether formation of partial oxidation products of PCB was linear with increasing dilution in a solvent. This question appears to be answered. Within the constraints of variations in analytical conditions and recoveries during cleanup and analyses of extremely small quantities of material, the linearity should be considered good; in most cases, there was no more than a factor of 10 variation in a range of concentrations from 50 ppm to 100% of the askarel tested. Improvements in the pyrolysis and combustion schemes during the course of the project provided this degree of resolution. No doubt, this can be improved upon with further sophistication in the work.

The results of these laboratory trials are found to be significantly different from those of other workers in the field (5-9). This fact brings with it a word of caution in applying any of these results to real-world PCB fires except for use as guiding principles because each real PCB fire, under completely random conditions, is different from all others. Even

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within a given fire, there will be an infinite range of competing reactions. It can be assumed that only a small portion of the combustion process has optimum conditions, and that the combustion process varies with time, leading to the partial destruction of the various combustion by-products. Thus, all of the research being done must be considered as setting a boundary or worst-case condition that gives a general direction to the investigation of the real world.

A number of new avenues for exploration have been seen here and in the work of others. Among the more significant ones are the finding of relatively large quantities of other chlorinated polycyclic aromatic compounds (PCAs). A second is the need for a more rapid method of analysis. A bioassay designated the Flat-Cell Assay (10), based on a change in in vitro growth and morphology of a line of skin cells has been shown to be very sensitive and relatively specific for the more toxic of the PCDF and PCDD compounds. The biological mechanism of this effect is considered to be related to the development of chloracne in humans after 2,3,7,8-TCDF exposure and is thus, a relevant end point. This method is already applicable to certain products of combustion. It is being modified to detect and assay these products in the presence of solvents such as mineral oil, which currently interfere with the test. Calibration against a range of chlorinated PCAs would follow.

ACKNOWLEDGMENTS

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GENP 010799

PYROLYSIS AND COMBUSTION OF CHLORORSANICS IN DIELECTRIC FLUIDS

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New York State Department of Health
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Numerous laboratory simulations and several real world incidents have demonstrated that, under certain conditions, heating of concentrated solutions of polychlorinated biphenyls (PCBs), or tri- and tetrachlorobenzenes (TCBs) can produce significant concentrations of polychlorinated dibenzodioxins (PCDDs) and/or polychlorinated dibenzofurans (PCDFs). However, at the outset of this EPRI-supported project, little was known about the thermal behavior of these compounds when diluted to the 5000-50 ppm concentration range with various retrofill fluids or askarel substitutes. This laboratory has selected two heating regimens for study with the goal of choosing conditions that were distinct from each other as well as those used by earlier and contemporary investigators and that might bear some similarity to potential real world events. At the outset, however, it was recognized that no feasible sequence of laboratory experiments could effectively model the range of possible real world incidents. One set of experiments ("Pyrolysis") involved heating 100 mg solutions of the mixture of interest in long vertically mounted pyrex tubes sealed at one end. The sealed end containing the sample was inserted for 15 minutes into a snugly fitting thermostatically controlled metal block, maintained at constant temperature in the range of 500°C - 650°C. Typically under these conditions, the solution refluxed vigorously about half-way up the tube; additional precautions were taken to assure that all materials were retained in the tube. The initial experimental design called for pyrolysis of neat Aroclor 1254 and 5000 ppm solutions of Aroclor 1254 in mineral oil, silicone oil, and tetrachloroethylene at a range of temperatures sufficient to establish the optimum for PCDF formation. Then, 5000, 500, and 50 ppm solutions of Aroclor 1254 in the three fluids were pyrolyzed at the optimal conditions. Similarly, 1,2,4-trichlorobenzene, 1,2,3,4-tetrachlorobenzene and the 2:1 mixture were pyrolyzed neat and as 5000, 500, and 50 ppm solutions in the three dielectric fluids. The result of greatest practical interest was the observation that the yield of PCDF, expressed as ug PCDF/g mixture pyrolyzed, decreased by a factor of 100 or more as PCB concentration was decreased from 1,000,000 to 5000 ppm, and that yields decreased monotonically as PCB concentrations were lowered from 5000 to 50 ppm. Much larger decreases were observed as TCB concentrations were lowered to 5000 ppm; in fact, no PCDF or PCDD could be detected in pyrolysates of 5000, 500, or 50 ppm solutions. An alternative way to discuss the PCB and TCB results is in terms of PCDF or PCDD yield per gram of PCB or TCB pyrolyzed. This mode of interpretation, though of less practical significance, can facilitate understanding of the mechanisms of PCDF and PCDD formation. On this basis, PCDF yields are only moderately influenced by

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dilution of Aroclor 1254 from 1,000,000 to 5000 ppm with mineral oil or silicone oil. The data contain equivocal evidence for improved yields from 50 ppm Aroclor 1254 solutions in mineral oil as compared to 500 or 5000 ppm, and clearer evidence for this phenomena in the corresponding silicone oil solutions. This result implies the existence of a destructive pathway for PCB and/or PCDF that is higher than first order for PCB. Interestingly, no evidence for PCB conversion to PCDF was noted in the 5000 ppm TCE solutions; destruction of pre-existing PCDF in the PCB appeared predominant. The TCB data, expressed again as yield PCDF or PCDD per gram of TCB pyrolyzed, suggests that at 5000 ppm concentrations, the rate or yield limiting step must be kinetically bimolecular in TCB concentration.

Combustion experiments were performed by motorized syringe-drive introduction of solution into a natural gas fueled flame mounted in a one meter horizontal quartz tube itself mounted in a thermostatically controlled oven. The entire gas flow was drawn through a series of traps arranged to ensure complete removal of organics. Some interesting observations include the fact that combustion of TCE alone can form very small amounts (ng/ml) of dibenzofurans. (The pyrolysis of TCE itself at 650°C also formed low amounts of PCDFs.) The solvent/chloroorganic combination that was most prone to PCDF formation during these combustion experiments was Aroclor 1254 in TCE. Aroclor 1254 in TCE, mineral oil, or silicone oil gave yields of PCDF (expressed as ug PCDF/g PCB) which varied only modestly, if at all, over the 5000-50 ppm concentration range. Yields from combustion of 5000 ppm solutions of TCBs in mineral oil, hexane, or silicone oil were markedly lower than obtained from the corresponding PCB solutions, again suggesting the rate or yield limiting step may be second order in TCB and thus sharply affected by dilution. In contrast, TCB in TCE forms considerable PCDF on combustion, probably resulting from reaction of one TCB derived molecule with multiple TCE-derived molecules.

The observation that combustion of PCB or TCB in TCE forms higher yields of PCDFs than the corresponding combustion in mineral oil or silicone oil is in contrast to results obtained during pyrolysis. In those experiments, pyrolysis of Aroclor 1254 in mineral oil or silicone oil clearly gave higher yields than the corresponding reaction in TCE. This dichotomy illustrates what may be the most important result of these studies. Qualitatively very different results can be obtained from a particular chloroorganic/dielectric combination depending on the experimental parameters and heating regimem used.

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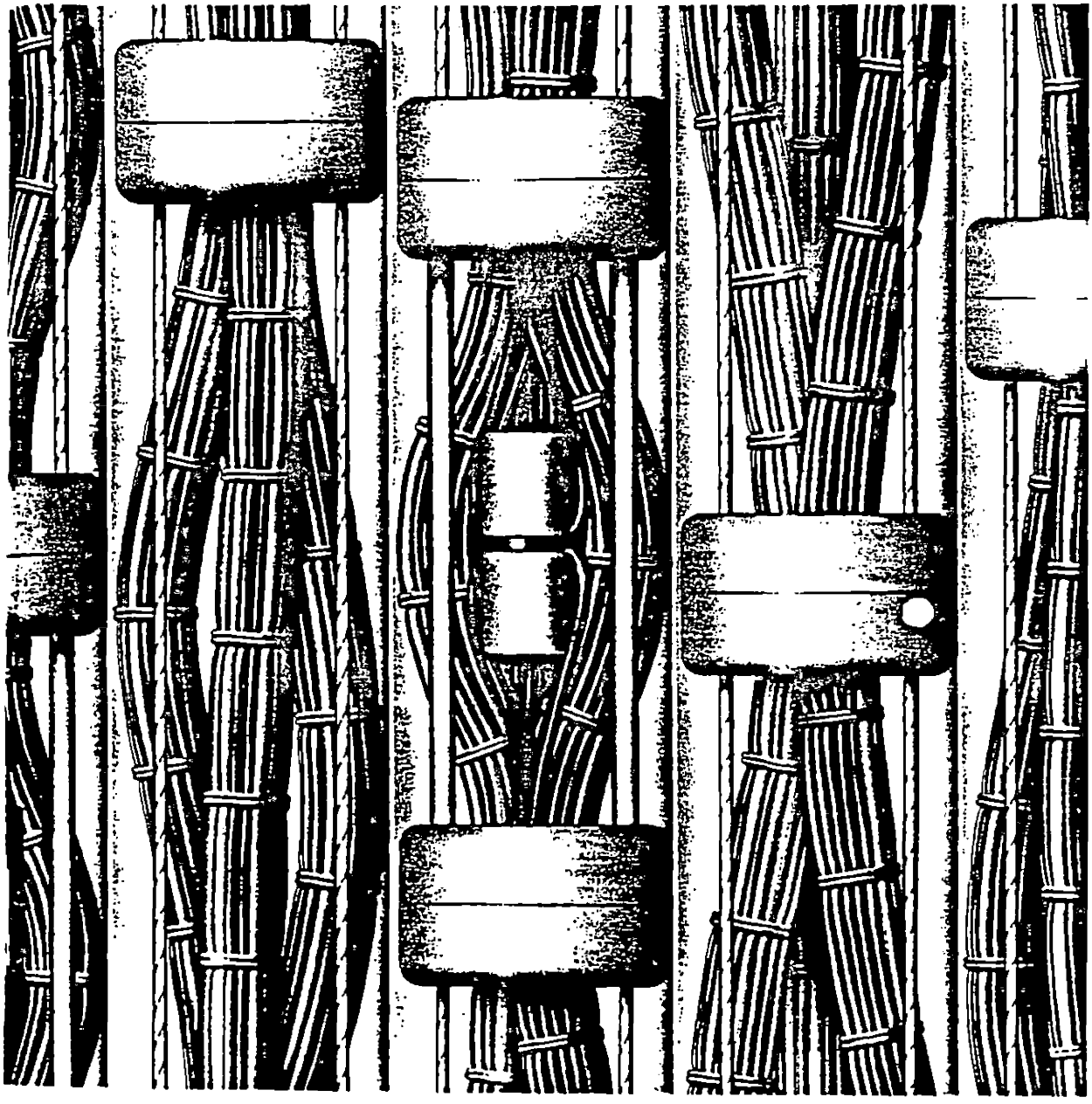

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Dioxin

Concern that this material is harmful to health or the environment may be misplaced. Although it is toxic to certain animals, evidence is lacking that it has any serious long-term effect on human beings

by Fred H. Tschirley

Dioxin. The word evokes a variety of reactions. Much of the public worries that the compound will cause poisoning even at minimal exposure. Toxicologists, knowing the severe toxic effects of dioxin in experimental animals but being uncertain about comparable serious effects on people, call for more research. Regulators, who must make decisions based on this conflicting evidence, are left wondering what to do. As one who has spent many years studying the dioxin issue, I hope in this article to provide a useful perspective for making judgments about the potential hazard of the material.

Dioxin is actually a name for a family of chemical compounds. The name refers to their basic structure: two oxygen atoms joining a pair of benzene rings. Substitution of chlorine atoms for hydrogen atoms on the rings produces a chlorinated dioxin, of which there are many. The chlorinated dioxin of interest here is 2,3,7,8-tetrachlorodibenzo-p-dioxin, usually abbreviated to TCDD. It is a by-product of the manufacture of trichlorophenol, which serves in the manufacture of two herbicides (the best-known being 2,4,5-trichlorophenol, or 2,4,5-T, one of the ingredients of Agent Orange) and the antibacterial agent hexachlorophene. TCDD is also produced in a variety of combustion processes.

The legacy of Agent Orange, Times Beach, Seveso, several industrial accidents and other instances of human exposure to significant amounts of TCDD is widespread concern about adverse health effects resulting from minimal exposure to the material. Yet none of the many studies directed at this question have demonstrated that TCDD causes severe chronic human effects. Moreover, not one human death has been attributed to TCDD, even though exposure has been high in a number of cases. The issue points up the broader problem of the difficulty faced by regulators who must make judgments on the basis of incomplete scientific knowledge on the one hand and public fear on the other.

TCDD was first recognized in 1957 as a contaminant of 2,4,5-T, when 31 workers involved in the manufacture of the herbicide in West Germany developed the dermatologic affliction now called chloracne. It is a skin eruption resembling acne and takes its name from the fact that it is caused by exposure to various chlorinated organic chemicals.

General awareness that TCDD is a potential hazard to health and the environment arose in 1970, when a House subcommittee held a hearing on "Effects of 2,4,5-T on Man and the Environment." The hearing dealt among other things with a study by the Bionetics Research Institute showing that 2,4,5-T caused birth defects in animals. Testimony suggested that the teratogenic component of 2,4,5-T may have been TCDD. In the sample of 2,4,5-T tested by Bionetics the dioxin occurred as a contaminant at an extremely high concentration: 27 ± 8 parts per million.

Since then there has been a steady accumulation of information about the sources of TCDD, its environmental fate and its toxic effects. One well-defined source is the formation of TCDD during the manufacture of 2,4,5-T. The amount of TCDD formed increases as the temperature of the reaction and the *p*H (degree of alkalinity) increase.

In 1977 investigators in the Netherlands reported that polychlorinated dibenzo-p-dioxins (PCDD's) were present in the fly ash from a municipal incinerator. Typically such an incinerator burns, among other things, organic wastes containing chlorine. Similar reports soon came from Switzerland, Canada and Japan. It was believed the compounds resulted from the condensation of chlorophenols. Later quantitative data demonstrated from a wide variety of combustion sources the presence of TCDD's that could not be explained on the basis of preexisting polychlorinated phenols.

These findings led R. R. Bumb of the Dow Chemical Company and 12 of his co-workers to put forward in 1980 the hypothesis that PCDD's can result from trace chemical reactions in fire. The hypothesis has been challenged because the reactions have not been defined. Nevertheless, PCDD's have now been found in the effluent and ash of so many combustion processes that there is no longer serious argument about their formation during combustion, even though the precise nature of the process remains obscure.

Moreover, TCDD has been specifically identified in soil and dust from numerous places, in soot from the chimneys of wood furnaces, as residues in river fishes (some from rivers whose watersheds do not have industrial operations known to form

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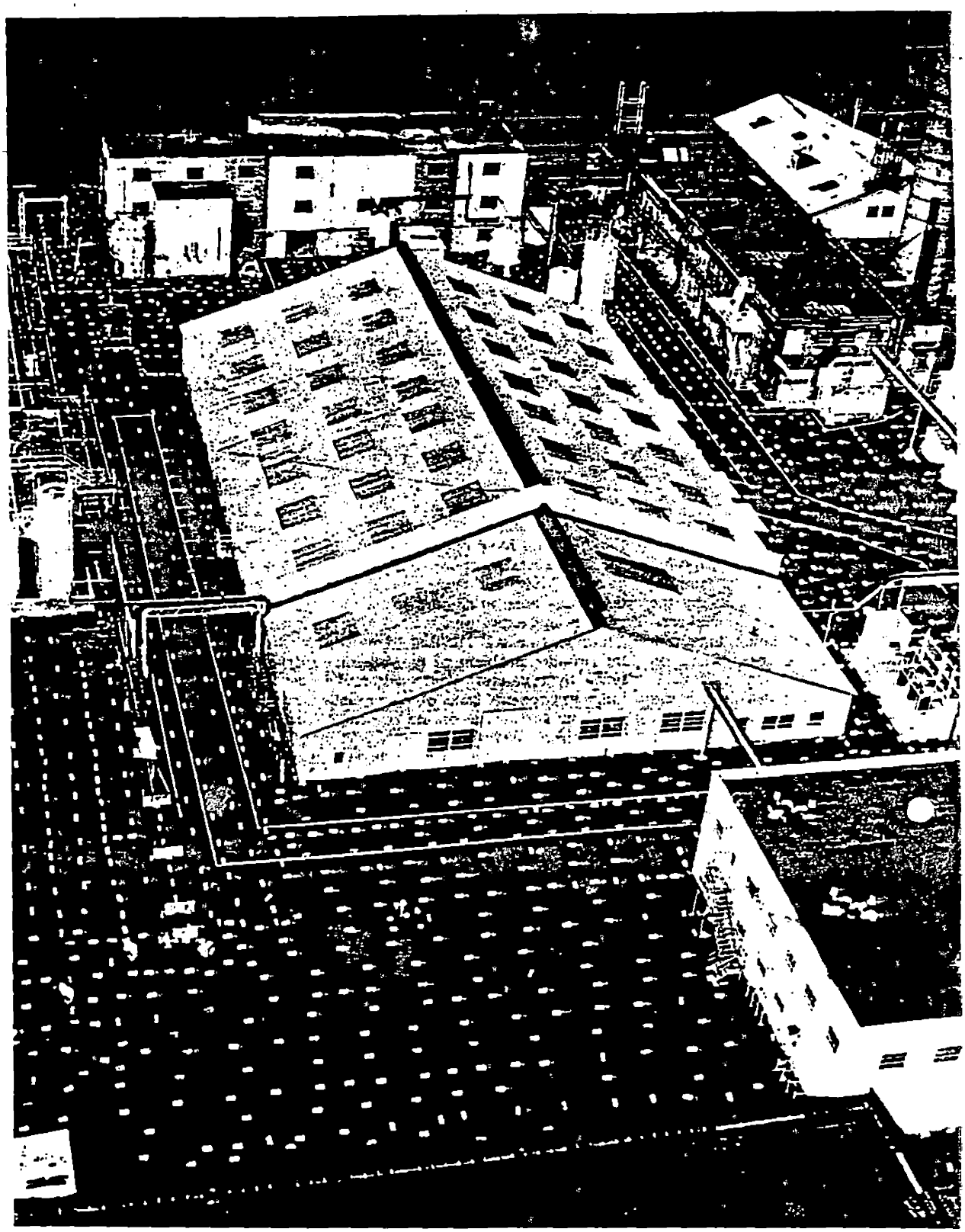

PLASTIC TARPAULINS cover much of the open area of the Diamond Alkali Co. plant in Newark, N.J., because the land may be contaminated by a chlorinated dioxin: 2,3,7,8-tetrachlorodibenzo-p-dioxin, usually abbreviated as TCDD. From 1943 through 1968 the company manufactured chemicals at the plant, including the herbicide 2,4,5-T. TCDD is a by-product of the manufacture of 2,4,5-T. Federal and state officials had the tarpaulins put down as a precaution because TCDD is toxic to a number of animals tested in experiments. The material has not been shown to have severe chronic effects on human beings. It does produce some short-term effects

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TCDD), as residues in the eggs of herring gulls and recently in adipose tissue from more than 100 people in Canada, the U.S. and Vietnam. It appears that TCDD is a ubiquitous chemical, particularly in industrialized nations.

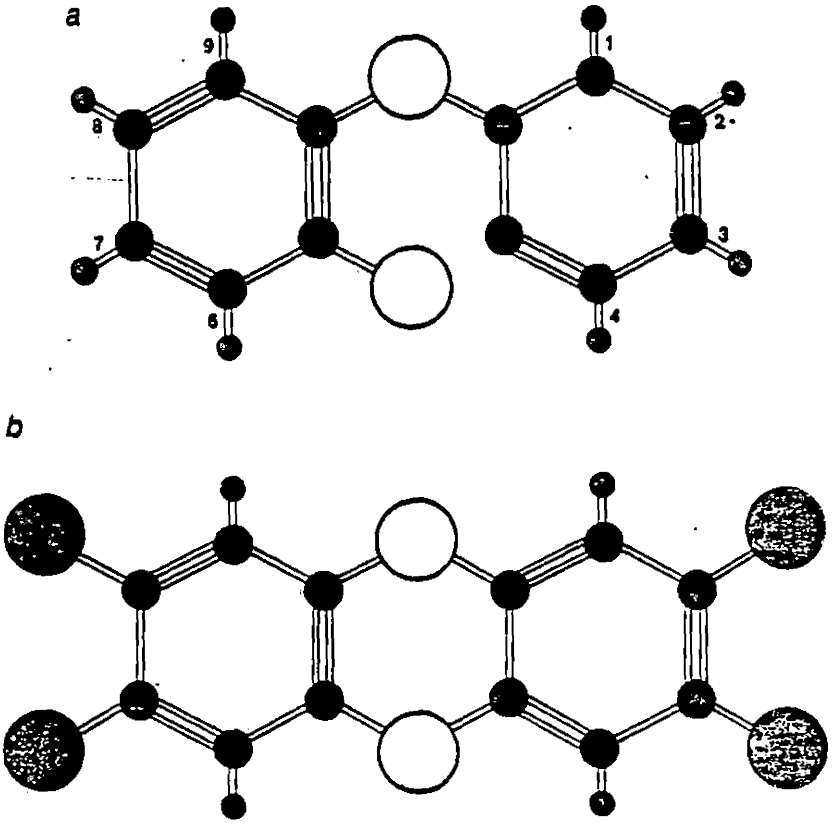
One might then ask why it was not detected sooner. For one thing, no one looked for it seriously before the 1970's. At the time it would not have been found anyway, except in unusual circumstances, because analytic chemists were only able to detect concentrations of a few parts per million. Since then the detecting equipment has improved at least a millionfold, so that concentrations of a few parts per trillion are now detected routinely. In addition dilution and the destruction of TCDD by light may reduce concentrations to undetectable levels. As analytic technology improves, one can expect that TCDD will be found in many more sites than are currently known.

In places where TCDD is protected from light it is an extremely persistent material. In the early 1970's it was thought the half-life of TCDD (the time required for half of a given amount to be degraded) was about a year; later studies in the U.S. suggested the half-life might be as long as three years. Recent reports from Italy raise the possibility that the half-life of TCDD in soil might be 10 years or even more.

Although precise data are meager, TCDD is known to be strongly held by most soils. The strength of the binding is inferred from the fact that known concentrations of TCDD applied to soil have remained near the surface. Even in the sandy soil examined in Florida the concentration of TCDD in the upper 15 centimeters was as high as 1,500 nanograms per kilogram 10 to 12 years after application.

In the most heavily contaminated zones near Seveso TCDD has been found at a depth of 136 centimeters. Moreover, the concentrations well below the surface were slightly but significantly higher in 1977 than they were in 1976, soon after the accident took place. The presence of soil fissures does not adequately explain this unexpected vertical distribution.

The processes that degrade TCDD in soil are poorly known. Microorganisms do degrade the substance, but at a low rate. S. D. Aust of Michigan State University found a wood-decaying fungus (the white mold *Phanerochaete chryosporium*) that breaks down TCDD without observable mortality of the organism. The rate of degradation is low, but it is conceivable that contaminated sites could be inoculated with the mold to speed up the degradation process.



CHEMICAL STRUCTURE of a dioxin and of TCDD is depicted. The dibenzo-p-dioxin molecule (*a*) consists of two benzene rings joined by two oxygen atoms (*light color*). From one chlorine atom to eight atoms can substitute for hydrogen atoms (*gray*) attached to carbons (*black*) at the numbered positions to form any of the 75 chlorinated dioxins. The chlorinated dioxin known as TCDD (*b*) is shown with its four chlorine atoms (*dark color*).

Sunlight degrades TCDD rapidly by splitting off the chlorine atoms. The reaction requires a hydrogen donor, which is usually available in water or in the wax on leaves. Experiments by Donald G. Crosby of the University of California at Davis showed that 40 percent of the TCDD layered on a glass plate remained after six hours in sunlight; the amount was from 25 percent to negligible when the material was applied as drops on leaves of the rubber plant, but on a loam soil the figure was 85 percent.

TCDD is a highly toxic chemical in experimental animals. The first animal-toxicity test performed is usually a determination of the LD_{50}: the dose that kills half of a test population. Between 1973 and 1978 the LD_{50} of TCDD was determined for eight species. The guinea pig was by far the most sensitive species tested: its LD_{50} for an oral dose was .6 microgram per kilogram of body weight. The hamster was the least sensitive animal tested: its oral-dose LD_{50} was about 1,900 times as high as the guinea pig's. Its intraperitoneal level, 3,000 micrograms per kilogram, was about 5,000 times as high as the oral dose for the guinea pig.

The reasons for the extreme range of acute (short-term) toxicity may relate to the relative speed of clearance from the body. In the hamster half of a dose is removed within 15 days, compared with 30 days for the other species tested. Even though the hamster is much less sensitive than the guinea pig, an intraperitoneal LD_{50} of only 3,000 micrograms per kilogram signifies an extremely toxic material: the toxicity is comparable to that of the insecticide parathion.

Many essentially acute symptoms have been observed in human beings. They include chloracne, digestive disorders, effects on some essential enzyme systems, aches and pains of muscles and joints, effects on the nervous system and psychiatric effects. These symptoms have been transitory except for a few severe cases of chloracne.

Additional tests have measured the chronic, or long-term, effects of

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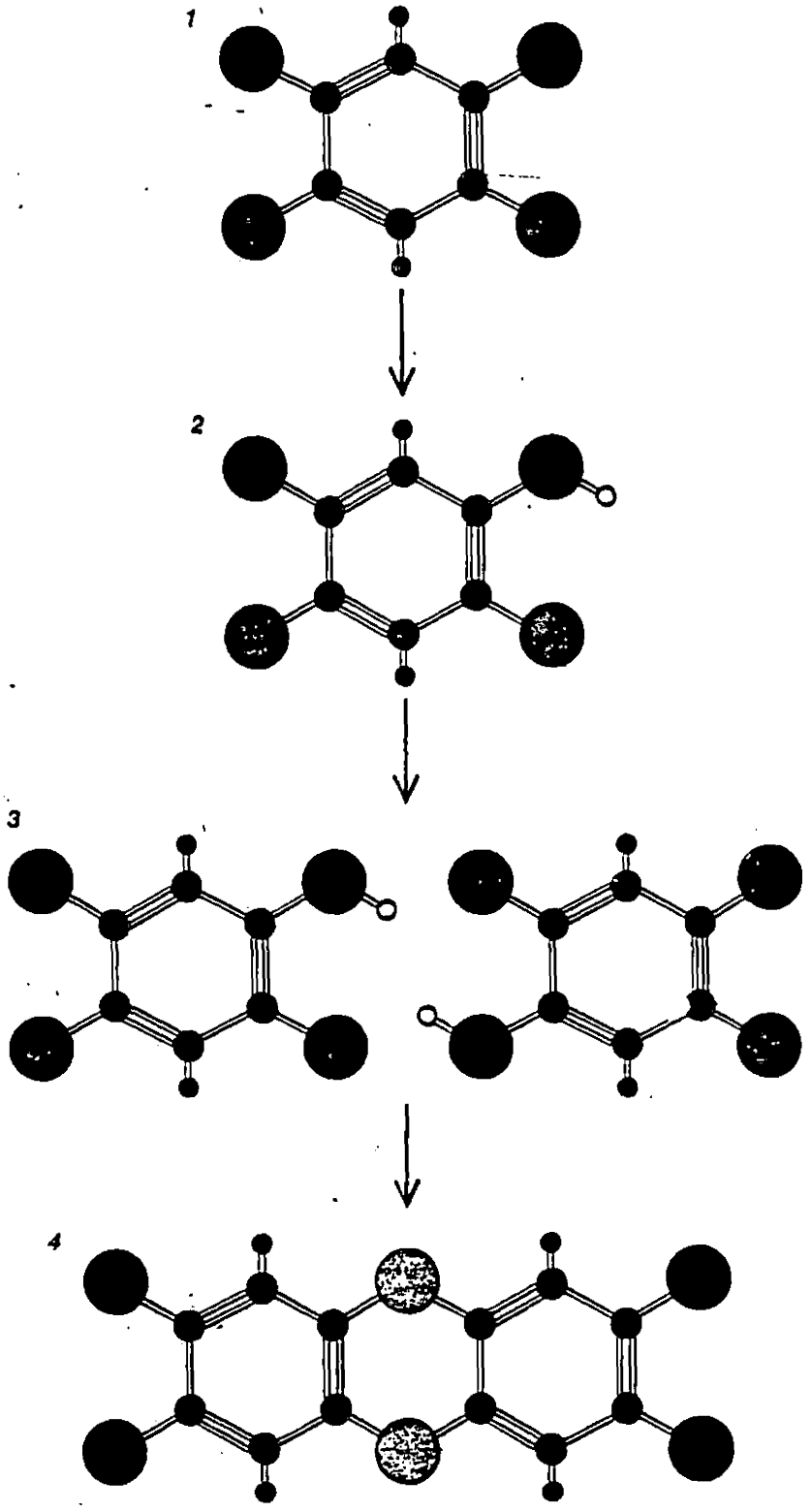

STEPS IN FORMATION of TCDD during the manufacture of 2,4,5-trichlorophenol (TCP) are shown. (TCP is used to make the herbicides 2,4,5-T and Silvex and the antibacterial agent hexachlorophene.) In the process of making TCP a molecule of 1,2,4,5-tetrachlorobenzene is hydrolyzed (*1*) in the presence of caustic soda to form 2,4,5-trichlorophenate (*2*). Two molecules of the phenate (*3*) combine to form TCDD (*4*). They lose atoms of sodium (*white*) and chlorine. The higher the temperature and the alkalinity are, the greater is the amount of TCDD that is formed. When the mixture temperature is above 180 degrees Celsius, a heat-forming reaction occurs; it has been the cause of industrial accidents.

TCDD in rodents, rabbits and nonhuman primates. Chloracne, the most sensitive indicator of human exposure to TCDD, has also appeared in rabbits, nonhuman primates and hairless mice. The skin and body can become dry and scaly. A few species lose hair. Some nonhuman primates lose fingernails and toenails without apparent evidence of pain.

TCDD also causes reproductive effects in experimental animals. Cleft palate and abnormalities of the kidney were caused in the offspring of exposed mice at dosage levels of from one nanogram to three nanograms per kilogram of body weight per day. Similar doses in rats caused the death of fetuses. In monkeys a dose of 1.7 nanograms per kilogram per day for two years caused abortions in four out of seven pregnancies.

TCDD is a proved carcinogen in rats and mice. Although the test results vary somewhat, there is fairly good agreement among tests by different investigators. The liver is the primary target in both rats and mice, although the brain, the respiratory system and the thyroid gland have also been involved in a few studies. It is important to recognize that an oncogenic response was reported only after the animal had ingested high doses of TCDD over a long period of time. Rats showed no oncogenic response at dosage levels of from one nanogram to 1.4 nanograms per kilogram per day; mice showed none at dosage levels ranging from one nanogram to 30 nanograms. Moreover, a study by R. J. Kociba of Dow Chemical and his co-workers showed that rats tolerated a daily dose of one nanogram per kilogram per day for two years without showing toxicologic effects.

Nevertheless, the findings from the tests on animals intensified concern about the effects of TCDD on people. On several occasions people have been exposed to "high" levels of TCDD. In this context "high" is a relative term because with one exception—a group of prisoners who volunteered for tests with TCDD—the amount of the material to which a person was exposed is not accurately known. The criterion I employ here to distinguish high from low exposure is whether or not the exposure results in chloracne.

The case of the prisoners is important because known amounts of TCDD were applied to their skin. In the first experiment 60 volunteers were treated with concentrations ranging from 200 to 8,000 nanograms (from three to 114 nanograms per kilogram for a 70-kilogram person), and the

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dose was repeated two weeks later. The dosages chosen were those that had caused chloracne when they were applied to the ears of rabbits. None of the volunteers developed chloracne, and no other symptoms were observed. The second experiment involved 10 volunteer prisoners who were treated with 107,000 nanograms of TCDD per kilogram. Eight of them developed chloracne, but no other symptoms were noted.

From these experiments one can conclude only that TCDD does cause chloracne in humans when the dose is sufficiently high but that people are less sensitive than rabbits. The tests did not identify a threshold for the development of chloracne in human beings—a piece of information that would be of great value.

The number of people who have been exposed to high levels of TCDD cannot be determined accurately, but it must be in the thousands. Alistair Hay of the University of Leeds has estimated that in the chemical industry alone about 2,000 workers have had high exposure. Low levels of exposure have undoubtedly been experienced by people who handle the herbicides 2,4,5-T and Silvex, in which TCDD was a contaminant; by Vietnam veterans exposed to Agent Orange (50 percent of which was 2,4,5-T); by residents of Times Beach, Mo., where waste oil that contained TCDD was spread on the ground in several places; by chemical-industry workers making the products that include the material, and by many thousands of people who have eaten food (notably fish) containing trace amounts of TCDD or have been exposed to fallout from combustion processes that form TCDD. The total number of individuals with such low exposures probably runs well into the millions.

The possibility of chronic effects from exposure to TCDD causes far greater public concern than that of acute effects. An aspect of this problem about which little is known is the effect of protracted exposure to low levels of the material, as might occur in an occupational setting or from incidental exposures to, for example, the fallout of combustion effluents or to fish that contain low levels of TCDD.

A look at some of the major exposures to TCDD, approximately in order of their severity, reveals few if any unambiguous chronic effects. The industrial accident at Seveso in 1976 exposed some 37,000 people of all ages to considerable amounts of TCDD. A relatively small number of them showed transient effects such as chloracne (184 cases, 164 of them children under the age of 15), headaches and digestive upsets, but no long-term effects such as birth defects and chromosomal damage have been identified. It is too early to tell whether the incidence of cancer is abnormal.

SPECIES	ROUTE	LD_{50} (micrograms per kilogram)
GUINEA PIG (MALE)	ORAL	.6
GUINEA PIG (FEMALE)	ORAL	2.1
RABBIT (MALE,FEMALE)	ORAL	115
RABBIT (MALE,FEMALE)	DERMAL	275
RABBIT (MALE,FEMALE)	INTRAPERITONEAL	252–500
MONKEY(FEMALE)	ORAL	<70
RAT (MALE)	ORAL	22
RAT (FEMALE)	ORAL	45–500
MOUSE (MALE)	ORAL	<150
MOUSE (MALE)	INTRAPERITONEAL	120
DOG (MALE)	ORAL	30–300
DOG (FEMALE)	ORAL	>100
FROG	ORAL	1,000
HAMSTER (MALE,FEMALE)	ORAL	1,157
HAMSTER (MALE,FEMALE)	INTRAPERITONEAL	3,000

ACUTE TOXICITY of TCDD in experimental animals is ascertained on the basis of the LD_{50}: the dose (in micrograms per kilogram of body weight) that kills half of a test group.

An accident in a Monsanto plant in Nitro, W.Va., in 1949 exposed more than 200 workers to TCDD. Of 122 who developed chloracne, 121 were monitored for the next 30 years. The total number of deaths in that group did not differ significantly from that expected in the population at large, and there were no excess deaths due to cancer or diseases of the circulatory system. Similar findings have been made after other industrial accidents, except for two in which an excess of deaths from cancer was found in small groups of the people exposed.

A particular type of cancer (soft-tissue sarcoma, a generic term for more than 100 different types of rare cancer) has become a focus of concern because of a survey of Swedish forestry workers by Lennart Hardell of the University of Umea. He concluded that their exposure to 2,4,5-T (and thus to TCDD) had caused six times the normal incidence of soft-tissue sarcoma.

This study led to an investigation of chemical-plant workers in the U.S. who had been exposed to 2,4,5-T and other chemicals. Seven apparent cases of soft-tissue sarcoma were discovered, raising the level of concern substantially. Subsequent events have emphasized the difficulties in accurate diagnosis of soft-tissue sarcoma and in accurate identification of exposed individuals. At a conference in 1983 Marilyn A. Fingerhut of the National Institute of Occupational Safety and Health reported that two of the seven people had in fact died of cancers other than soft-tissue sarcoma. Moreover, the exposure of three others to TCDD could not be documented. Such findings fall far short of being hard evidence for the proposition that TCDD causes soft-tissue sarcoma.

Other studies also fail to support Hardell's hypothesis. In the state of Washington no consistent pattern of death due to soft-tissue sarcoma was found among occupations in which workers would have been exposed to TCDD. A study in Finland found no cases of the disease among 1,900 people who applied herbicides, nor was their death rate from any natural cause different from that of the total male population in Finland. The U.S. Air Force, in its Ranch Hand study of about 1,200 military personnel who sprayed Agent Orange in Vietnam, found no cases of soft-tissue sarcoma. Finally, examinations by the Veterans Administration of 85,000 self-selected veterans showed fewer cases of these cancers than the national average would suggest.

Reproductive effects are also a subject of concern because of the animal findings. The most celebrated case alleging such effects in humans is commonly known as the Alsea II study, made by the U.S. Environmental Protection Agency. The study reported a link between the spraying of 2,4,5-T on foliage and spontaneous abortion among pregnant women in Alsea, Ore.

This study has come in for much criticism, notably by an interdisciplinary group at Oregon State University. The group concluded that an asso-

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ciation between herbicide spraying and spontaneous abortion could not be shown from the data relied on by the agency. Other studies—in Australia, Hungary, New Zealand and the U.S.—failed to find a link between the use of 2,4,5-T and birth defects.

Because of the extreme acute toxicity and the multiple chronic effects of TCDD in animals, regulatory agencies have had to consider what to do in order to protect people from exposure to the material. Such agencies must extrapolate animal data to human beings in all but a few instances, in spite of the fact that the validity of this type of extrapolation has not been ascertained. Compounding the difficulty is the lack of a simple, accurate method for determining whether and at what level TCDD occurs in the tissues of exposed individuals. (The present test requires a surgical procedure to obtain samples of the liver and fat tissues where TCDD resides.) Without such information a dose-response relation cannot be established.

TCDD has been called the most toxic synthetic chemical known to man. If its acute toxicity to the guinea pig, and even the rat and the mouse, is the criterion, the statement is probably correct. If its considerably lower toxicity to the hamster is the criterion, however, the statement would surely not be true. Yet there is no need to quibble: TCDD is unquestionably a chemical of supreme toxicity to experimental animals. Moreover, severe chronic effects from low dosages have also been demonstrated in experimental animals. Therefore the concern about its effects on human health and the environment is understandable.

When toxic chemicals are at issue, a regulatory agency has few options beyond extrapolating animal data to humans. Yet health effects on humans are rarely proved in the case of environmental chemicals to which the public is variably exposed at subacute levels that can only be estimated (and then only in the crudest approximation). A case in point is aflatoxin, the product of a mold that develops commonly in stored oilseed crops such as peanuts. In animal tests aflatoxin is one of the most potent carcinogens known, but it has not yet been proved to have this effect in human beings.

Diversity in reaction to stimuli is a hallmark of biological organisms. Reactions to toxins are no exception to the general rule. People may be more, less or equally sensitive to a given toxin than an experimental animal is. Extrapolation is neither art nor science; it is simply the most rational way to assess a hazard in the absence of definitive data. Hence regulatory actions continue to be based on the animal data even when the human data, although they are not definitive, may be sufficiently compelling to allow a scientific judgment that the hazard to people has been overestimated.

That appears to be the case with TCDD. Investigators are in general agreement that TCDD is less toxic to humans than it is to experimental animals, but the available information is not sufficiently compelling to stimulate a change in regulatory posture toward either more or less restriction of exposure to the material. I suspect that the direct evidence of TCDD's effects on humans will never be either more or less compelling than it now is.

The public's perception of a toxin is an important determinant of the posture taken by a regulatory agency. The public has heard a great deal about both the acute and the chronic effects of TCDD on experimental animals but little about the substantial body of data showing that human beings are less sensitive. The initial reports of TCDD's acute toxicity, followed by reports of its carcogenicity and reproductive effects, have instilled a public fear that probably cannot be dispelled even by adequate information about the countervailing experience with human beings. The regulatory agency is therefore left in the position of having to deal with not only the available evidence but also the public's fear.

The U.S. Environmental Protection Agency has responded to the public's fear with a number of regulations intended to control the formation and release of TCDD and to limit individual exposure to it. Those regulations could be made stronger or weaker on the basis of new evidence. What the

DATE	WORKERS EXPOSED	LOCATION OF ACCIDENT	REMARKS
1949	250	Monsanto plant in Nitro, W.Va.	122 cases of chloracne being studied; 32 deaths v. 46.4 expected; no excess deaths from malignant neoplasms or circulatory disease
1953	75	BASF plant in Ludwigshafen	55 cases of chloracne, 42 severe; 17 deaths v. 11 to 25 expected (four gastrointestinal cancers and two oat-cell lung cancers); most common injuries were impaired senses and liver damage
1956	?	Rhone-Poulenc plant in Grenoble	17 cases of chloracne, also elevated lipid and cholesterol levels in the blood
1963	106	NV Philips plant in Amsterdam	44 chloracne cases (42 severe), of whom 21 also had internal damage or central-nervous-system disturbances; eight deaths (six possible myocardial infarctions); some symptoms of fatigue
1964	61	Dow Chemical plant in Midland, Mich.	48 cases of chloracne; four v. 7.8 expected deaths; three cancer deaths v. 1.5 expected, one a soft-tissue sarcoma
1965–69	78	Continuing leaks in Spolana plant near Prague	78 cases of chloracne; five deaths; many of the 50 workers studied for more than 10 years have hypertension, elevated blood levels of lipid and cholesterol, prediabetes; significant amounts of severe liver and neurologic damage
1966	?	Rhone-Poulenc plant in Grenoble	21 chloracne cases
1968	90	Coalite & Chemical plant in Derbyshire	79 chloracne cases; one death from coronary thrombosis
1976	158	ICMESA plant in Seveso, Italy	Workers are being studied along with exposed townspeople; more than 500 residents treated for presumed toxic symptoms; 134 confirmed chloracne cases; overall mortality rate normal

INDUSTRIAL ACCIDENTS have exposed more than 800 workers to significant amounts of TCDD. The accident at Seveso in 1976 also exposed some 37,000 residents of nearby communities. The data are based on a study made by the American Medical Association.

GENP 010808

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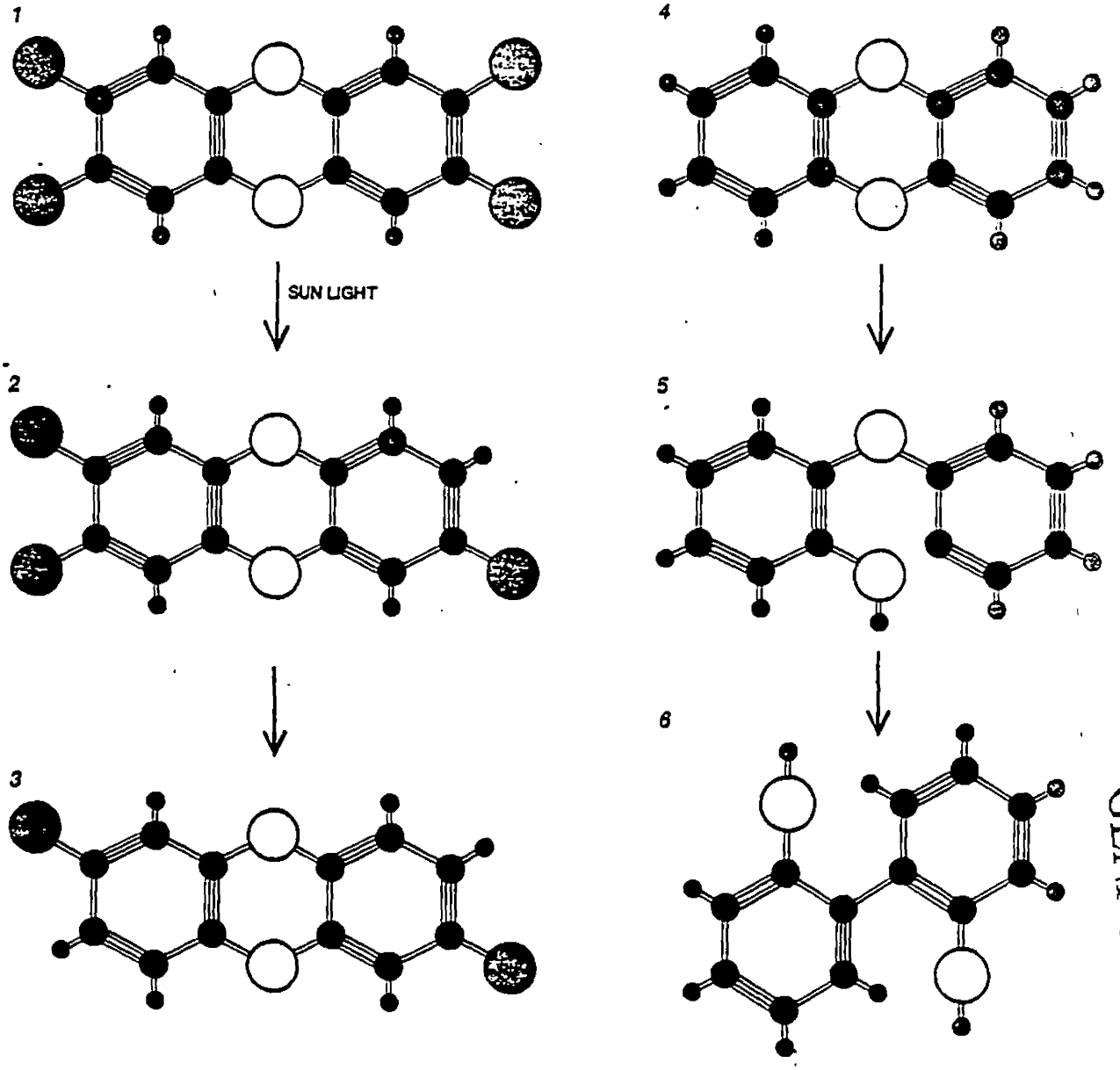
agency has not done—and might be said to have a responsibility to do—is to try to dispel the public's fear on the basis of the evidence that exposure to low concentrations of TCDD in the environment appears not to have serious chronic effects on human beings.

The TCDD case is further exacerbated by its relation to the defoliation program in Vietnam, an unpopular program in an unpopular war. The many and diverse health effects alleged by Vietnam veterans to have been caused by exposure to Agent Orange have been widely publicized. The public is generally aware that the complaints were settled out of court for $180 million, and many people believe the settlement was an admission of guilt by the chemical companies that manufactured Agent Orange. Apparently few people know of Federal Judge Jack B. Weinstein's statement to the attorneys for the plaintiffs that "in no case have you shown causality for the health effects alleged."

A troublesome matter exemplified by the TCDD issue is the appropriate utilization of scientific resources. A. L. Young of the Office of Science and Technology Policy has calculated that more than a billion dollars will have been spent by the Federal Government for research and other dioxin-related matters before all the major studies now in progress have been completed. Additional expenditures of both time and money have been made by chemical companies, private organizations and government agencies. The total outlay is a tremendous amount for an issue of questionable importance.

Two years ago a conference on dioxin at Michigan State concluded that the TCDD case is relatively less important than a number of other issues and that the nation's limited scientific resources should be devoted to the issues posing a greater threat. On the basis of the evidence turned up so far, the conclusion is still valid.



DECOMPOSITION OF TCDD takes place when the ultraviolet in sunlight splits off the molecule's chlorine atoms. Six steps in the reaction are shown. In soil, where sunlight cannot get at the material, TCDD tends to be quite persistent, enduring as long as 10 years

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CHEMICAL MANUFACTURERS ASSOCIATION

RECEIVE
AUG 2 8 198
S. B. HAMILT

August 26, 1986

TO: Dibenzofurans/Dibenzodioxins Program Panel

FROM: C. Stack

RE: Recent Filings and Documents

For your files the following is enclosed:

* Panel comments on the EPA Health Assessment Document on Dibenzofurans and cover letters to Drs. Mukerjee and Byrd;

* Panel comments on EPA's Toxicity Equivalency Factors Scheme and cover letter to Dr. Yosi;

* The Panel's "Considerations for Assessing Potential Human Risk from Exposures to 2,3,7,8-Tetrachlorodibenzodioxin" sent to all of above;

* The April 1986 draft of EPA's "Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-dioxins and Dibenzofurans (CDDs and DCFs)"

GENP 010810

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CHEMICAL MANUFACTURERS ASSOCIATION

GERALDINE V. COX, Ph.D.
Vice President
Technical Director

August 25, 1986

BY HAND

Terry F. Yosie, Director
Science Advisory Board, A-101-F
Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Dear Mr. Yosie:

The Dibenzofurans and Dibenzodioxins Panel of the Chemical Manufacturers Association (CMA) requests the opportunity to make a five-minute presentation at the Science Advisory Board's (SAB's) Dioxin Toxic Equivalency Methodology Subcommittee meeting on September 8-9, 1986. Dr. James Wilson will make the presentation on behalf of the Panel and will comment on EPA's "Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-Dioxins and Dibenzofurans (CDDs and CDFs), the subject of the SAB Subcommittee meeting.

We are submitting with this letter a copy of the Panel's written comments on EPA's Toxic Equivalency Factor (TEF) Scheme for predicting risks from chlorinated dibenzodioxins and dibenzofurans. Dr. Wilson's presentation will briefly summarize these comments.

We are also enclosing with this letter (Attachment I) a copy of a working paper prepared by the CMA Panel entitled "Considerations for Assessing the Potential Human Risk from Exposures to 2,3,7,8-Tetrachlorodibenzodioxin." EPA's Toxic Equivalency Factor Scheme represents an effort by EPA to develop risk values for a number of chlorodibenzodioxins and chlorodibenzofurans in relationship to the toxicity of 2,3,7,8-TCDD. It thus is obviously important that EPA accurately and reliably determine that underlying TCDD value.

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The Panel's working paper discusses the variance between EPA's 1985 risk assessment of TCDD and assessments of other international expert panels. It concludes that EPA overestimates the potency of TCDD by approximately three orders of magnitude. Because the underlying TCDD risk is an integral part of the TEF Scheme, we invite the Subcommittee's attention to this second document.

We are sending copies of these comments directly to all members of the Toxic Equivalency Factor Methodology Subcommittee. If there are any questions, please call Dr. Carol Stack (887-1196) of my staff.

Sincerely yours,

Geraldine V. Cox

Geraldine V. Cox, Ph.D.
Vice President -
Technical Director

Enclosures

cc w/ encs.: Members of the TEF
Methodology Subcommittee

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GENP 010812

Attachment I

COMMENTS OF THE
CHEMICAL MANUFACTURERS ASSOCIATION
DIBENZOFURANS/DIBENZODIOXINS PANEL ON
EPA'S TOXICITY EQUIVALENCY FACTORS (TEF) SCHEME

August 25, 1986

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GENP 010813

COMMENTS OF THE
CHEMICAL MANUFACTURERS ASSOCIATION
DIBENZOFURANS/DIBENZODIOXINS PANEL ON
EPA'S TOXICITY EQUIVALENCY FACTORS (TEF) SCHEME

The Chemical Manufacturers Association (CMA) Dibenzofurans/Dibenzodioxins Panel urges the Scientific Advisory Board to recognize two features of the Toxicity Equivalency Factor (TEF) schemes used by EPA to estimate relative toxicity of different polychlorinated dibenzodioxins and dibenzofurans (PCDDs and PCDFs).

First, none of them is truly valid, nor strongly supported by scientific evidence. The relative activity of different compounds in the dibenzodioxin and dibenzofuran series varies, from species to species and from biological endpoint to biological endpoint, in ways that are not predictable with our present state of knowledge.

Second, the TEF system proposed by Barnes and Bellin, which EPA has used in several regulatory contexts, provides an approximation that can be useful for risk management purposes until more precise information can be developed. At the same time, several downward adjustments to the values attributed to certain congeners by the scheme are necessary to make it more consistent with the available data base.

I. ALL TEF SCHEMES LACK SCIENTIFIC VALIDITY.

No strong scientific support exists for a TEF scheme for chlorinated dibenzodioxins and dibenzofurans. We focus on the chlorodibenzofurans in this discussion, because they

make up a substantial fraction of the PCDD/PCDF compounds present in hot exit gas streams. However, similar remarks also apply to the chlorodibenzodioxins.

The compound 2,3,7,8-tetrachlorodibenzofuran (TCDF) is closely similar to 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in size and shape, and according to the paradigm currently accepted by many scientists working in this field, should exhibit similar biological activity. This follows from the theory of receptor-modulated toxicity, in which binding to a receptor protein is the first step in the process that eventually leads to the very unusual toxicities exhibited by these compounds. In his cell-culture systems, Dr. William Greenlee of the Chemical Industry Institute of Toxicology indeed found 2,3,7,8-TCDF exhibits the same potency as 2,3,7,8-TCDD.

On the other hand, in several systems studied by Safe, et al., TCDF exhibits only one-tenth to three-tenths the activity of TCDD. Birnbaum, et al., also found in the B6C3F1 mouse that 2,3,7,8-TCDF had one-thirtieth the activity of TCDD when the endpoint was hydronephrosis or cleft palate in the fetuses of treated dams. Birnbaum and her co-workers also observed that the relative activities of these two compounds in that mouse strain parallels the relative rates at which they are metabolized and cleared from the body, and that clearance activity was higher in pregnant mice.

Accordingly, at a minimum, the relative activities of different compounds in the dibenzofurans series depend on two factors -- the intrinsic receptor-binding activity and the clearance rates in the organism involved. Other factors not yet identified may also contribute. Greenlee's cell-culture system is capable of little or no metabolic activity, and expresses something nearer to the intrinsic activity than do most other systems yet studied. Conversely, the studies of Birnbaum and her colleagues at NIEHS show the maximal effects of clearance rate on activity.

Safe and his co-workers, as well as many others, have made numerous attempts to derive a self-consistent, regular relationship between the activities of PCDDs and PCDFs so that reliable predictions could be made. These efforts are doomed to failure. EPA should not be encouraged to pursue this line of inquiry.

II. THE EPA TEF SCHEME NONETHELESS HAS REGULATORY RISK MANAGEMENT UTILITY AND THUS SHOULD BE AMENDED SLIGHTLY TO REFLECT AVAILABLE DATA MORE CLOSELY.

CMA recognizes that from time to time circumstances will arise that require risk management decisions to be made on health risks posed by mixtures of PCDDs and PCDFs. Because good data will often not be available on a mixture close in composition to the one at issue, use of a TEF scheme can be justified.

CMA supports use of the Barnes-Bellin TEF scheme for regulatory risk management purposes. Among the several schemes which have been proposed, this one best reflects the complexity of the toxicologic issues and makes use of more of the information available than other schemes. At the same time CMA urges the Science Advisory Board to recommend minor revisions to this EPA scheme so that it will better account for well-known toxicity differences in the tetrachlorodibenzodioxins and tetrachlorodibenzofurans.

The present scheme assigns a single "toxicity equivalent" value for all TCDDs other than the 2,3,7,8-isomer. Existing data indicate, however, that the non-2,3,7,8-tetrachlorodibenzodioxins are not equivalently potent.

It is well known that certain non-2,3,7,8-TCDD isomers, such as the 1,3,7,8-isomer, exhibit activity in several in vitro tests. (Few in vivo results have been reported). The value assigned by Barnes-Bellin for all other TCDDs may be appropriate for these compounds.

However, it is also well known that other members of this series, such as 1,3,6,8-TCDD, exhibit activities orders of magnitude lower than 2,3,7,8-TCDD. In guinea pigs, for instance, the LD_{50} for 2,3,7,8-TCDD is 0.5 to 2.0 µg/kg, while the LD_{50} for 1,3,6,8-TCDD is >1.5 x 10 µg/kg, that is greater than 15 grams 1,3,6,8-TCDD per kg body mass. Probably two-thirds of the 22 different TCDDs will be found, like 1,3,6,8-TCDD, to be practically non-toxic.

If all of the nearly non-toxic TCDDs were rare and the more toxic isomers common, a scheme that treated all TCDDs equivalently would have little adverse consequence. However, the 1,3,6,8-/1,3,7,9-TCDD pair together predominate among tetra isomers in environmental sources of mixed PCDDs and PCDFs. At least these two TCDD isomers should be assigned a TEF value of zero.

Similar considerations apply to the tetrachlorodibenzofurans. If these compounds are to be lumped in one category in a TEF scheme, the values assigned should reflect the fact that in most mixtures the non-toxic isomers make up the majority. Similarly, the data now available on pentachlorodibenzofurans should be reviewed to see if the Barnes-Bellin value assigned to this class reflects their activity.

Finally, CMA urges the SAB to recognize that the composition of PCDDs/PCDFs on incinerator-derived particulates to which humans are ultimately exposed differs markedly from the compositions measured in hot gas streams. It is likely that the particulate composition is nearly enough constant that biological effects of this mixture could be measured directly. This would, of course, obviate the need for any indirect TEF estimate of toxicity.

CHEMICAL MANUFACTURERS ASSOCIATION

GERALDINE V. COX, Ph.D.
Vice President
Technical Director

August 22, 1986

BY HAND

Dr. Daniel Byrd, III
Science Advisory Board, A-101-F
Environmental Protection Agency
499 South Capitol St., S.W.
Washington, D.C. 20460

Dear Dr. Byrd:

The Dibenzofurans and Dibenzodioxins Panel of the Chemical Manufacturers Association (CMA) requests the opportunity to make a ten to fifteen minute presentation at the September 2, 1986, meeting of the Halogenated Organics Subcommittee of the Environmental Health Committee of the Science Advisory Board (SAB) to be held in Kansas City. The presentation will be made on behalf of the Panel by Dr. James Wilson.

The Panel has submitted comments to Dr. Debdas Mukerjee of EPA's Office of Environmental Criteria and Assessment on the revised draft Health Assessment Document for Chlorinated Dibenzofurans, which will be the subject of the SAB meeting on September 2. A copy of those comments is attached to this letter. At the SAB meeting, Dr. Wilson intends to summarize briefly CMA's concerns about the draft Health Assessment Document.

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We are sending copies of the CMA Panel comments on the draft Health Assessment Document directly to members of the Halogenated Organics Subcommittee. If there are any questions, please call Dr. Carol Stack (887-1196) of my staff.

Sincerely yours,

Geraldine V. Cox

Geraldine V. Cox, Ph.D.
Vice President-Technical Director

Enclosure

cc w/enc.: Dr. Debdas Mukerjee
Members of the Halogenated
Organics Subcommittee

783631

CHEMICAL MANUFACTURERS ASSOCIATION

GERALDINE V. COX, Ph.D
Vice President
Technical Director

August 22, 1986

VIA FEDERAL EXPRESS

Dr. Debdas Mukerjee
Project Officer for
Polychlorinated Dibenzofurans
Environmental Criteria
and Assessment Office
Environmental Protection Agency
26 W. St. Clair Street
Cincinnati, Ohio 45268

Re: Health Assessment Document for
Polychlorinated Dibenzofurans
(51 Fed. Reg. 26052, July 18, 1986)

Dear Dr. Mukerjee:

The Chemical Manufacturers Association Dibenzofurans and Dibenzodioxins Panel (CMA) appreciates this opportunity to comment on the June 1986 External Review Draft of the "Health Assessment Document for Polychlorinated Dibenzofurans" prepared for the Office of Air Quality Planning Standards.

CMA's comments focus upon the risk assessment in Chapter 8 of the document and the underlying health data upon which this risk assessment is based. We find that the risk assessment overestimates potential risks to chlorinated dibenzofurans by:

(1) Misinterpreting the toxicology data on which it relies (the Murray three-generation 2,3,7,8-TCDD study) to conclude that 0.001 g/kg/day is a Lowest-Observed-Adverse-Effect Level (LOAEL), when in fact that dose was a No-Observed-Adverse Effect Level (NOAEL); and

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(2) Underestimating exposures to chlorinated dibenzofurans of persons involved in the Yusho, Japan, incident, thus overestimating potency per unit exposure.

In addition, we find that the document overstates by a factor of approximately 100 the likely dibenzofuran concentration in polychlorinated biphenyls found in the United States. Each of these points are detailed in our Comments (Attachment I).

The draft document does not attempt to determine a unit potency value for potential carcinogenicity of any chlorinated dibenzofurans. CMA agrees development of any such value would be inappropriate given the absence of any long-term studies indicating chlorinated dibenzofurans are carcinogenic. Nonetheless, we are aware that a number of scientists have proposed development of such potency values by analogy to the carcinogenic potency of 2,3,7,8-TCDD. We invite your attention to this document.

We would appreciate your sending copies of our comments to members of the peer review panel that met in late May to discuss the chlorinated dibenzofurans criteria document. If there are any questions, please call Dr. Carol Stack of my staff (202/887-1196).

Sincerely,

Geraldine V. Cox

Geraldine V. Cox, Ph.D.
Vice President-Technical Director

Enclosures

cc w/encs.: Members of the EPA
Science Advisory Board
Environmental Health Committee
Halogenated Organics Subcommittee

Attachment I

COMMENTS OF THE
CHEMICAL MANUFACTURERS ASSOCIATION
ON
EPA'S HEALTH ASSESSMENT DOCUMENT
FOR CHLORINATED DIBENZOFURANS

August 22, 1986

GENP 010823

COMMENTS OF THE
CHEMICAL MANUFACTURERS ASSOCIATION
ON
EPA'S HEALTH ASSESSMENT DOCUMENT
FOR CHLORINATED DIBENZOFURANS

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GENP 010824

COMMENTS OF THE
CHEMICAL MANUFACTURERS ASSOCIATION
ON
EPA'S HEALTH ASSESSMENT
DOCUMENT FOR CHLORINATED DIBENZOFURANS

INTRODUCTION

EPA's Health Assessment Document for Chlorinated Dibenzofurans (HAD) concludes with a risk assessment that derives Reference Doses (RfDs) for two chlorinated dibenzofurans -- 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF. These RfDs are derived by analogy to results of a three-generation reproductive study, not of any chlorinated dibenzofuran, but rather of 2,3,7,8-TCDD. EPA also compares the values determined from the TCDD data base to data from the Yusho incident in which humans ingested chlorinated dibenzofurans. The Agency further determines some potential exposure routes, including exposures from polychlorinated biphenyls contaminated with chlorinated dibenzofurans. The CMA Dibenzofurans/Dibenzodioxins Panel finds flaws in each of these three aspects of the HAD.

CMA considers the appropriateness of deriving any Reference Doses for chlorinated dibenzofurans from data on TCDD to be less than optimal. Great uncertainty exists in attempting to estimate risks of one chemical based on data from another chemical. At the same time, we recognize that EPA has often in recent years used a Toxic Equivalency Factor (TEF) scheme in risk management decision-making for estimating the potency of dibenzofurans and dibenzodioxins

other than 2,3,7,8-TCDD given the limited data on these other compounds.

Employment of a TEF scheme is appropriate for some regulatory purposes, but it always must be recognized that the use of such schemes is merely a surrogate and should not be misinterpreted as a conclusion that there is in fact evidence that non-tested chemicals cause the predicted effects. Accordingly, we discuss in these comments the manner in which EPA employs the TCDD data to reach conclusions about chlorinated dibenzofuran potency. As detailed below, we find the Agency has misinterpreted the TCDD data on which it relies and that the effect is to calculate an RfD that is at least ten times too low.

We also comment on the Agency's employment of the human data from the Yusho incident. As our detailed discussion shows, the Agency's incorrect interpretation of these data leads to an overestimate of risk by more than 10 times and further demonstrates the likelihood the RfD derived from the TCDD animal data may be too low.

Finally, we comment on the exaggerated estimate of chlorinated dibenzofuran exposures from potential exposures to polychlorinated biphenyls. The HAD both incorrectly states the contamination level found in U.S. commercial PCB products and inappropriately constructs exposure scenarios that fail to take into account the significant decline in potential PCB exposures that has occured over the past 15

years since manufacture was voluntarily curtailed and legislatively prohibited.

I. THE DOSE OF 0.001 µg/kg/DAY IN THE MURRAY THREE-GENERATION TCDD REPRODUCTIVE STUDY WAS A NOAEL, RATHER THAN A LOAEL.

Central to EPA's development of a Reference Dose (RfD) for chlorinated dibenzofurans is its declaration (at p. 8-4) that the three-generation Murray et al. (1979) study found a LOAEL for 2,3,7,8-TCDD of 0.001 µg/kg/day. To the contrary, 0.001 µg/kg/day was a NOAEL in this study.

The erroneous interpretation of the Murray TCDD study is based solely on statistical re-evaluation of the data by Nisbet and Paxton (1982) and is contrary to the interpretations of the study rendered by the original authors (Murray et al. 1979), the FIFRA Scientific Advisory Panel (1979), the Ontario Ministry of the Environment Expert Panel on Dioxins (1984), and essentially all experts who have evaluated that study. The overwhelming scientific opinion is that the Murray study defined the dose level of 0.001 µg TCDD/kg/day to be a NOAEL.

A NOAEL of 0.001 µg TCDD/kg/day as defined in the Murray multi-generation rat study also agrees with the NOAEL of 0.001 µg TCDD/kg/day established in the most definitive lifetime study, by Kociba et al. (1978), which subsequently has been used by most regulatory agencies in the derivation of long-term human exposure control limits for TCDD. As

discussed in Attachment II to today's submission by CMA the same NOAEL has also been demonstrated in a variety of other toxicology studies of 2,3,7,8-TCDD. (See Attachment II, Section II).

Accordingly, if one uses a long-term or lifetime exposure level of 0.001 µg TCDD/kg/day as a NOAEL, application of the 3-fold and 20-fold estimates by EPA of the lesser degrees of toxicity for 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF, would lead to estimation of lifetime NOAELs for 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF in the rat of 0.003 µg PeCDF/kg/day (3 times the TCDD value) and 0.02 µg TCDF/kg/day (20 times), respectively.

The RfD values for human exposure to 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF can thus be established by application of a composite uncertainty factor of 100 to the NOAEL projected for the rat species. This uncertainty factor of 100 is considered scientifically appropriate to represent a factor of 10 for extrapolation from animal to man and another factor of 10 to account for possible interhuman response variability. Thus, the RfD value for 2,3,4,7,8-PeCDF would be 0.03 nanograms/kg/day (30 picograms/kg/day). For 2,3,7,8-TCDF, the RfD value would be 0.2 nanograms/kg/day (200 picograms/kg/day).

The human LOAEL for 2,3,4,7,8-PeCDF of 0.007 µg/kg/day estimated on p. 8-6 of the HAD from Yusho data could also be used to derive short-term and long-term human exposure

control limits. 1/ As the LOAEL of 0.007 µg/kg/day was estimated for a 71-day period of exposure in humans, application of an uncertainty factor of 10 will estimate a short-term (71 day) NOAEL RfD for 2,3,4,7,8-PeCDF of 0.0007 µg/kg/day (or 700 pg/kg/day) for humans. To extrapolate from the 71-day short-term RfD to lifetime RfD, application of an additional 10 times uncertainty factor would lead to a lifetime RfD of 70 pg/kg/ day for 2,3,4,7,8-PeCDF. This calculated lifetime RfD value of 70 pg/kg/day derived from EPA's human exposure estimates for 2,3,4,7,8-PeCDF is quite similar to the calculated RfD value of 30 pg/kg/day derived from the chronic lifetime animal toxicity data on 2,3,7,8-TCDD, using the 3-fold TEF estimated by EPA for 2,3,4,7,8-PeCDD.

II. HUMAN DATA FROM THE YUSHO INCIDENT CONFIRM THAT THE REFERENCE DOSES FOR 2,3,4,7,8-PeCDF AND 2,3,7,8-TCDF DERIVED FROM ANIMAL DATA ARE QUITE PROTECTIVE.

To attempt to confirm the accuracy of the Reference Dose developed from the 2,3,7,8-TCDD three-generation study, the criteria document compares the animal-derived value to a determination of the LOAEL for 2,3,4,7,8-PeCDF in the

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1/ We note in Section II of these comments, however, that this value is not appropriate reflection of the Yusho data of human effect levels.

Western Japan poisoning incident. In so doing, however, EPA incorrectly assesses that incident. As detailed below, the human data suggest a lifetime RfD for total PCDFs in the Yusho episode of 500 picograms/kg/day, thus corroborating the appropriateness of RFD values of at least 30 and 200 pg/kg/day for 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF, as derived from the animal data discussed above.

A. Detailed Data Exist on Human Exposures to and Effects of PCDFs in the Yusho Incident.

Persons in southern Japan ("Yusho" - 1968) and Taiwan ("Yu-Chen" - 1976) ingested cooking oil that had been become contaminated with heat-transfer fluids during processing. Originally thought to result from the chlorobiphenyls present in the oil, Yusho symptoms have now been shown by Japanese investigators to be largely due to PCDFs present at ppm levels in the oil. (Masuda, et al., 1985; Chen et al., 1985; Miyata, et al., 1985; Kashimoto, et al., 1985; Kunita, et al., 1985.)

More than 1,000 patients with the Yusho syndrome had been identified in Japan by 1971; a larger number were affected in Taiwan. People intoxicated in both incidents exhibited a complex of symptoms very similar to what has been called "chloracne" when seen in industrial situations in the United States and Europe. (Kimbrough 1983; Kuratsune, et al., 1972.)

To estimate the amount ingested by persons with Yusho symptoms, Kuratsune, et al., surveyed 146 users of the contaminated oil, which was produced February 5, 1968, and was distributed in 16.5 kg cans. They estimated the level of consumption of contaminated oil from cooking and eating habits. The population was divided into three dose groups: those who consumed: (1) less than 720 ml, (2) between 720 and 1,440 ml, and (3) more than 1,440 ml, of the contaminated oil. Ten of 80 in the low-dose group were without symptoms; all 66 in the mid- and high-dose groups exhibited either "light" or "severe" symptoms. Thirty-one of 80 in the low-dose group (38.8%), 31 of 45 in the mid-dose group (68.9%), and 18 of 21 in the high dose group (85.7%), exhibited "severe" symptoms. From these data and knowledge of the concentration of PCDFs in the oil that was ingested, an approximate ED_{50} value for grade III Yusho (clinical chloracne) can be estimated. 2/

The concentration of PCDFs in the contaminated oil has been studied by Buser, et al., (1978), Miyata, et al., (1985), Morita, et al., (1972). Each group used comparable

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2/ By Kuratsune's terminology, "light" symptoms correspond to clinical grades I and II Yusho symptoms, while "severe" corresponds to clinical grades III and IV. Grade III corresponds approximately to chloracne as it would have been diagnosed by physicians seeing workers exposed to TCDD in 2,3,5-trichlorophenol production accidents.

techniques -- medium-to-high resolution gas chromatography with mass spectrometric detection, and their results are comparable. All reported concentrations of total PCDFs in the range of 1.9 to 7.4 mg/kg oil. The high report of Miyata, et al., comes from a sample described as "5 February 1968 production"; this matches the date on which the oil consumed by patients surveyed by Kuratsune, et al., was reported to have been produced and shipped. Samples described as having been produced on February 9 and 10, 1968, were estimated to contain 1.9 and 2.3 mg/kg PCDFs, respectively. The sample examined by Morita, et al., was described as having been produced February 12, 1968. Buser, et al., gave no description of the origin of the sample they examined. Because of the coincidence of production dates, and the evident decline in PCDF concentration with time over the period from February 5 to February 9-12, 7.4 mg PCDF/kg rice oil is a reasonable estimate of the concentration of these agents in the oil ingested by the survey population.

B. EPA Misinterprets the Yusho Data.

As a means of checking the reliability of the RFD determined from animal data, EPA employs the Yusho data. In so doing, however, it fails to employ the relevant data correctly.

EPA's calculation is based on the following factors:

(1) minimum quantity to cause a grade III response of 121 ml;

(2) a mean latency period of 71 days;

(3) PCDF concentration in the oil of 3.85 ppm; and

(4) a concentration of 2,3,4,7,8-PeCDF of 8.4% of total PCDFs in the oil.

Based on these factors, EPA concludes the LOAEL for 2,3,4,7,8-PeCDF was 0.007 µg/kg/day, thus leading it to conclude that the LOAEL it derived from the animal studies of 0.003 µg/kg/day is reasonable. EPA's calculations contain several errors.

First, no source is given for the value of 121 ml that is cited as "the minimim quantity ... that led to an observed effect ... ." As detailed above, in the studies reported by Kuratsune, et al. (1972), only estimates of the amount of oil ingested were given, and the lowest range cited was "less than 720 ml." Over the period of ten or so days during which the contaminated rice-bran oil was produced, the concentration varied by at least a factor of three. Data from the Yusho reports show that oil processed February 5, 1968 had about 7.4 ppm of total PCDFs and that this had fallen to about 2 ppm by February 10. If it were not possible to relate the amount of oil ingested to the effects seen, use of the mean value would be acceptable. However, since good data are available on a population known to have ingested oil produced on a particular date, oil for

which an analysis is available, the concentration given for this known oil should be used.

Second, in using these data from the Yusho episode, the LOAEL value can be considered an approximation of the dose level that gave low incidence of the effects seen in studies of the population. Use of a single extreme value, as EPA has done, however, is improper. It incorrectly mixes risk management judgments into the risk assessment. When proper dose-response information can be extracted from the available data, as is the case here, it should be used to calculate a population mean dose for the effect.

Third, the work of Birnbaum (1985) and others has shown that the effects of compounds in the chlorinated dibenzofuran class are additive. In a series of experiments, effects seen were directly proportional to the sum of the products of concentration of each compound times its relative activity in producing that effect in the animal being tested. Thus it is entirely incorrect to ignore the contributions to Yusho symptoms of compounds such as 1,2,3,7,8-penta, 2,3,4,6,8-penta, and 1,2,4,6,7,8-hexa chlorodibenzofurans. Individually, their contributions may be less than that of the 2,3,4,7,8 congener, but their total contributions may well exceed those of the latter. As no data are available to estimate reliably the relative activities of these compounds in whole animals, no attempt should

be made to estimate the relative contribution of a single compound to the activity of this mixture. 3/

We discuss next appropriate use of the Yusho data to determine acceptable daily intakes for total chlorinated dibenzofurans involved in the Yusho episode. We encourage EPA to review this assessment closely.

C. Properly Interpreted, the Yusho Data Show an Acceptable Lifetime Average Daily Dose of 500 Picograms Total PCDFs/Kilogram/Day.

Combining the Yusho analytical, consumption and effects data demonstrates the following Effective Doses (EDs):

(1) From the low-dose group: 4/

$ED_{39} < (0.72\ 1)(0.91\ kg\ oil/1)(7.4\ mg\ PCDF/kg\ oil)/60\ kg\ body\ weight = 81\ \mu g/kg.$

$ED_{39} < 81\ \mu g/kg.$

3/ In addition, it is known that the rate at which a compound is metabolized and thus deactivated affects its activity in the whole animal. In a situation such as Yusho, where a large number of very similar compounds are present, the activity of individual compounds cannot be assessed because enzymes that would deactivate them if present alone may preferentially react with more reactive compounds also present. Because easily-metabolized compounds are present in the body along with the more toxic, activity of the less easily metabolized 2,3,4,7,8-pentachlorodibenzofuran may be greater than it would appear to be when tested alone.

4/ We assume that the rice-bran oil of Yusho has the same specific gravity as cottonseed and corn oils (0.91 kg/1) and that the mean body weight of the Yusho population studied by Kuratsune, et al., was 60 kg.

(2) From the mid-dose group:

$81 < ED_{69} < (1.44\ l)(0.91\ kg/l)$
$(7.4\ mg/kg\ oil/60\ kg) = 162\ \mu g/kg.$

$81\ \mu g/kg < ED_{69} < 162\ \mu g/kg$

(3) From the high-dose group:

$ED_{86} > 162\ \mu g/kg.$

From analyses of these data by a normal probit method, an estimate of the ED_{50} value for grade III Yusho can be made:

$46 < ED_{50} < 105\ \mu g/kg.$ 5/

It would be desirable to relate the activity of this mixture to that of some single standard, such as 2,3,7,8-TCDD. However, that is problematic in this case, for three reasons.

First, no isomer-specific quantitative analysis of Yusho oil has been published. The results of Buser, et al., suggest that more than 30 tetra-, penta-, and hexachlorodibenzofurans were present in the oil.

Second, the relative activities of these different congeners are unknown, even in in vitro systems.

Finally, such a congener assessment would require determining the contribution of chlorobiphenyls and -quaterphenyls to the activity since these were present in

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5/ An ED_{01} estimate can also be made: 2.6 to 10 µg/kg. However, we have more confidence in the ED_{50} value, as it is almost independent of the form of the dose-response curve assumed.

the oil at more than one hundred times greater concentration, and Birnbaum, et al. (1985), as previously noted, have shown that some compounds from these classes contribute to effects from PCDFs in an additive manner. It is reasonable to conclude that at least some of the PCDFs present in the Yusho oil would be more active than this aggregate dose-response estimate suggests, but it is unlikely that any would be more than ten times more so. Using the estimated low ED_{50} value from the above analysis (46 µg/kg) as a point of departure for standard-setting, we thus incorporate, as detailed below, an extra order of magnitude for this uncertainty.

Employing the ED_{50} value of 46 µg/kg, short-term and lifetime health advisory levels can be developed from the Yusho data. Each is explained below.

Single Dose Health Advisory (SDHA)

The above estimates of Effective Doses in the Yusho incident were made from exposures that occurred over a period of about 50 days. This period approximates the estimated half-life for these compounds in the body (King, et al., 1983). Because of poorer absorption and more rapid excretion at higher dose rates, a single dose of twice that accumulated at Yusho would have approximately the same effect.

Because human data are used as the basis here for determining a single dose health advisory (SDHA) guideline, an

uncertainty factor of 1,000 applied to the ED_{50} would be a quite conservative approach. One factor of ten is applied because the base dose is not a NOAEL; a second factor of ten is applied to take into account conservatively the distribution of susceptibilities in the population; and a third factor of ten to allow for uncertainties in the activity of the various PCDFs present in the Yusho oil, as mentioned above. Thus:

$$SDHA = 2 \times ED_{50}/1000 = (2)(46)/1000 = 0.09 \text{ µg total PCDF/kg body weight}$$

$$= 9 \times 10^{-5} \text{ mg total PCDF/kg, or 90 ng PCDF total/kg}$$

Short-Term Health Advisory (STHA)

For cumulative exposure over a 10 to 100 day period, one one-thousandth of the low ED_{50} dose at Yusho can be considered to be protective of human health for determination of a short-term health advisory (STHA) guideline. Thus:

$$STHA = ED_{50}/1000 = 0.046 \text{ µg total PCDF/kg body weight.}$$

$$= 4.6 \times 10^{-5} \text{ mg total PCDF/kg, or 46 ng total PCDF/kg}$$

Long-Term (Lifetime) Health Advisory (LADD)

A lifetime health advisory or lifetime acceptable daily dose (LADD) should be estimated taking possible accumulation of chlorinated dibenzofurans into account. That is, the intake required to reach serum concentrations equal to those

implied by the STHA dose should be estimated. However, analysis of Yusho data shows that such an analysis would require more data on human pharmacodynamics than is now available. Absent these data, use of an additional conservative uncertainty factor of 100 applied to the STHA is appropriate. Here, that is appropriately represented by LADD = STHA/100. Thus: 6/

LADD = 0.046 µg total PCDF/kg body weight/100

= 4.6×10^{-7} mg total PCDF/kg/day, or 460 total PCDF pg/kg/day.

6/ In 1973 FDA estimated the mean oil intake of all Yusho patients to be 15 grams/day for 50 days, or 750 grams. The mean PCDF concentration is not known, but we know it to be greater than about 2 ppm (the minimum concentration reported). Thus the mean intake of PCDFs was > $(2 \times 10^{-6})(750\ g) = >1.5 \times 10^{-3}$ g = >1.5 mg. FDA assumes a mean body weight of 50 kg, and further assume that one-tenth the mean dose could be taken as a tolerable dose of PCBs. (This logic was used to derive a tolerance level for PCBs.)

Following similar logic, we could define a tolerable dose for PCDFs to be $>1.5 \times 10^{-3}$ g/50/10 = 3×10^{-6} gm PCDF/kg = 3 µg/kg. FDA reasoned that a total accumulation of less than the dose so calculated would cause no effect. This quantity was divided by 675 days (22 months) to obtain an acceptable average daily intake (AADI). Applying a similar procedure here, one would obtain an LADD of 4×10^{-9} gm PCDF/kg/day = 4 ng/kg/day.

Our lower value differs from FDA's in three respects: we started with a higher "effect" dose (46µg/kg vs. 30 µg/kg) and included a much more conservative uncertainty factor to arrive at a tolerable accumulated dose (0.046 µg/kg vs. 3 µg/kg), but allowed a shorter time to reach equilibrium body burden. On balance, we believe an LADD of 500 pg/kg/day to be quite protective of human health.

This LADD of 460 picograms/kg/day derived from the Yusho data for total polychlorinated dibenzofurans is higher than the LADDs derived both by analogy to the TCDD animal data (30 and 200 picograms/kg/day for 2,3,4,7,8 and 2,3,7,8, respectively, see page 4 above) and by calculations based on what EPA says is the LOAEL in the Yusho incident (70 picograms/kg/day for 2,3,4,7,8, see page 4 above). It thus supports the clear adequacy of 30 to 70 and 200 picograms/kg/day RFD values derived for 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF from animal data.

III. THE CONCENTRATION OF CHLORINATED DIBENZOFURANS IN, AND POTENTIAL EXPOSURES TO, PCBS ARE OVERESTIMATED.

Section 4 of the HAD also requires comment, especially in light of the misuse of some of the exposure information set forth in other sections of the report, most notably Section 6.3. The Section 4 discussion of chlorinated dibenzofuran (PCDF) contamination of polychlorinated biphenyls (PCBs) is not relevant to the situation in the United States. American PCB products have consistently been shown to contain extremely low levels of PCDFs, compared to normal foreign materials, let alone the thermally stressed materials involved in the Yusho and Yu-Cheng episodes. Numerous exaggerations and misleading statements need to be eliminated from the HAD.

The errors on this issue begin in the HAD Chapter 4 discussion of potential exposures of PCDFs. On page 4-11, the statement that less than ppt levels of PCDFs were detected in Aroclor 1016 is misleading. The correct statement would be that no PCDFs were detected in Aroclor 1016, at a detection limit of X ppt. The next sentence is also erroneous, in that Bowes, et al., did not have in 1975 the analytical capability to detect 2,3,7,8-TCDF or 2,3,4,7,8-PeCDF in PCB formulations. At that time, as acknowledged on page 3-6, there were neither columns available to separate 2,3,7,8-TCDF from coeluting isomers nor standards to determine the retention time of potential interferents.

On page 4-12, the statement that the composition of Kanechlor 400 resembled that of Aroclor 1248 is also erroneous and misleading, especially since the statement implies that the Aroclor product contained the same level of contaminants as "Yusho" fluid. In fact, the fluid responsible for the Yusho incident did not even resemble Kanechlor 400, having been thermally stressed and exposed to oxygen, as later acknowledged expressly in the same paragraph.

On page 4-12, the HAD attributes a 2,3,7,8-TCDF concentration of 1.25 ppm to a thermally stressed heat exchange fluid, but notes in the next sentence that at least one other isomer coeluted under the conditions used. Nonetheless, the 1.25 ppm value is used in Section 6.3 to calculate exposure to PCDFs from any exposure to PCBs.

All of pages 4-13 is a compilation of "suspecteds", "probablies" and "may haves". This section of the HAD thus exaggerates the contribution of PCB exposure to potential effects of PCDFs.

The calculations in Section 6.3 of the HAD, purporting to estimate maximum exposure levels associated with PCB exposure, are not realistic estimations, even assuming the desirability of developing a worst-case scenario.

First, the dietary intake value, stated to represent present intake is taken from an eight-year-old paper. The quoted paper relies on data obtained before the voluntary cessation of PCB manufacture in the United States, and thus bears little relation to current potential exposures. More recent EPA and FDA evaluations clearly reflect the fact that PCB levels in fish from the Great Lakes are decreasing.

A more serious error, and substantial exaggeration, is the use of 2,3,7,8-TCDF concentration in PCBs of 1.25 ppm. This value is not representative of 2,3,7,8-TCDF concentrations in American PCB products and in fact represents the reported concentration in a severely heat-stressed and oxygen-exposed Japanese product, used under conditions known to maximize PCDF concentrations in PCBs. (See Rappe, C., et al., 1985). Nor is it correct to maintain that this material is "similar to Aroclor 1248".

Japanese PCB products have consistently been shown to contain higher concentrations of PCDFs, even ignoring the

issue of heat stress. The same author who reported the value of 1.25 ppm in the heat-stressed Japanese product has also reported levels of PCDFs in truly representative American PCB products. (Rappe, C. et al., 1985). The 2,3,7,8-TCDF concentrations in Aroclor 1254 and Aroclor 1260 were reported as 19 and 13 ppb respectively. Although these data are presented in Table 4-8 of the HAD, they are later ignored in the exposure assessments. Accordingly, the HAD exaggerates potential PCDF exposures due to PCBs by a factor of almost 100 by using data not representing PCBs that could be the source of exposure to the American public. Using more realistic values for 2,3,7,8-TCDF in American PCB products reduces the value for stored 2,3,7,8-TCDF to about 0.002 ng/day, if one assumes no excretion, another exaggerated assumption.

The same factor of 100 should be applied to all of the other calculated exposure levels in Section 6.3 of the HAD. The entire section thus needs to be rewritten to reflect representative exposures, even in developing worst case scenarios so popular in EPA risk assessments.

The mention of exposure based on pentachlorophenol ingestion on page 6-19 is also misleading given the fact that the rest of the paragraph is a discussion of exposure to 2,3,7,8-TCDF resulting from exposure to PCBs. In the next paragraph, PCDD exposure is discussed, if only to conclude that "no firm conclusions could be reached." The rest of

the paragraph is pure speculation, typified by the doublet "might possibly."

The second paragraph on page 6-20 contains further examples of unwarranted speculation. Although generation of PCDFs as by-products of the incomplete combustion of PCBs in fire-related situations has been demonstrated in the laboratory and is substantiated by measured levels of PCDFs in building fires, the other exposure scenarios in the paragraph are not realistic and certainly do not support the statement that "exposures should be investigated." The use of PCBs in casting waxes, carbonless copy papers, and heat transfer systems was voluntarily curtailed by 1972 and was expressly banned under TSCA in 1979. Such exposure scenarios are thus very unlikely. 7/

In sum, a consistent pattern exists in Chapters 4 and 6 of the HAD of over-estimation of the contribution of PCDF contamination of PCBs to potential human exposures. A complete rewrite of these sections of the report is necessary to reflect accurately both the actual concentrations of

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7/ In addition, there is an apparent misunderstanding of the chemistry and physical properties of PCDFs. The HAD evidences apparent surprise at the fact the PCDFs were not extracted by water from latex nipples, "unlike pentachlorophenol." Pentachlorophenol is a polar material with a measurable solubility in water, while PCDFs are insoluble in water -- thus it should clearly not be surprising that water is not a good extractant for PCDFs.

PCDFs in commercial U.S. PCBs and the limited current potential for human exposures to PCBs. Unless such changes are made in the HAD, it will inaccurately express unwarranted cause for concern about PCDF/PCB exposures.

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Attachment II

CONSIDERATIONS FOR ASSESSING POTENTIAL HUMAN RISK FROM EXPOSURES TO 2,3,7,8-TETRACHLORODIBENZODIOXIN

A Working Paper of the
Dibenzofurans/Dibenzodioxins
Panel of the Chemical Manufacturers Association

August 22, 1986

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CONSIDERATIONS FOR ASSESSING POTENTIAL HUMAN RISK FROM EXPOSURES TO 2,3,7,8-TETRACHLORODIBENZODIOXIN

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CONSIDERATIONS FOR ASSESSING POTENTIAL HUMAN RISK FROM EXPOSURES TO 2,3,7,8-TETRACHLORODIBENZODIOXIN

A Working Paper of the
Dibenzofurans/Dibenzodioxins
Panel of the Chemical Manufacturers Association

INTRODUCTION

Numerous regulatory initiatives are being driven by the Environmental Protection Agency's (EPA's) assessment of the potential human risk posed by 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD). Because EPA's Cancer Assessment Group has determined that a 1 in 1,000,0000 cancer risk exists for a Lifetime Average Daily Dose (LADD) as low as 6 femtograms (6 x 10^{-15} grams)/kilogram/day (EPA 1985), numerous regulatory initiatives have sought to control TCDD to extremely low levels.

By contrast, many other nations have derived human exposure control limits of 1 to 10 picograms (1 to 10 x 10^{-12} grams/kilogram/day). It is therefore important to recognize, as detailed in this paper, the relevant scientific evidence that demonstrates the advisability of revising EPA's LADD to fall in line with the views of international experts.

EPA has also employed a Toxicity Equivalency Factor (TEF) scheme to determine control limits for other chlorinated dibenzodioxins, as well as for chlorinated dibenzofurans. The Agency has further indicated the same

scheme may be applied to brominated dibenzodioxins and dibenzofurans. Because the TCDD toxicity determination is the base number from which these other control limits are derived, it is vitally important that the TCDD assessment be scientifically appropriate and technically supportable.

The driving regulatory force of EPA's extremely low recommended control limit for TCDD is symptomatic of a general over-concern about potential human toxicity of chlorinated dibenzodioxins and dibenzofurans. The United States and Europe have expended huge resources in an effort to determine if there has been any impact on human health of exposures to such substances, but have not found any obvious concerns given current limited exposures. Scarce and valuable resources are being devoted to examine non-obvious and less major concerns with full knowledge that zero impact can never be scientifically proven. There will always be uncertainty in the numbers, and additional effort can only reduce the uncertainty by small increments -- never eliminate it. At the same time, EPA is implementing ever more stringent rules and requirements for dibenzodioxins and dibenzofurans under FIFRA, TSCA, RCRA, and CERCLA.

CMA is concerned that there is no plan for deciding when society has reached a point of diminishing return on the use of national resources investigating alleged human problems related to dibenzodioxin and dibenzofuran exposure. Before further substantial expenditures are mandated, EPA

should reassess the health criteria upon which these regulatory initiatives are based. As one reviewer (Tschirley 1986) recently concluded:

> What the agency has not done -- and might be said to have a responsibility to do -- is to try to dispel the public's fear on the basis of the evidence that exposure to low concentrations of TCDD in the environment appears not to have serious chronic effects on human beings.

The importance of EPA's current determination of TCDD toxicity is illustrated by the fact that the control limits employed by EPA are about 1,000 times more restrictive than the levels determined by other international expert groups who have reviewed the same evidence. Specifically, its TCDD recommended human exposure control level is 160 to 1,600 times more restrictive than the levels recommended by various non-U.S. regulatory agencies and expert panels, including a Swiss expert group (Schlatter, et al., 1985); a Canadian expert panel (Ontario Criteria Document 1985); a West German expert group (Appel, et al., 1985); and a Dutch expert group (Van der Heijden, et al., 1982). The EPA control limits are also about 10 times more restrictive than a U.S. Food and Drug Administration assessment of the same data (FDA 1983).

Accordingly, the CMA Panel on Dibenzodioxins and Dibenzofurans has prepared this Working Paper discussing the

scientific evidence that has led other expert groups to disagree with EPA's assessment of TCDD. In this paper, we:

(1) Note the extensive human data on exposures to TCDD and their results, which find some toxic effects (especially chloracne) in highly exposed manufacturing workers and industrial accident victims, but no consistent data indicating any carcinogenic or other life-threatening diseases in humans.

(2) Review the extensive animal data on TCDD with special emphasis on the lifetime studies that evaluated chronic toxicity and carcinogenic potential. We also review the mechanistic and dose-response data, relied on heavily by other review panels, that indicate TCDD carcinogenicity is most appropriately considered a threshold-based phenomenon to which an "uncertainty" or "margin of safety" factor can be applied in deriving an acceptable human LADD.

I. EPIDEMIOLOGY STUDIES HAVE NOT DEMONSTRATED THAT TCDD CAUSES CANCER OR OTHER LIFE-THREATENING DISEASES IN HUMANS.

In the late 1970's concern that TCDD impurities in various pesticides caused cancer, especially soft tissue sarcomas, was raised by a series of Swedish epidemiology studies (Hardell 1981; Hardell, et al., 1982; Hardell and Sandstrom 1979; Eriksson, et al., 1981; Hardell and Eriksson 1981; Axelson 1980). More recent data from studies

of similarly exposed pesticide applicators in New Zealand (Smith, et al., 1983; Smith, et al., 1984) and Vietnam (U.S. Air Force 1983; U.S. Air Force 1985) and forestry and agricultural workers in Sweden (Wiklund & Holm, 1986) have found no such association.

In addition, critical scientific review of the original Swedish studies, as well as examination of results of studies of manufacturing workers exposed to TCDD in the U.S. (Cook, et al., 1980; Ott, et al., 1980; Zack and Suskind 1980; Zack and Gaffey 1983) and in Europe (Theiss, et al., 1982; Dallderup and Zellenrath 1983), have led most reviewers to conclude that there is no significant epidemiology evidence of an association between TCDD and human cancer or other life-threatening disease.

The Expert Committee (including Drs. O. Hutzinger, G. L. Plaa, S. Safe, E. Y. Spencer and B. Birmingham) that reported to the Ontario Canada Ministry of Environment in its Scientific Criteria Document on PCDDs and PCDFs, for example, concluded (at 1-13):

> Epidemiological studies to date do not present sufficient evidence to establish a causal relationship between exposure to PCDDs or PCDFs and a chronic human health effect relating to carcinogenesis, coronary disease or impairment of the immune system.

The American Medical Association's Council on Scientific Affairs (1984) similarly concluded that although many

adverse effects of dioxins had been found in exposed animals:

> Except for chloracne, ... TCDD has not demonstrated comparable levels of biologic activity in man; that is to say, no long-term effects on the cardiovascular and central nervous systems, the liver, the kidney, the thymus and immunologic defenses, and the reproduction function -- in the male, female or offspring -- has been demonstrated.

It is true that even this extensive body of epidemiologic data cannot prove an absence of risk for low level TCDD exposures, but no such body of data ever will exist. On the other hand, these data provide assurance that significant adverse human effects are unlikely at low exposures. These data, moreover, caution strongly against exaggerated interpretation of the laboratory animal evidence, as discussed next, to establish guidelines for human exposure limits.

II. LIFETIME STUDIES OF TCDD IN ANIMALS SHOW A CONSISTENT NO-OBSERVED-ADVERSE-EFFECT LEVEL OF AT LEAST 1 NANOGRAM/KILOGRAM/DAY.

Given the absence of data indicating long-term debilitating effects of TCDD exposure in man, most expert review groups and governments have sought to establish human control limits based on the substantial animal toxicity data base. TCDD has been tested in a variety of toxicity tests using various animal species.

The animal studies considered most appropriate for determining lifetime exposure control limits for man are the lifetime animal studies evaluating chronic toxicity and carcinogenic potential. These long-term animal studies consistently have shown a no-observed-adverse-effect level (NOAEL) of at least 1 nanogram/kilogram/day. The preponderance of data thus supports derivation of a lifetime human exposure control limit of approximately 10 picograms/kilogram/day based on the existence of these NOAELs and application of a 100 times "uncertainty" or "margin of safety" factor.

A. Animal Toxicity Studies Have Adequately Defined the No-Observed-Adverse-Effect Levels for Non-Cancer Effects of TCDD.

TCDD has been administered to a variety of species in standard toxicologic studies. Apart from the lifetime animal studies discussed next, the studies of reproductive and teratogenic effects are most significant to human health risk assessment. The Ontario Expert Committee summarized those studies (Ontario Criteria Document 1985 at 3-132):

> Data from teratological (reproductive effects) studies in rats and mice indicate that 2,3,7,8-T_4CDD causes embryolethality and/or fetotoxicity. A 3-generation reproduction study of rats was associated with decreased fertility and neonatal survival in the first and second generations.

> NOELs for these teratogenic and reproductive effects over the range 0.001 to 0.03 ug 2,3,7,8-T_4CDD/kg have been reported.

As the Ontario Report further notes, TCDD has been found to affect the fetus only at doses equivalent to those which produce adult toxicity.

An additional margin of safety should be inherent in any extrapolation from TCDD animal results given the data indicating the lesser sensitivity of humans. Reviews by both Neal (1983) and Tschirley (1986) found man to be less sensitive to acute TCDD effects than tested laboratory animals, including rats, mice and guinea pigs.

Data concerning actual human exposures to TCDD confirms this analysis. Perhaps the most valuable human data for evaluating teratogenic, fetotoxic, and reproductive risk are those obtained in the Seveso region following the 1976 accident. Unlike other human exposures to TCDD, Seveso involved severe exposure to women of child-bearing age. The Seveso exposures caused no increase in spontaneous abortion, birth defects, or any abnormalities in the growth and development of children in utero at the time of the accident. (Marni, et al., 1982.) In addition, the Kligman study which demonstrated that humans voluntarily exposed to TCDD by dermal application suffered no adverse effects at doses well above those which produce chloracne in test rabbits, also suggests that people are less sensitive than test animals.

Taking all of these considerations into account, the application of a 100-fold "uncertainty" or "safety margin" for TCDD to the NOAEL defined in the animal studies is a conservative approach to establishment of lifetime human control limits.

B. Lifetime Animal Studies of Chronic Toxicity/Carcinogenicity Have Established a No-Observed-Adverse-Effect Level for TCDD of at Least 1 Nanogram/Kilogram/Day.

Almost all reviews of the evidence of TCDD carcinogenicity in animals have focused on the Kociba, et al. (1978) study in Sprague-Dawley rats. This study, in agreement with other bioassays conducted in other rat strains and in mice, established a lifetime NOAEL for chronic toxicity/carcinogenic response of at least 1 nanogram/kilogram/day. The Kociba study is considered the most pertinent animal study for deriving long-term human exposure control limits because it was conducted with state-of-the-art procedures to evaluate the dose-response pattern for both chronic toxicity and potential carcinogenicity.

Detailed pathologic examination in the Kociba study of an extensive list of tissues from rats given the high dose level of 0.1 µg/kg/day revealed the presence of toxicologic lesions. The liver was the primary target organ. Only at this highest and overly-toxic dose level, which exceeded the maximal tolerated dose and shortened the lifespan of female

rats, was there a carcinogenic response following lifetime treatment.

At the intermediate dose level of 0.01 µg/kg/day, there was no definite carcinogenic response, despite the fact that this dose was high enough to produce some toxicity. In the primary target organ, the liver, the maximal response was the formation of hyperplastic nodules originating from the liver cells. At the lowest dose level of 0.001 µg/kg/day, there was no carcinogenic response, and no other adverse effect was associated with this lifetime exposure to TCDD.

NCI bioassay results in Osborne-Mendel rats are in agreement with the results observed in the Sprague-Dawley rats in the Kociba study. In the NCI study, TCDD induced liver neoplasms in female rats only at the high dose of 0.07 µg/kg/day. No carcinogenic effects were observed at a daily dose of 0.0014 µg/kg/day.

TCDD has also been administered to mice in long-term studies. The report is ambiguous (Toth 1979), but even if a true carcinogenic response was produced at the middle dose level of 0.7 µg TCDD/kg/week, this dose is equivalent to the daily dose of 0.1 µg/kg/day demonstrated to be carcinogenic to rats in the Kociba study. The Toth study found no carcinogenic effects in mice at the lower dose of 0.03 µg/kg/day.

Upon reviewing these lifetime studies, the Canadian experts concluded (at 1-11):

> Those studies involving oral administration (2,3,7,8-T_4CDD is most toxic using this route of exposure) have reliable dose-response data and indicate clear NOELs (0.001 to 0.0014 µg/kg/day) for tumour incidence.
>
> In general, 2,3,7,8-T_4CDD administration was associated with relatively few histologically different tumour types. The onset of these tumours appeared late in the study and generally at similar time to onset of similar, but less frequent, tumors in the control animals.
>
> Although 2,3,7,8-T_4CDD has been found to be carcinogenic in rats and mice, it is difficult at this stage to predict the ability of this compound to induce tumours in humans.

In sum, long-term cancer studies of TCDD in several strains of both rats and mice have shown neither chronic toxicity nor carcinogenicity at lifetime doses of 1 nanogram/kilogram/day.

C. The Animal Bioassays and Other Studies on the Mechanism of Action of TCDD Show That the Carcinogenic Response Observed in Animals Is Reflective of a Promotion Mechanism Rather than a Genotoxic Mechanism, Indicating Lifetime Human Exposure Control Limits Are Most Appropriately Derived on an "Uncertainty" or "Margin of Safety" Basis.

Recent reviewers have deliberated on the proper interpretation for human risk assessment of carcinogenic responses from administration of TCDD to animals. Each of these assessments has focused on the absence of genotoxic potential and have identified an indirect promotion mechanism as the likely cause of the tumors seen in laboratory animals.

Accordingly, these reviewers have all applied an "uncertainty" or "margin of safety" analysis to the NOAELs found in the definitive and sensitive long-term animal studies to determine human control limits.

Many scientists, including Pereira (1985) of EPA, have recognized the need for differentiation between carcinogens that mechanistically interact directly with DNA (genotoxic) and those that do not (non-genotoxic). Initiators are genotoxic agents that penetrate the cell nucleus and damage or alter the DNA genetic material of the cell. By contrast, promoters or modifying agents produce a carcinogenic response as an indirect effect of organ toxicity or by impairing the body's defense mechanisms against carcinogenic initiators.

Current theory of some scientists assumes that the genotoxic potential of initiators may indicate the lack of a threshold for carcinogenic effects. For promoters, on the other hand, it is generally agreed that a threshold for the carcinogenic response exists (Pereira 1985). This threshold would be indicated by the dose-response data defining the NOAEL for organ toxicity or impairment of bodily defense mechanisms. In the absence of detailed quantitative information about the exact mechanism of action, most authorities thus recommend, in line with an increasing scientific consensus, using a conservative "uncertainty" or "margin of safety" factor to protect human health.

These considerations apply to TCDD, as it is generally agreed it is not a cancer initiator. Short term assays indicate TCDD is not genotoxic. Various mechanisms of TCDD carcinogenicity have been investigated, each of which points to the conclusion that TCDD is acting as a promoting or modifying agent, for example, through tissue damage found at the same doses at which a carcinogenic response is observed.

TCDD does not covalently bind to DNA or RNA (Poland and Glover 1979), does not induce DNA repair synthesis (Althaus, et al., 1982), and is essentially devoid of any mutagenic activity (Wassom 1977; Kociba 1984). In an in vivo cytogenetics screening study conducted for FDA, Green and Moreland (1975) found no chromosomal aberrations in the bone marrow of male rats treated with TCDD. A subsequent study by Green, et al., (1977), limited by the fact that controls were not used, reported "only weakly positive" results, while an earlier study by Khera and Ruddick (1973) was negative for dominant lethal mutations in Wistar rats.

In addition, Poland and Glover (1979) tested the DNA binding potential of TCDD in the liver of Sprague-Dawley rats and found that the maximum potential DNA binding by TCDD is 10,000 to 1,000,000 times less than that of most chemical carcinogens. They thus concluded, "it is unlikely that TCDD-induced oncogenesis is through a mechanism of covalent binding to DNA and somatic mutation."

Taken together, these studies provide strong evidence that TCDD does not interact with or damage cellular genetic material to produce heritable genetic changes. TCDD is therefore unlikely to cause cancer via a genotoxic mechanism.

As the Ontario Expert Committee concluded (at 1-10):

> 2,3,7,8-T4CDD does not form DNA adducts, i.e., it does not chemically bind to DNA. Therefore it is unlikely to cause mutations by chemically changing DNA directly.
>
> This compound has been extensively tested in in vitro microbial mutagenicity tests, such as the Ames test, with mainly negative results. More limited information is available from in vitro and in vivo mammalian test systems for mutagenicity and clastogenicity.

On the other hand, considerable evidence indicates that TCDD acts through one or more non-genetic mechanisms as a promoting or modifying agent. These mechanisms depend upon the occurrence of TCDD-induced toxicity or physiological alterations which, in turn, enhance or promote the growth of tumors. Dose levels below those needed to produce toxicity or physiological alterations are unlikely to increase tumor incidence.

The lifetime study of TCDD rats by Kociba, et al. (1978), for example, showed a carcinogenic response only at dose levels that induced toxicity. As detailed above, the lowest dose level (0.001 µg/kg/day) was a NOAEL for such toxicity, and no carcinogenic effects were observed. It is

well-known that recurrent tissue injury and repair commonly leads to tumors in affected organs of laboratory animals. Thus, one very likely mechanism for TCDD production of tumors is recurrent tissue injury and regenerative repair within the affected organ.

The non-genetic mechanisms involved in the intermediate toxic or physiological effect occurred only above the 0.001 µg/kg/day NOAEL defined in the lifetime toxicity studies. Accordingly, with lifetime exposure levels at or below 0.001 µg/kg/day, one would expect no intermediate toxicity or physiological alteration and therefore no tumor promotion.

The subsequent study by Pitot, et al. (1980), supports the non-genotoxic interpretation. Pitot tested "the hypothesis that the liver cancer associated with chronic administration of TCDD might arise from the promoting activity of the compound, presumably stimulating cells already spontaneously initiated by dietary and other environmental carcinogens." The strain of "rats and the TCDD dosage regimen" used "were chosen to resemble closely the conditions" in the Kociba study. Dr. Pitot concluded: "TCDD is a potent promoting agent for hepatocarcinogenesis."

In addition to the promotion of liver tumors in rats reported by Pitot, another study by Poland, et al. (1982) reported TCDD to be a promoter of skin tumors in hairless mice after treatment with known cancer-initiating agents

such as N-methyl-N-nitrosoguanidine (MNNG) or dimethylbenzonthrocene (DMBA). Another more recent study by Abernethy, et al. (1984) reported TCDD to be a promoter of the transformation of $C_3H/10T$ 1/2 cells that had been initiated with MNNG. Overall, these studies provide compelling evidence that TCDD acts as a cancer promoter in animal bioassays.

In sum, strong evidence exists that TCDD acts not by a genotoxic mechanism but rather by one or more non-genetic mechanisms that promote or enhance tumor growth. As the Canadian expert group concluded:

> Results of short term genotoxicity tests suggest that 2,3,7,8-T_4CDD is not genotoxic in a classical sense and does not act directly on the chromosomes or the DNA molecule itself. Consequently, 2,3,7,8-T_4CDD appears to produce tumours in rodents by an indirect mechanism.

The Ontario expert committee thus concluded that an acceptable LADD for TCDD could be derived by applying an "uncertainty" or "margin of safety" factor of 100 to the NOAEL of 1 nanogram/kg/day. This LADD of 10 picograms/kg/day is, as noted above, consistent with other Swiss, West German and Dutch expert conclusions.

In sum, based on detailed review of the available data, international experts have determined the appropriate level of concern for lifetime exposure to TCDD is 1,000 times higher than EPA has determined. Given the many Agency initiatives being driven by this unduly high estimate of TCDD

potency, it is imperative that EPA reassess its 1985 determination.

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INTERIM PROCEDURES FOR ESTIMATING RISKS ASSOCIATED WITH EXPOSURES TO MIXTURES OF CHLORINATED DIBENZO-p-DIOXINS AND DIBENZOFURANS (CDDs AND CDFs)

DRAFT

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INTERIM PROCEDURES FOR ESTIMATING RISKS ASSOCIATED WITH EXPOSURES TO MIXTURES OF CHLORINATED DIBENZO-p-DIOXINS AND -DIBENZOFURANS (CDDs and CDFs) 1/

I. SUMMARY

The U.S. Environmental Protection Agency (EPA) is often confronted with the need to determine the risks associated with exposure to materials such as soot, incinerator fly ash, industrial wastes, and soils which contain complex mixtures of chlorinated dibenzo-p-dioxins (CDDs) and chlorinated dibenzofurans (CDFs). Recognizing the public and toxicological concern generated by these chemicals and the significant gaps in our ability to evaluate the human health potential of these compounds by existing procedures, the CDD/CDF Technical Panel of the Risk Assessment Forum (Forum) is recommending an interim method to aid in the assessment of the human health risks posed by mixtures of CDDs/CDFs until data gaps are filled.

The Technical Panel has reviewed a spectrum of approaches for making such assessments, consistent with EPA's Guidelines for the Health Risk Assessment of Chemical Mixtures and has concluded that a direct biological assessment of the toxicity of complex mixtures of CDDs/CDFs is preferred. A validated bioassay that can plausibly be applied to such mixtures is not now available, although promising research is in progress in the area. An alternative approach involves explicit analysis and toxicological determination of each of the constituent CDD/CDF congeners. The data required for such an approach also need to be developed and are not likely to be generated soon. The Forum therefore concludes that, as an interim science policy measure, a reasonable estimate of the toxic

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1/ See Appendix A for the nomenclature and conventions used in this paper.

risks associated with a mixture of CDDs/CDFs can be made by taking into account the distribution of CDD/CDF congeners or homologues and the likely relative toxicity of these compounds. This document describes the recommended interim procedure for generating the "2378-TCDD equivalence" of complex mixtures of CDDs/CDFs, based on congener- or homologue-specific data, and for using such information in assessing risk. (The recommendations are summarized in the rightmost column of Table 1.)

The Forum acknowledges that this procedure is not based on a thoroughly established scientific foundation. Instead, the approach represents a consensus recommendation for interim science policy, subject to change as additional data are available. The approach is judged to be applicable to mixtures of CDDs/CDFs, but should not be construed as being applicable as well to mixtures of other chemicals.

The basis of this approach, i.e., the assignment of toxicity equivalence factors (TEFs) is subject to revision as new scientific data become available in the future. Consequently, risk assessors and risk managers are urged to use informed discretion, noting specific problems on a case-by-case basis, when deciding to which situations the procedure can be applied. The Forum urges the support of research to broaden the scientific basis for this approach and develop the more preferred approaches.

II. THE NEED FOR A PROCEDURE FOR ASSESSING THE RISK ASSOCIATED WITH EXPOSURE TO COMPLEX MIXTURES OF CDDs/CDFs

During the late 1970s, the Agency was faced with assessing the human health significance of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD).

In preparation for the cancellation hearings for the herbicides 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and Silvex, the Agency generated risk assessments for several toxic responses for 2,3,7,8-TCDD. The quantitative cancer risk assessment developed by the Carcinogen Assessment Group was later adapted for use in the Water Quality Criteria (WQC) Document for 2,3,7,8-TCDD (U.S. EPA, 1984a). In addition to carcinogenicity concerns, the WQC document contains an assessment of systemic toxicity based on reproductive effects resulting from exposure to 2,3,7,8-TCDD.

Later, it became clear that exposure situations exist in the country which involve more than 2,3,7,8-TCDD alone. Data on emissions from combustion sources (e.g., hazardous waste and municipal waste incinerators) and contents of waste from certain industrial production processes indicate that the majority of the 75 CDDs and 135 CDFs can be detected in the environment.

In recent years, the reporting of at least homologue-specific data for the CDDs and CDFs has become commonplace, and the Agency has taken some steps to address the significance of these findings. For example, the current draft of the Health Assessment Document for Polychlorinated Dibenzo-p-Dioxins prepared for the Office of Air Quality, Planning, and Standards (U.S. EPA, 1984b) contains a quantitative risk assessment for a mixture of hexachlorodibenzo-p-dioxins (HxCDDs) based on carcinogenicity studies conducted by the National Cancer Institute. These concerns have also led to regulatory action; e.g., several industrial wastes containing tetra-, penta-, hexachlorodioxins, and -dibenzofurans were recently designated by the Agency as EPA hazardous wastes.

Faced with increasing amounts of isomer- and homologue-specific data, and recognizing the significant potency and structure-activity relationships exhib-

ited in in vivo and in vitro studies of CDDs and CDFs, the Technical Panel perceives a need to address more generally the potential risks posed by the congeners other than 2,3,7,8-TCDD and the mixture of HxCDDs. 2/ Detailed consideration of the toxicity of the vast majority of the CDDs/CDFS is limited by the lack of a complete toxicological data base on most of the congeners. Further, it is unlikely that many long-term test results will be available soon. For example, research on 2,3,7,8-TCDD has been under way for more than two decades at an estimated cost of more than one hundred million dollars. Although this chemical has been investigated to a much greater extent than any of the other CDDs/CDFs, unanswered questions remain. Therefore, the Forum believes that an interim science policy position should be adopted for use in assessing risks associated with CDD/CDF mixtures, until more definitive scientific data are available.

III. APPROACHES TO HAZARD ASSESSMENT FOR CDD/CDF MIXTURES

A. The Ideal Approach -- Long-Term, Whole-Animal Toxicity Assay of Mixtures

Under ideal conditions, an assessment of the toxicity of a mixture of chemicals is best accomplished by direct evaluation of its toxic effects, e.g., by determining the effects of chronic exposure in an experimental animal (U.S. EPA, 1985). Such an assessment is time-consuming and costly and would theoretically

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2/ In the early 1980s, the Agency developed a method for an approximate assessment of the risks of the emission of CDDs/CDFs associated with the high-temperature incineration of PCBs and combustion of municipal waste (U.S. EPA, 1981; U.S. EPA, 1982); see Table 1. The procedure presented in this document is a refinement of that approach. A comparison of a variety of methods is included in Appendix B.

have to be performed for each of the many mixtures of environmental importance. Therefore, this idealized approach would cause unacceptable delays in addressing the potential health risks associated with exposures to CDD/CDF mixtures.

B. A Promising Approach -- Short-Term, Biological Assay of Mixtures

An alternative, and perhaps more achievable, approach to hazard assessment of a mixture is a short-term assay that indirectly provides a measure of the mixture's potential toxicity. In the case of mixtures containing CDDs and CDFs, short-term assays are under development that directly determine the 2,3,7,8-TCDD-like response which could be used as a measure of the toxicity of the mixture as a whole. Such assays, which take advantage of the similar toxic end points induced by CDDs and CDFs, and have been used to assess the potential health hazards of exposure to CDD/CDF-contaminated soot from PCB fires (Eadon, 1982; Gierthy and Crane, 1984; Gravitz et al., 1983), and for predicting the potential toxicity of incinerator fly ash (Rizzardini, et al., 1983; Sawyer, et al., 1983).

The development of such "mixture assays" is progressing rapidly. While additional work is required to more fully validate the assay findings for specific toxic end points, especially chronic effects, and aspects of pharmacokinetics need to be considered, an increasing body of data demonstrate correlations with subchronic effects of CDDs/CDFs (Safe et al., 1985). The Forum, recognizing the importance of this approach in implementing its regulatory strategy for 2,3,7,8-TCDD-like chemicals, strongly encourages research in this area.

C. A Reductionist Approach -- Additivity of Toxicity of Components

In the absence of a fully developed "mixture assay," the components in a mixture of CDDs and CDFs could theoretically be identified and quantified

by analytical chemists. Then the toxicity of the mixture could be estimated by adding the toxicity contributed by each of its components. In the case of most environmental mixtures, however, this method would be of limited value since congener-specific analyses for the 75 CDDs and 135 CDFs potentially present in the mixture are seldom available. In addition, there is little information available on the toxic potency of most of these congeners. Therefore, this approach is not viable at this time; nor is it likely to be feasible in the near future.

D. An Interim Approach -- 2378-TCDD Toxicity Equivalence Factors (TEFs)

The Forum recommends a fourth alternative for estimating the risks associated with exposure to complex mixtures of CDDs/CDFs. In this approach, as in approach C above, information is obtained on the concentrations of homologues and/or congeners present in the mixture. Then, using the available toxicological data and reasoning on the basis of structure-activity relations, the significance of the exposure to each of the components is estimated and expressed as an "equivalent amount of 2378-TCDD." Combining this information with hazard information on 2,3,7,8-TCDD, and assuming additivity of effects, the risks associated with the mixture of CDDs/CDFs can be estimated if exposure is known. Key to the approach are the 2378-TCDD Toxicity Equivalence Factors (TEFs) which are derived in Section IV.

The general approach using TEFs as outlined here is not unique; several organizations have used similar approaches (see Table 1).

At one extreme, all CDDs/CDFs could be assumed to be as toxic as 2,3,7,8-TCDD (all TEFs = 1). This position is not recommended since the limited long-term data (2-year cancer bioassays) on 2,3,7,8-TCDD and a mixture of 2378-HxCCDs

(and the greater body of short-term data on many CDDs/CDFs) indicate that such an assumption is overly conservative. At the other extreme one could totally ignore the presence of CDDs/CDFs other than those for which adequate long-term data are available (most TEFs = 0). This position is not recommended in light of the similar toxic properties of several of these compounds and the structure-activity relationship demonstrated for effects resulting from less than lifetime exposures.

Instead, the Forum recommends that the TEF procedure presented below be adopted as a matter of science policy on an interim basis, subject to revision as new experimental data become available. Based on the available scientific information, the Forum believes that this approach represents an appropriate means of approximating the potential risk of exposure to mixtures of CDDs and CDFs for purposes of risk management.

The approach will enable the Agency to deal with many, but not all, of its problems; e.g., assigning priority to Superfund sites, estimating the extent to which a hazardous waste site should be cleaned up, guiding decisions on which manufacturing wastes can be delisted as EPA hazardous wastes, and estimating risks associated with the emission of CDDs/CDFs from combustion sources.

The remainder of this document discusses the TEF approach in greater detail, illustrates its use in risk assessment, and identifies additional research, the results of which would provide information for adjustments to this interim approach.

IV. THE 2378-TCDD TOXICITY EQUIVALENCE FACTORS (TEFs) APPROACH TO ASSESSING THE TOXICITY OF COMPLEX MIXTURES OF CDDs/CDFs

2,3,7,8-TCDD is one of 75 CDDs. Exceptionally low doses of this compound elicit a wide range of toxic responses in many animals., e.g., adverse reproduc-

tive effects, thymic atrophy, and a "wasting syndrome" leading to death. The EPA's Carcinogen Assessment Group (CAG) has determined that there is sufficient evidence to conclude that 2,3,7,8-TCDD and a mixture of two 2378-HxCDDs are probable human carcinogens. The CAG quantitative assesssment indicates that these chemicals are among the most potent animal carcinogens evaluated by the Agency to date. Limited data suggest that some of the other CDDs may have other toxic effects similar to those of 2,3,7,8-TCDD, again at very low doses.

Moreover, these toxicity concerns are not restricted to CDDs. Limited experimental data, supplemented by structure/activity relationships in in vitro tests that are correlated with in vivo toxic effects of CDFs indicate that some of these compounds exhibit "2,3,7,8-TCDD-like" toxicity (Bandiera et al., 1984; Okey et al., 1984; Safe et al., 1985).

The biochemical mechanisms leading to the toxic response resulting from exposure to CDDs and CDFs are not known in detail. However, experimental data have accumulated which suggest that an important role in the development of systemic toxicity resulting from exposure to these chemicals is played by an intracellular protein, the Ah receptor, the putative product of a gene locus designated Ah. This receptor binds halogenated polycyclic aromatic molecules, including CDDs and CDFs. It has been postulated that the Ah locus controls several pleiotropic responses: a limited, but widely expressed gene complex that includes the structural genes for AHH expression, and, in a few organs, such as skin and thymus, a second gene complex regulating cell proliferation and differentiation (Knutson and Poland, 1980; Neal, et al., 1982; Greenlee et al., 1985a).

In several mouse strains, the expression of toxicity of 2,3,7,8-TCDD-related compounds, including cleft palate formation, liver damage, effects on

body weight gain, thymic involution, and chloracnegenic response, has been correlated with their binding affinity for the Ah receptor, and with their ability to induce several enzyme systems, some of which have been linked to the expression of carcinogenicity (Poland and Knutson, 1982; Bandiera et al., 1984; Madhukar et al., 1984; Poland et al., 1985; Safe et al., 1985; Vickers et al., 1985). Structure-activity studies also link the enhanced in vitro cell differentiation caused by these compounds to the presence of the Ah receptor (Greenlee et al., 1985b).

However, it has also been noted that the cytosolic receptor concentration alone may not be the sole determinant of the capacity for AHH induction (Neal, 1985; Okey and Vella, 1984). In interspecies comparisons there are poor correlations between the amount of cellular Ah receptor, its ability to bind 2,3,7,8-TCDD, and AHH induction (Denison and Wilkinson, 1985; Gasiewicz and Rucci, 1984; Neal, 1985); and in the mouse the development of TCDD-induced liver toxicity cannot be ascribed solely to the presence of the Ah receptor (Greig et al., 1984).

A recent review concludes that although there are inconsistencies across species in the Ah receptor's being the sole mechanism of toxicity of CDDs and CDFs, the data suggest that the binding of these compounds to the receptor is in some way related to some of the biological effects they cause in experimental animals (Neal, 1985)

Table 2 summarizes information on a variety of end points elicited by CDDs/CDFs: acute toxicity, carcinogenicity, reproductive effects, receptor binding, enzyme induction, and in vitro cell transformation. For ease of comparison, the data are normalized to unity for 2,3,7,8-TCDD. For example, 2378-HxCDDs have about 5% the Ah receptor binding strength of 2,3,7,8-TCDD. Their repro-

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ductive toxicity and carcinogenic potency are, respectively, about 1% and 4% that of 2,3,7,8-TCDD. Kociba and Cabey (1985) recently presented similar data.

The structure/activity generalizations based on the data in Table 2 support the generalizations in the literature concerning the congeners that are most likely to be of toxic concern (Poland and Knutson, 1982; Gasiewicz and Rucci, 1984; Bandiera et al., 1984). That is, congeners that are substituted in the lateral 2, 3, 7, and 8 positions are likely to exhibit toxic effects at lower doses than other congeners. This includes the 15 tetra-, penta-, hexa- and heptachlorinated CDDs and CDFs listed in Table 3. 3/

3/ The Technical Panel is aware that some investigators (e.g., Grant, 1977; Olie et al, 1983; Commoner et al., 1984; and Ontario, 1982, 1984) have broadly defined congeners of concern to include those tri- to hepta- congeners which are substituted with at least three chlorines in the four lateral (2, 3, 7, and 8) positions. The toxicity data (Table 2) do not argue strongly for this extended range of concern. Further, the increased level of complexity invoked by including these additional congeners is to suggest a greater level of accuracy and resolution than the Technical Panel believes is presently warranted by the TEF approach.

The Technical Panel is also aware that receptor binding data suggest a relatively high potential toxicity for 1,2,4,6,7-PeCDF. Examination of stereochemical models show that the 4 and 6 positions of CDFs exhibit partial overlap with the lateral chlorine groups of 2,3,7,8-TCDD (Bandiera et al., 1984). However, this increased receptor binding activity is not reflected in an increased potency of 1,2,4,6,7-PeCDF as an enzyme inducer (cf. Table 2), an end point which has been shown to correlate with subchronic toxicity (Safe et al., 1985). Therefore, the Technical Panel is treating 1,2,4,6,7-PeCDF as a "non-2378-congener" at this time; however, additional data could lead to a change in this position.

1,2,3,6,7- and 2,3,4,6,7-PeCDF are almost as potent as 2378-PeCDF in the induction of AHH activity in human lymphoblastoid cells in vitro (see Table 2). However, because this assay seems to yield relative potencies that do not agree with other short-term tests, and because dose-response data are not available for this assay, these data are not included in the overall evaluation at the present time.

The "2378-TCDD equivalence factors" (TEFs) listed in Tables 1 and 3 were assigned using the following criteria:

1. Information on carcinogenic potency based on long-term animal studies takes precedence.

2. Where carcinogenic activity has not been demonstrated, information on systemic effects is used in the following manner:

   a. Data on reproductive effects take priority because of the significance of the end point in humans and because the estimated human exposure levels potentially resulting in reproductive and carcinogenic effects are very similar.

   b. Data on acute lethality is accorded very low priority since a correlation between LD_{50}s chronic effects has not been established for these compounds.

   c. The data currently available on systemic effects (e.g., body or organ weight losses, hepatotoxicity) are not sufficient for use in developing TEFs. Such data are used to provide qualitative support for these assignments.

3. In the interpretation of the data from in vitro test systems, more weight is placed on data from receptor binding interaction and oxidative enzyme induction, because for these effects the correlations with in vivo systemic effects (immunotoxicity, body weight loss) are strongest.

The above criteria were applied as follows:

1. Since the primary concern is with chronic effects, the relative carcinogenicity responses (Table 2) for 2,3,7,8-TCDD and the mixture of two 2378-HxCDDs 4/ were used to generate the TEF for 2378-PeCDD.

   The TEF for 2378-PeCDD (0.5) is the arithmetic mean of the carcinogenic potency values for 2,3,7,8-TCDD (1) and 2378-HxCDDs (0.04). Data on receptor binding, enzyme induction, and cell keratinization generally support this value.

2. 2,3,7,8-TCDF is assigned a TEF of 0.1 primarily because it is 1 to 2 orders of magnitude (OMs) less potent than 2,3,7,8-TCDD in reproductive toxicity tests. Also, it is about one OM less potent than 2,3,7,8-TCDD in the in vitro tests.

3. The 2378-PeCDF congeners are assigned a TEF of 0.1 due to the responses seen in in vitro tests. Greater reliance was placed on the animal

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4/ See Appendix A, item 6, for explanation of notation.

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enzyme induction studies due to the more significant correlations observed between this end point and subchronic responses than have been observed with the receptor binding end point. The human cell data were accorded less weight because these experiments were conducted at only one exposure concentration.

4. Because in vitro data in general show HxCDFs to be about one tenth as potent as PeCDFs, their TEF is assigned a value of 0.01 (0.1/10). Further, the data generally suggest that CDFs are somewhat less toxic than the analogous CDDs. Therefore, the TEF for 2378-HxCDFs should be less than that of the 2378-HxCDDs (0.04).

5. The 2378-HpCDDs and 2378-HpCDFs are assigned TEFs 3 OM less than that for 2,3,7,8-TCDD because the enzyme induction potencies of these congeners differ from that of 2,3,7,8-TCDD by about this factor.

6. Based on the data in Table 2, the non-2378-substituted isomers are 1 to 3 OMs less potent than the 2378-substituted isomers. Since these data are limited to in vitro systems, a factor of 0.01 is applied to the non-2378-substituted, as compared to the 2378-substituted congeners.

With the exception of 2,3,7,8-TCDD, the 2378-HxCDDs, and 2378-TCDF, the TEFs are not based on the results of major animal (reproductive, carcinogenic) studies. Generally, TEFs are based on estimates of the relative toxicity in in vitro tests whose relationship to the chronic effects of concern is largely presumptive. However, as discussed above, studies on systemic effects continue to reinforce the view that the short-term assays provide important fundamental information on the toxicity of the CDDs/CDFs.

In summary, the Forum concludes that there is a sufficiently plausible basis for the TEF approach of estimating risks associated with exposures for CDDs/CDF and recommends that the Agency adopt the approach, on an interim basis, as a matter of science policy. The TEFs should be revised as additional scientific information is developed. It should be noted that this general approach to estimating such CDD/CDF risks has been taken by other regulatory groups (see Table 1 and Appendix B).

V. APPLICATIONS TO RISK ASSESSMENT

In general, an assessment of the human health risk of a mixture of CDDs and CDFs, using the TEF approach, involves the following steps:

1. Analytical determination of the CDDs and CDFs in the sample.
2. Multiplication of congener concentrations in the sample by the TEFs in Table 1 to express the concentration in terms of 2378-TCDD equivalents.
3. Summation of the products in step 2 to obtain the total 2378-TCDD equivalents in the sample.
4. Determination of human exposure to the mixture in question, expressed in terms of 2378-TCDD equivalents.
5. Combination of exposure from step 4 with toxicity information on 2,3,7,8-TCDD (usually carcinogenicity and/or reproductive effects) to estimate risks associated with the mixture.

In cases in which the concentrations of the 15 congeners of concern are known:

2378-TCDD Equivalents = (TEF of each 2378-CDD/CDF congener x the concentration of the respective congener) + (TEF of each non-2378 CDD/CDF congener x the concentration of the respective congener)

Samples of this calculation for several environmental mixtures are provided in Table 4.

In cases where only the concentration of homologous groups is known, i.e., no isomer-specific data are available, different approaches are possible. For example, the assumption that the 2378-congeners of concern constitute all of the CDDs and CDFs present in the mixture is likely to provide an upper-bound, most conservative estimate of the toxicity. Alternatively, one could assume

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that the occurrence of each of the congeners in the mixture has equal probability (Olie et al., 1983; Commoner et al., 1984). For instance, 2,3,7,8-TCDD is one of 22 possible TCDDs and would constitute about 4% of a mixture of isomers occurring with equal probability. In other situations particular knowledge of chemical reaction parameters, process conditions, and results from related studies (e.g., congener distributions in emissions from combustion sources) might enable one to estimate the relative occurrence of 2378-congeners. However, one must be careful to explicitly explain and justify whatever assumptions are made. Table 5 illustrates the results obtained using different methods to estimate the proportion of 2378 to non-2378 isomers in the absence of analytical data for individual isomers.

The calculated 2378-TCDD equivalents can then be used to assess the health risk of a mixture. As an explicit example, consider a municipal solid waste (MSW) combustor whose particulate emissions, the CDD/CDF mixture in question, are the same as the electrostatic precipitator (ESP) catch cited in columns 5 and 6 of Table 4. The sample is estimated to contain 32 ppb 2378-TCDD equivalents; i.e., 32 picograms of 2378-TCDD equivalents per milligram of mixture. Suppose that an exposure analysis indicates that a person living downwind from the incinerator receives an average daily dose of 1 ng of the mixture/kg body weight resulting from inhalation (i.e., without consideration of other possible routes of exposure). This exposure estimate is combined with the upper-bound carcinogenic potency of 2,3,7,8-TCDD (1.6×10^5 per mg/kg-day [U.S. EPA, 1984c]) to generate the upper 95% limit of the excess risk of developing cancer (from inhalation exposure alone) for a person living downwind from the facility emitting the mixture under consideration, assuming lifetime exposure:

Upper 95% limit of excess cancer risk resulting from inhalation exposure

= [potency] x [exposure]

= [1.6 x 10^5 per mg 2,3,7,8-TCDD/kg-day] x

[32 pg TCDD/mg mixture x 10^{-9} mg 2,3,7,8-TCDD/pg x 1 ng mixture/kg-day

x 10^{-6} mg mixture/ng mixture]

VI. COMPARISON OF TEF APPROACH WITH RESULTS OF BIOLOGICAL TESTING

A limited number of *in vivo* and *in vitro* approaches have been employed in assessing the toxicity of complex mixtures of CDDs and CDFs. While the results from these attempts are not definitive, it is instructive to compare those results with the results from the TEF approach proposed here.

Eadon et al. (1982) investigated the toxicity of CDD/CDF-contaminated soot associated with a fire involving PCB-containing electrical equipment. Using the results from acute *in vivo* toxicity (LD_{50}) studies in which the soot was the test substance, the researchers determined that it had the acute toxicity expected of material containing about 60 times the amount of 2,3,7,8-TCDD actually found by GC/MS analysis.

Table 5 illustrates the results of employing the TEF approach through three different procedures, each of which depends upon the results of GC/MS analysis of the soot. In the first instance (A, in Table 5), the analytical data have been consolidated to totals within a homologous class. These concentrations are treated as if they consisted completely of 2378-members of the class and, therefore, are multiplied by the TEF appropriate for the 2378-members of the class. The resulting estimate of 2378-TCDD equivalents by this procedure is about 80.

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In procedure B the assumption is made that the occurrence of each of the congeners in a homologous class is equally probable; e.g., the concentration of 2,3,7,8-TCDD is 1/22 (about 5%) of the concentration of the total TCDDs. This approach leads to an estimate of the total 2378-TCDD equivalents of 8.

A rather unique data base exists in the case of the soot from this fire in that an extensive isomer-specific analysis of the sample is available (as cited in Des Rosiers, 1984). Therefore, the full array of TEFs from Table 1 (using the current EPA recommendations) can be applied. This procedure (C in Table 5) results in an estimate of roughly 50 for the total 2378-TCDD equivalents in the sample.

As might be expected, the most conservative of these procedures, A, leads to the highest estimate. Approach B (using theoretical probability of occurrence) leads to an estimate that is about 10-fold lower than the isomer-specific results C, reflecting the fact that the 2378-congeners are present in somewhat higher than "equal probability" proportions in this particular soot sample. Given the complexity of the analysis involved, the approximate nature of the TEF method, and the vagaries of the assay, a major feature of note in Table 5 regarding the soot samples is that the results of procedures A, B, and C span a range of only one order of magnitude and bracket the bioassay estimate.

In a separate study, Sawyer et al. (1983) published results of homologue-specific CDD and CDF concentrations in fly ash from four municipal solid waste combustors which are amenable to treatment by the TEF methodology. In addition, extracts from the fly ash samples were analyzed by three bioassay techniques (AHH induction, EROD induction, and receptor binding).

These data suggest that the TEF approach is likely to be a useful interim tool for the rough (order of magnitude) estimation of the toxicity of complex

mixtures of CDDs and CDFs. The availability of additional data comparing the results of analytical and biological assays will enable a conclusion regarding the preferred method of estimating TEFs (e.g., method A or B of Table 5).

VII. RESEARCH NEEDS

The Forum recommends that research be conducted that will enable the development of assays to directly assess the toxicity of mixtures of CDDs and CDFs. In addition, research should be conducted in order to provide a firmer basis for, and to guide appropriate modification of the TEF approach. The following areas of research are appropriate for these purposes:

1. Validation and completion of the *in vitro* test data such as those listed in Table 2.

2. Investigation of the relationships between short-term *in vivo* and *in vitro* tests and the toxic end points of concern; i.e., carcinogenicity, reproductive toxicity, immunotoxicity, and other significant human health effects resulting from CDD/CDF exposure.

3. Determination of the toxic potencies of metabolites of CDDs and CDFs in *in vitro* tests, relative to the potencies of the parent compounds. As pointed out by several reviewers, this would enable a refinement of the TEF approach.

4. Investigation of additional short-term assays which can test the mechanistic hypotheses underlying the TEF approach.

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TABLE 1. SOME APPROACHES TO ESTIMATING RELATIVE TOXICITIES OF PCDDs AND PCDFs

Basis/ compound	Swiss[a]	Grant[b] Olie[c] Commoner[d]	New York State[e]	Ontario[f]	FDA[g]	CA[h]	EPA[i] 1981	EPA current recommend.
(Basis)	Enzyme		LD_{50}	Various effects	Various effects			Various effects
Mono thru di	0	0	0	0	0	0	0	0
tri	0	0	0	1	0	0	0	0
2378-TCDD	1	1	1	1	1	1	1	1
other TCDDs	0.01	0	0	0.01	0	0	1	0.01
2378-PeCDDs	0.1	0.1	1	1	0	1	0	0.5
other PeCDDs	0.1	0	0	0.01	0	0	0	0.005
2378-HxCDDs	0.1	0.1	0.03	1	0.02	1	0	0.04
other HxCDDs	0.1	0	0	0.01	0.02	0	0	0.0004
2378-HpCDDs	0.01	0.01	0	1	0.005	1	0	0.001
other HpCDDs	0.01	0	0	0.01	0.005	0	0	0.00001
OCDD	0	0	0	0	<0.00001	1	0	0

[a]Swiss Government, 1982.
[b]Grant, 1977.
[c]Olie et al., 1983.
[d]Commoner et al., 1984.
[e]Eadon et al., 1982.
[f]Ontario, 1982.
[g]U.S. DHHS, 1983.
[h]Gravitz et al., 1983.
[i]U.S. EPA, 1981.

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TABLE 1. (continued)

Basis/ compound	Swiss[a]	Grant[b] Olie[c] Commoner[d]	New York State[e]	Ontario[f]	FDA[g]	CA[h]	EPA[i] 1981	EPA current recommend.
(Basis)	Enzyme		LD_{50}	Various effects	Various effects			Various effects
2378-TCDFs	0.1	0.1	0.33	0.02	0	1	0	0.1
other TCDFs	0.1	0	0	0.0002	0	0	0	0.001
2378-PeCDFs	0.1	0.1	0.33	0.02	0	1	0	0.1
other PeCDFs	0.1	0	0	0.0002	0	0	0	0.001
2378-HxCDFs	0.1	0.1	0.01	0.02	0	1	0	0.01
other HxCDFs	0.1	0	0	0.0002	0	0	0	0.0001
2378-HpCDFs	0.1	0.01	0	0.02	0	1	0	0.001
other HpCDFs	0	0	0	0.0002	0	0	0	0.00001
OCDF	0	0	0	0	0	0	0	0

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TABLE 2. POTENCIES OF DIOXINS RELATIVE TO 2,3,7,8-TCDD

Chemical	Guinea pig LD_{50}	Carcinogenicity	Reproductive/ teratogenic effects	Receptor binding	Enzyme Induction: AHH: Animal cells	AHH: Human cells	EROD	Cell keratin.	Flat (XB) cell assay	Immunotoxicity in vitro
CDDs:										
Mono thru tri	$<10^{-4}$[e]	-	--	.001-.01[e]	<.001[f]	--	--	.01[e] [l]	--	.00[n]
2378-TCDD	1[a]	1[b]	1[c,l]	1[e]	1[e]	1[m]	1[g]	1[e]	1[j]	1[o,p]
TCDDs	< .001[a]	--	< .001[k]	<.01-.16[e]	<.001-.029	--	--	<.001-.01[e]	--	--
2378-PeCDD	.67[a]	--	--	1[e]	.02-.29	--	--	.5[e]	--	--
PeCDDs	.002[a]	--	--	--	<.0019	--	--	--	--	--
2378-HxCDDs	.03[a]	.04[b]	.01[c]	.05[e]	.001-.19	--	--	.005[e]	--	--
HxCDDs	--	--	--	--	<.0019	--	--	--	--	--
2378-HpCDDs	.004[a]	--	--	--	.002-.0049[f]	--	--	--	--	--
HpCDDs	.002[a]	--	--	--	<.001[f]	--	--	--	--	--
OCDD	--	--	<.00001[k]	--	<.001[f]	--	--	--	--	--

[a] McKinney and McConnell, 1982; Moore et al., 1979.
[b] U.S. EPA, 1984a.
[c] Murray et al., 1979; Schwetz et al., 1973; Weber et al., 1984.
[d] Bandiera et al., 1983.
[e] Knutson and Poland, 1980.
[f] Bradlaw et al., 1979.
[g] Bradlaw et al., 1980.
[h] Bandiera et al., 1984.
[i] Hassoun et al., 1984.
[j] Glerthy and Crane, 1985.
[k] Weber et al., 1984.
[l] Poland et al., 1979.
[m] Nagayama et al., 1985a,b.
[n] Poland et al., 1976.
[o] Dencker et al., 1985.
[p] Greenlee et al., 1985b.

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TABLE 2(continued)

Chemical	Guinea pig LD_{50}	Carcino-genicity	Reproductive/ teratogenic effects	Receptor binding	Enzyme Induction: AHH: Animal cells	AHH: Human cells	EROD	Cell keratin.	Flat (XB) cell assay	Immuno-toxicity in vitro
CDFs:										
Mono thru tri	--	--	--	≤.001-.02[d,h]	<.001[d]	<.001[d]	--	.001[e]	--	--
2378-TCDF	.28;.5[a]	--	.3-.13[i,k]	.3[e];.24[h];.4[l]	.01-.4[f,h,m]	.4[m]	.1[h]	.05[e]	.1[j]	.1[o,lp]
TCDFs	--	--	--	.001-.05[d,e]	≤.001[d];.04[m]	.4[m]	≤.005[d]	--	--	--
2378-PeCDF		--	--	.13[d];.7[e];.6[h]	<.3[d];.4[m]	.8[m]	.1[d]	--	--	--
12467-PeCDF			--	.15[h]	.002[h]	--	<.001[h]	--	--	--
PeCDFs		--	--	.001-.1[d,e]	≤.001-2[d,n,m]	.6[m]	≤.001[h]	--	--	--
2378-HxCDFs	.017[a]	--	--	.04-.5[e,h]	.05-.2[h,m]	.9[m]	.1-.5[h]	--	--	--
HxCDFs	--	--	--	.001[e,h]	.001[m];.002[h]	--	.006[h]	--	--	--
2378-HpCDFs	--	--	--	--	.004[g]	--	--	--	--	--
HpCDFs	--	--	--	<.001[h]	<.001[f]	--	--	--	--	--

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TABLE 3. CDD/CDF ISOMERS OF MOST TOXIC CONCERN[a]

Dioxin		Dibenzofuran	
Isomer	TEF[b]	Isomer	TEF[b]
2,3,7,8-TCDD	1	2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDD	0.5	1,2,3,7,8-PeCDF	0.1
		2,3,4,7,8-PeCDF	0.1
1,2,3,4,7,8-HxCDD	0.04	1,2,3,4,7,8-HxCDF	0.01
1,2,3,7,8,9-HxCDD	0.04	1,2,3,7,8,9-HxCDF	0.01
1,2,3,6,7,8-HxCDD	0.04	1,2,3,6,7,8-HxCDF	0.01
		2,3,4,6,7,8-HxCDF	0.01
1,2,3,4,6,7,8-HpCDD	0.001	1,2,3,4,6,7,8-HpCDF	0.001
		1,2,3,4,7,8,9-HpCDF	0.001

[a] In each homologous group, the relative toxicity factor for the isomers not listed above is 1/100 of the value listed above.

[b] TEF = Toxicity Equivalence Factor = relative toxicity assigned.

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TABLE 4. PCDDs/PCDFs IN SOME ENVIRONMENTAL SAMPLES

Isomer	TEF	Air partics. St. Louis[e] COD/F conc. (ppb)	Air partics. St. Louis[e] TCDD eqts.	MSW ESP dust[e] COD/F conc. (ppb)	MSW ESP dust[e] TCDD eqts.	Lake sediment[e] COD/F conc. (ppb)	Lake sediment[e] TCDD eqts.	1982 Milorganite[d] COD/F conc. (ppt)	1982 Milorganite[d] TCDD eqts.	MSW fly ash[f] Ontario COD/F conc. (ppt)	MSW fly ash[f] Ontario TCDD eqts.	MSW fly ash[f] Oslo COD/F conc. (ppt)	MSW fly ash[f] Oslo TCDD eqts.
TCDDs	1	0.2	0.2	5	5	0	0	206	206	541	541	ND	--
PeCDDs	0.5	1	0.5	10	5	0.1	0.05	--	--	467	234	11	5.5
HxCDDs	0.04	1.2	0.048	160	6.4	0.34	0.014	2768	110.7	591	24	51	2
HpCDDS	0.001	25	0.025	120	0.12	0.5	0.001	7600	7.6	434	0.43	119	0.1
OCDD	0	170	--	260	--	1.3	--	60000	--	467	--	186	--
TCDFs	0.1	--	--	40	4	0.13	0.013	--	--				
PeCDFs	0.1	--	--	80	8	0.14	0.014	--	--				
HxCDFs	0.01	--	--	280	2.8	0.38	0.004	--	--				
HpCDFs	0.001	--	--	160	0.16	1.13	0.001	--	--				
OCDF	0	--	--	40	--	0.14	--	--	--				
Total TCDD eqts.			0.08		32		0.10		324		799		7.7

[a]U.S. EPA, 1984d.
[b]Cooper Engineers, 1984.
[c]Rappe, 1984.
[d]Lamparski et al., 1984.
[e]Czuwa and Hites, 1984.
[f]Tong et al., 1984.
[g]Des Rosiers, 1984.

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TABLE 4. (continued)

Isomer	TEF	Thermal degradation prods. from dielectric fluids[a] Run 8-13-40 CDD/F conc. (ng)	TCDD eqts.	Run 8-30-61 ASKL CDD/F conc. (ug)	TCDD eqts.	Jap. MSW[b] Pt. A TEF CDD/F conc. [lb/MMBTU(x10^{-6})]	TCDD eqts.	Pt. B TEF CDD/F conc.	TCDD eqts.	Commercial CPs 246TCP[c] CDD/F conc. (ppm)	TCDD eqts.	PCP[c] CDD/F conc. (ppm)	TCDD eqts.	Soot from PCB fire[g] CDD/F conc. (ppm)	TCDD eqts.
TCDDs															
2378	1	0	0	0	0	0.1	0.1	0.58	0.58	<0.1	--	<0.1	--	0.6	0.6
other	0.01													0.6	0.01
PeCDDs															
2378	0.5	0	0	0	0	0.07	0.035	0.47	0.24	<0.1	--	<0.1	--	2.5	1.25
other	0.002													2.5	0.01
HxCDDs															
2378	0.04	0	0	0	0	0.04	0.002	0.36	0.014	<1	--	2.5	0.1	1.1	0.04
other	0.0004													3.6	0
HpCDDS															
2378	0.001	0	0	330	0.33	0.02	<0.001	0.08	<0.001	<1	--	175	0.18	3	0
other	0.00001													4	0
OCDD	0	0	0	37	0	0.01	0	0.04	0	<1	--	500	--	2	--

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TABLE 4. (continued)

		Thermal degradation prods. from dielectric fluids[a]				Jap. MSW[b]				Commercial CPs				Soot from PCB fire[g]	
		Run 8-13-40		Run 8-30-61 ASKL		Pt. A TEF		Pt. B TEF		246TCPC[c]		PCPC[c]			
Isomer	TEF	CDD/F conc.	TCDD eqts.	CDD/F conc.	TCDD eqts.	CDD/F conc.	TCDD eqts.	CDD/F conc.	TCDD eqts.	CDD/F conc.	TCDD eqts.	CDD/F conc.	TCDD eqts.	CDD/F conc.	TCDD eqts.
		(ng)		(ug)		[lb/MMBTU(10^{-6})]				(ppm)		(ppm)		(ppm)	
TCDFs															
2378	0.1	690	69	1400	140	1.31	0.131	1.25	0.125	1.5	0.15	<0.1	--	12	1.2
other	0.001													16	0.01
PeCDFs															
2378	0.1	43	4.3	6400	640	0.38	0.038	0.46	0.046	17.5	1.75	<0.1	--	358	35.8
other	0.001													312	0.3
HxCDFs															
2378	0.01	7	0.07	910	9.1	0.06	0.006	0.06	0.006	36	3.6	<0.3	--	670	6.7
other	0.0001													295	0.03
HpCDFS															
2378	0.001	0	0	29	0.029	0.01	<.001	0.02	<.001	4.8	0.005	19	0.019	285	0.29
other	0.00001													172	0
OCDF	0	0	0	3.4	0	0.004	0	0.01	0	<1	--	25	--	40	--
Total TCDD eqts.			73		789		0.3		1.02		5.5		0.3		46

TABLE 5. USE OF THE TEF APPROACH

Isomer	TEF	Propn. factor	PCB fire soot[a] CDD/F conc. (ppm)	PCB fire soot[a] TCDD eqts. (ppm) A[c]	PCB fire soot[a] TCDD eqts. (ppm) B[c]	PCB fire soot[a] TCDD eqts. (ppm) C[c]	MSW fly ash[b] Sample 1 CDD/F conc. (ppb)	MSW fly ash[b] Sample 1 TCDD eqts. (ppb) A[c]	MSW fly ash[b] Sample 1 TCDD eqts. (ppb) B[c]	MSW fly ash[b] Sample 2 CDD/F conc. (ppb)	MSW fly ash[b] Sample 2 TCDD eqts. (ppb) A[c]	MSW fly ash[b] Sample 2 TCDD eqts. (ppb) B[c]
Total TCDDs	1	1	1.2	1.2			85	85		2.7	2.7	
2378 TCDDs	1	0.05	1.2		0.2	0.6	85		4.3	2.7		0.1
other TCDDs	0.01	0.95	1.2		--[d]	--	85		0.8	2.7		--
Total PeCDDs	0.5	1	5.0	2.5			213	107		6.6	3.3	
2378 PeCDDs	0.5	0.07	5.0		0.2	1.3	213		7.0	6.6		0.2
other PeCDDs	0.005	0.93	5.0		--	--	213		1.0	6.6		--
Total HxCDDs	0.04	1	4.7	0.2			354	14.2		11.6	0.5	
2378 HxCDDs	0.04	0.3	4.7		0.1	--	354		4.3	11.6		0.1
other HxCDDs	0.0004	0.7	4.7		--	--	354		0.1	11.6		--
Total HpCDDs	0.001	1	7	--		--	184	0.2		5.7	--	
2378 HpCDDs	0.001	0.5	7		--		184		0.1	5.7		--
other HpCDDs	0.00001	0.5	7		--		184		--	5.7		--

[a] Des Rosiers, 1984, assuming only homologue-specific concentrations are known (for isomer-specific analyses; see Table IV).
[b] Sawyer et al., 1983.
[c] A = estimated assuming 2378-isomers constitute 100% of a homologous group.
B = estimated assuming occurrence of all isomers in a homologous group is equally probable (thus using the proportionality factor in column three).
C = estimated by utilizing isomer-specific analyses (see Table IV).
[d] Values rounding off to less than 0.1 are omitted.

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TABLE 5. (continued)

Isomer	TEF	Propn. factor	PCB fire soot[a] CDD/F conc. (ppm)	PCB fire soot[a] TCDD eqts. (ppm) AC	PCB fire soot[a] TCDD eqts. (ppm) BC	PCB fire soot[a] TCDD eqts. (ppm) CC	MSW fly ash[b] Sample 1 CDD/F conc. (ppb)	MSW fly ash[b] Sample 1 TCDD eqts. (ppb) AC	MSW fly ash[b] Sample 1 TCDD eqts. (ppb) BC	MSW fly ash[b] Sample 2 CDD/F conc. (ppb)	MSW fly ash[b] Sample 2 TCDD eqts. (ppb) AC	MSW fly ash[b] Sample 2 TCDD eqts. (ppb) BC
Total TCDFs	0.1	1	28	2.8			209	20.9		7.0	0.7	
2378 TCDFs	0.1	0.03	28		0.1	1.2	209		0.6	7.0		--
other TCDFs	0.001	0.97	28		--	--	209		0.2	7.0		--
Total PeCDFs	0.1	1	670	67			549	54.9		17.8	1.8	
2378 PeCDFs	0.1	0.07	670		4.7	35.8	549		3.8	17.8		0.1
other PeCDFs	0.001	0.93	670		0.6	0.3	549		0.5	17.8		--
Total HxCDFs	0.01	1	965	9.7			1082	10.8		32.1	0.3	
2378 HxCDFs	0.01	0.25	965		2.4	6.7	1082		2.7	32.1		0.1
other HxCDFs	0.0001	0.75	965		0.1	--	1082		0.1	32.1		--
Total HpCDFs	0.001	1	460	0.5			499	0.5		10.9		--
2378 HpCDFs	0.001	0.50	460		0.2	0.3	499		0.2	10.9		--
other HpCDFs	0.00001	0.50	460		--	--	499		--	10.9		--
Total TCDD equivalents (TEF):												
TEF estimate:				84	9	46		294	26		9	1
AHH bioassay:				--	--	--		4			--	--
EROD bioassay:				--	--	--		5			--	--
Receptor binding assay:				--	--	--		32			4	--
Acute toxicity bioassay:				58	--	--		--			--	--

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Table 5. (continued)

			MSW fly ash[b]					
			Sample 3			Sample 4		
				TCDD eqts. (ppb)			TCDD eqts. (ppb)	
Isomer	TEF	Propn. factor	CDD/F conc. (ppb)	A[c]	B[c]	CDD/F conc. (ppb)	A[c]	B[c]
Total TCDDs	1	1	12.9	12.9		2.4	2.4	
2378 TCDDs	1	0.05	12.9		0.6	2.4		0.1
other TCDDs	0.01	0.95	12.9		0.1	2.4		--
Total PeCDDs	0.5	1	37.5	18.8		7.9	4.0	
2378 PeCDDs	0.5	0.07	37.5		1.3	7.9		0.3
other PeCDDs	0.005	0.93	37.5		0.2	7.9		--
Total HxCDDs	0.04	1	75	3		9.7	0.4	
2378 HxCDDs	0.04	0.3	75		0.9	9.7		0.1
other HxCDDs	0.0004	0.7	75		--	9.7		--
Total HpCDDs	0.001	1	41.9	--		9.1	--	
2378 HpCDDs	0.001	0.5	41.9		--	9.1		--
other HpCDDs	0.00001	0.5	41.9		--	9.1		--
Total TCDFs	0.1	1	8.2	0.8		4.4	0.4	
2378 TCDFs	0.1	0.03	8.2		--	4.4		--
other TCDFs	0.001	0.97	8.2		--	4.4		--
Total PeCDFs	0.1	1	19.8	2.0		21.0	2.1	
2378 PeCDFs	0.1	0.07	19.8		0.1	21.0		0.1
other PeCDFs	0.001	0.93	19.8		--	21.0		--
Total HxCDFs	0.01	1	38.7	0.4		21.6	0.2	
2378 HxCDFs	0.01	0.25	38.7		0.1	21.6		0.1
other HxCDFs	0.0001	0.75	38.7		--	21.6		--
Total HpCDFs	0.001	1	20.6	--		16.6	--	
2378 HpCDFs	0.001	0.50	20.6		--	16.6		--
other HpCDFs	0.00001	0.50	20.6		--	16.6		--
Total TCDD equivalents (TEF):								
TEF estimate:				38	2		9	0.7
AHH bioassay:				4	--		2	--
EROD bioassay:				5	--		2	--
Receptor binding assay:				65	--		11	--
Acute toxicity bioassay:				--	--		--	--

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TABLE 6. EXAMPLE OF A CALCULATION OF TCDD EQUIVALENTS WHERE ISOMER-SPECIFIC CONCENTRATIONS ARE AVAILABLE

Isomer	Concentration (ppb)	Relative potency	TCDD equivalents (ppb)
2,3,7,8-TCDD	0.5	1	0.5
Other TCDDs	20	0.01	0.2
1,2,3,7,8-PeCDD	0.10	0.5	0.05
1,2,3,6,7,8-HxCDD	0.05	0.04	0.002
1,2,3,7,8,9-HxCDD	0.5	0.04	0.02
1,2,3,4,7,8-HxCDD	0.1	0.04	0.004
Other HxCDDs	50	0.004	0.02
2,3,7,8-TCDF	10.5	0.1	1.05
1,2,3,7,8-PeCDF	0.1	0.1	0.01
2,3,4,7,8-PeCDF	0.01	0.1	0.01
1,2,3,6,7,8-HxCDF	0.01	0.1	0.001
1,2,3,7,8,9-HxCDF	0.01	0.1	0.001
1,2,3,4,7,8-HxCDF	0.01	0.1	0.001
2,3,4,6,7,8-HxCDF	0.01	0.1	0.001
Other HxCDFs	1	0.001	0.001
		Total	1.8

TABLE 7. CALCULATION OF TCDD EQUIVALENTS
WHERE ONLY HOMOLOGUE-SPECIFIC ANALYSES ARE AVAILABLE

Homologue	Concentration (ppb)	Relative potency	TCDD equivalents (ppb)
TCDDs	0.5	1	0.5
PeCDDs	0.5	0.5	0.25
HxCDDs	13	0.04	0.42
TCDFs	100	0.1	10.0
PeCDFs	150	0.1	15.0
HxCDFs	10	0.1	10.0
		Total	36.0

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APPENDIX A

NOMENCLATURE

The following terminology and abbreviations are used in this document:

1. The term "congener" refers to any one particular member of the same chemical family; e.g., there are 75 congeners of chlorinated dibenzo-p-dioxins.

2. The term "homologue" refers to a group of structurally related chemicals that have the same degree of chlorination. For example, there are eight homologues of CDDs, monochlorinated through octochlorinated.

3. The term "isomer" refers to substances that belong to the same homologous class. For example, there are 22 isomers that constitute the homologues of TCDDs.

4. A specific congener is denoted by unique chemical notation. For example, 2,4,8,9-tetrachlorodibenzofuran is referred to as 2,4,8,9-TCDF.

5. Notation for homologous classes is as follows:

Dibenzo-p-dioxin	D
Dibenzofuran	F

No. of halogens	Acronym	Example
1	M	
2	D	2,4-DCDD
3	Tr	
4	T	1,4,7,8-TCDD
5	Pe	
6	Hx	
7	Hp	
8	O	
1 through 8	CDDs and CDFs	

6. Dibenzo-p-dioxins and dibenzofurans that are chlorinated at the 2,3,7, and 8 positions are denoted as "2378" congeners, except when 2,3,7,8-TCDD is uniquely referred to; e.g., 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF are both referred to as "2378-PeCDFs."

APPENDIX-B

COMPARISON OF DIFFERENT APPROACHES TO CALCULATING

2378-TCDD EQUIVALENTS

Table III lists a number of different approaches to calculating 2378-TCDD toxicity equivalents. Five of the approaches (those that deal with 4-position 2378-substituted congeners, but not the 3-position substituted congeners) were applied to

1. The data in Table IV.

2. Some of the data included in Table I of the Report of the Citizens Advisory Committee on Resource Recovery in Brooklyn (March, 1985), produced by Ketcham and the Mt. Sinai School of Medicine.

A summary comparison of the relative results is found in Table B-1, with the supporting tables attached. (Note that the units of mass emission are not the same for all of the facilities. Therefore, comparison of absolute numbers between facilities may be invalid.)

These data indicate that, in general, the methods used by the Swiss government, New York State, and the U.S. EPA (the 1981 approach and the 1985 proposal) all generate results which are within an order of magnitude of each other. This suggests that, within the range considered, the results are not particularly sensitive functions of the TEFs selected.

The procedure recommended by the state of California, however, gives results which are roughly an order of magnitude higher than those generated by the other approaches. In general, the greater the contribution from the TCDDs, the greater the similarity in the results of the methods. This is due to the fact that all methods assign a TEF of 1 for 2,3,7,8-TCDD (and 1 to all TCDDs, when isomer-specific analyses are not available). Because higher chlorinated

CDDs and CDFs contribute significantly to the total, the disparity is greater between the CA results and those produced by the other methods, since CA assumes that all 2378-substituted CDDs/CDFs are as potent as 2,3,7,8-TCDD. The other methods acknowledge, to one degree or another, the reduced toxicity in higher chlorinated species; cf. Table 2.

TABLE B-1. RELATIVE 2378-TCDD EQUIVALENTS[a]

Source	EPA '85	EPA '81	Swiss	NY	CA
St. Louis air part.	1	0.3	1	2	40
PCB fire soot (isomer-specific)	1	0.03	4	3	30
MSW ESP dust	1	0.2	3	2	30
Lake sediment	1	--	2	2	30
Milorganite	1	0.6	2	0.9	30
Oslo MSW flyash	1	--	1	2	20
Ontario MSW flyash	1	0.8	1	2	3
Jap. plant A	1	0.3	1	2	7
Jap. plant B	1	0.6	0.8	2	3
Albany	1	0.3	0.4	2	5
Wright-Pat. (best)	1	0.2	2	3	20
Wright-Pat. (worst)	1	0.4	2	2	20

[a]Calculated using the Toxicity Equivalence Factors shown in Table 1.

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TABLE B-2. CALCULATION OF 2378-TCDD TOXICITY EQUIVALENTS FOR ST. LOUIS AIR PARTICULATES USING HOMOLOGUE-SPECIFIC DATA

Compound	CDD/F conc. (ppb)	EPA 1985 TEFs	EPA 1985 TEs (ppb)	EPA 1981 TEFs	EPA 1981 TEs (ppb)	Switzerland TEFs	Switzerland TEs (ppb)	New York TEFs	New York TEs (ppb)	California TEFs	California TEs (ppb)
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDD	0.2	1	0.2	1	0.2	1	0.2	1	0.2	1	0.2
TCDDs	0	0.01	0	1	0	0.01	0	0	0	0	0
2378-PeCDD	1	0.5	0.5	0	0	0.1	0.1	1	1	1	1
PeCDDs	0	0.005	0	0	0	0.1	0	0	0	0	0
2378-HxCDD	1.2	0.04	0.048	0	0	0.1	0.12	0.03	0.036	1	1.2
HxCDDs	0	0.0004	0	0	0	0.1	0	0	0	0	0
2378-HpCDD	25	0.001	0.025	0	0	0.01	0.25	0	0	1	25
HpCDDs	0	0.00001	0	0	0	0.01	0	0	0	0	0
OCDD	170	0	0	0	0	0	0	0	0	0	0
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDF	NA	0.1	0	0	0	0.1	0	0.33	0	1	0
TCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-PeCDF	NA	0.1	0	0	0	0.1	0	0.33	0	1	0
PeCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-HxCDF	NA	0.01	0	0	0	0.1	0	0.01	0	1	0
HxCDFs	0	0.0001	0	0	0	0.1	0	0	0	0	0
2378-HpCDF	NA	0.001	0	0	0	0.1	0	0	0	1	0
HpCDFs	0	0.00001	0	0	0	0	0	0	0	0	0
OCDF	NA	0	0	0	0	0	0	0	0	0	0
Total 2378-TCDD equivalents			0.7		0.2		0.7		1.2		27.4

TABLE B-3. CALCULATION OF 2378-TCDD TOXICITY EQUIVALENTS FOR PCB FIRE SOOT USING ISOMER-SPECIFIC DATA

Compound	CDD/F conc. (ppm)	EPA 1985 TEFs	EPA 1985 TEs (ppm)	EPA 1981 TEFs	EPA 1981 TEs (ppm)	Switzerland TEFs	Switzerland TEs (ppm)	New York TEFs	New York TEs (ppm)	California TEFs	California TEs (ppm)
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDD	0.6	1	0.6	1	0.6	1	0.6	1	0.6	1	0.6
TCDDs	0.6	0.01	0.006	1	0.6	0.01	0.006	0	0	0	0
2378-PeCDD	2.5	0.5	1.25	0	0	0.1	0.25	1	2.5	1	2.5
PeCDDs	2.5	0.005	0.0125	0	0	0.1	0.25	0	0	0	0
2378-HxCDD	1.1	0.04	0.044	0	0	0.1	0.11	0.03	0.033	1	1.1
HxCDDs	3.6	0.0004	0.00144	0	0	0.1	0.36	0	0	0	0
2378-HpCDD	3	0.001	0.003	0	0	0.01	0.03	0	0	1	3
HpCDDs	4	0.00001	0.00004	0	0	0.01	0.04	0	0	0	0
OCDD	2	0	0	0	0	0	0	0	0	0	0
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDF	12	0.1	1.2	0	0	0.1	1.2	0.33	3.96	1	12
TCDFs	16	0.001	0.016	0	0	0.1	1.6	0	0	0	0
2378-PeCDF	358	0.1	35.8	0	0	0.1	35.8	0.33	118.14	1	358
PeCDFs	312	0.001	0.312	0	0	0.1	31.2	0	0	0	0
2378-HxCDF	670	0.01	6.7	0	0	0.1	67	0.01	6.7	1	670
HxCDFs	295	0.0001	0.0295	0	0	0.1	29.5	0	0	0	0
2378-HpCDF	285	0.001	0.285	0	0	0.1	28.5	0	0	1	285
HpCDFs	172	0.00001	0.00172	0	0	0	0	0	0	0	0
OCDF	40	0	0	0	0	0	0	0	0	0	0
Total 2378-TCDD equivalents			46		1.2		196		132		1332

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TABLE B-4. CALCULATION OF 2378-TCDD TOXICITY EQUIVALENTS FOR MSW ESP DUST USING HOMOLOGUE-SPECIFIC DATA AND 2378 TEFs

Compound	CDD/F conc. (ppb)	EPA 1985 TEFs	EPA 1985 TEs (ppb)	EPA 1981 TEFs	EPA 1981 TEs (ppb)	Switzerland TEFs	Switzerland TEs (ppb)	New York TEFs	New York TEs (ppb)	California TEFs	California TEs (ppb)
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDD	5	1	5	1	5	1	5	1	5	1	5
TCDDs	0	0.01	0	1	0	0.01	0	0	0	0	0
2378-PeCDD	10	0.5	5	0	0	0.1	1	1	10	1	10
PeCDDs	0	0.005	0	0	0	0.1	0	0	0	0	0
2378-HxCDD	160	0.04	6.4	0	0	0.1	16	0.03	4.8	1	160
HxCDDs	0	0.0004	0	0	0	0.1	0	0	0	0	0
2378-HpCDD	120	0.001	0.12	0	0	0.01	1.2	0	0	1	120
HpCDDs	0	0.00001	0	0	0	0.01	0	0	0	0	0
OCDD	260	0	0	0	0	0	0	0	0	0	0
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDF	40	0.1	4	0	0	0.1	4	0.33	13.2	1	40
TCDFs	0	.001	0	0	0	0.1	0	0	0	0	0
2378-PeCDF	80	0.1	8	0	0	0.1	8	0.33	26.4	1	80
PeCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-HxCDF	280	0.01	2.8	0	0	0.1	28	0.01	2.8	1	280
HxCDFs	0	0.0001	0	0	0	0.1	0	0	0	0	0
2378-HpCDF	160	0.001	0.16	0	0	0.1	16	0	0	1	160
HpCDFs	0	0.00001	0	0	0	0	0	0	0	0	0
OCDF	40	0	0	0	0	0	0	0	0	0	0
Total 2378-TCDD equivalents			31		5		79		62		855

TABLE B-5. CALCULATION OF 2378-TCDD TOXICITY EQUIVALENTS FOR LAKE SEDIMENT USING HOMOLOGUE-SPECIFIC DATA

Compound	CDD/F conc. (ppb)	EPA 1985		EPA 1981		Switzerland		New York		California	
		TEFs	TEs (ppb)	TEFs	TEs (ppb)	TEFs	TEs (ppb)	TEFs	TEs (ppb)	TEFs	TEs (ppb)
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDD	0	1	0	1	0	1	0	1	0	1	0
TCDDs	0	0.01	0	1	0	0.01	0	0	0	0	0
2378-PeCDD	0.1	0.5	0.05	0	0	0.1	0.01	1	0.1	1	0.1
PeCDDs	0	0.005	0	0	0	0.1	0	0	0	0	0
2378-HxCDD	0.34	0.04	0.0136	0	0	0.1	0.034	0.03	0.0102	1	0.34
HxCDDs	0	0.0004	0	0	0	0.1	0	0	0	0	0
2378-HpCDD	0.5	0.001	0.0005	0	0	0.01	0.005	0	0	1	0.5
HpCDDs	0	0.00001	0	0	0	0.01	0	0	0	0	0
OCDD	1.3	0	0	0	0	0	0	0	0	0	0
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDF	0.13	0.1	0.013	0	0	0.1	0.013	0.33	0.0429	1	0.13
TCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-PeCDF	0.14	0.1	0.014	0	0	0.1	0.014	0.33	0.0462	1	0.14
PeCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-HxCDF	0.38	0.01	0.0038	0	0	0.1	0.038	0.01	0.0038	1	0.38
HxCDFs	0	0.0001	0	0	0	0.1	0	0	0	0	0
2378-HpCDF	1.13	0.001	0.00113	0	0	0.1	0.113	0	0	1	1.13
HpCDFs	0	0.00001	0	0	0	0	0	0	0	0	0
OCDF	0.14	0	0	0	0	0	0	0	0	0	0
Total 2378-TCDD equivalents			0.1		0		1.2		0.2		2.7

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TABLE B-6. CALCULATION OF 2378-TCDD TOXICITY EQUIVALENTS FOR MILORGANITE USING HOMOLOGUE-SPECIFIC DATA

Compound	CDD/F conc. (ppt)	EPA 1985 TEFs	EPA 1985 TEs (ppt)	EPA 1981 TEFs	EPA 1981 TEs (ppt)	Switzerland TEFs	Switzerland TEs (ppt)	New York TEFs	New York TEs (ppt)	California TEFs	California TEs (ppt)
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDD	206	1	206	1	206	1	206	1	206	1	206
TCDDs	0	0.01	0	1	0	0.01	0	0	0	0	0
2378-PeCDD	0	0.5	0	0	0	0.1	0	1	0	1	0
PeCDDs	0	0.005	0	0	0	0.1	0	0	0	0	0
2378-HxCDD	2768	0.04	110.72	0	0	0.1	276.8	0.03	83.04	1	2768
HxCDDs	0	0.0004	0	0	0	0.1	0	0	0	0	0
2378-HpCDD	7600	0.001	7.6	0	0	0.01	76	0	0	1	7600
HpCDDs	0	0.00001	0	0	0	0.01	0	0	0	0	0
OCDD	60000	0	0	0	0	0	0	0	0	0	0
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDF	NA	0.1	0	0	0	0.1	0	0.33	0	1	0
TCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-PeCDF	NA	0.1	0	0	0	0.1	0	0.33	0	1	0
PeCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-HxCDF	NA	0.01	0	0	0	0.1	0	0.01	0	1	0
HxCDFs	0	0.0001	0	0	0	0.1	0	0	0	0	0
2378-HpCDF	NA	0.001	0	0	0	0.1	0	0	0	1	0
HpCDFs	0	0.00001	0	0	0	0	0	0	0	0	0
OCDF	NA	0	0	0	0	0	0	0	0	0	0
Total 2378-TCDD equivalents			324		206		559		289		10600

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TABLE B-7. CALCULATION OF 2378-TCDD TOXICITY EQUIVALENTS FOR OLSO MSW FLY ASH USING HOMOLOGUE-SPECIFIC DATA

Compound	CDD/F conc. (ppt)	EPA 1985 TEFs	EPA 1985 TEs (ppt)	EPA 1981 TEFs	EPA 1981 TEs (ppt)	Switzerland TEFs	Switzerland TEs (ppt)	New York TEFs	New York TEs (ppt)	California TEFs	California TEs (ppt)
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDD	NA	1	0	1	0	1	0	1	0	1	0
TCDDs	0	0.01	0	1	0	0.01	0	0	0	0	0
2378-PeCDD	11	0.5	5.5	0	0	0.1	1.1	1	11	1	11
PeCDDs	0	0.005	0	0	0	0.1	0	0	0	0	0
2378-HxCDD	51	0.04	2.04	0	0	0.1	5.1	0.03	1.53	1	51
HxCDDs	0	0.0004	0	0	0	0.1	0	0	0	0	0
2378-HpCDD	119	0.001	0.119	0	0	0.01	1.19	0	0	1	119
HpCDDs	0	0.00001	0	0	0	0.01	0	0	0	0	0
OCDD	186	0	0	0	0	0	0	0	0	0	0
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDF	NA	0.1	0	0	0	0.1	0	0.33	0	1	0
TCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-PeCDF	NA	0.1	0	0	0	0.1	0	0.33	0	1	0
PeCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-HxCDF	NA	0.01	0	0	0	0.1	0	0.01	0	1	0
HxCDFs	0	0.0001	0	0	0	0.1	0	0	0	0	0
2378-HpCDF	NA	0.001	0	0	0	0.1	0	0	0	1	0
HpCDFs	0	0.00001	0	0	0	0	0	0	0	0	0
OCDF	NA	0	0	0	0	0	0	0	0	0	0
Total 2378-TCDD equivalents			7.7		0		7.4		12.5		181

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TABLE B-8. CALCULATION OF 2378 CDD TOXICITY EQUIVALENTS FOR ONTARIO MSW FLY ASH USING HOMOLOGUE-SPECIFIC DATA

Compound	CDD/F conc. (ppt)	EPA 1985 TEFs	EPA 1985 TEs (ppt)	EPA 1981 TEFs	EPA 1981 TEs (ppt)	Switzerland TEFs	Switzerland TEs (ppt)	New York TEFs	New York TEs (ppt)	California TEFs	California TEs (ppt)
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDD	541	1	541	1	541	1	541	1	541	1	541
TCDDs	0	0.01	0	1	0	0.01	0	0	0	0	0
2378-PeCDD	467	0.5	233.5	0	0	0.1	46.7	1	467	1	467
PeCDDs	0	0.005	0	0	0	0.1	0	0	0	0	0
2378-HxCDD	591	0.04	23.64	0	0	0.1	59.1	0.03	17.73	1	591
HxCDDs	0	0.0004	0	0	0	0.1	0	0	0	0	0
2378-HpCDD	434	0.001	0.434	0	0	0.01	4.34	0	0	1	434
HpCDDs	0	0.00001	0	0	0	0.01	0	0	0	0	0
OCDD	467	0	0	0	0	0	0	0	0	0	0
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDF	NA	0.1	0	0	0	0.1	0	0.33	0	1	0
TCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-PeCDF	NA	0.1	0	0	0	0.1	0	0.33	0	1	0
PeCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-HxCDF	NA	0.01	0	0	0	0.1	0	0.01	0	1	0
HxCDFs	0	0.0001	0	0	0	0.1	0	0	0	0	0
2378-HpCDF	NA	0.001	0	0	0	0.1	0	0	0	1	0
HpCDFs	0	0.00001	0	0	0	0	0	0	0	0	0
OCDF	NA	0	0	0	0	0	0	0	0	0	0
Total 2378-TCDD equivalents			799		541		651		1026		2023

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TABLE B-9. CALCULATION OF 2378-TCDD TOXICITY EQUIVALENTS FOR MSW AT JAPANESE PLANT A USING HOMOLOGUE-SPECIFIC DATA

Compound	CDD/F conc.[a]	EPA 1985		EPA 1981		Switzerland		New York		California	
		TEFs	TEs[a]	TEFs	TEs[a]	TEFs	TEs[a]	TEFs	TEs[a]	TEFs	TEs[a]
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDD	0.1	1	0.1	1	0.1	1	0.1	1	0.1	1	0.1
TCDDs	0	0.01	0	1	0	0.01	0	0	0	0	0
2378-PeCDD	0.07	0.5	0.035	0	0	0.1	0.007	1	0.07	1	0.07
PeCDDs	0	0.005	0	0	0	0.1	0	0	0	0	0
2378-HxCDD	0.04	0.04	0.0016	0	0	0.1	0.004	0.03	0.0012	1	0.04
HxCDDs	0	0.0004	0	0	0	0.1	0	0	0	0	0
2378-HpCDD	0.02	0.001	0.00002	0	0	0.01	0.0002	0	0	1	0.02
HpCDDs	0	0.00001	0	0	0	0.01	0	0	0	0	0
OCDD	0.01	0	0	0	0	0	0	0	0	0	0
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDF	1.31	0.1	0.131	0	0	0.1	0.131	0.33	0.4323	1	1.31
TCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-PeCDF	0.38	0.1	0.038	0	0	0.1	0.038	0.33	0.1254	1	0.38
PeCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-HxCDF	0.06	0.01	0.0006	0	0	0.1	0.006	0.01	0.0006	1	0.06
HxCDFs	0	0.0001	0	0	0	0.1	0	0	0	0	0
2378-HpCDF	0.01	0.001	0.00001	0	0	0.1	0.001	0	0	1	0.01
HpCDFs	0	0.00001	0	0	0	0	0	0	0	0	0
OCDF	0.004	0	0	0	0	0	0	0	0	0	0
Total 2378-TCDD equivalents			0.3		0.1		0.3		0.7		2.0

[a]Units = lb/MM BTU($X10^{-6}$)

GENP 010920

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GENP 010921

TABLE B-10. CALCULATION OF 2378-TCDD TOXICITY EQUIVALENTS FOR MSW AT JAPANESE PLANT B USING HOMOLOGUE-SPECIFIC DATA

Compound	CDD/F conc.[a]	EPA 1985		EPA 1981		Switzerland		New York		California	
		TEFs	TEs[a]	TEFs	TEs[a]	TEFs	TEs[a]	TEFs	TEs[a]	TEF	TEs[a]
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDD	0.58	1	0.58	1	0.58	1	0.58	1	0.58	1	0.58
TCDDs	0	0.01	0	1	0	0.01	0	0	0	0	0
2378-PeCDD	0.47	0.5	0.235	0	0	0.1	0.047	1	0.47	1	0.47
PeCDDs	0	0.005	0	0	0	0.1	0	0	0	0	0
2378-HxCDD	0.36	0.04	0.0144	0	0	0.1	0.036	0.03	0.0108	1	0.36
HxCDDs	0	0.0004	0	0	0	0.1	0	0	0	0	0
2378-HpCDD	0.08	0.001	0.00008	0	0	0.01	0.0008	0	0	1	0.08
HpCDDs	0	0.00001	0	0	0	0.01	0	0	0	0	0
OCDD	0.04	0	0	0	0	0	0	0	0	0	0
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDF	1.25	0.1	0.125	0	0	0.1	0.125	0.33	0.4125	1	1.25
TCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-PeCDF	0.46	0.1	0.046	0	0	0.1	0.046	0.33	0.1518	1	0.46
PeCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-HxCDF	0.06	0.01	0.0006	0	0	0.1	0.006	0.01	0.0006	1	0.06
HxCDFs	0	0.0001	0	0	0	0.1	0	0	0	0	0
2378-HpCDF	0.02	0.001	0.00002	0	0	0.1	0.002	0	0	1	0.02
HpCDFs	0	0.00001	0	0	0	0	0	0	0	0	0
OCDF	0.01	0	0	0	0	0	0	0	0	0	0
Total 2378-TCDD equivalents			1.0		0.6		0.8		1.6		3.3

[a]Units = lb/MM BTU($X10^{-6}$)

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GENP 010922

TABLE B-11. CALCULATION OF 2378-TCDD TOXICITY EQUIVALENTS FOR MSW AT ALBANY USING HOMOLOGUE-SPECIFIC DATA

Compound	CDD/F conc. (ng/m^3)	EPA 1985 TEFs	EPA 1985 TEs (ng/m^3)	EPA 1981 TEFs	EPA 1981 TEs (ng/m^3)	Switzerland TEFs	Switzerland TEs (ng/m^3)	New York TEFs	New York TEs (ng/m^3)	California TEFs	California TEs (ng/m^3)
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDD	0.45	1	0.45	1	0.45	1	0.45	1	0.45	1	0.45
TCDDs	14	0.01	0.14	1	14	0.01	0.14	0	0	0	0
2378-PeCDD	97	0.5	48.5	0	0	0.1	9.7	1	97	1	97
PeCDDs	0	0.005	0	0	0	0.1	0	0	0	0	0
2378-HxCDD	53	0.04	2.12	0	0	0.1	5.3	0.03	1.59	1	53
HxCDDs	0	0.0004	0	0	0	0.1	0	0	0	0	0
2378-HpCDD	71	0.001	0.071	0	0	0.01	0.71	0	0	1	71
HpCDDs	0	0.00001	0	0	0	0.01	0	0	0	0	0
OCDD	10	0	0	0	0	0	0	0	0	0	0
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDF	2.1	0.1	0.21	0	0	0.1	0.21	0.33	0.693	1	2.1
TCDFs	33	0.001	0.033	0	0	0.1	3.3	0	0	0	0
2378-PeCDF	21	0.1	2.1	0	0	0.1	2.1	0.33	6.93	1	21
PeCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-HxCDF	4	0.01	0.04	0	0	0.1	0.4	0.01	0.04	1	4
HxCDFs	0	0.0001	0	0	0	0.1	0	0	0	0	0
2378-HpCDF	1	0.001	0.001	0	0	0.1	0.1	0	0	1	1
HpCDFs	0	0.00001	0	0	0	0	0	0	0	0	0
OCDF	2	0	0	0	0	0	0	0	0	0	0
Total 2378-TCDD equivalents			54		14		22		107		250

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TABLE B-12. CALCULATION OF 237[illegible]CDD TOXICITY EQUIVALENTS FOR WP AFB (BEST) USING HOMOLOGUE-SPECIFIC DATA

Compound	CDD/F conc. (ng/m^3)	EPA 1985 TEFs	EPA 1985 TEs (ng/m^3)	EPA 1981 TEFs	EPA 1981 TEs (ng/m^3)	Switzerland TEFs	Switzerland TEs (ng/m^3)	New York TEFs	New York TEs (ng/m^3)	California TEFs	California TEs (ng/m^3)
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDD	0.4	1	0.4	1	0.4	1	0.4	1	0.4	1	0.4
TCDDs	0	0.01	0	1	0	0.01	0	0	0	0	0
2378-PeCDD	0.4	0.5	0.2	0	0	0.1	0.04	1	0.4	1	0.4
PeCDDs	0	0.005	0	0	0	0.1	0	0	0	0	0
2378-HxCDD	1	0.04	0.04	0	0	0.1	0.1	0.03	0.03	1	1
HxCDDs	0	0.0004	0	0	0	0.1	0	0	0	0	0
2378-HpCDD	3	0.001	0.003	0	0	0.01	0.03	0	0	1	3
HpCDDs	0	0.00001	0	0	0	0.01	0	0	0	0	0
OCDD	3	0	0	0	0	0	0	0	0	0	0
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDF	8	0.1	0.8	0	0	0.1	0.8	0.33	2.64	1	8
TCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-PeCDF	3	0.1	0.3	0	0	0.1	0.3	0.33	0.99	1	3
PeCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-HxCDF	4	0.01	0.04	0	0	0.1	0.4	0.01	0.04	1	4
HxCDFs	0	0.0001	0	0	0	0.1	0	0	0	0	0
2378-HpCDF	9	0.001	0.009	0	0	0.1	0.9	0	0	1	9
HpCDFs	0	0.00001	0	0	0	0	0	0	0	0	0
OCDF	2	0	0	0	0	0	0	0	0	0	0
Total 2378-TCDD equivalents			1.8		0.4		3.0		4.5		28.8

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TABLE B-13. CALCULATION OF 23.7CDD TOXICITY EQUIVALENTS FOR WP AFB (WORST) USING HOMOLOGUE-SPECIFIC DATA

Compound	CDD/F conc. (ng/m^3)	EPA 1985 TEFs	EPA 1985 TEs (ng/m^3)	EPA 1981 TEFs	EPA 1981 TEs (ng/m^3)	Switzerland TEFs	Switzerland TEs (ng/m^3)	New York TEFs	New York TEs (ng/m^3)	California TEFs	California TEs (ng/m^3)
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDD	4	1	4	1	4	1	4	1	4	1	4
TCDDs	0	0.01	0	1	0	0.01	0	0	0	0	0
2378-PeCDD	3	0.5	1.5	0	0	0.1	0.3	1	3	1	3
PeCDDs	0	0.005	0	0	0	0.1	0	0	0	0	0
2378-HxCDD	6	0.04	0.24	0	0	0.1	0.6	0.03	0.18	1	6
HxCDDs	0	0.0004	0	0	0	0.1	0	0	0	0	0
2378-HpCDD	32	0.001	0.032	0	0	0.01	0.32	0	0	1	32
HpCDDs	0	0.00001	0	0	0	0.01	0	0	0	0	0
OCDD	16	0	0	0	0	0	0	0	0	0	0
Mono to tri	x	0	0	0	0	0	0	0	0	0	0
2378-TCDF	31	0.1	3.1	0	0	0.1	3.1	0.33	10.23	1	31
TCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-PeCDF	15	0.1	1.5	0	0	0.1	1.5	0.33	4.95	1	15
PeCDFs	0	0.001	0	0	0	0.1	0	0	0	0	0
2378-HxCDF	23	0.01	0.23	0	0	0.1	2.3	0.01	0.23	1	23
HxCDFs	0	0.0001	0	0	0	0.1	0	0	0	0	0
2378-HpCDF	93	0.001	0.093	0	0	0.1	9.3	0	0	1	93
HpCDFs	0	0.00001	0	0	0	0	0	0	0	0	0
OCDF	8	0	0	0	0	0	0	0	0	0	0
Total 2378-TCDD equivalents			11.0		4		21.4		22.6		207

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STEPHEN B. HAMILTON, JR.
MANAGER - ENVIRONMENTAL SCIENCE AND TECHNOLOGY

(203) 373-3316

January 12, 1987

Dr. Ruth Arisman
Rt. 1, Box 21
Burkville, AL 36725

Dear Ruth:

Here are the two items on inhibition of dioxin toxicity by PCBs. I think they are pretty exciting. I am also enclosing an abstract of a paper given by Steve Safe last October in Montreal. The bottom line conclusions on PCB health effects are very similar to those drawn by Seymour Friess back in 1982-83.

Safe, as you may recall, has been one of EPA's main consultants on PCB toxicity, and what he says on the subject should be very influential.

I will plan on taking you up on your offer to see the Burkville plant sometime when I'm traveling to the South.

Very truly yours,

SBH:cas
Enclosures

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SOCIETY OF TOXICOLOGY

ABSTRACT FORM FOR THE 1987 ANNUAL MEETING

Any correspondence regarding your abstract *must* reference the above number.

Follow format for heading. Leave double space between heading and abstract. Text of abstract *must* be single spaced. There will be a charge of $25 for any abstract that must be retyped. Leave no margins — type *must* be within rectangle. Single space, elite type, except for double space between body of text and authors. Deadline for receipt in Executive Secretary's office is October 24, 1986. Follow instructions accompanying this form. Submit original and one copy only. Original *must* be on this form. Proof carefully.

TERATOGENICITY OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN: ANTAGONISM BY AROCLOR 1254: J.M. Haake, F. Mayura, T. Phillips and S. Safe, Departments of Veterinary Physiology and Pharmacology and Veterinary Public Health, Texas A&M University, College Station, TX.

The dose-response teratogenic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were determined in the C57BL/6J mouse. Administration of a single dose of 2,3,7,8-TCDD (20 ug/kg) in corn oil to the pregnant female on day 10 resulted in 70% cleft palate and 85% hydronephrosis in the fetuses. In contrast, administration of the commercial polychlorinated biphenyl, Aroclor 1254 (750 umol/kg), to the pregnant mice did not result in any cleft palate or hydronephrosis in the fetuses. Cotreatment of the pregnant C57BL/6J mice with Aroclor 1254 (750 umol/kg) and 2,3,7,8-TCDD (20 ug/kg) resulted in only 8% fetal cleft palate and was significantly lower than the 70% fetal cleft palate observed using 2,3,7,8-TCDD alone. These results are comparable to other studies in our laboratory which demonstrate that Aroclor 1254 antagonizes receptor-mediated responses to 2,3,7,8-TCDD. (Sponsored by N.I.H. ES03554.)

1. Name and address of presenting author:

Name: Ms. J.M. Haake

Organization: Texas A&M University

Address: College of Veterinary Medicine

College Station TX 77843
City State Zip Code

U.S.A.
Country

Telephone (409) 845-9181

2. Membership status: ____ SOT member ___X___ Non-member
Abstracts must have an SOT member as an author or be sponsored by a member. If sponsored, the SOT member sponsor must sign below:

Signature of SOT member sponsor

Dr. S. Safe
Typed name of SOT member sponsor

Undergraduate and graduate students and postdoctoral fellows are also requested to check one of the spaces below:

____ Undergraduate ___X___ Graduate ____ Postdoctoral

3. The Program Committee reserves the right to assign papers to either a platform or poster session. Please indicate your preference below and whether you wish to withdraw your paper if your choice cannot be met:

Platform ___X___ Poster _____

Either _____ Withdraw _____
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4. Select from the list below the session topic that best suits your paper. Specify your first and second choice or specify another possible session topic area under "other." Specify only two choices.

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___ 4. Biotransformation/Disposition
___ 5. Reactive Intermediates
_____ 6. Genotox/Mutagenesis
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_____ 14. Neurochemistry
_____ 15. Neuropathology
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_____ 17. Behavior
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Return this form by October 24, 1986, to: Program Committee, c/o Executive Secretary, SOT, 1133 Fifteenth Street, N.W., Suite 620, Washington, D.C. 20005 (202/293-5535).
Submit abstract form and one copy ONLY. Do not enclose any other materials or requests. If more than one abstract is submitted in the same envelope, indicate

GENP 010926

PCBs may counteract dioxin, chemist says

JACK MILLER
Science

If you take a bath in PCBs, you might notice some effect. Maybe a little acne-type skin trouble which will go away.

Then again, you may see nothing. Any exposure less extreme than that probably won't do anything to you at all, say scientists expert in these things.

There, now — do we have your attention?

It's taken years for the general public to become convinced that polychlorinated biphenyls are deadly poisons, so it probably will take just as many more years to convince the public that this has all been blind, unquestioning, gross misinformation, these people say.

But if that's what the job calls for, let's start.

Oh, and while we're at it — you've heard of dioxins? Or to use the more common term, "deadly dioxins?"

Six poisonous

Well, the proper term in this case is chlorinated dibxins, although it's equally correct to call them chlorodioxins.

We make the distinction because there are thousands of other dioxins that are not involved with chlorine, which means they are not part of this family of chemicals that people worry so much about, even though they can all properly be called dioxins.

Well, there are 75 known chlorodioxins. And yes, of those 75, six are considered harmful enough to people to be called poisons, and one of those six is rated as the third most poisonous substance in the world for humans (a form of botulism is the Number 1 poison, but that's another story).

Four more of those 75 chlorodioxins are somewhat toxic, and the other 65 seem to have no bad effects whatever on humans.

That worst chlorodioxin of them all (worst for humans, that is) carries the cumbersome chemical title of 2378-TCDD, which is short for 2378-tetrachlorodibenzo-para-dioxin (the name alone is enough to scare you off).

None of this information, up till now, is new. It has been generally missed by the media and the public, but lots of top chemists have known it all along.

But here's something that is new. Research under chemist Stephen Safe at Texas A and M University, soon to be published, has concluded that many of those relatively harmless PCBs actually can work as a protection, if not an outright antidote, against the bad chlorodioxins.

And, yes, this does work against 2378-TCDD, the worst of the bunch.

Last week the Ontario environment ministry staged a forum in Metro on dioxins in the environment to a full house, and some of what the crowd heard seemed to surprise many of them.

Government advisor

Safe was brought up from Texas to be keynote speaker. His credentials are good. He's a Belleville native who studied at Queen's University and then Oxford on his way to becoming an advisor on these subjects for both the U.S. and Canadian governments.

PCBs, he said, are almost totally harmless to people. They may be damaging to some plants or other life forms in the food chain, and they do not break down readily, so they should not be dumped into the environment.

But if there's an accidental exposure, like that spill of PCBs from a truck on the Trans-Canada Highway in Ontario some time ago, it's nothing to worry about.

People driving their cars through the stuff were not in any danger. The near-panic that followed that spill, which wound up with a long stretch of pavement being dug up and carted away, was ridiculous and pure hysteria, Safe said.

Several years ago, there were bad mass poisonings, one in Japan and another in Korea, of people who consumed rice oil contaminated with chemicals. There were PCBs in the chemicals, and the outcome was that all PCBs (there are several) were labelled deadly.

In fact, Safe said, some of the other components in the chemicals must have done the damage, because he has studied PCBs exposure cases for years and found you practically have to drown a human in the stuff to do even a little harm.

Then he switched to the subject of chlorodioxins and dropped his bombshell.

"In our studies of the effects of the few toxic forms of these chemicals, we felt there must be some anti-dioxins at work in the body. And we found that there were.

"We have now developed a number of compounds that give this effect on laboratory test animals. When administered along with the worst of the chlorodioxins, they have cut the incidence of harm by 80 per cent."

One of those magic anti-dioxin compounds is Aroclor 1254.

What's Aroclor 1254? "It's a mixture of PCBs. It's well known. It's been available commercially for years," Safe said.

Any more bulletins? Well, it may turn out that you can also fight dioxin with dioxin. Some of those 65 non-toxic chlorodioxins seem to offer the same sort of protection from 2378-TCDD as the PCBs do.

Stay tuned to the good-news channel.

STAR
THE TORONTO STORE

10/27/86

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GENP 010927

IEEE MONTECH'86 Conférence sur les BPC et les fluides de remplacement • 29 septembre – 1er octobre 1986
Conference on PCBs and replacement fluids • September 29 – October 1, 1986

HEALTH EFFECTS OF PCBs AND RELATED SUBSTANCES

Stephen H. Safe, Kathy Farrell, Roy Bannister
Michael Kelley, Stelvio Bandiera and Grant Mason
Veterinary Physiology and Pharmacology
Texas A&M University, College Station, Tx 77843

Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are halogenated aromatic chemicals which have been used as industrial compounds (PCBs), or have been identified as by-products in the production of organic compounds or their combustion (PCBs, PCDDs and PCDFs) (1,2). These chemicals are characterized by their chemical stability, resistance to chemical and biological breakdown and high lipophilicity. These chemical properties contribute to the adverse environmental effects of the class of compounds which have been widely identified in the global ecosystem and preferentially bioconcentrate in fish, wildlife and humans(3). Since the commercial PCBs are complex mixtures of isomers and congeners, it is not suprising that the PCB composition of extracts from environmental samples and occupationally exposed humans is also complex. Moreover it is also apparent that the relative composition of the individual PCB compounds which have been identified in various matrices are highly variable and do not resemble the distribution patterns observed for the commercial PCB products (4). It is apparent from several studies that the environmental persistence and bioaccumulation of individual PCBs are dependent on several factors including their degree of chlorination, lipophilicity, volatility, and biodegradability. Hazard assessment of commercial and environmental PCB mixtures requires analytical data on the composition of these mixtures and the toxicology of individual PCB isomers and congeners and their interactive effects.

PCBs and Related Halogenated Aryl Hydrocarbons – Toxicology and Structure – Activity Relationships (SARs).

Toxicology

Although the acute LD_{50} values for commercial PCB mixtures are relatively high, it is apparent from subacute and chronic exposures studies that much lower dose levels of some PCB mixtures elicit diverse biologic and toxic effects which include (2):

1. the development of skin lesions (which resemble human chloracne) in several laboratory animal species and humans;
2. immunotoxic effects;
3. a wasting syndrome;
4. reproductive and fetal toxicity;
5. carcinogenesis (initiation and promotion)
6. porphyria and hepatotoxicity;
7. the induction of diverse drug-metabolizing enzymes including aryl hydrocarbon hydroxylase (AH), a cytochrome P-450-dependent monooxygenase enzyme.

It was also evident that the spectrum of toxic effects elicited by PCBs quantitatively were similar to those observed after treatment with several related classes of halogenated aryl hydrocarbons which include the dibenzofurans (PCDFs)(2,5). Several studies with individual PCBs indicated that the three most toxic PCB congeners, namely 3,3'4,4'-tetra-, 3,3',4,4',5-penta, and 3,3',4,4',5,5'-hexachlorobiphenyl are minor to trace components of the commercial PCB formulations and are unlikely to be the sole contributors to the toxicity of these mixtures (2,4). Our research has focused on the identification of the toxic PCBs, the determination of their presence or absence in commercial and environmental PCB mixtures and the use of this data to develop risk-assessment of PCBs.

PCBs – SARs The observed correlation between the toxicity and the AHH induction potencies for the toxic halogenated aryl hydrocarbons suggests that the induction assay can be used as indicator of potential PCB toxicity. Our research initially focused on the activity of PCBs as inducers of AHH in immature male Wistar rats, and rat hepatoma H-4-II E cells in culture. A comprehensive study of all 209 PCBs was not feasible. However, several reports indicated that the most potent AHH inducers are substituted at both para and two or more meta positions. Initial studies confirmed that only 4 of these compounds (3,3',4,4'-tetra-, 3,4,4',5-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl) induced AHH and its associated cytochrome P-450 isozyme and elicited the toxic effects associated with halogenated aryl hydrocarbons. In subsequent research we synthesized and reported the biologic and toxic effects of all the monoortho chloro-substituted analogs of the 4 highly toxic coplanar PCBs (See Figure 1). The results demonstrated that this group of 8 PCB congeners elicited a spectrum of biologic and toxic effects comparable to those observed for the coplanar PCBs although it was apparent that the introduction of the ortho-chloro substituent substantially reduced the activity of these compounds (2,6). The monoortho coplanar PCBs have been identified in the commercial PCBs and environmental extracts and appear to be the major toxic components of these mixtures (4).

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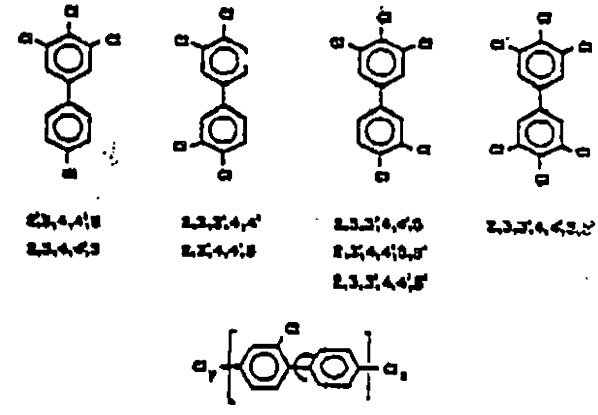

PCBs - Human Toxicology Human populations have been exposed to PCBs and PBBs via three major pathways, i.e., environmental, occupational, and accidental. The effects of PCBs and PBBs on humans have been determined by studying the occupationally and accidentally exposed individuals, since it has been acknowledged that these two groups have been exposed to the highest levels of PCBs. The major effects and symptoms observed during the Yusho poisoning in Japan and Taiwan include chloracne and several related dermal changes, ocular damage, increased serum triglycerides, reproductive problems, alterations in steroid metabolism, increased serum (and adipose tissue) levels of PCBs, and immunologic effects (7). In contrast, only a few of these symptoms have been noted in the occupationally exposed workers. It is not clear why this latter group does not present more severe symptoms of PCB poisoning since the serum levels of workers exposed to PCBs are comparable and often higher than the levels observed in victims of accidental poisonings.

The toxic Yusho oil contained a mixture of polychlorinated biphenyls (PCBs) which were contaminated by other halogenated aromatics including the highly toxic PCDFs. It has been reported (7,8) that the PCBs and PCDFs which persist in the liver of victims still suffering from Yusho poisoning include the following compounds; 2,3',4,4',5-penta-, 2,2',4,4',5,5'-, 2,2',3',4,4',5- and 2,3,3',4,4',5-hexa, 2,2',3,4,4',5,5'- and 2,2',3,3',4,4',5-heptachlorobiphenyls and the 2,3,7,8-tetra-, 1,2,4,7,8-, 1,2,3,7,8- and 1,2,3,4,7,8-penta- and 1,2,3,4,7,8-hexachlorodibenzofurans. All of these PCBs and PCDFs have been synthesized in my laboratory and reconstituted to approximate their composition in human liver (8). A comparison of the dose-response effects of the reconstituted PCB and PCDF mixtures in causing weight loss, thymic atrophy and the induction of cytochrome P-448-dependent monooxygenases indicated that the PCDF mixture was at least 700 times more active than the PCBs. Since the ratio of PCBs/PCDFs existing in Yusho patients' blood and liver was less than 600:1 and 5:1 respectively, the results suggest that the PCDFs are the major etiologic agent in Yusho poisoning. Comparable studies on the toxicity of the PCB and PCDF fractions isolated from Yusho oil confirm that the PCDFs were by far the most toxic fraction present and must make the major contribution to the toxicity of the contaminated rice oil (9). These results explain the toxic response differences in the Yusho and occupationally exposed humans. Yusho oil contained relatively high levels of toxic PCDFs whereas most commercial PCBs contain lower levels of these toxic impurities. Based on these results, risk assessment of environmental PCB exposure should be extrapolated from epidemiologic evaluation of occupationally exposed individuals. Since workers exposed to high levels of PCBs exhibit relatively few adverse health effects this would suggest that the potential adverse human health effects resulting from environmental exposure to PCBs are very low.

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MANAGER - ENVIRONMENTAL SCIENCE AND TECHNOLOGY

(203) 373-3316

November 24, 1986

FEDERAL EXPRESS

Dr. Stephen H. Safe, Professor of Toxicology
Texas A&M University
Veterinary Physiology and Pharmacology
Highway 60
College Station, TX 77843-4466

Dear Dr. Safe:

Thank you for the papers on PCBs as dioxin antagonists. I find them extremely interesting and would like to see this work progress further to look at the carcinogenic response, and also look at the effectiveness of certain individual congeners.

Regarding our meeting on December 1, I will be accompanied by Dr. Joseph Rodericks of Environ, who has personal knowledge of the PCB regulatory process, and Dr. John F. Brown, Manager of GE's Health Research at our Corporate Research and Development Center. We will be arriving at the College Station Airport at 11:50 a.m. and departing at 4:45 p.m., same day.

I would like to start the meeting by summarizing the reasons why we think that a new approach to quantitating PCBs in fish, i.e., quantitation of specific toxic congeners rather than total PCBs is more scientifically sound than FDA's current approach.

I would then like to discuss with you the kind of toxicity testing that might make this a convincing argument to the Agency.

I would also like to discuss toxicity tests that would demonstrate the differences between the Aroclors that were released to the environment, and those largely dechlorinated mixtures found in the sediments of certain rivers. For your interest, I am enclosing a copy of chromatograms of Aroclor 1242 and of PCBs found in Hudson River sediments.

I trust we will also have time to discuss your dioxin "antagonist" work as well.

We should have a very interesting and productive meeting.

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Sincerely yours,

Stan Hamilton

SBH:cas
Enclosure
cc: Dr. J.F. Brown
Dr. J. Rodericks

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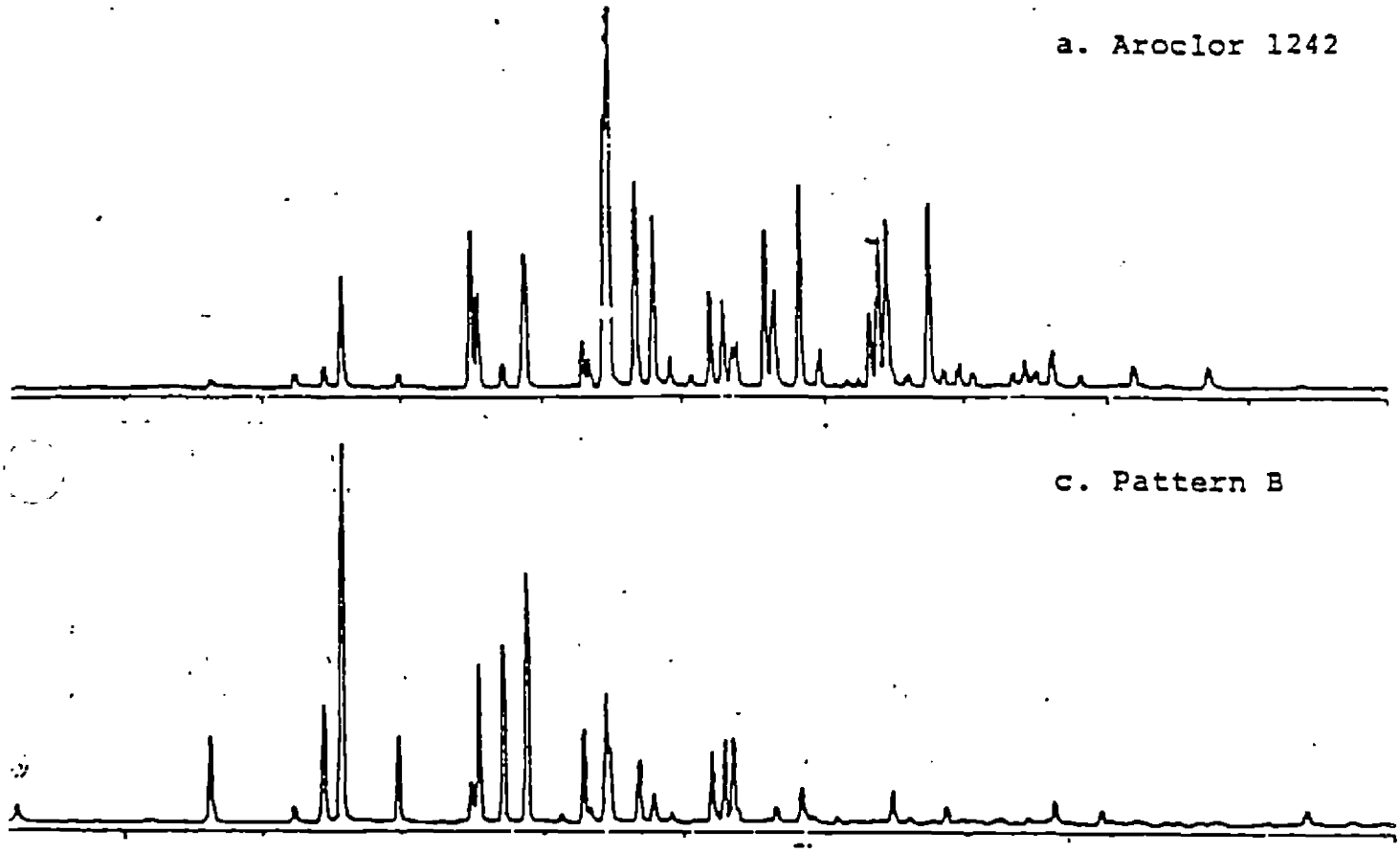
PCB Dechlorination in Hudson River Sediment



jure 1. Capillary gas chromatograms of PCB specimens illustrating patterns
st commonly seen in upper Hudson sediments: (a) Aroclor 1242 standard;
c) "Pattern B," dechlorinated Aroclor 1242± from Core 18-6, 16-17"

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AROCLOR 1254 AS A 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN ANTAGONIST: EFFECTS ON ENZYME INDUCTION AND IMMUNOTOXICITY

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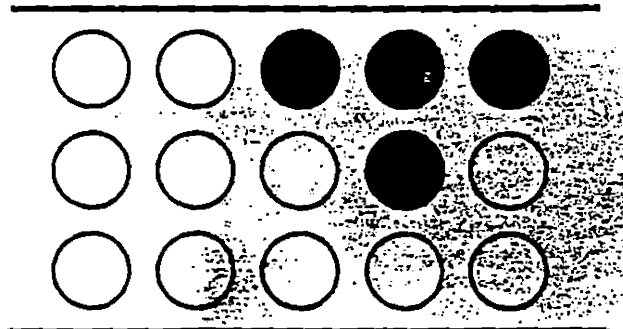

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TOXICOLOGY

An international journal concerned with the effects of chemicals on living systems

Toxicology is a journal for the publication of original scientific papers on the biological effects arising from the administration of chemical compounds, principally to animals, tissues or cells, but also to man. Such compounds include industrial chemicals and residues, chemical contaminants, consumer products, drugs, metals, pesticides, food additives, cosmetics, and additives to animal feeding stuffs. Preference will be given to investigations dealing with the mechanisms of action of toxic agents. Papers describing molecular interactions with cellular and genetic processes will be welcomed.

Quantitative toxicological studies will be published that are of relevance to risk assessment and regulatory management of exposure hazards and safety evaluation. This applies particularly to carcinogenicity, mutagenicity, embryotoxicity and related areas, as well as to alternatives to the use of animals in toxicological experimentation. Epidemiological studies bearing toxicological significance to man fall within the scope of the journal. The Editors would also welcome the submission of concise and pertinent reviews on current issues in toxicology.

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AROCLOR 1254 AS A 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN ANTAGONIST: EFFECTS ON ENZYME INDUCTION AND IMMUNOTOXICITY

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SUMMARY

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and Aroclor 1254 induced the cytochrome *P*-450 dependent monooxygenases, aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin *O*-deethylase (EROD) in rat hepatoma H-4-II E cells and C57BL/6J mice. It has been proposed that both Aroclor 1254 and 2,3,7,8-TCDD induce these enzymes via a common mechanism which features initial binding to the aryl hydrocarbon (Ah) cytosolic receptor protein. The major difference between these compounds was the relative potency (i.e. 2,3,7,8-TCDD ≫ Aroclor 1254). Cotreatment of rat hepatoma H-4-II E cells or C57BL/6J mice with a dose of 2,3,7,8-TCDD which submaximally induces AHH and EROD and a dose of Aroclor 1254 which exhibited little or no induction activity resulted in significant antagonism of the induction effects of 2,3,7,8-TCDD. For example, cotreatment of C57BL/6J mice with 2,3,7,8-TCDD (15 nmol/kg) and Aroclor 1254 (25, 75 and 150 μmol/kg) resulted in up to 23% antagonism of AHH induction by 2,3,7,8-TCDD. Moreover, cotreatment with a higher dose of the 2,3,7,8-TCDD agonist (30 or 50 nmol/kg) partially reversed some of the antagonism by Aroclor 1254. In vivo antagonism was observed only at Aroclor 1254/2,3,7,8-TCDD molar ratios of 1667:1, 5000:1 and 10 000:1. Administration of 2,3,7,8-TCDD (3.72 nmol/kg) to C57BL/6J mice resulted in a 76% decrease in the splenic plaque forming cell response to sheep red blood cells. This T-cell mediated immunotoxic effect of 2,3,7,8-TCDD segregates with

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Abbreviations: Ah, aryl hydrocarbon; AHH, aryl hydrocarbon hydroxylase; EROD, ethoxyresorufin *O*-deethylase; HCBP, hexachlorobiphenyl; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; SARs, structure-activity relationships; 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDF, 2,3,7,8-tetrachlorodibenzofuran.

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the Ah locus. In contrast, administration of 5, 15, 75 and 150 μmol/kg of Aroclor 1254 resulted in impairment of the immune response only at the highest dose level. However, cotreatment of mice with 2,3,7,8-TCDD (3.72 nmol/kg) and Aroclor 1254 (5, 15 or 75 μmol/kg) resulted in no significant decrease in the plaque forming cell response and complete protection from the immunotoxicity of 2,3,7,8-TCDD. Cotreatment of the mice with Aroclor 1254 (75 μmol/kg) and a higher dose of the 2,3,7,8-TCDD agonist resulted in partial reversal of the protective effects of Aroclor 1254. The in vitro and in vivo data suggest that within specific antagonist/agonist dose ratios, Aroclor 1254 can antagonize at least 2 Ah receptor-mediated effects of 2,3,7,8-TCDD, namely AHH induction and immunotoxicity.

Key words: 2,3,7,8-TCDD; Aroclor 1254; Antagonism.

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related halogenated aryl hydrocarbons elicit a number of common toxic and biologic responses including chloracne and other dermal lesions, porphyria, reproductive effects and teratogenicity, endocrine disorders, tissue-dependent hypoplastic and hyperplastic responses, immunotoxicity, a wasting syndrome and the induction of diverse enzymes including several cytochrome *P*-450-dependent monooxygenases [1—4]. The overall mechanisms associated with the toxic effects elicited by 2,3,7,8-TCDD have not been delineated; however, it has been proposed that initial binding of the toxin (or related agonist) to a cytosolic (aryl hydrocarbon, Ah) receptor protein is a common obligatory step in initiating the pleiotropic responses noted above [1—7].

Although the Ah receptor protein has not been fully characterized or purified, there is considerable evidence which supports a receptor-mediated mechanism of action for 2,3,7,8-TCDD and related compounds. For example, the Ah receptor exhibits saturable high affinity binding with [^{3}H]2,3,7,8-TCDD (K_D = 0.7 nM) [5,7]; the competitive receptor binding affinities of polychlorinated dibenzo-*p*-dioxin (PCDD), biphenyl (PCB) and dibenzofuran (PCDF) isomers and congeners are structure-dependent and illustrate the highly stereoselective interaction between the receptor protein and different classes of chemical agonists [2,8—11]. The most avid ligands for the receptor are approximate isostereomers of 2,3,7,8-TCDD and are substituted in their lateral positions. Genetic studies with responsive C57BL/6 (high receptor levels) and non-responsive DBA/2J (low receptor levels) inbred mice and their backcrosses demonstrate that tissue responsiveness to 2,3,7,8-TCDD is, in part, dependent on receptor levels. Moreover, mouse genetic inbreeding studies have shown that teratogenicity, porphyria, body weight loss, immunotoxicity and the induction of cytochrome P_1-450, segregate with the Ah locus [6,12—19]. Quantitative in vitro and in vivo structure-activity rela-

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tionships (SARs) have recently been reported for several structurally diverse PCDDs, PCDFs and PCBs [9—11,20]. Comparable SARs were observed for cytosolic receptor binding, the in vitro induction of aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin *O*-deethylase (EROD), the in vivo induction of these same monooxygenase enzymes, body weight loss and thymic atrophy (in the rat).

Previous studies with agonists for the estrogen receptor have demonstrated that some compounds which exhibit moderately high estrogen receptor binding affinities but low estrogenic activities (e.g. estriol and some triphenylethylene analogs) can antagonize the effects of estradiol [21,22]. Similarly, recent studies in our laboratory have shown that both 1,3,6,8- and 2,4,6,8-tetrachlorodibenzofuran (TCDF) exhibit relatively high cytosolic receptor binding affinities (1.25×10^{-7} M and 1.5×10^{-6} M, respectively) but are only weakly active as inducers of AHH and EROD in rat hepatoma H-4-II E cells in culture [23]. Cotreatment of the cells with 2,3,7,8-TCDD and either of the 2 PCDF isomers demonstrated that both compounds antagonized the induction of the monooxygenases by 2,3,7,8-TCDD. Previous studies [8] have reported that the commercial PCB, Aroclor 1254 also exhibited moderate binding affinity to the cytosolic receptor protein (6.6×10^{-6} M) but was a weak agonist for most receptor-mediated responses in vivo and in vitro [24]. This paper reports the activity of Aroclor 1254 as a 2,3,7,8-TCDD antagonist in rat hepatoma H-4-II E cells and in C57BL/6 mice.

MATERIALS AND METHODS

Chemicals

2,3,7,8-TCDD and ethoxyresorufin were synthesized in this laboratory to greater than 99% purity as determined by gas chromatography [10]; Aroclor 1254 was a gift from Dr. O. Hutzinger; NADPH and NADH were purchased from the United States Biochemical Corporation (Cleveland, OH). Benzo[*a*]pyrene was purchased from Sigma Chemical Co.; DNA-grade hydroxylapatite was purchased from Bio-Rad Laboratories (Richmond, CA). All other chemicals were of the highest quality commercially available. [^{3}H]2,3,7,8-TCDD was synthesized in the laboratory by the chlorination of 1,6[$^3H_2$]dibenzo-*p*-dioxin followed by chlorination and HPLC purification of the product (> 98% pure) [5].

In vivo enzyme induction studies

Male C57BL/6J, 3—4 weeks of age, were obtained from the Jackson Laboratories, Bar Harbor, ME. The mice were housed in plastic cages with hardwood bedding, allowed free access to Teklab Laboratory Rodent Chow, (Teklab, Madison, WI) and water and maintained on a diurnal 12-h light/12-h dark cycle. Aroclor 1254 or 2,3,7,8-TCDD were dissolved in corn oil and administered by intraperitoneal injection (10 ml/kg). The C57BL/6J mice treated with Aroclor 1254 and/or 2,3,7,8-TCDD were sacrificed after 72 h.

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Corn oil (10 ml/kg) served as a vehicle control for all studies. Livers were perfused via the hepatic portal vein with ice cold saline containing 0.1 mM EDTA, weighed and placed in beakers containing homogenization buffer. Livers were washed and then homogenized using a Potter-Elvehjem homogenizer in 4 volumes of 0.25 M sucrose with 0.1 mM EDTA. A microsomal pellet was prepared by centrifuging the homogenate at 10 000 *g* for 20 min and then centrifuging the resultant supernatant at 100 000 *g* for 1 h. The pellet was resuspended in homogenization buffer and stored at −80 °C until used for the enzyme assays. Ethoxyresorufin o-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) were determined as previously described [25,26]. Protein concentrations were determined [27] using bovine serum albumin as a protein standard. All data are expressed as means ± S.D. Significance was determined by a two-way ANOVA and Dunnet's *t*-test [28].

In vitro enzyme induction

Rat hepatoma H-4-II E cells were grown in minimum essential medium without ribo- or deoxyribonucleotides but with L-glutamine. The medium was supplemented with 10% fetal calf serum, 10% calf serum, gentamycin sulfate (50 μg/ml) and Fungizone (2.5 μg/ml) and cultures were maintained in a humidified 5% CO_2 atmosphere at 37 °C. At confluency stock cultures were trypsinized and seeded in 25-cm² Corning culture flasks at a density of 0.6×10^6 cells/flask (Day 1). On Day 2, the spent medium was aspirated off and replaced with fresh medium containing the test compound in DMSO to a final DMSO concentration of 0.5%. Dose-response induction studies with at least 6 different concentrations of each inducer were carried out. On Day 3, the cultures reached confluency and were harvested. Cultures were washed 3 times with PBS (pH 7.4) and scraped off with a rubber policeman in Tris-sucrose (0.05—0.02 M) buffer (pH 8.0). The cell suspensions were centrifuged and resuspended in buffer. After protein determination [27], the cell suspensions were adjusted to a final protein concentration of 1 mg/ml and whole cell suspensions were assayed for enzyme activity. EROD and AHH activities were measured fluorimetrically [25,26] and the EC_{50} values determined as previously described [9—11].

Receptor binding assays

Hepatic cytosol ~3—5 mg/ml) was incubated with different concentrations of [³H]2,3,7,8-TCDD and Aroclor 1254 (100, 500 and 667 nM) for 2 h at 20 °C. Each assay was performed in duplicate. A hydroxylapatite slurry (Bio Rad Lab; 3 parts gel in 5 parts phosphate or HEDG buffer) was freshly prepared. The phosphate buffer was 50 mM Tris—HCl, 1 mM KH_2PO_4. After incubation, 200 μl aliquot of the cytosol was transferred to a second tube containing 250 μl of the hydroxylapatite slurry. The tubes were incubated on ice for 30 min with shaking every 10 min. The mixture was then resuspended in 2 ml phosphate buffer and centrifuged at 800 *g* for 2 min. The hydroxylapatite pellets were washed 3 additional times with phosphate buffer (2.0 ml buffer) and then resuspended in 1.0 ml absolute ethanol. The

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hydroxylapatite-ethanol solution was vortexed and transferred to scintillation vials and any remaining hydroxylapatite was washed into the vial with an additional 1 ml of ethanol; 10 ml of scintillation cocktail was added and samples were counted for radioactivity. Saturation binding of [^{3}H]2,3,7,8-TCDD with C57BL/6J hepatic cytosolic receptor protein was determined in the presence of different concentrations of the competitive ligand, Aroclor 1254 and the results are summarized in Fig. 2.

Plaque forming cell (PFC) assay

The method employed was the "Cunningham" variant of the Jerne PFC assay [29,30]. C57BL/6J mice at 8 weeks were purchased from the Jackson Laboratories, Bar Harbor, ME. Upon arrival the animals were allowed to acclimate for 5—7 days before treatment. On day 1 the animals were injected (i.p.) with the appropriate chemical as described for the enzyme induction studies (Aroclor 1254 and/or 2,3,7,8-TCDD) and sheep red blood cells (SRBC, 4×10^8 cells) were injected after 5 days. The animals were sacrificed by cervical dislocation 10 days after treatment with the chemicals and the spleens were removed and placed individually in 60-mm petri dishes containing 10 ml of cold Eagle's MEM media supplemented with Hank's salts (KC Biological). Each spleen was then teased through a 70-μm nylon screen (Spectrum Medical, Inc.) which was saturated with 2 ml of the Eagle's media. The screens were washed of remaining cells by the addition of 8 ml of media. The cell solution was then spun at 220 *g* for 10 min and the pellets resuspended in 2 ml of fresh media. Appropriate dilutions of the suspensions were then made with 0.1 ml of a dilution mixed with 0.2 ml of a 20% SRBC solution and 0.1 ml of a 1—5 guinea pig serum dilution. Through capillary action, microliter aliquots of the mixtures were placed in "Cunningham" slide chambers and incubated for 60 min at 37 °C. Viable cell counts were determined by Eosin Y staining.

RESULTS

2,3,7,8-TCDD evoked a dose-dependent induction of AHH and EROD in rat hepatoma H-4-II E cells at concentrations of 10^{-12}—10^{-8} M. At higher dose levels ($> 10^{-8}$ M), 2,3,7,8-TCDD was toxic to the cells and this was evidenced by a dose-dependent decrease in the AHH induction maximum with increasing concentrations of 2,3,7,8-TCDD (Fig. 1). The dose-dependent increase and subsequent 'decrease of AHH and EROD induction has previously been reported for other halogenated aromatics [24]. Moreover, for some weak agonists such as Aroclor 1254, cellular toxicity is noted at concentrations which are insufficient to elicit maximum induction of the cytochrome *P*-450-dependent monooxygenases (i.e. see Fig. 1). Cotreatment of the rat hepatoma cells with a non-toxic concentration of Aroclor 1254

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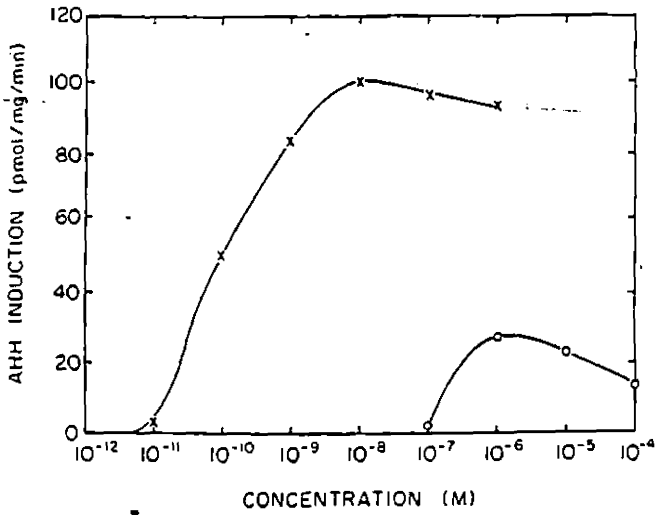

Fig. 1. Dose response induction of AHH (pmol/mg protein/min) in rat hepatoma H-4-II E cells by Aroclor 1254 (O) and 2,3,7,8-TCDD (×). Cellular toxicity by Aroclor 1254 and 2,3,7,8-TCDD was observed at concentrations > 10^{-6} M and 10^{-8} M, respectively.

(1×10^{-7} M) and a concentration of 2,3,7,8-TCDD which elicited a submaximal AHH and EROD induction response (1×10^{-9} M) resulted in the antagonism of the induction activity of 2,3,7,8-TCDD by Aroclor 1254 (Table I). Cotreatment of the cells with the same concentration of Aroclor 1254 (1×10^{-7} M) and a higher concentration of 2,3,7,8-TCDD (2.25×10^{-8} M) par-

TABLE I

EFFECTS OF 2,3,7,8-TCDD, AROCLOR 1254 AND 2,3,7,8-TCDD PLUS AROCLOR 1254 (COTREATED) AS INDUCERS OF AHH AND EROD IN RAT HEPATOMA H-4-II E CELLS IN CULTURE

Inducer	Concentration (M)	EROD[b]	AHH[b]
Solvent (control)	—	0	0
Aroclor 1254	1×10^{-7}	0	0
2,3,7,8-TCDD	1×10^{-9}	74.0 ± 20.1	155.9 ± 14.6
2,3,7,8-TCDD	2.25×10^{-8}	77.9 ± 19.9	179.0 ± 19.7
Aroclor 1254 plus 2,3,7,8-TCDD	1×10^{-7} 1×10^{-9}	46.8 ± 3.99[a]	105.7 ± 11.7[a]
Aroclor 1254 plus 2,3,7,8-TCDD	1×10^{-7} 2.25×10^{-8}	65.6 ± 2.21	117.0 ± 7.36

[a]Significantly lower ($P < 0.01$) than observed after treatment with 2,3,7,8-TCDD (1.0×10^{-9} M) alone.
[b]pmol/mg protein/min.

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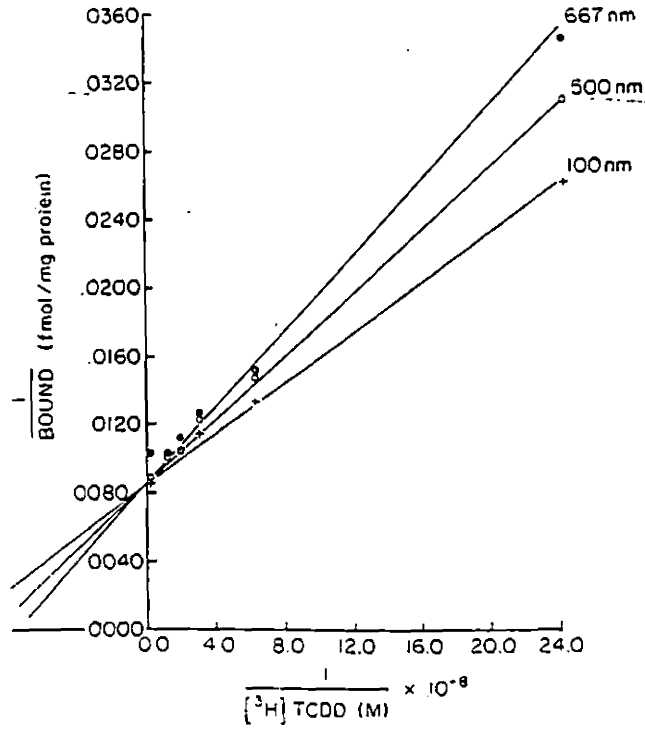

Fig. 2. Double reciprocal plot analysis of the saturation binding of [^{3}H]2,3,7,8-TCDD in the presence of different concentrations of Aroclor 1254 (100, 500 and 667 nM). The y intercepts of the double-reciprocal plots of the saturation binding of [^{3}H]2,3,7,8-TCDD with the Ah receptor in the presence of 100, 500 and 667 nM Aroclor 1254 were 0.0091, 0.0088 and 0.0092, respectively. The linear correlation coefficients were 0.9925, 0.9984 and 0.9995, respectively. In the absence of Aroclor 1254, the y intercept of the double-reciprocal plot of the saturation binding of [^{3}H]2,3,7,8-TCDD with the Ah receptor was 0.0074 and the correlation linear correlation coefficient was 0.9899 (data not shown). The incubations were carried out using hepatic cytosol preparation (3—5 mg/ml) and a range of [^{3}H]2,3,7,8-TCDD concentrations (from 0.8 to 11 nM).

tially overcame the antagonism by Aroclor 1254. Double reciprocal plot analysis of the saturation binding curves of [^{3}H]2,3,7,8-TCDD and rat hepatic cytosolic receptor in the presence of different concentrations of Aroclor 1254 is summarized in Fig. 2 and demonstrates that Aroclor 1254 acts as a competitive antagonist for the receptor-2,3,7,8-TCDD interaction.

Table II summarizes the dose-response effects of 2,3,7,8-TCDD and Aroclor 1254 as inducers of hepatic microsomal AHH and EROD in immature male C57BL/6J mice. Maximal induction of AHH (4.06 nmol/mg/min) and EROD (4.86 nmol/mg/min) were observed at a dose level of 50 nmol/kg of 2,3,7,8-TCDD. No overt toxicity (e.g. body weight loss, hepatotoxicity or thymic atrophy) was observed at this dose level. In contrast, administration of Aroclor 1254 to C57BL/6J mice at doses of 5, 25, 75, 150 and 400 μmol/kg significantly induced EROD only at the highest dose of Aroclor 1254

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TABLE II

THE DOSE-RESPONSE EFFECTS OF 2,3,7,8-TCDD AND AROCLOR 1254 AS INDUCERS OF HEPATIC MICROSOMAL AHH AND EROD IN MALE C57BL/6J MICE (n = 5)

Compound	Dose (μmol/kg)	EROD[b]	AHH[b]
Corn oil	—	0.06 ± 0.004	0.21 ± 0.08
2,3,7,8-TCDD	0.001	0.33 ± 0.08[a]	0.64 ± 0.16[a]
2,3,7,8-TCDD	0.003	1.39 ± 0.54[a]	1.67 ± 0.71[a]
2,3,7,8-TCDD	0.010	3.31 ± 0.50[a]	3.36 ± 0.07[a]
2,3,7,8-TCDD	0.015	3.96 ± 0.16[a]	3.56 ± 0.43[a]
2,3,7,8-TCDD	0.03	4.19 ± 0.30[a]	3.67 ± 0.30[a]
2,3,7,8-TCDD	0.05	4.86 ± 0.61[a]	4.06 ± 0.37[a]
Aroclor 1254	5	0.06 ± 0.006	0.18 ± 0.06
Aroclor 1254	25	0.05 ± 0.03	0.41 ± 0.06[a]
Aroclor 1254	75	0.06 ± 0.01	0.39 ± 0.06[a]
Aroclor 1254	150	0.09 ± 0.04	0.40 ± 0.08[a]
Aroclor 1254	400	0.25 ± 0.05[a]	0.55 ± 0.07[a]

[a]Significantly different ($P < 0.01$) from the corn oil controls.
[b]nmol/mg protein/min.

TABLE III

COTREATMENT OF C57BL/6J MICE WITH AROCLOR 1254 AND 2,3,7,8-TCDD: EFFECTS ON AHH AND EROD INDUCTION (n = 5)

Inducers	Dose (μmol/kg)	EROD[c]	AHH[c]
Aroclor 1254 plus 2,3,7,8-TCDD	5 0.015	3.79 ± 0.58	3.34 ± 0.42
Aroclor 1254 plus 2,3,7,8-TCDD	25 0.015	3.05 ± 0.47[a]	2.84 ± 0.33[a]
Aroclor 1254 plus 2,3,7,8-TCDD	75 0.015	3.09 ± 0.34[a]	2.88 ± 0.21[a]
Aroclor 1254 plus 2,3,7,8-TCDD	150 0.015	3.22 ± 0.07[b]	3.09 ± 0.27[a]
Aroclor 1254 plus 2,3,7,8-TCDD	400 0.015	3.96 ± 0.45	3.55 ± 0.18
Aroclor 1254 plus 2,3,7,8-TCDD	25 0.03	4.19 ± 0.39	3.78 ± 0.25
Aroclor 1254 plus 2,3,7,8-TCDD	25 0.05	4.93 ± 0.25	4.10 ± 0.20

Significantly lower [a]($P < 0.01$) [b]($P < 0.05$) than induction values observed after treatment with 2,3,7,8-TCDD (15 nmol/kg) alone; the induction values for 2,3,7,8-TCDD (0.015, 0.03 and 0.5 μmol/kg) are shown in Table II.
[c]nmol/mg protein/min.

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and marginally increased AHH at doses above 25 μmol/kg. Table III summarizes the effects of coadministration of Aroclor 1254 (5, 25, 75, 150 or 400 μmol/kg) with a dose of 2,3,7,8-TCDD (15 nmol/kg) which elicited a submaximal AHH and EROD induction response. Cotreatment of 2,3,7,8-TCDD with 25 or 75 μmol/kg of Aroclor significantly inhibited the induction of AHH and EROD by 2,3,7,8-TCDD. Coadministration of Aroclor 1254 (25 μmol/kg) with higher doses of 2,3,7,8-TCDD (30 and 50 nmol/kg) reversed the antagonistic effects of Aroclor 1254.

Table IV summarizes the effects of 2,3,7,8-TCDD and Aroclor 1254 on the T-cell dependent plaque-forming cell (PFC) assay after exposure of

TABLE IV

EFFECTS OF 2,3,7,8-TCDD, AROCLOR 1254 AND 2,3,7,8-TCDD PLUS AROCLOR 1254 ON THE PLAQUE FORMING CELL ASSAY IN MICE PRETREATED WITH SHEEP RED BLOOD CELLS

Treatment (dose)	PFC/spleen × 10^5	PFC/10^6 viable spleen cells	% Control (PFC/spleen × 10^5)
Corn oil[a]	1.61 ± 0.17	562 ± 76	100
2,3,7,8-TCDD			
(3.72 nmol/kg)[b]	0.38 ± 0.03[c]	156 ± 40[c]	24
(11.2 nmol/kg)	0.20 ± 0.03[c]	62 ± 12[c]	12
Aroclor 1254			
(5 μmol/kg)	1.57 ± 0.11	561 ± 140	98
(15 μmol/kg)[b]	1.60 ± 0.87	465 ± 114	100
(75 μmol/kg)	1.57 ± 0.23	594 ± 35	98
(150 μmol/kg)	1.20 ± 0.22	394 ± 35	75
2,3,7,8-TCDD (3.72 nmol/kg) plus Aroclor 1254 (5 μmol/kg)	1.70 ± 0.30	491 ± 62	106
2,3,7,8-TCDD (3.72 nmol/kg) plus Aroclor 1254 (15 μmol/kg)[b]	1.49 ± 0.08	480 ± 48	93
2,3,7,8-TCDD (3.72 nmol/kg) plus Aroclor 1254 (75 μmol/kg)	1.66 ± 0.28	558 ± 52	103
2,3,7,8-TCDD (3.72 nmol/kg) plus Aroclor 1254 (150 μmol/kg)	0.92 ± 0.08[c]	372 ± 81[c]	57
2,3,7,8-TCDD (11.2 nmol/kg) plus Aroclor 1254 (75 μmol/kg)	0.58 ± 0.10[c]	170 ± 10[c]	36

[a]11 animals.
[b]8 animals; all other 4 animals/group.
[c]Significantly different ($P < 0.01$) than the corn oil controls.

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C57BL/6J mice to sheep red blood cells. Administration of 2,3,7,8-TCDD (3.72 and 11.2 nmol/kg) resulted in only 24 and 12% PFCs/10^6 spleen cells respectively, compared to the corn oil-treated animals. In contrast, administration of Aroclor 1254 at dose levels of 5, 15, and 75 μmol/kg did not significantly impair the immune response although administration of a higher dose of Aroclor 1254 (150 μmol/kg) did evoke some decrease in the plaque forming cell response. Cotreatment of the mice with 2,3,7,8-TCDD (3.72 nmol/kg) and 5, 15, or 75 μmol/kg Aroclor 1254 resulted in significant protection of these animals from the immunotoxic effects of 2,3,7,8-TCDD (Table IV). Significant antagonism of 2,3,7,8-TCDD was also observed in animals cotreated with the highest dose of Aroclor 1254 (150 μmol/kg) which was also immunotoxic in this assay when administered alone. Coadministration of a higher dose of 2,3,7,8-TCDD (11.2 nmol/kg) with Arcolor 1254 (75 μmol/kg) reversed some of the antagonistic effects of the commercial PCB mixture.

DISCUSSION

This study describes the in vitro and in vivo interactive effects of 2,3,7,8-TCDD and Aroclor 1254, 2 compounds which individually elicit a number of common biologic and toxic effects in diverse animal species. For example, both of these halogenated aryl hydrocarbons induce the same monooxygenase enzymes (e.g. AHH and EROD) and their associated cytochrome *P*-450 isozymes, cause body weight loss, hepatotoxicity and porphyria, thymic atrophy and immunotoxicity [1—3,24]. In addition, 2,3,7,8-TCDD and Aroclor 1254 competitively bind to the Ah or 2,3,7,8-TCDD receptor protein [8]. These results support the proposed common mechanism of action for both compounds; however, it is apparent from this study and others that the major differences between Aroclor 1254 and 2,3,7,8-TCDD are their toxic and biologic potencies. The results summarized in Tables I and II clearly illustrate the quantitative differences between 2,3,7,8-TCDD and Aroclor 1254 as inducers of AHH and EROD. In rat hepatoma cells, the EC_{50} for 2,3,7,8-TCDD as an inducer of AHH and EROD was 9.78×10^{-11} and 1.90×10^{-10} M respectively whereas the EC_{50} for Aroclor 1254 as an inducer of these enzymes was $> 10^{-5}$ M. Moreover in C57BL/6J mice, 2,3,7,8-TCDD was > 50 000 times more potent than Aroclor 1254 as an inducer of hepatic microsomal AHH and EROD. 2,3,7,8-TCDD and Aroclor 1254 and several PCB congeners are immunotoxic and inhibit the plaque forming antibody response in C57BL/6J mice challenged with sheep erythrocytes [13—17,31]. The ED_{50} for inhibition of this response by Aroclor 1254 in C57BL/6N mice was between 250 and 500 mg/kg [31] whereas the ED_{50} for 2,3,7,8-TCDD was <1.2 μg/kg [14].

Previous in vitro studies have reported that 1,3,6,8- and 2,4,6,8-tetrachlorodibenzofuran antagonize the induction of AHH and EROD in rat hepatoma H-4-II E cells in culture [23]. Like Aroclor 1254, both of these isomers exhibit moderate competitive receptor binding affinities and are weak

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inducers of AHH and EROD in the cell culture system. Table I summarizes the interactive effects of 2,3,7,8-TCDD and Aroclor 1254 in the rat hepatoma H-4-II E system and at Aroclor 1254/2,3,7,8-TCDD concentration ratios of 100:1 there was a significant inhibition of the induction activity of 2,3,7,8-TCDD by Aroclor 1254. Moreover at a higher dose of the agonist (2.25×10^{-8} M) some of the inhibitory effects of Aroclor 1254 were overcome. A comparable interaction study was carried out in C57BL/6J mice. The mice were cotreated with a dose of 2,3,7,8-TCDD which elicited a submaximal induction response for hepatic microsomal AHH and EROD (15 nmol/kg) and one of several doses of Aroclor 1254. The results showed the Aroclor significantly reduced the induction of AHH and EROD by 2,3,7,8-TCDD (15 nmol/kg) only at doses of 25, 75 and 150 μmol/kg. The role of Aroclor 1254 as a 2,3,7,8-TCDD antagonist was further supported by the effects of cotreating the animals with the most effective "inhibitory" dose of Aroclor 1254 (25 μmol/kg) and higher doses of 2,3,7,8-TCDD (30 and 50 nmol/kg) (Table III). These results showed that at higher dose levels of the agonist the partial antagonist activity of Aroclor 1254 was decreased complementing the results obtained in the in vitro studies. It was also apparent that Aroclor 1254 was a more effective partial antagonist of the induction responses by 2,3,7,8-TCDD in cell culture. Significant antagonism of AHH was observed at Aroclor 1254/2,3,7,8-TCDD ratios of 100:1 in cell cultures whereas antagonism was only observed in the mice at ratios between 1667:1 and 10 000:1.

Several reports have shown that 2,3,7,8-TCDD and related compounds are immunotoxic and impair many T-cell mediated functions [13—17,31—34]. Moreover the T-cell dependent plaque forming cell response to sheep red blood cells is one of the most sensitive indicators of exposure to toxic halogenated aryl hydrocarbons and studies in genetically inbred mice have provided strong evidence that this response is Ah receptor-mediated. Table IV summarizes the immunosuppressive effects of 2,3,7,8-TCDD and Aroclor 1254 in C57BL/6J mice using the plaque forming cell assay response to sheep red blood cells. 2,3,7,8-TCDD was highly immunotoxic whereas doses of 5, 15 and 75 μmol/kg of Aroclor 1254 were inactive in this assay system. This latter result was not surprising since Aroclor 1254 was previously reported to be inactive at doses of < 125 mg/kg (382 μmol/kg) [31]. Cotreatment of the mice with 2,3,7,8-TCDD (3.72 nmol/kg) and different doses of Aroclor 1254 (5, 15 or 75 μmol/kg) resulted in complete protection from the immunotoxic effects of 2,3,7,8-TCDD by Aroclor 1254 (5, 15 or 75 μmol/kg). Moreover, the antagonistic effects of Aroclor 1254 (75 μmol/kg) can be partially overcome by a higher dose of the 2,3,7,8-TCDD agonist (11.2 nmol/kg).

The interactive effects of 2,3,7,8-TCDD, a potent Ah receptor agonist, and Aroclor 1254 suggest that this commercial PCB mixture can antagonize at least 2 Ah receptor mediated responses, namely immunotoxicity and the induction of cytochrome P_1-450-dependent monooxygenases (AHH and EROD). These effects are dependent on the dose of both the antagonist and

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agonist and their respective antagonist/agonist ratios. For antagonism of AHH and EROD induction by 2,3,7,8-TCDD in C57BL/6J mice, an Aroclor 1254/2,3,7,8-TCDD ratio of 1667:1 gave the maximum antagonism (23%) of the EROD induction response. In contrast, Aroclor 1254/2,3,7,8-TCDD ratios from 1340:1 to 20 160:1 resulted in complete protection from the immunotoxic effect of 2,3,7,8-TCDD (3.72 nmol/kg). The results demonstrate that the "window of antagonism" for Aroclor 1254 is clearly dependent not only on the absolute and relative doses of Aroclor 1254 and 2,3,7,8-TCDD but on the specific receptor-mediated response which is being measured. However, it was observed that for Aroclor 1254/2,3,7,8-TCDD ratios which result in antagonism by Aroclor 1254 of AHH and EROD induction (in vivo and in vitro) and immunotoxicity, an increased dose of 2,3,7,8-TCDD overcame some of the partial antagonism by Aroclor 1254. These results, coupled with the double-reciprocal plot analysis of the in vitro binding data (Fig. 2) suggest that the antagonist activity of Aroclor 1254 is related to the competitive inhibition of 2,3,7,8-TCDD binding to the Ah receptor and is not due to cellular toxicity caused by Aroclor 1254.

A previous study has reported that 2,3-dimethyl-5-*t*-butyl-1,3-benzodioxole (DBBD) inhibits EROD induction by 3-methylcholanthrene [35]. However, it is unlikely that the mechanism of antagonism for this compound involves competitive inhibition of receptor binding since DBBD exhibits a low binding affinity for the Ah receptor [36]. A more recent study suggested that the activity of DBBD may be associated with down regulation of the Ah receptor [37]. A recent paper [38] reported that 1-amino-3,7,8-trichlorodibenzo-*p*-dioxin was also an effective antagonist for 2,3,7,8-TCDD induced myelotoxicity (in vivo) and enzyme induction (in vitro). They also proposed that this compound acts as a competitive antagonist and their results complement the data reported in this study which demonstrates that Aroclor 1254 antagonizes 2,3,7,8-TCDD-mediated enzyme induction and immunotoxicity in C57BL/6J mice.

It is interesting to note that the relatively high ratios of Aroclor 1254/2,3,7,8-TCDD which result in antagonist activity are comparable to the ratios of PCBs/PCDFs or PCBs/PCDDs which have been identified in the analysis of human tissues and environmental samples [39,40]. It is conceivable that environmental or accidental exposure to these mixtures within certain exposure levels may result in some protection from the highly toxic PCDDs and PCDFs by PCBs and this possibility is currently being investigated in our laboratory.

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AROCLOR 1254 AS AN ANTAGONIST OF THE TERATOGENICITY OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN

(Aroclor 1254; 2,3,7,8-TCDD; antagonism)

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SUMMARY

Administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, 20 μg/kg) to pregnant C57BL/6J mice (on day 10) resulted in 62% fetuses with cleft palate per litter without any observable maternal toxicity. In contrast, Aroclor 1254 administered at a dose of 750 μmol/kg was not teratogenic. Cotreatment of the pregnant mice with both Aroclor 1254 (244 mg/kg) and 2,3,7,8-TCDD (20 μg/kg) resulted in an 8.2% incidence of cleft palate per litter. In contrast, Aroclor 1254 did not afford any protection from the teratogenicity of dexamethasone in C57BL/6J mice. Previous studies have shown that Aroclor 1254 can act as a partial antagonist of the microsomal enzyme induction and immunotoxic effects of 2,3,7,8-TCDD in C57BL/6J mice and this paper demonstrates that the commercial polychlorinated biphenyl mixture also antagonizes 2,3,7,8-TCDD-mediated teratogenicity in this strain of mice.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are widely used industrial compounds which have been identified in almost every component of the global ecosystem including fish, wildlife and human adipose tissue, blood and milk [1–4]. During the past few years, the more toxic halogenated aryl hydrocarbons, namely the polychlorinated

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Abbreviations: Ah, aryl hydrocarbon; AHH, aryl hydrocarbon hydroxylase; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; TCDD, tetrachlorodibenzo-*p*-dioxin.

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dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs) have also been identified in the environment [5–8] and in human tissue including milk samples [9–16]. Since the PCDFs and PCDDs are industrial or combustion byproducts [17–19], the levels of these compounds are usually $>10^3$-fold lower in most environmental samples. Moreover, high resolution analyses of the human milk and adipose tissue have confirmed the presence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic halogenated aryl hydrocarbon and several other toxic 2,3,7,8-substituted PCDDs and PCDFs [9–16].

The toxicity and mechanism of action of halogenated aryl hydrocarbons including PCBs, PCDDs and PCDFs have been extensively investigated [20–26]. Laboratory animal studies show that these compounds elicit a number of common toxic responses including: body weight loss, thymic atrophy and immunotoxicity, teratogenicity and reproductive problems, hepatotoxicity and porphyria, carcinogenicity, effects on the endocrine system and dermal toxicity. All of these responses are not evoked in any single animal species; however, body weight loss and thymic atrophy are the signs of toxicity most frequently observed in laboratory animals.

Most incidents involving environmental or accidental human exposure to the toxic PCDDs and PCDFs are associated with concomitant exposure to higher levels of PCBs and these are generally reflected in human tissue levels in which the PCBs/PCDDs, PCDFs ratios are >1000:1. For example in the Yusho and Yu Cheng poisoning in Japan and Taiwan respectively, several thousand individuals were poisoned with rice oil contaminated with PCBs and PCDFs [27,28]. The ratios of PCBs/PCDFs in the contaminated oil and the blood of exposed individuals were generally in the order of 184–667:1 and 1500–1890:1 respectively [27]. Since the interactive effects of concurrent exposure to halogenated aryl hydrocarbons is not well understood, this study reports teratogenicity of combined exposure to 2,3,7,8-TCDD and Aroclor 1254, a commercial PCB formulation.

MATERIALS AND METHODS

Chemicals

2,3,7,8-TCDD was prepared in our laboratory and the purity was > 99% as determined by gas-liquid and high-pressure liquid chromatographic analysis. Dexamethasone was purchased from Sigma Chemical Co., St. Louis, MO. Aroclor 1254 was a generous gift of Mr. B. Chittim, Wellington Environmental Consultants, Guelph, Ont., Canada.

Animal treatment

Mature male C57BL/6J mice and virgin female C57BL/6N mice (20–25 g) were purchased from Harlan Sprague-Dawley, Indianapolis, IN and maintained on food and water ad libitum at the Texas A&M University Laboratory Animal Resources

and Research facility. At the end of a 7-day acclimation period, females were mated with the males overnight. The following morning, females were checked for the presence of vaginal plugs. The presence of a vaginal plug was denoted as day 0 of pregnancy. Females with vaginal plugs were weighed and marked. Pregnancies were confirmed by measuring weight gain. The dams were treated by oral gavage with either corn oil (10 ml/kg), Aroclor 1254 (244 mg/kg), 2,3,7,8-TCDD (20 μg/kg), Aroclor 1254 (244 mg/kg) followed by 2,3,7,8-TCDD (20 μg/kg), dexamethasone (90 mg/kg), or Aroclor 1254 (244 mg/kg) followed by dexamethasone (90 mg/kg). All these compounds or mixtures were administered in corn oil (10 ml/kg) by oral gavage. Aroclor 1254 alone was administered on day 9 of pregnancy; corn oil and 2,3,7,8-TCDD were given on day 10 and dexamethasone on day 13. The animals receiving 2 chemicals were given Aroclor 1254 on the day prior to either 2,3,7,8-TCDD or dexamethasone, i.e. Aroclor 1254 on day 9 and 2,3,7,8-TCDD on day 10 or Aroclor 1254 on day 12 and dexamethasone on day 13 of pregnancy. Controls were either untreated or treated with corn oil (10 ml/kg). On day 17, the females were killed with CO_2 or by cervical dislocation. The weights of the dams and their respective liver weights were recorded. The uterine horns were exposed and the number of resorptions, dead and live fetuses were counted. Live fetuses were removed from the uterus, weighed and examined for gross abnormalities. The fetuses were fixed in Bouin's solution for at least 1 week and examined for internal soft tissue anomalies as previously described [29,30]. Statistical differences were determined using an analysis of variance procedure (ANOVA).

RESULTS AND DISCUSSION

Table I summarizes the effects of 2,3,7,8-TCDD, Aroclor 1254, 2,3,7,8-TCDD plus Aroclor 1254, dexamethasone and dexamethasone plus Aroclor 1254 on C57BL/6J females and their fetuses. No apparent signs of maternal toxicity were observed in any of the treatment groups. The percentages of fetuses with hydronephrosis per litter for the treatment groups were: untreated (1.3 ± 4.3); corn oil (5.7 ± 9.9), Aroclor 1254 (33.4 ± 18.0); 2,3,7,8-TCDD (87.8 ± 20.4); Aroclor 1254 + 2,3,7,8-TCDD (96.0 ± 11.0); dexamethasone (3.2 ± 7.3); and Aroclor 1254 + dexamethasone (3.7 ± 5.5). The dose of Aroclor 1254 (224 mg/kg) caused considerable hydronephrosis and, not surprisingly, did not antagonize the effects of 2,3,7,8-TCDD. Hydronephrosis is a more sensitive indicator of exposure to toxic halogenated aryl hydrocarbons [31,38,39] and at a dose level of 244 mg/kg, Aroclor 1254 acts as an agonist for this response. At a dose of 20 μg/kg, 2,3,7,8-TCDD caused 61.8% fetal cleft palate per litter whereas Aroclor 1254 (244 mg/kg) did not cause any cleft palate in the litters. In contrast, hydronephrosis was observed in animals exposed to 2,3,7,8-TCDD or Aroclor 1254 (data not shown). Cotreatment of the mice with a teratogenic dose of 2,3,7,8-TCDD (20 μg/kg) plus Aroclor 1254 (244 mg/kg) resulted in only 8.2% fetal cleft palate per litter which was significantly

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TABLE I

EFFECTS OF AROCLOR 1254 PRETREATMENT ON 2,3,7,8-TCDD-INDUCED TERATOGENESIS

Treatment	No. of litters	Fetal body weight (g)	Maternal weight gain (g)	Maternal liver/body weight (× 100)	Live fetuses per litter (%)	Resorptions of deaths per litter (%)	No. of litters with cleft palate	Fetuses with cleft palate per litter (%)
Untreated	11	0.79 ± 0.20	13.40 ± 3.68	5.60 ± 0.42	88.9 ± 11.5	11.1 ± 11.5	1	1.3 ± 4.3
Corn oil (10 ml/kg)	11	0.80 ± 0.20	15.58 ± 3.40	5.74 ± 0.33	91.8 ± 14.7	8.2 ± 14.7	0	0.0 ± 0.0
2,3,7,8-TCDD (20 μg/kg)	10	0.94* ± 0.21	15.70 ± 3.11	6.38 ± 0.67*	87.8 ± 14.8	12.1 ± 14.8	10	61.8 ± 23.1*,a
Aroclor 1254 (244 mg/kg) plus 2,3,7,8-TCDD (20 μg/kg	17	0.80[a] ± 0.18	14.47 ± 3.69	9.25 ± 0.93*,a,c	82.1 ± 16.4	17.9 ± 16.4	6	8.2 ± 1.5[a]
Aroclor 1254 (244 mg/kg)	11	0.76[b] ± 0.20	15.65 ± 2.15[a]	7.41 ± 5.6	93.2 ± 5.6	6.8 ± 5.6	0	0.0 ± 0.0[b]
Dexamethasone (90 mg/kg)	10	0.65* ± 0.21	11.59 ± 1.30*	6.02 ± 0.37[b]	94.6 ± 7.7	5.4 ± 7.7	10	69.9 ± 18.2*,b
Aroclor 1254 (244 mg/kg) plus dexamethasone (90 mg/kg)	9	0.64* ± 0.21	12.50 ± 2.23*,a	7.78 ± 9.3	88.8 ± 9.3	11.2 ± 9.3	9	85.8 ± 29.1*,b,c

*Significantly different from corn oil-treated group at $P<0.05$.

a,b,cValues with such signs are significantly different from each other at $P<0.05$.

decreased from the teratogenic effects observed with 2,3,7,8-TCDD alone.

These results contrast with previous studies on the interactive effects of individual PCBs and 2,3,7,8-TCDD as teratogens [31]. For example, the teratogenicity of 2,3,7,8-TCDD was unaffected by cotreatment with 2,2',4,4',5,5'-hexachlorobiphenyl whereas 2,3,3',4,4',5-hexachlorobiphenyl potentiated the teratogenicity of 2,3,7,8-TCDD. Previous reports have shown that 2,3,3',4,4',5-hexachlorobiphenyl evokes several typical 2,3,7,8-TCDD-like responses such as the induction of cytochrome *P*-450$_c$ and related monooxygenases, body weight loss, thymic atrophy and immunotoxicity [32–36]. Moreover, this PCB congener binds with moderate affinity to the Ah receptor protein [37]. In contrast, 2,2',4,4',5,5'-hexachlorobiphenyl does not elicit Ah receptor-mediated effects in laboratory animals [20,33]. Subsequent studies on the interactive effects of 2,3,7,8-TCDD and 2,3,3',4,4',5-hexachlorobiphenyl as teratogens suggest that the interactive effects are additive [38] and recent work shows that the interactive effects of 2,3,7,8-TCDD and 2,3,7,8-tetrachlorodibenzofuran (TCDF) as teratogens are also additive [39].

Although Aroclor 1254 is a complex mixture of PCB isomers and congeners, this mixture, like 2,3,3',4,4',5-hexachlorobiphenyl, also elicits a broad spectrum of Ah receptor-mediated responses including immunotoxicity, thymic atrophy, body weight loss, porphyria and the induction of cytochrome *P*-450$_c$ and related monooxygenases and exhibits moderate affinity for the Ah receptor protein [20,32–37]. In fact, both Aroclor 1254 and 2,3,3',4,4',5-hexachlorobiphenyl are weak Ah receptor agonists compared to 2,3,7,8-TCDD and their competitive binding affinities for the hepatic Ah receptor are comparable [37]. One major difference between 2,3,3',4,4',5-hexachlorobiphenyl and Aroclor 1254 is the relative potency of the former compound. For example, the in vivo ED_{50} values for body weight loss, thymic atrophy and hepatic microsomal aryl hydrocarbon hydroxylase in the rat for 2,3,3',4,4',5'-hexachlorobiphenyl are 180, 180 and 7 μmol/kg [36]; the corresponding values for Aroclor 1254 are at least 10 times higher [40]. Moreover, Aroclor 1254 exhibits a competitive Ah receptor binding affinity only 500 times lower than 2,3,7,8-TCDD whereas the toxicity and enzyme induction activity of the latter compound is at least 10^4 times greater in the rat [41]. Although a direct comparison of the teratogenic potency of Aroclor 1254 and 2,3,7,8-TCDD has not been made, the ED_{50} for teratogenicity (cleft palate) in this study was < 20 μg/kg whereas the ED_{50} for Kanechlor 500 in the ddY strain of mice was >2000 μmol/kg [42]. We therefore propose that Aroclor 1254 may act as a partial 2,3,7,8-TCDD antagonist by competing for the Ah receptor and thus lowering the cellular levels of the Ah receptor available for interaction with the potent agonist, 2,3,7,8-TCDD. Evidence which supports this hypothesis include the following: (a) cotreatment of C57BL/6J mice with Aroclor 1254 and 2,3,7,8-TCDD at Aroclor 1254/2,3,7,8-TCDD ratios >1000:1 showed that Aroclor 1254 can significantly antagonize the induction of 2 Ah receptor-mediated responses, namely the induction of aryl hydrocarbon hydroxylase (AHH) and the plaque-forming cell response to sheep

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erythrocytes [43]; (b) Aroclor 1254 also antagonizes the induction of AHH by 2,3,7,8-TCDD in rat hepatoma H-4-II E cells in culture [43]; (c) double-reciprocal plot analysis of the Ah receptor-2,3,7,8-TCDD binding isotherms in the presence of different concentrations of Aroclor 1254 gave data which are consistent with competitive inhibition of 2,3,7,8-TCDD-receptor binding by Aroclor 1254 [43]; (d) Aroclor 1254 does not act as a non-specific teratogen antagonist since Aroclor 1254 does not antagonize the teratogenicity of dexamethasone, a synthetic glucocorticoid hormone analog which acts through initial binding with the glucocorticoid receptor (see Table I).

These results further extend the spectrum of 2,3,7,8-TCDD-mediated responses which can be antagonized by Aroclor 1254. Moreover since human environmental exposure to the toxic PCDD and PCDF congeners occurs in concert with simultaneous exposure and bioaccumulation of PCBs [1–4,9–16], it is possible that the non-toxic environmental PCB levels may afford some measure of protection from these toxins since the environmental ratios of PCB/toxic PCDDs + PCDFs [7–9,12–15] are comparable to ratios in which Aroclor 1254 exhibits antagonistic activity (this paper; [43]). Current studies in our laboratory are focused on further delineating the mechanism of the interactive effects of commercial PCBs and individual PCB congeners with the toxic 2,3,7,8-substituted PCDDs and PCDFs.

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Analysis of Polychlorinated Dibenzofurans and Polychlorinated Dibenzo-*p*-Dioxins in Transformers and Capacitors

Volume 1: PCDF/PCDD at New York State Health Department

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- Use of the ^{13}C compounds allowed successful separation of the components in several closely eluting pairs of physiologically active PCDF and less-active compounds.
- Analyses of samples from utility equipment suggested that the PCDF–PCDD present did not result from high-load, high-temperature operation.

Volumes 1 and 2 describe several analytic techniques, with the appendix of Volume 2 including a report on the analysis by the University of Umeå in Sweden, as well. Volume 3 summarizes all the laboratory techniques, presents comprehensive statistical analyses, and provides an executive summary.

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This research is a landmark in PCDF–PCDD analysis. One important result is the synthesis of new PCDFs for use as spiking compounds in gas chromatography–mass spectrometry analysis. The ability to separate many of the active PCDF compounds from more innocuous materials is also of great value. The high correlation of results from several laboratories indicates that researchers can exercise great flexibility in selecting techniques and developing facilities.

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Analysis of Polychlorinated Dibenzofurans and Polychlorinated Dibenzo-*p*-Dioxins in Transformers and Capacitors

Volume 1: PCDF/PCDD at New York State Health Department

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ABSTRACT

Seven samples of in-service utility fluids were analyzed for nineteen different polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzo-p-dioxin (PCDD) compounds. After spiking with five different ^{13}C-labeled internal standards, the samples were chromatographically simplified (semi-automated). Analyses were conducted with high resolution gas chromatography-mass spectrometry (GC-MS) using a 50-m DB-5 or an OV-225 fused-silica capillary column. Generated calibration plots for eight different individual compounds were linear over four orders of magnitude concentrations. Analyses in triplicate provided some insight on reproducibility of method.

Spiked samples were analyzed with an average error of 28.9%, indicating that for the chosen analytes in this type of oily matrix, the method was quite reproducible and accurate. The relative standard deviation was less than 30% for more than 92% of the analyte determinations. In general, the study showed that the samples of in-service utility fluids have considerably different PCDF-PCDD profiles. Maximum concentrations of tetrachlorodibenzofuran (TCDF) and tetrachlorodibenzo-p-dioxin (TCDD) were 1.2 and 0.001 mg/kg, respectively. Maximum concentrations of octachlorodibenzofuran (OCDF) and octachlorodibenzo-p-dioxin (OCDD) were 40 and 0.02 mg/kg, respectively. And two fluids contained barely detectable quantities of any PCDD or PCDF (maximum concentration: 0.003 mg/kg).

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SUMMARY

Much concern has been attached to the occurrence of polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzo-p-dioxin (PCDD) in the environment. Several environmental catastrophes were precipitated by the identification and quantitation of these two classes of compounds. 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), one of 210 possible compounds within these classes, is generally regarded as the most toxic man-made compound. Much of the controversy generated at the Love Canal was a result of finding 2,3,7,8-TCDD. The Federal government literally purchased the town of Times Beach, Missouri when 2,3,7,8-TCDD was found widely dispersed in the soil. A transformer fire in the State Office Building in Binghamton, New York spread high concentrations of PCDF and PCDD throughout the building's eighteen stories. Consequently, the building has been closed since February 1981 with ongoing cleanup costs of several million dollars.

The Binghamton Office Building disaster helped to emphasize that PCDF and PCDD contamination is a potential utility problem. This unfortunate event and other research revealed that PCDF and PCDD can be found in some transformers as a result of contamination, severe operating conditions, or fire. And hundreds of thousands of Askarel and polychlorinated biphenyl (PCB)-contaminated mineral oil transformers are still in use in the Unites States.

An obvious alternative to replacing currently operating electrical equipment suspected of contamination is to identify the equipment with the greatest environmental concentrations of PCDF and PCDD. This will require an understanding of the factors that control the degree of contamination. It will also be necessary to have accurate methods of measurement. Establishing accurate measurement methods for PCDF and PCDD in PCB matrices is a goal of this study. In addition, the study measures compound-specific concentrations of PCDF and PCDD in relatively well-known insulating fluids from commonly used electrical equipment. This determines the measurement precision. Also, this work constitutes part of a larger inter-laboratory study that will provide insight regarding the accuracy and measurement methods for assessing PCDF and PCDD contamination in utility equipment.

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Seven different pieces of electrical equipment (i.e., load center network transformers, capacitors, precipitators, distribution transformers, and arc furnace transformers) were analyzed for nineteen PCDF and PCDD compounds. Historical information such as manufacturer, age, use, and maintenance was collected on each piece of equipment. Prior to analysis, the fluid samples were chromatographically simplified, concentrating the PCDF and PCDD fractions while eliminating interfering compounds. Each fluid sample was prepared in triplicate and each extract was chemically analyzed by gas chromatography-mass spectrometry (GC-MS). In addition, three common insulating fluids (mineral oil, Aroclor 1016, Aroclor 1260) were spiked with known quantities of compounds of interest. Then, these were concentrated and subjected to a similar "cleanup" procedure as used for the samples from the in-service equipment. This latter analytical procedure served as quality control for the analysis of unknown samples.

Spiked samples were analyzed with an average error of 28.9% indicating that for the chosen analytes in this type of matrix, the method was quite reproducible and accurate. The relative standard deviation was less than 30% for more than 92% of the analyte determinations. In general, the study showed that the samples of in-service utility fluids have considerably different PCDF-PCDD profiles. Maximum concentrations of tetrachlorodibenzofuran (TCDF) and tetrachlorodibenzo-p-dioxin (TCDD) were 1.2 and 0.001 mg/kg, respectively. Maximum concentrations of octachlorodibenzofuran (OCDF) and octachlorodibenzo-p-dioxin (OCDD) were 40 and 0.02 mg/kg, respectively. And two fluids contained barely detectable quantities of any PCDD or PCDF (maximum concentration: 0.003 mg/kg).

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Section 1

INTRODUCTION

Considerable interest has been focused on the analysis of various environmental samples for the closely related families of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). This interest originated with the documented occurrence of the most toxic isomer, 2,3,7,8-tetrachloro-dibenzo-p-dioxin (2,3,7,8-TCDD). This highly toxic species has been detected in Lake Ontario fish (1), in human adipose tissue (2), and turtles (3) , landfills (4) and municipal incinerators (5),(6),(7) and the combustion products of askarels and transformer fluids (8). Rappe has presented a comprehensive review of the occurrence of 2,3,7,8-TCDD and related isomers (9). Increased activity in this area stimulated the availability of pure native (^{12}C) and labelled (^{13}C) PCDD and PCDF compounds for use as analytical standards. This development coincided with investigations of improved compound separation techniques and was driven by the known fact that other PCDD and PCDF compounds have significant toxicity (10),(11),(12),(13).

PCDD and PCDF compounds have been detected in Utility Askarel fluids and combusted or pyrolyzed Utility Askarel fluids (8),(9),(8). The occurrence of these contaminants in dielectric fluids raises several questions. Fundamental to this work is the question, "What are the predominant isomers and their concentrations in utility fluids in equipment now in use?". Once the specific compounds and their concentrations are known the relative health risk posed by continued use of these fluids can be assessed.

To this end seven samples of dielectric fluids from in-service electrical equipment and three baseline samples were analyzed for the presence of 19 different compounds or groups of isomers. The samples were analyzed using five different ^{13}C-labelled internal standards. In all, 13 different standards were synthesized and used for calibration and quantitation. Fourteen different individual PCDD and PCDF compounds were quantitated along with the total concentration of tetra- through hepta-CDF and tetra-CDD congener classes. The determination of each analyte was replicated three times for each sample in order to generate an average concentration, standard deviation of the concentration, and

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percent relative standard deviation (coefficient of variation). Three of the ten fluid samples were spiked with known amounts of individual compounds as a check on the accuracy of this method. Furthermore, the same ten fluid samples were subjected to an interlaboratory round robin analysis. The results of that study will be published elsewhere. Thus, besides providing an initial view of PCDD and PCDF compound concentrations in in-service utility fluids this work represents the most rigorous study of PCDD and PCDF compounds in an environmental matrix.

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Section 2

METHODS

IN-SERVICE FLUID SAMPLES

Samples of in-service dielectric fluids were collected by various utilities and shipped to the Battelle Columbus Laboratories. BCL was responsible for accessioning the fluids and developing a catalogue of background information. The type of equipment from which each in-service fluid used in this study was obtained is listed below:

1. 1962 Load Center Network Transformer
2. Load Center Network Transformer
3. Substation Distribution Transformer
4. 1946 Load Center Network Transformer
5. Precipitator
6. 1976 Arc Furnace Transformer
7. Capacitor

A listing of accumulated background information on each of these pieces of equipment is given in Appendix A. In addition three typical virgin dielectric fluids were blindly spiked to test the method accuracy. These fluids were:

1. Mineral oil
2. Aroclor 1016
3. Aroclor 1260

PCDD AND PCDF STANDARDS

PCDD and PCDF standard compounds including ^{13}C-labelled standard compounds were synthesized by Radian Corp., Austin. The standards were dissolved in solvent at the various concentration levels and shipped in sealed glass vials. Seven vials were received each containing all of the following compounds:

2,3,7,8-TCDD	2,3,7,8-TCDD-$^{13}C_{12}$
2,3,7,8-TCDF	2,3,7,8-TCDF-$^{13}C_{12}$
1,2,3,7,8-PnCDF	
2,3,4,7,8-PnCDF	1,2,3,7,8-PnCDF-$^{13}C_{12}$

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1,2,3,4,7,8-HxCDF	
2,3,4,6,7,8-HxCDF	1,2,3,4,7,8-HxCDF-$^{13}C_{12}$
1,2,3,4,6,7,8-HpCDF	
1,2,3,4,6,7,8,9-OCDF	1,2,3,4,6,7,8,9-OCDD-$^{13}C_{12}$

Each vial contained all of the native compounds at one of the following concentration levels:

7,500 ng/ml
2,500 ng/ml
500 ng/ml
150 ng/ml
40 ng/ml
10 ng/ml
2.5 ng/ml

Each standard solution contained all of the ^{13}C-compounds at 500 ng/ml each.

SAMPLE EXTRACTION AND EXTRACT PREPARATION

Samples of electrical insulating fluids and mineral oil dissolved in hexane were received from Radian Corp. in sealed vials. The volume of a portion of each vial was measured and extracted by adding 25 ml of acetone and mixing thoroughly. Hexane (25 ml) was added and the sample was stirred and extracted for a minimum of 1 h. To remove PCBs and nonplanar aromatics and isolate one fraction containing all tetra- to octa-CDF and -CDD the sample extract was chromatographed on acid alumina, PX-21 carbon and neutral alumina. A preprogrammed Hamilton valve control system designed in our lab was used to reproducibly select solvents and chromatographic columns (9). The recovery of all tetra- to octa-CDFs and -CDDs (as verified with available standards) from an individual chromatographic column is >95%.

The sample extract was applied to an activated acidic alumina column, followed by 30 ml of 3% CH_2Cl_2 in hexane. The CDF/CDD fraction was eluted with 70 ml of 50% CH_2Cl_2 in hexane onto a carbon column followed by 50 ml of 10% benzene in hexane. The CDF/CDD fraction was eluted in the reverse direction with 30 ml of 50% xylene in hexane onto a neutral alumina column (replaces xylene with a volatile solvent) followed by 30 ml of 3% CH_2Cl_2 in hexane. The final purified CDF/CDD fraction was eluted with 70 ml of easily volatilized 50% CH_2Cl_2 in hexane and concentrated by heating to 100°C and then vacuum evaporating to 4-10 µl (8). Prior to GC/MS, extracts were stored in the dark in sealed 150 µl borosilicate capillary tubes.

HIGH RESOLUTION CAPILLARY GAS CHROMATOGRAPHY/LOW RESOLUTION MASS SPECTROMETRY(HRGC/LRMS)

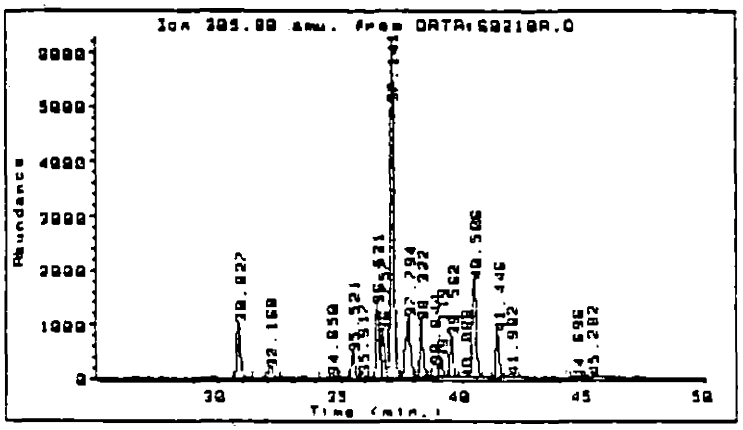
All sample extracts were analyzed with a Hewlett-Packard 5970 Mass Selective Detector. A portion of each extract was injected into the split-splitless injection port(250°C) and eluted through a 50m(0.25mm i.d.;0.33μm film thickness) Hewlett-Packard 5% phenyl methyl silicone fused silica capillary column. The following temperature program was used:

Table 2-1

H-P 5890 GAS CHROMATOGRAPH TEMPERATURE PROGRAM

Level	Initial Temp (°C)	Initial Time (min)	Rate (°C/Min)	Final Temp (°C)	Final Time (min)	Total Time (min)
1	190	1.00	5.0	220	16.00	23.00
2			5.0	235	7.00	33.00
3			5.0	250	18.00	54.00
4			5.0	270	7.00	65.00
5			5.0	300	24.00	95.00

The gas chromatograph was interfaced directly to the source of the mass selective detector and the transfer line and ion source were heated to 275°C and 150°C, respectively. The mass selective detector was operated in the selective ion monitoring (SIM) mode. The m/z values characteristic of native TCDD, TCDF, PnCDF, HxCDF, OCDF and OCDD and TCDD-$^{13}C_{12}$, TCDF-$^{13}C_{12}$, PnCDF-$^{13}C_{12}$, HxCDF-$^{13}C_{12}$ and OCDD-$^{13}C_{12}$ were monitored at various times during the 95 minute runs. The time windows used for monitoring the different congener classes (TCDD, TCDF, etc.) were determined by the previous injection of either window standards (TCDD) or a mixture of combusted PCB. Raw data were collected into and processed by an H-P Quicksilver data system. The data system was used to generate electronic copies of reports of retention times and areas at each m/z value monitored (Figure 2-1). Additional custom software was used to identify G.C. peaks and quantitate analyte levels. This software was written by NYSDOH staff (H. Valente) and runs on an IBM PC or XT. The H-P reports are input in ASCII and transferred by using a



Mass 305.90 amu

Ret Time	Area
30.827	107155
32.167	22962
34.650	7919
35.521	44916
35.917	8558
36.521	129702
36.757	80014
37.141	508644
37.794	151286
38.322	96838
38.844	38905
39.119	38741
39.562	73974
40.088	2030
40.506	220688
41.446	83751
41.982	12165
44.696	3228
45.282	11243

Figure 2-1: Typical MSD Raw data. The retention time window for TCDF is shown. The Quicksilver data system automatically integrates the gas chromatographic peaks and labels each with its retention time. The list of retention times for each mass monitored is generated by a separate program.

customized H-P Pascal operating system (sender) and the program called Kermit (receiver).

HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY(HRGC/HRMS)

Some extracts were analyzed with a Kratos MS-50 high resolution GC/MS/DS. In this system, portions of the extracts were injected into the on-column injection port of a Carlo-Erba 4160 high resolution gas chromatograph and eluted through a 50m (0.20mm i.d.;0.33µm film thickness) Hewlett-Packard 5% methyl phenyl silicone fused silica capillary column. The oven was temperature programmed as follows:

Table 2-2

CARLO-ERBA GAS CHROMATOGRAPH 4160 TEMPERATURE PROGRAM

Level	Initial Temp (°C)	Initial Time (min)	Rate (°C/Min)	Final Temp (°C)	Final Time (min)	Total Time (min)
1	190	1.0	5.0	220	16.00	23.00
2			5.0	235	7.00	33.00
3			5.0	300	50.00	86.00

The gas chromatograph was interfaced to the MS-50 through a direct line heated to 250°C. The ion source temperature was 250°C. The high resolution mass spectrometer was operated in either of two SIM type modes. In normal multiple peak monitoring mode (NMPM), the m/z values of a particular analyte and its corresponding internal standard were observed at 10,000 RP (10% valley) (10) by integrating all the signal detected within 50 ppm of their exact mass values. In high resolution multiple peak monitoring mode (HRMPM) the mass spectrometer is operated at the same high resolving power but the analyte and internal standard exact masses are profiled. The data system adjusts the accelerating and electric sector voltages to focus a mass slightly below the mass of interest. The computer then triggers a 0.3 s analog scan through a 300 ppm section of the mass range which includes the exact mass of the ion of interest (11). In both ion monitoring modes, the raw data were acquired and processed by the Kratos DS-55 data system. Quantitation was done manually by calculating concentrations using peak areas obtained from the DS-55 output.

Section 3

DISCUSSION

PRELIMINARY BASELINE STUDY DATA

In an initial phase of this project, five fluids were analyzed. These five fluids included the Aroclors 1016, 1242, 1260, tri- and tetrachlorobenzenes, and mineral oil. Each of these fluids was spiked with four ^{13}C-labelled internal standards, i.e. 2,3,7,8-TCDF-$^{13}C_{12}$, 1,2,3,7,8-PnCDF-$^{13}C_{12}$, 2,3,7,8-TCDD-$^{13}C_{12}$, OCDD-$^{13}C_{12}$. Native 1,2,3,4,7,8-HxCDF and 1,2,3,4,6,7,8-HpCDF were also added since the ^{13}C-labelled version of these compounds was not available at the time the samples were prepared. Additionally, one of the fluids, aged mineral oil, was spiked with "blind" levels of certain native PCDD and PCDF compounds and approximately 500 ppm each of Aroclor 1260 and tri- and tetrachlorobenzene and 100 ppb each of 1,2,3,4,7,9-HxCDF, 2,3,4,7,8-PnCDF, 1,2,3,4,8-PnCDF and 2,3,4,8-TCDF. The results of these preliminary analyses are given in Appendix D Tables D-1 and D-2.

The levels of PCDD and PCDF compounds added as a blind spike to the aged mineral oil sample were quantitated with reasonable accuracy. Three of the four compounds spiked were the same compounds as the internal standards used for quantitation. 2,3,7,8-TCDD, 2,3,7,8-TCDF and 1,2,3,7,8-PnCDF were quantitated by comparing the areas for these isomers with 2,3,7,8-TCDD-$^{13}C_{12}$, 2,3,7,8-TCDF-$^{13}C_{12}$, and 1,2,3,7,8-PnCDF-$^{13}C_{12}$ respectively. The percentage error in the determination of the levels of these native isomers was 7.5%, 70% and 0% respectively. Although the level of 1,2,3,7,8-PnCDF is reported as 140 ppb, this includes 100 ppb of 1,2,3,4,8-PnCDF, which had been added. These two isomers were not separated on the SP2330 fused silica capillary column with the conditions that were used. 2,3,4,8-TCDF was added to the mineral oil sample but was not specifically determined. It was detected as part of the total TCDF that was reported (Total TCDF=120 ppb). Thus the percentage error in the determination of Total TCDF(2,3,7,8- and 2,3,4,8-TCDF) is 21%. The determination of 1,2,3,4,7,8-HxCDF (as Total HxCDF) is grossly in error. The total HxCDF added was 220 ppb and 1100 ppb was detected. The HxCDF added by the addition of 500 ppm of Aroclor 1260 and the chlorobenzenes is neglible and does not account for this discrepancy. The

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exact cause of this error is unknown but similar errors have been recorded whenever a compound is determined using an internal standard not of the same congener class. Total PnCDF and total HpCDF were not determined and OCDF was inadvertently destroyed in the clean-up. The spiked Nujol which represents a purified aliphatic hydrocarbon matrix was prepared in this laboratory and provides an additional check on the accuracy of the determinations. All of the compounds added to the aged mineral oil were added to the Nujol. In terms of accuracy the results were similar. In all cases except total HxCDF, the absolute value of the ratio of the difference between the spiked level and the measured level was .90 or less. As before total PnCDF and OCDF levels were not determined.

The results quoted for the Aroclor and chlorobenzene mixtures agree with later determinations of these mixtures (see section on In-service liquids). In general the detection limits for all these results were <1 ppb.

The data in these preliminary experiments were acquired at high resolution using the HRMPM mode of ion monitoring. Typical data are shown in Figures 3-1 and 3-2. The area of the mass peak shown in Figure 3-2 is used to calculate the amount of 2,3,7,8-TCDD. Although least susceptible to interferences, this instrumental technique addresses the largest amount of data and is least automated.

CALIBRATION AND QUALITY ASSURANCE DATA

A calibration curve for each of eight of the analytes of interest was constructed from HRGC/LRMS data. The curve for 2,3,7,8-TCDD is shown in Appendix B Figure 1. The log of the ratio of the area of native 2,3,7,8-TCDD to ^{13}C-labelled TCDD is plotted versus the log of the concentration of native 2,3,7,8-TCDD.

Seven solutions containing concentrations of native 2,3,7,8-TCDD of 2.5, 10, 40, 150, 500, 2500 and 7500 pg/µl and a constant ^{13}C-labelled 2,3,7,8-TCDD concentration of 500 pg/µl were used to construct this curve. The same concentrations of the other analytes and internal standards were used in other curves (Appendix B Figures 2-8). These curves demonstrate a linearity of response of almost four orders of magnitude.

The percentage fit (=100 x (correlation coefficient)^2) of the least squares line in each of the curves constructed with low resolution mass spectrometry data is:

2,3,7,8-TCDD - 99.7398
2,3,7,8-TCDF - 99.7614
1,2,3,7,8-PnCDF - 99.6357
2,3,4,7,8-PnCDF - 99.0483
1,2,3,4,7,8-HxCDF - 99.7262
2,3,4,6,7,8-HxCDF - 99.3756
1,2,3,4,6,7,8-HpCDF - 99.5848
OCDF - 99.7685

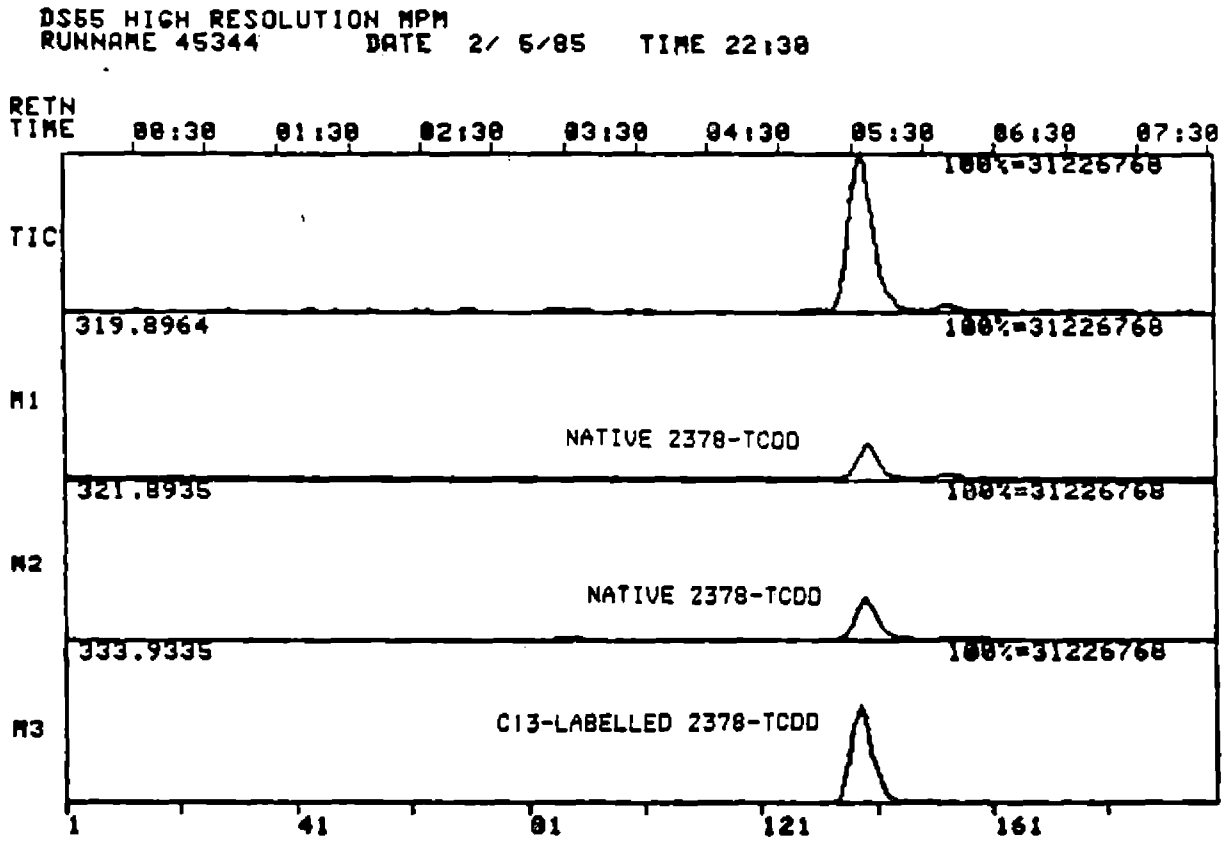

Figure 3-1: Exact Mass Chromatograms showing the retention time window for TCDD. Three 100 mmu. mass windows were monitored one each centered on m/z 321.8936 and m/z 319.8965 respectively for native TCDD and one centered on m/z 333.9338 for $^{13}C_{12}$-labelled-2378-TCDD.

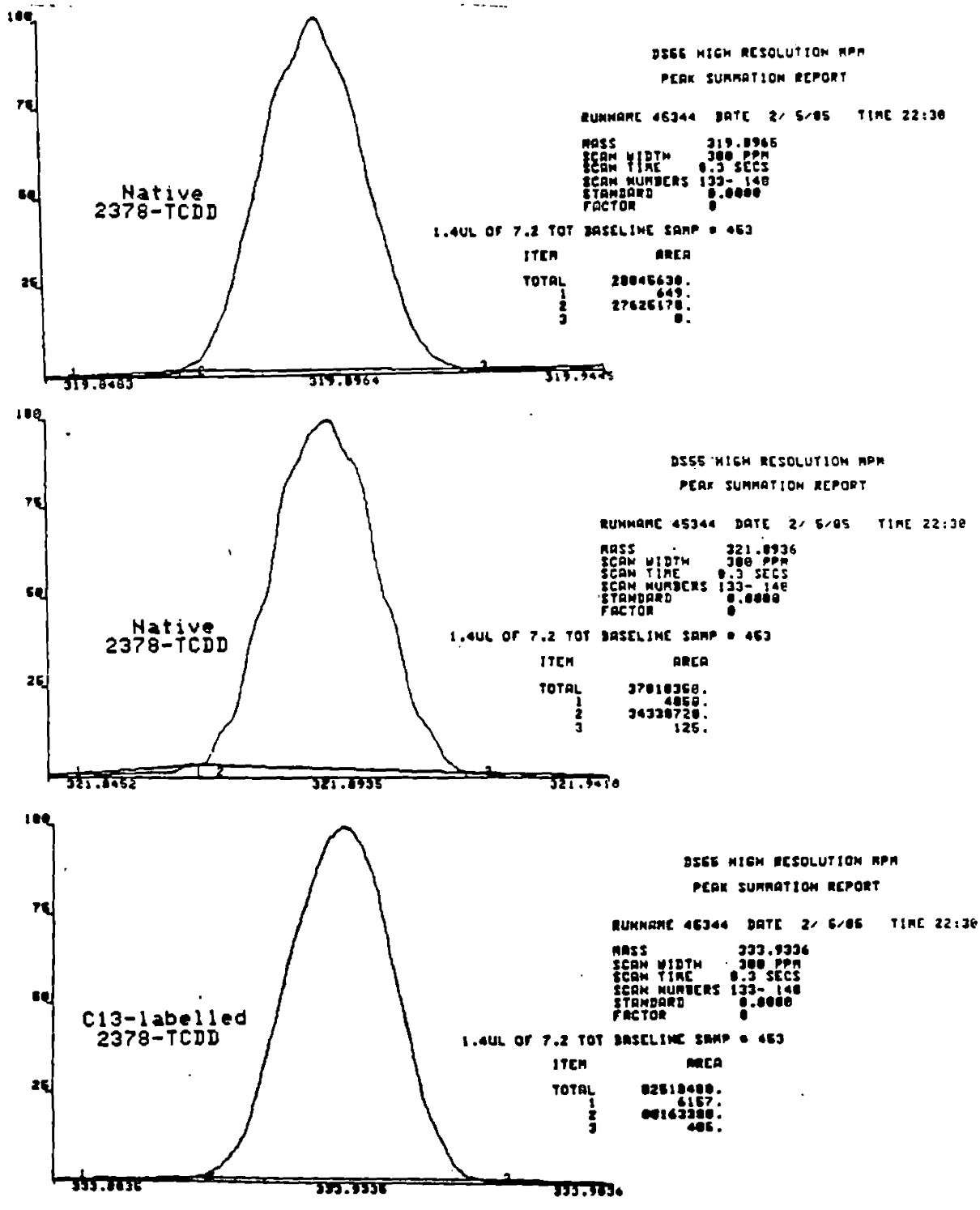

Figure 3-2: High Resolution Mass Scans & Summaries. This figure shows the sum of the mass scans which were taken during the elution of the 2378-TCDD peaks shown in Figure 3-1.

Each curve also shows the 99% confidence interval for the slope and intercept of the least squares regression line. These calculations were based on equations given in a recently published book on statistics for chemists(12).

Calibration curves derived from high resolution mass spectrometric data(HRGC/HRMS) for some of the analytes are drawn in Appendix B Figures 9-13. The curves were constructed using the same solutions as described above. They also demonstrate linearity over approximately four orders of magnitude.

The percentage fit of the least squares line in each of the curves constructed with high resolution mass spectrometry data is:

2,3,7,8-TCDD - 99.5395
2,3,7,8-TCDF - 99.4736
1,2,3,7,8-PnCDF - 97.0347
1,2,3,4,7,8-HxCDF - 99.949
1,2,3,4,6,7,8-HpCDF - 99.1012

Response factors were calculated daily from data collected from the injection of a portion of one of the standard solutions. The response factors (RF) are used in the following equation to calculate the concentration of analyte in the sample:

$$C_A = (A_N/A_{IS})(RF)(C_{IS}) \qquad (3\text{-}1)$$

where,
C_A = concentration of analyte in the sample
C_{IS} = concentration of internal standard added to the sample before the clean-up procedure
A_N = area counts of the signal due to the analyte
A_{IS} = Area counts of the signal due to the internal standard
RF = Response factor = S_{IS}/S_N
S_{IS} = Area counts per unit mass of the internal standard
S_N = Area counts per unit mass of the native compound

Ideally, for greatest precision, the response factors should not vary from analysis to analysis. The day-to-day variation in response factor(expressed as area ratio) can be demonstrated by comparing the daily area ratios to the area ratios generated for the calibration curve. In Appendix C Figure 14, the area ratios for the 2,3,7,8-TCDD, in the standard injection analyzed on each day of the project are plotted on the calibration curve. The area ratios for other analytes are plotted in Figures 15 through 21 Appendix C. For 2,3,7,8-TCDD only one area ratio fell outside the 99% confidence interval. Since no criteria had been

specifically established, this area ratio (and consequently, response factor) was adjusted based upon the average of area ratios generated by injections on the previous and following days. Errant ratios obtained for other analytes were treated in this same fashion. This was considered acceptable because it was observed that the area ratios fluctuated only rarely and randomly. These large apparently random changes in the area ratios constituted the greatest factor responsible for any large relative standard deviations in the sample determinations. At the time of analysis no mechanism had been established to efficiently check the daily response factors of so many analytes. In future work, daily checks of the response factors could be made by incorporating more currently available automation into the methodology. Instrument manufacturers are continually developing software and one could expect that among the developments would be an efficient and reliable check of the response factors. We can envision a relatively easy method of checking the response factors against the calibration curve using the relatively general and widely available Lotus 1-2-3 software package. When many response factors can be easily checked, a criteria for the reproducibility of response factors could be included as an analytical specification before analysis commences. Likely criteria could be typical confidence intervals such as the 90, 95 or 99% confidence interval or a percentage of the average response factor calculated from the responses generated during the construction of the calibration curve.

Analysis of blank samples is a final quality assurance point requiring some discussion. A laboratory blank was generated by carrying a sample of the solvents used in the analysis through the clean-up procedure. This sample was spiked with the appropriate internal standards. The internal standards were detected with reasonable recoveries. None of the analytes of interest were detected. The three baseline fluids, which constituted three of the ten samples in the project, were analyzed to quantify any of the PCDD and PCDF analytes which might be present before the blind spiking levels were added. None of the analytes were detected in the mineral oil or Aroclor 1016. However, the recovery of internal standards spiked into these samples was low. Some of the analytes were detected in Aroclor 1260. The results of these analyses are tabulated in Appendix D Tables 3, 4 and 5 and are used in conjunction with the results of the spiked Baseline fluids to determine the accuracy of the analysis.

BASELINE DATA

The actual project consisted of the analysis of ten samples of various utility fluids. Of these ten samples, six were dielectric fluids taken from in-service

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electrical equipment. Three of the samples were mineral oil, Aroclor 1016, and Aroclor 1260 that were spiked before analysis with both the internal standards and blind levels of various native PCDD-PCDF analytes. The results of these analyzes are tabulated in Appendix D Tables 3-5. The average percent error is 28.9%. In two cases, an analyte was measured where none had been spiked.

In the spiked Aroclor 1016, 1,2,3,4,7,8-HxCDF was detected even though it had not been spiked. In spiked Aroclor 1260, 1,2,3,7,8,9-HxCDF was detected but not spiked. It is impossible to determine with these results if these compounds were present in the unspiked fluids since the detection limit in the unspiked fluids is greater than the level detected in the spiked fluids.

The % error for the determination of OCDF in the spiked Aroclor 1260 was not included in the 28.9% average percent error calculation. The spike level was so low relative to the native amount of OCDF in Aroclor 1260 that the comparison of the spiked to measured level was not a reasonable indication of accuracy. The % error figure was eliminated from the average based upon the similarity in magnitude of the spiked level and the standard deviation for the triplicate determination (compare the OCDF values in Table D-15).

The average % error in each matrix is listed at the bottom of Tables D-1, D-2 and D-3. The % error in the OCDF determination in Aroclor 1260 (Table D-3) was not included in the local average as explained above. The large average % error in the case of Aroclor 1260 is due to the large error in the determination of 2,3,4,6,7,8-HxCDF and 1,2,3,4,6,7,8-HpCDF. This large error could be accounted for by a low recovery of these compounds in the blank Aroclor 1260. Recovery, as measured in this report, refers to the recovery of the internal standard used to quantitate a particular analyte and not to the recovery of the actual analyte. For these two compounds the internal standard was 1,2,3,4,7,8-HxCDF. This isomer may be different enough that slight differences in the performance of the chromatographic system will cause large differences in recovery. This phenomenon may account for many of the large relative standard deviations observed in this study.

IN-SERVICE LIQUIDS

The results of the triplicate analysis of each of the seven in-service dielectric fluids are tabulated in Appendix D, Tables D-6 to D-15. These results are

graphically displayed in Appendix E, Figures 1 through 10. In each table the result for each replicate for each analyte is listed along with the average of the three replicates, the standard deviation and the percent relative standard deviation (coefficient of variation). Analytes that were not detected are indicated by the detection limit enclosed in parentheses. In all ten samples, the concentrations range from 0.15 ppb of 2,3,7,8-TCDF in the Substation Distribution Transformer (Appendix D, Table D-11) to 39,600 ppb of OCDF in the Load Center Network Transformer (Appendix D, Table D-6). For all the analytes in all the samples, only 15 average values (out of 180) had percent relative standard deviations over 30%. None of these analyses involved the more toxic compounds namely 2,3,7,8-TCDD or 2,3,7,8-TCDF. The largest relative standard deviations (RSD) occurred in the analysis for Total TCDF or an isomer in one of the higher chlorinated congener classes. Some had percent relative standard deviations as low as 1%. Six of the analyzes with greater than 30% RSD were determinations of total congener concentrations, two were determinations of closely eluting PnCDF isomers and seven were determinations quantitated at < 10 ppb.

The detection limits and recoveries are tabulated in Appendix F Table 1 through 10. Tables 11 through 20 list % recovery versus 2,3,7,8-TCDD-$^{13}C_{12}$. The detection limits were calculated based upon a background response of 1000 counts in the diluted sample extract.
Thus the following equation was used:

$$\text{Detection Limit} = (1000/A_{IS})\ (RF)\ (100) \qquad (3\text{-}2)$$

where,

A_{IS} = area counts due to the internal standard
$RF = S_{IS}/S_N$ = response factor
S_{IS} = area counts per unit mass of internal standard
S_N = area counts per unit mass of native compound

A background area count of 1000 was chosen because this size peak was typical for G.C. peaks which are just distinguishable from background by the detector and computer combined. Smaller noise peaks are distinguishable in the detector output but these are not generally integrated by the computer and its integration algorithm. Furthermore 1000 counts is a conservative general estimate of the area noise observed just prior to the elution of the internal standards based upon the 2.5 pg/ml standard solution used for the calibration curves.

Some apparent discrepancies may arise when comparing these detection limit values with the results (and detection limits) listed in Appendix D Tables D-6 through D-15. However, the basis for any particular calculation in these two groups of tables may be different. The detection limit calculations in Appendix F Tables 1 through 10 are based on the previous equation for detection limits which includes the area count observed for each internal standard in a diluted extract. Most of the sample extracts were diluted because most contained one or two analytes which were highly concentrated relative to the other analytes. Many of the values listed in Appendix D Tables 4 through 13 were generated from data collected on the concentrated extracts before they were diluted.

In one sample, spiked Aroclor 1260, the replicate analysis of 2378-TCDD generated the following values: 61.7, 65.5 and 32.0 ppb. A possible reason for the discrepancy between the first two determinations and the third is an interference in the 2,3,7,8-TCDD-$^{13}C_{12}$ internal standard peak eluting at 42.194 minutes (see figure 3-3). To check for the occurrence of an interferant and generate the most accurate quantitation, the third extract was reanalyzed by high resolution G.C./high resolution M.S.(see Figure 3-4). The mass profile of the scans acquired during the elution of the 2,3,7,8-TCDD-$^{13}C_{12}$ internal standard shows the presence of an interferant (see Figure 3-5). The small peak in the profile was used with a typical response factor to quantitate 2,3,7,8-TCDD at a level of 106 ppb.

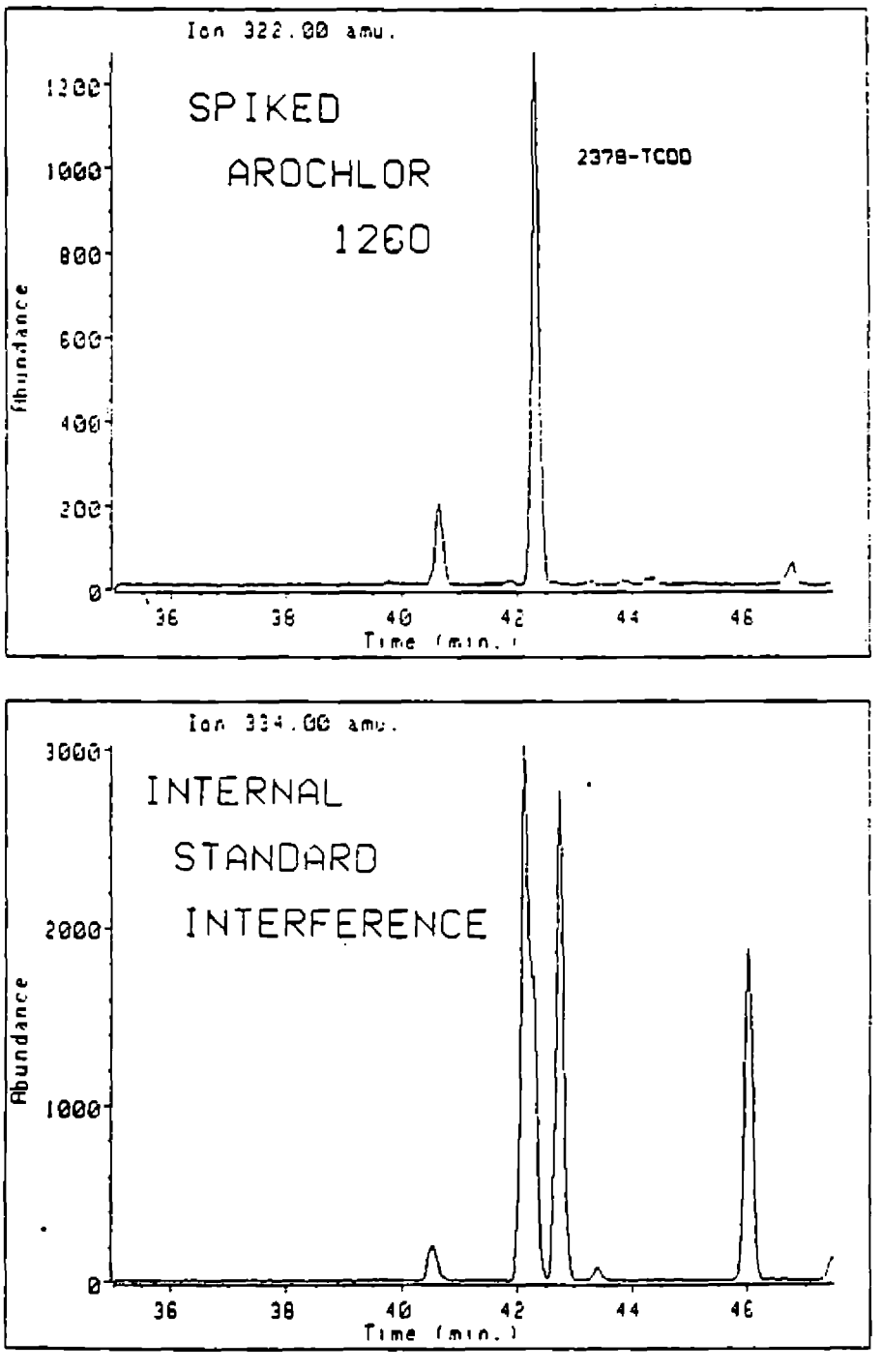

Figure 3-3. Interference in the determination of 2378-TCDD in spiked Aroclor 1260. The internal standard G.C. peak appears to have a coeluting interference which would cause the determination of 2,3,7,8-TCDD to be too low.

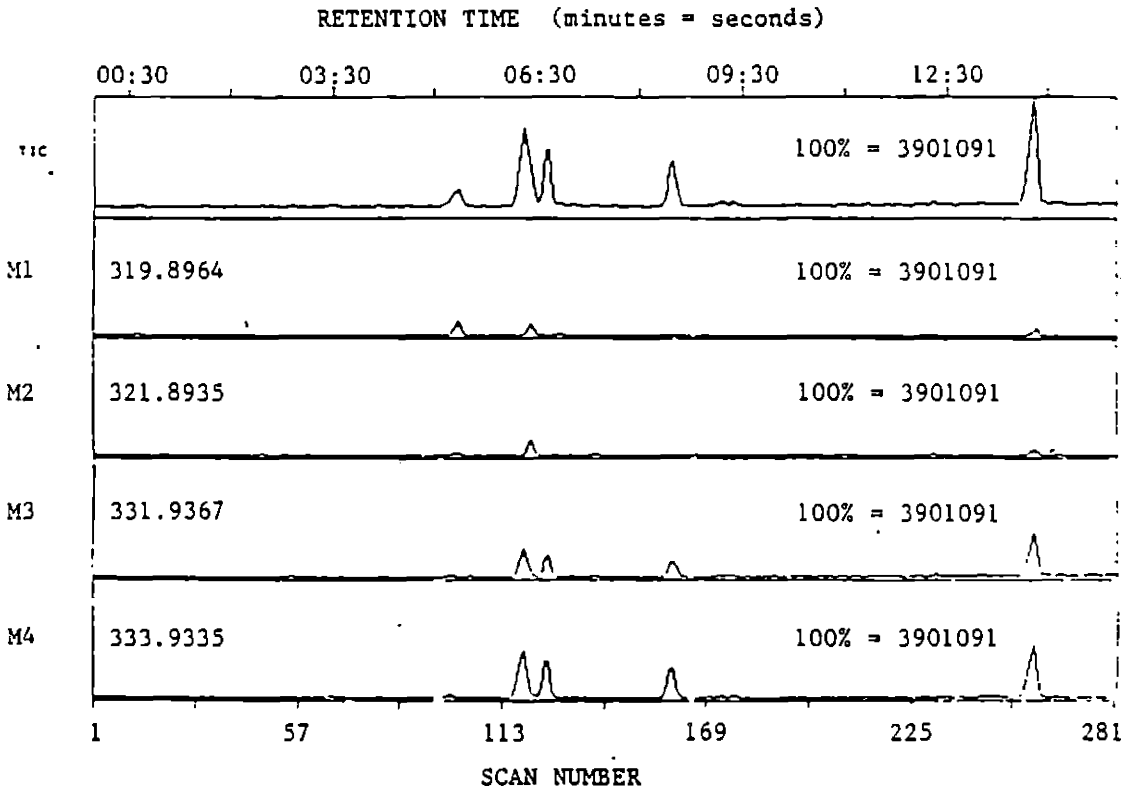

Figure 3-4. High resolution Mass Chromatogram of spiked Aroclor 1260. Notice the same pattern of G.C. peaks as in the low resolution data. This allows, in a sense, the analysis of isobaric components of the internal standard G.C. peak in the low resolution mass data.

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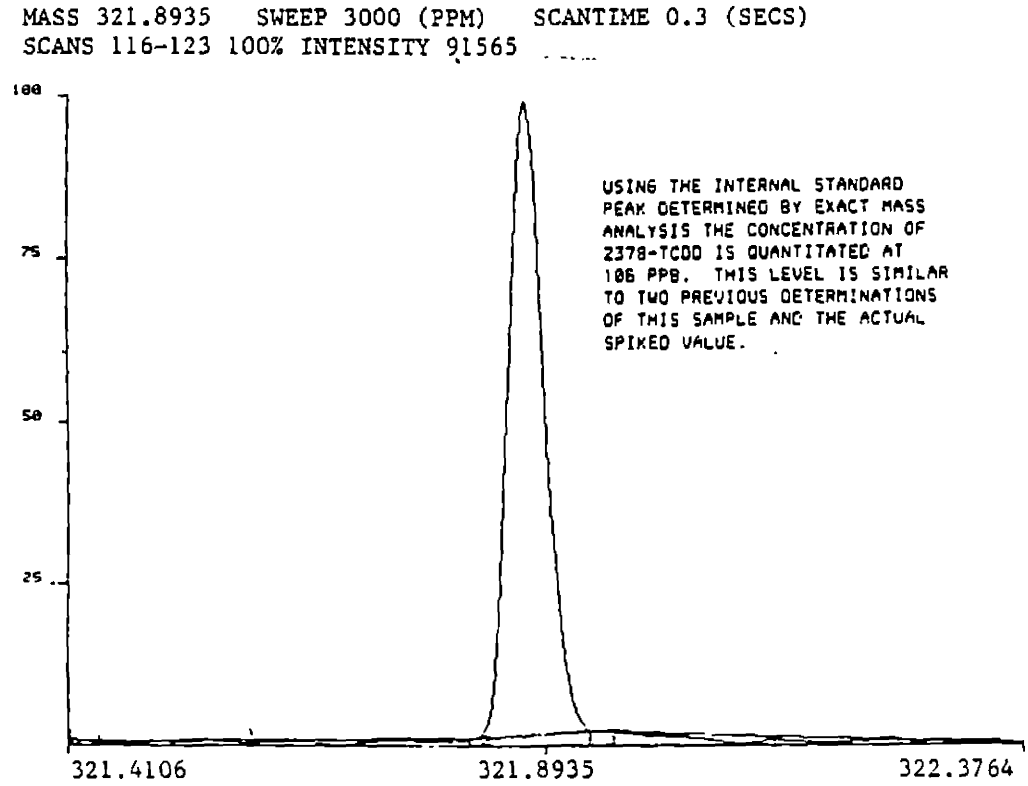

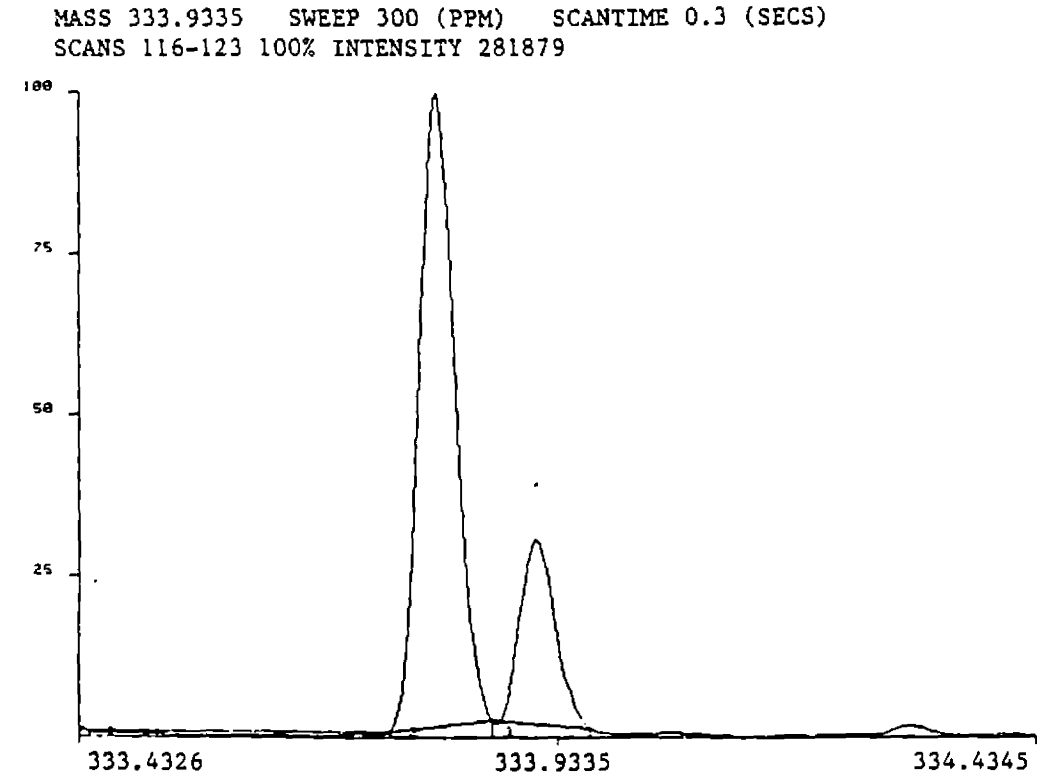

Figure 3-5. High resolution mass data from the 2,3,7,8-TCDD G.C. peak from the spiked Aroclor sample. The mass scan of the internal standard ion shows a coeluting interference responsible for the low determination of 2,3,7,8-TCDD.

Section 4

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Appendix A

IN-SERVICE LIQUIDS BACKGROUND INFORMATION

Type of equipment: Load Center Network Transformer
BCL Laboratory Code: ISL-02-A
Voltage Rating: 750 KVA 12 KV to 480/277 V
Liquid Volume: 2650 lbs.
Year: 1962
PCB/Askarel Type: Aroclor 1260 29% - T3CB 29% - T4CB 2%
PCB Levels (for Oil Filled):
Additional Equipment Identification: 65°C max. rise
Length of Actual Service: 20 yrs.
Major Maintenance:
Recorded Failure in Service:
Was Fluid Changed at Failure:
Any Record of Temperature Excursions:
Are Maintenance Records Available:
Any Other Pertinent Information:

Type of equipment: Load Center Network Transformer
BCL Laboratory Code: ISL-03-A
Voltage Rating: 500 KVA 13 KV to 213 V
Liquid Volume: ?
Year: 1953
PCB/Askarel Type: Aroclor 1260 70% - T3CB 29% - T4CB 1%
PCB Levels (for Oil Filled):
Additional Equipment Identification:
Length of Actual Service: 31 yrs.
Major Maintenance:
Recorded Failure in Service:
Was Fluid Changed at Failure:

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Any Record of Temperature Excursions:
Are Maintenance Records Available:
Any Other Pertinent Information:

Type of equipment: Substation Distribution Transformer
BCL Laboratory Code: ISL-04-0
Voltage Rating:
Liquid Volume: 200 gal.
Year:
PCB/Askarel Type:
PCB Levels (for Oil Filled): 100 ppm
Additional Equipment Identification: 65 ^{o}C max. rise
Length of Actual Service: 20 yrs.
Major Maintenance:
Recorded Failure in Service: Major arcing and in-service failure
Was Fluid Changed at Failure:
Any Record of Temperature Excursions:
Are Maintenance Records Available:
Any Other Pertinent Information:

Type of equipment: Load Center Network Transformer
BCL Laboratory Code: ISL-08-A
Voltage Rating: 500 KVA 12 KVA to 213 V
Liquid Volume: 3175 lbs.
Year: 1946
PCB/Askarel Type: Aroclor 1260 about 67% - T3CB 30% - T4CB 2%
PCB Levels (for Oil Filled):
Additional Equipment Identification: 55 ^{o}C max. rise
Length of Actual Service: 28 yrs.
Major Maintenance: None Noted
Recorded Failure in Service:
Was Fluid Changed at Failure:
Any Record of Temperature Excursions:
Are Maintenance Records Available:
Any Other Pertinent Information: Carbon present throughout the Askarel

Type of equipment: Precipitator
BCL Laboratory Code: ISL-17-A

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Voltage Rating: 480 to 53,500 V
Liquid Volume: 143 gal.
Year:
PCB/Askarel Type:
PCB Levels (for Oil Filled):
Additional Equipment Identification: Serial No. TH 5875, 50 KVA
Length of Actual Service: 1947 - 1974 (27 yrs.)
Major Maintenance:
Recorded Failure in Service:
Any Record of Temperature Excursions:
Are Maintenance Records Available:
Any Other Pertinent Information: Full load rise of 45°C.

Type of equipment: Arc Furnace Transformer
BCL Laboratory Code: ISL-23-0
Voltage Rating: 12470/280
Liquid Volume: 4500
Year: 1976
PCB/Askarel Type: Mineral Oil
PCB Levels (for Oil Filled): 150 ppm
Additional Equipment Identification: 7500/8750 KVA, 4.7% imp.,
No-load Secondary tap changer, FOA, 65°C rise insulation.
Length of Actual Service: 8 yrs.
Major Maintenance: Top cover of transformer and tap changer removed each year for internal visual inspection and check of connections.
Recorded Failure in Service: None
Was Fluid Changed at Failure:
Any Record of Temperature Excursions: None occurred
Are Maintenance Records Available: Yes
Any Other Pertinent Information: Oil chemical test and gas chromatography tests run yearly.

Type of equipment: Capacitor
BCL Laboratory Code: ISL-27-?
Voltage Rating: 7200 KVAR 100
Liquid Volume: 4" x 13.5" x 22"
Year:
PCB/Askarel Type:
PCB Levels (for Oil Filled):

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Additional Equipment Identification:

Length of Actual Service: ?

Major Maintenance:

Recorded Failure in Service:

Was Fluid Changed at Failure:

Any Record of Temperature Excursions:

Are Maintenance Records Available:

Any Other Pertinent Information:

Appendix B

ANALYTE CALIBRATION CURVES

Figures B-1 through B-8. Calibration curves constructed with data obtained from the low resolution mass selective detector. Along with the least squares line the 99% confidence interval is also plotted.

Figures B-9 through B-13. Calibration curves constructed with data obtained from the high resolution mass spectrometer.

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FIGURE B-1:EPRI BASELINE PROJECT

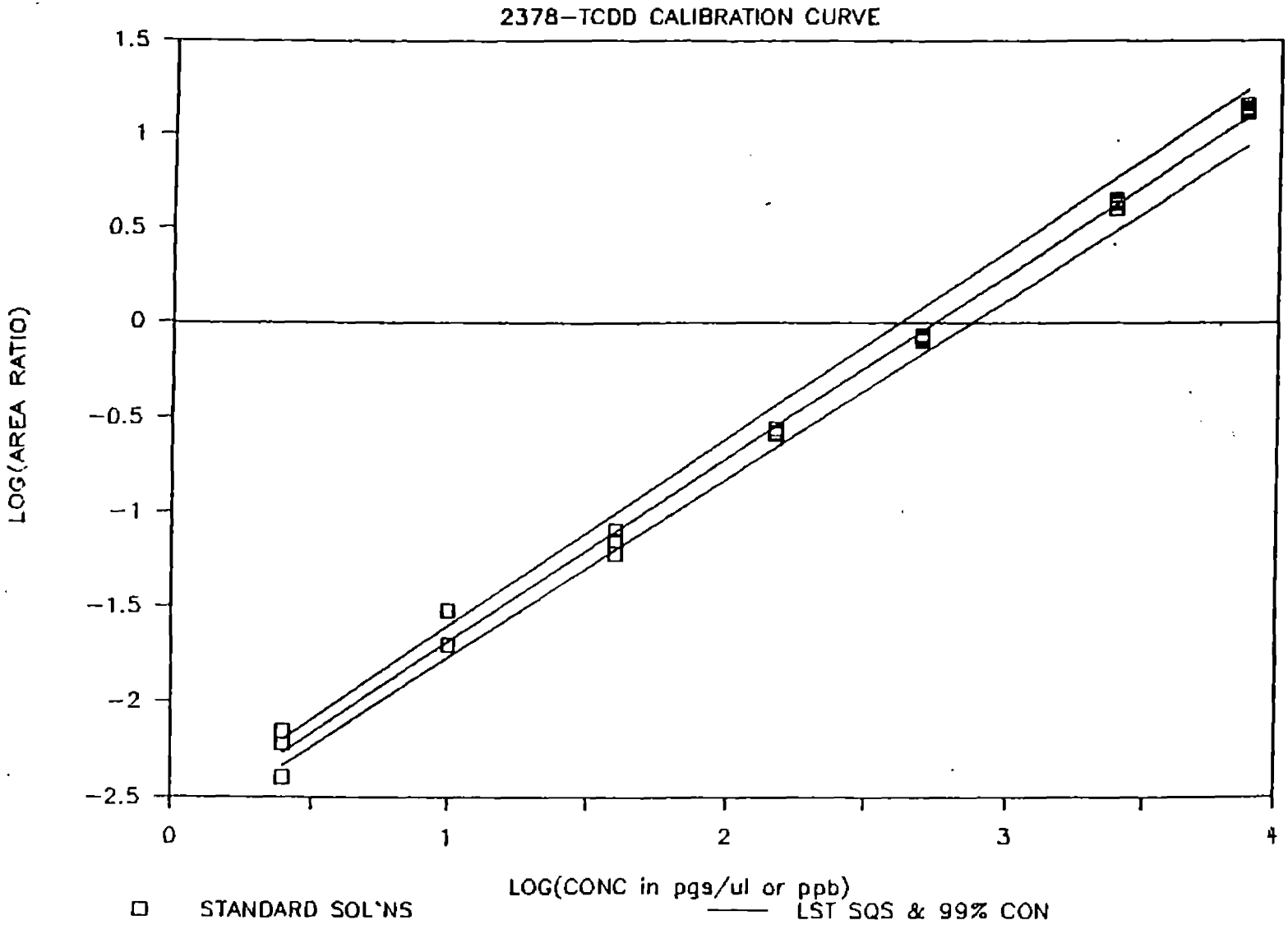

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FIGURE B-2: EPRI BASELINE PROJECT

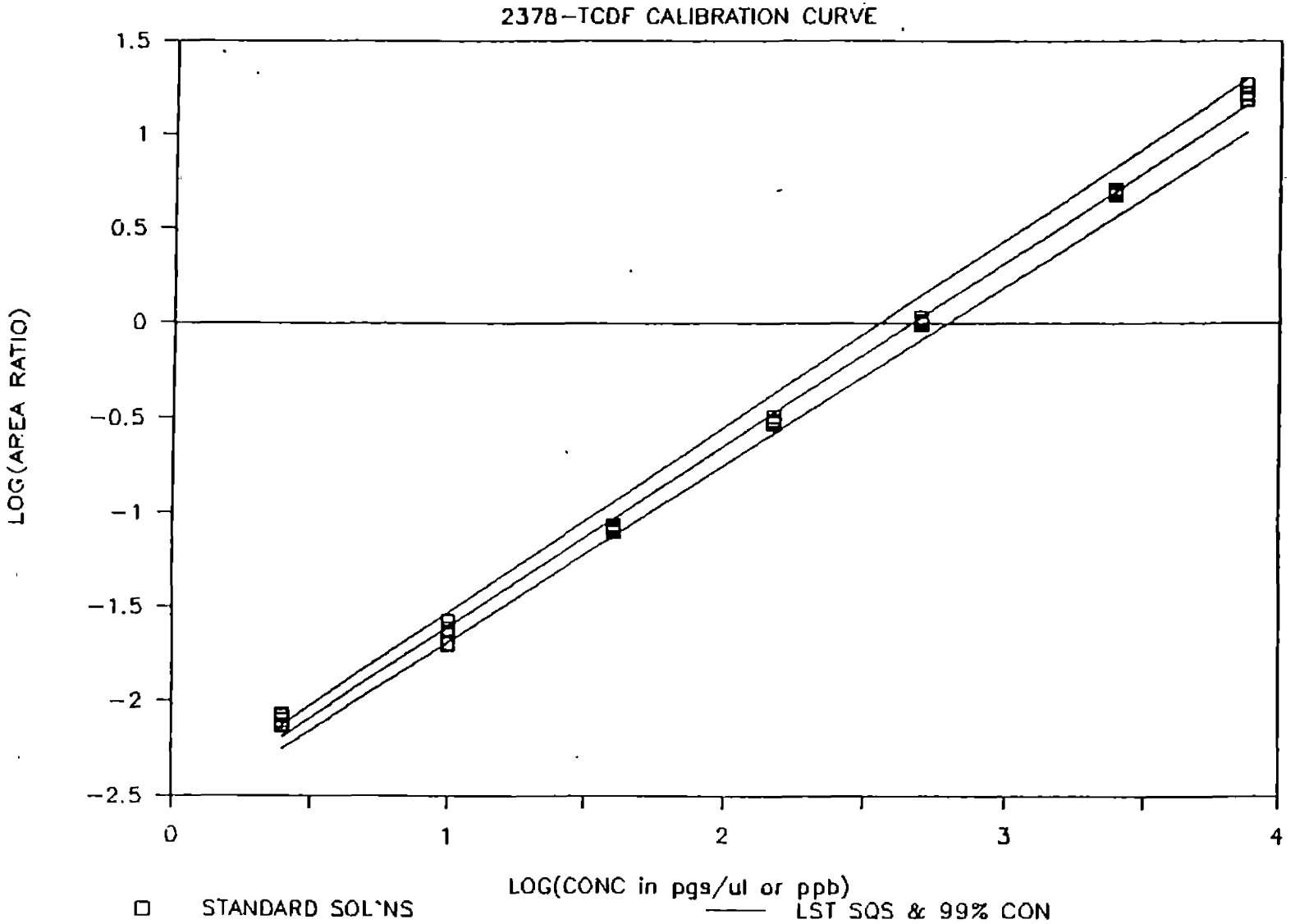

FIGURE B-3: EPRI BASELINE PROJECT

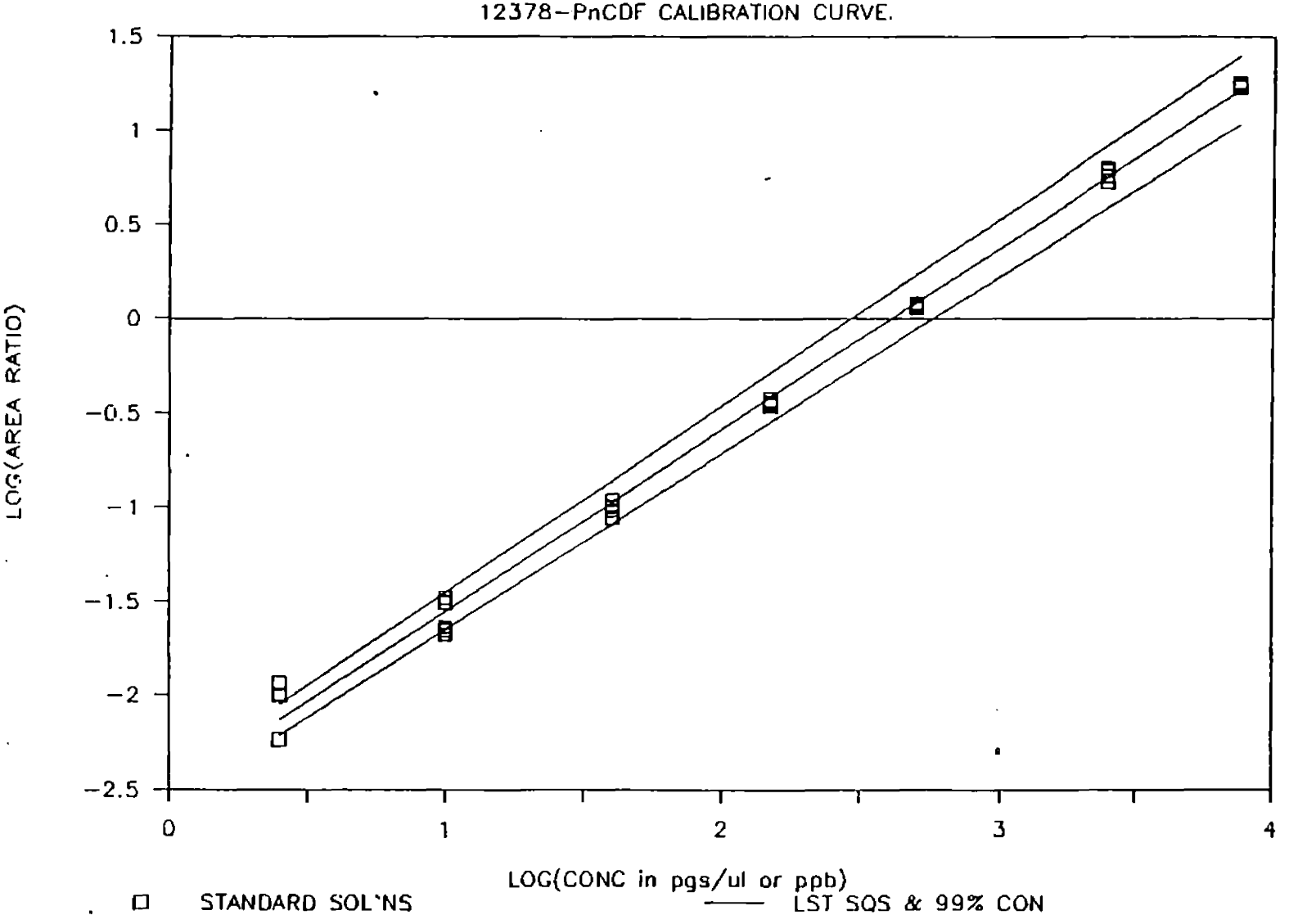

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FIGURE B-4: EPRI BASELINE PROJECT

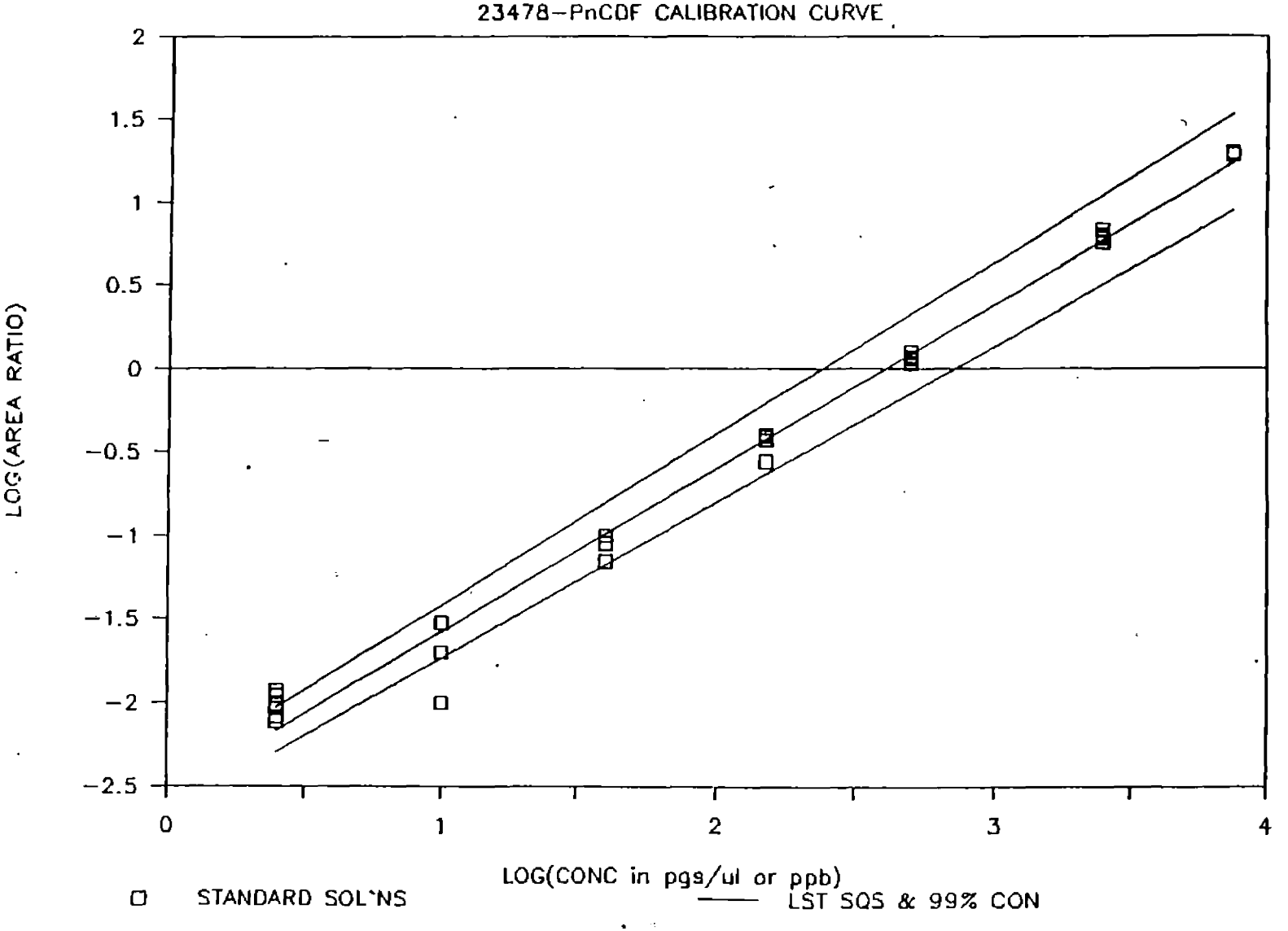

FIGURE B-5: EPRI BASELINE PROJECT

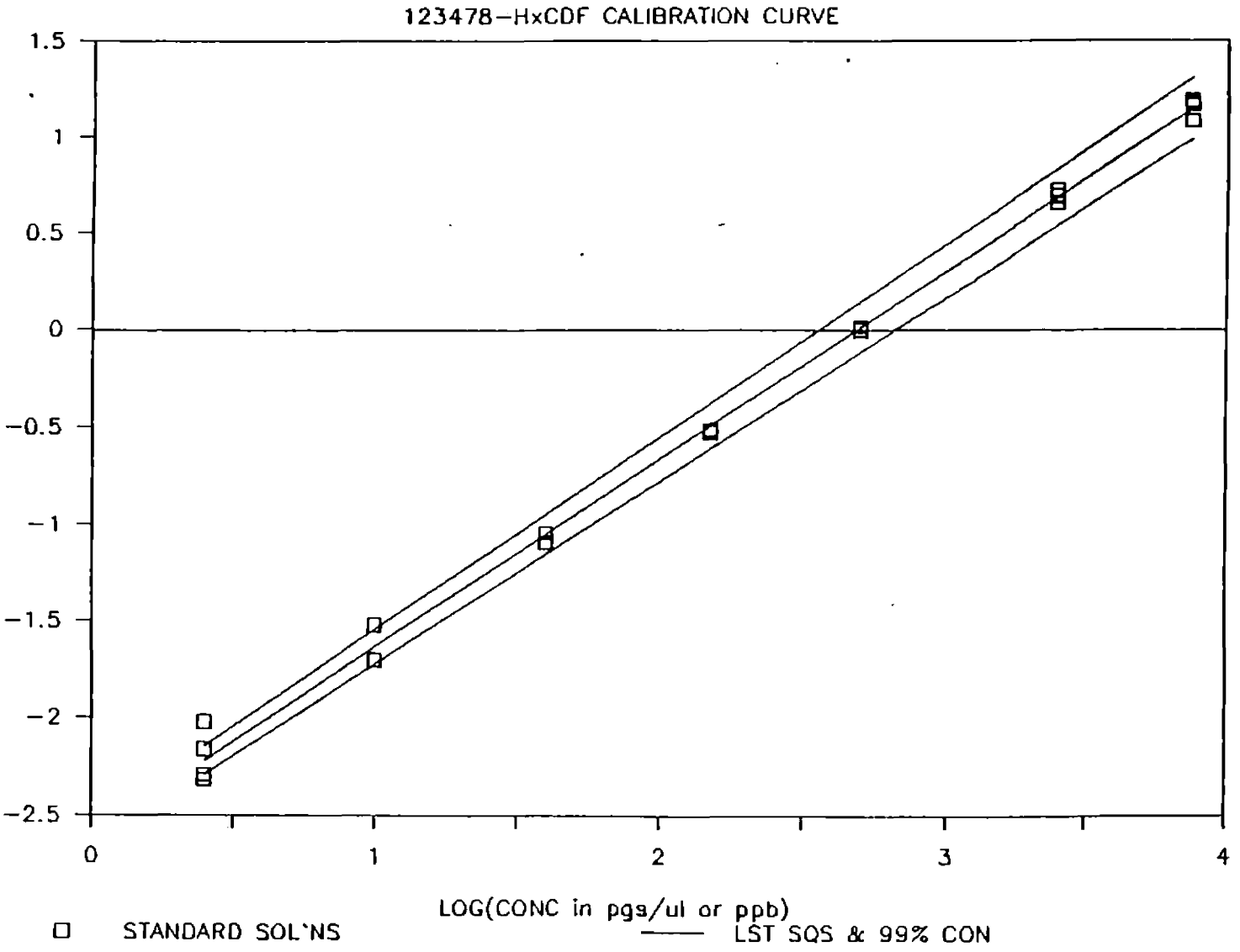

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FIGURE B-6: EPRI BASELINE PROJECT

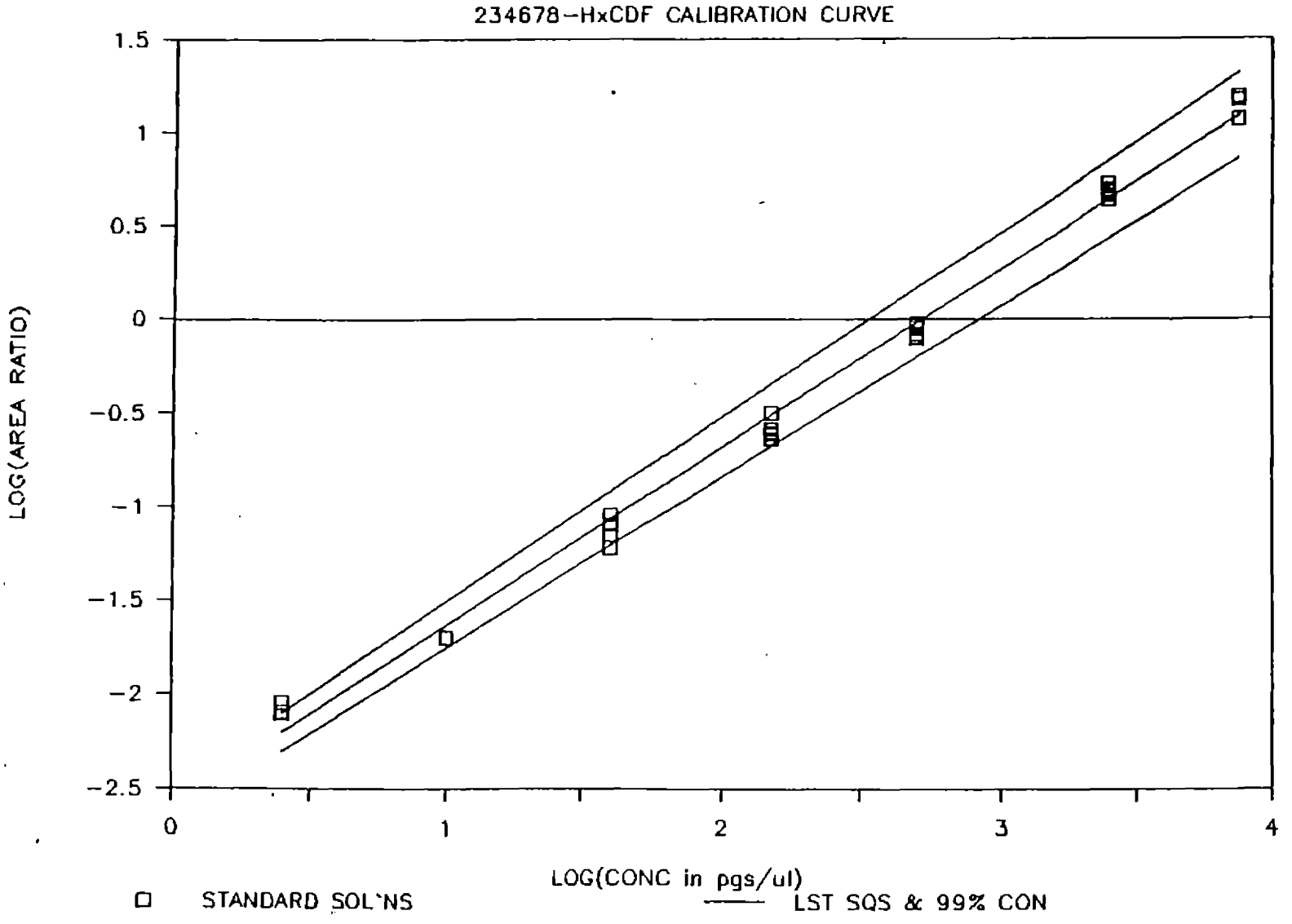

FIGURE B-7: EPRI BASELINE PROJECT

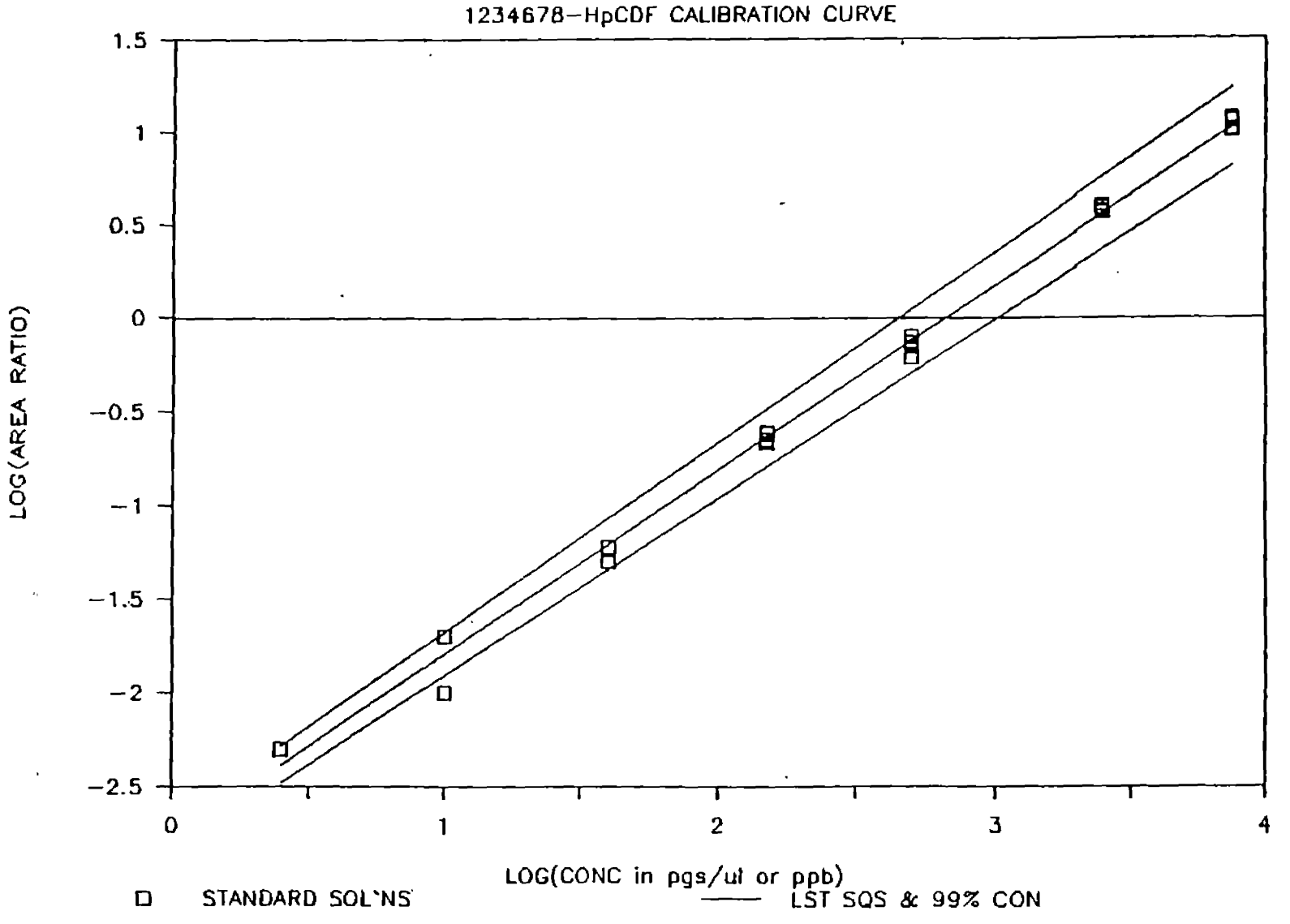

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FIGURE B-8: EPRI BASELINE PROJECT

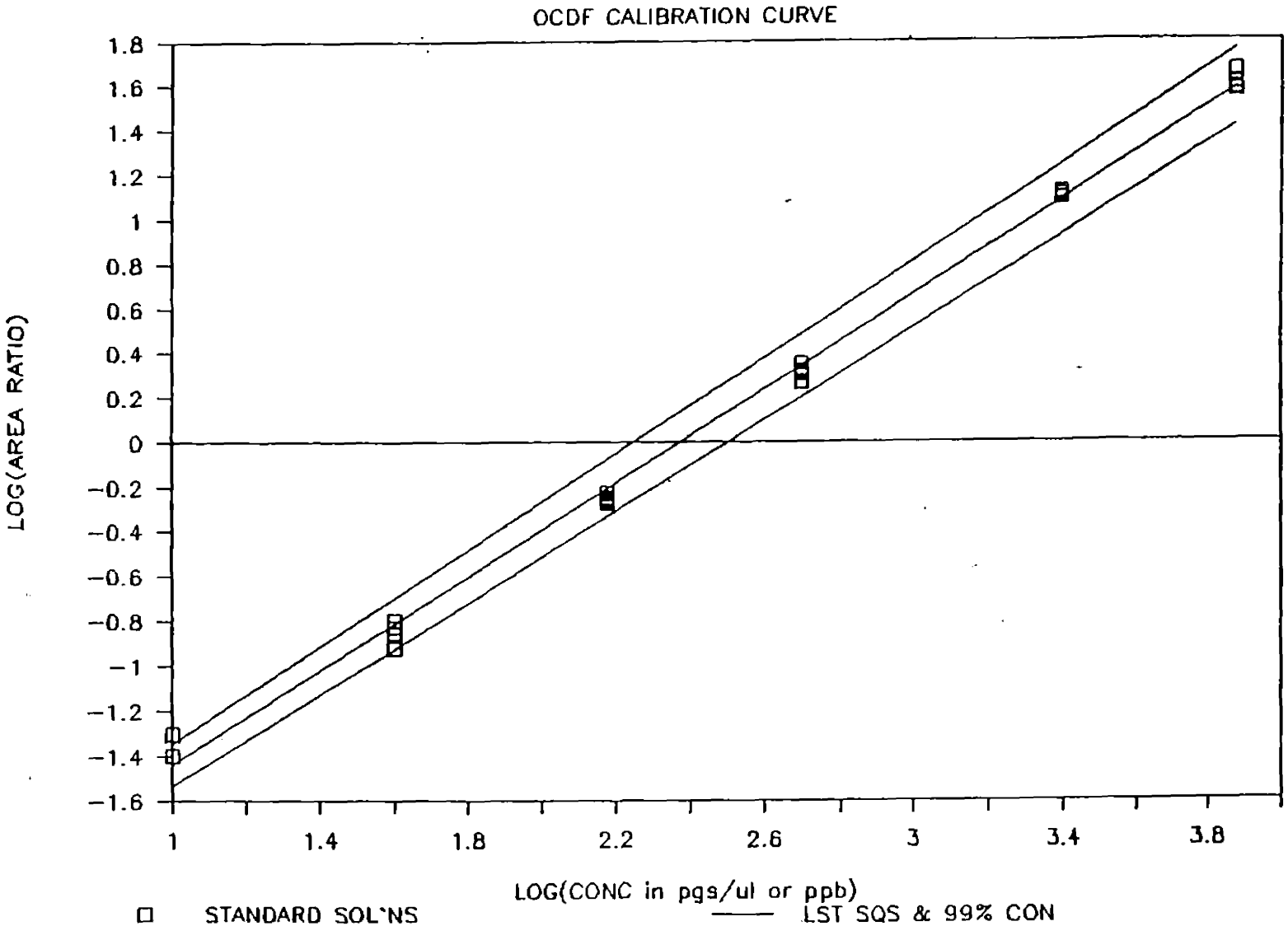

Figure B-9: CALIBRATION CURVE FOR 2,3,7,8-TCDD

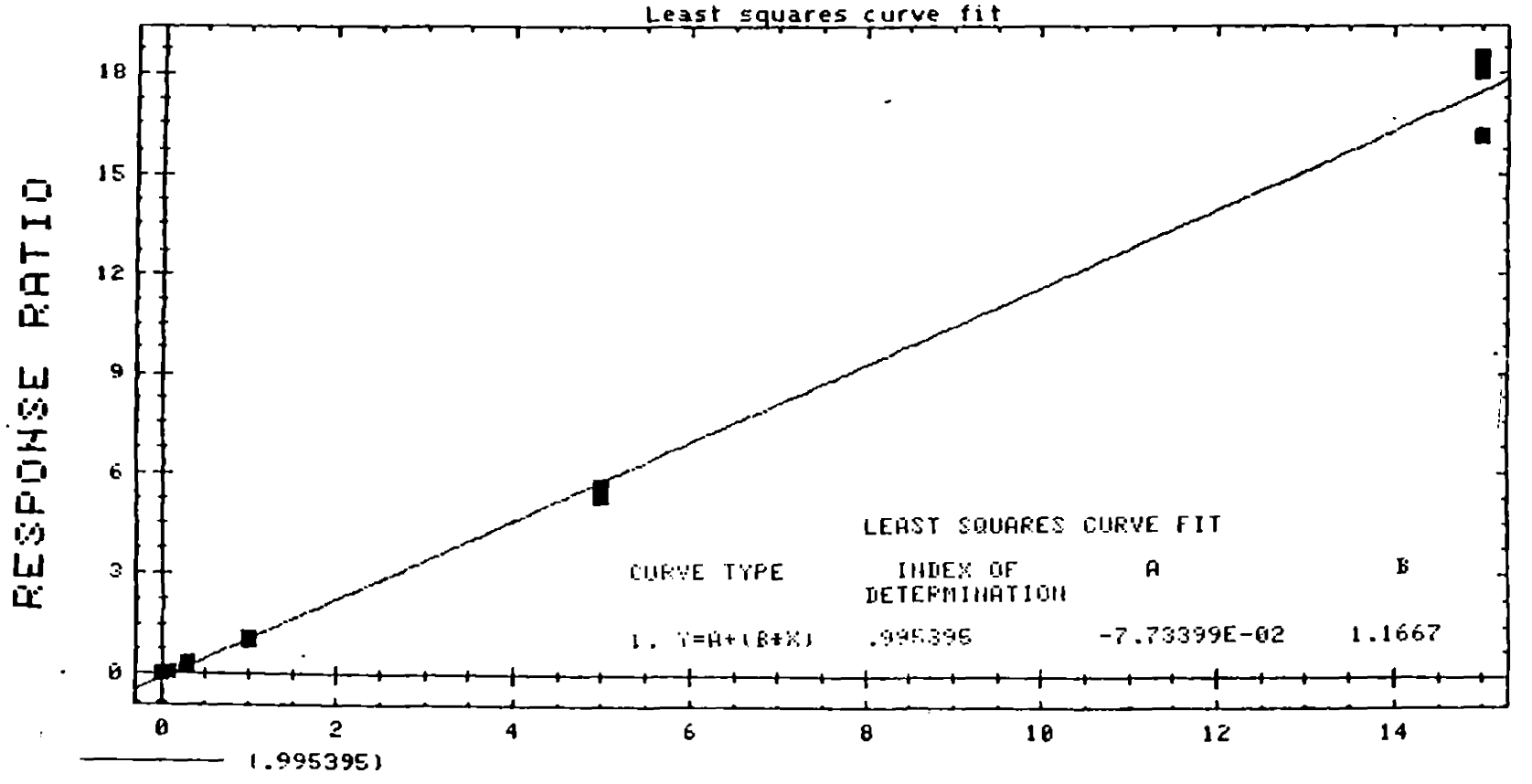

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Figure B-10: CALIBRATION CURVE FOR 2,3,7,8-TCDF

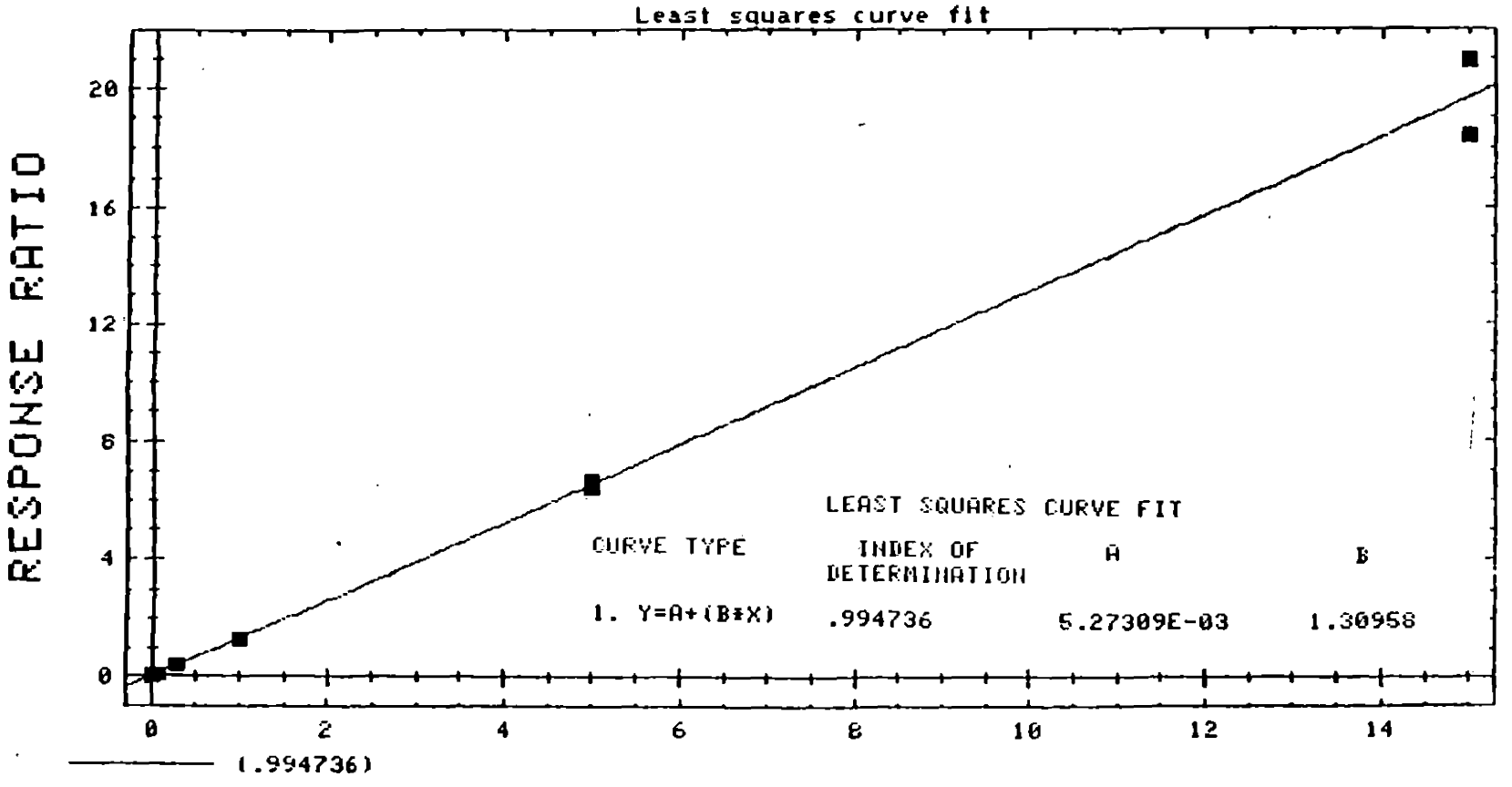


Figure B-11: CALIBRATION CURVE FOR 1,2,3,7,8-PnCDF

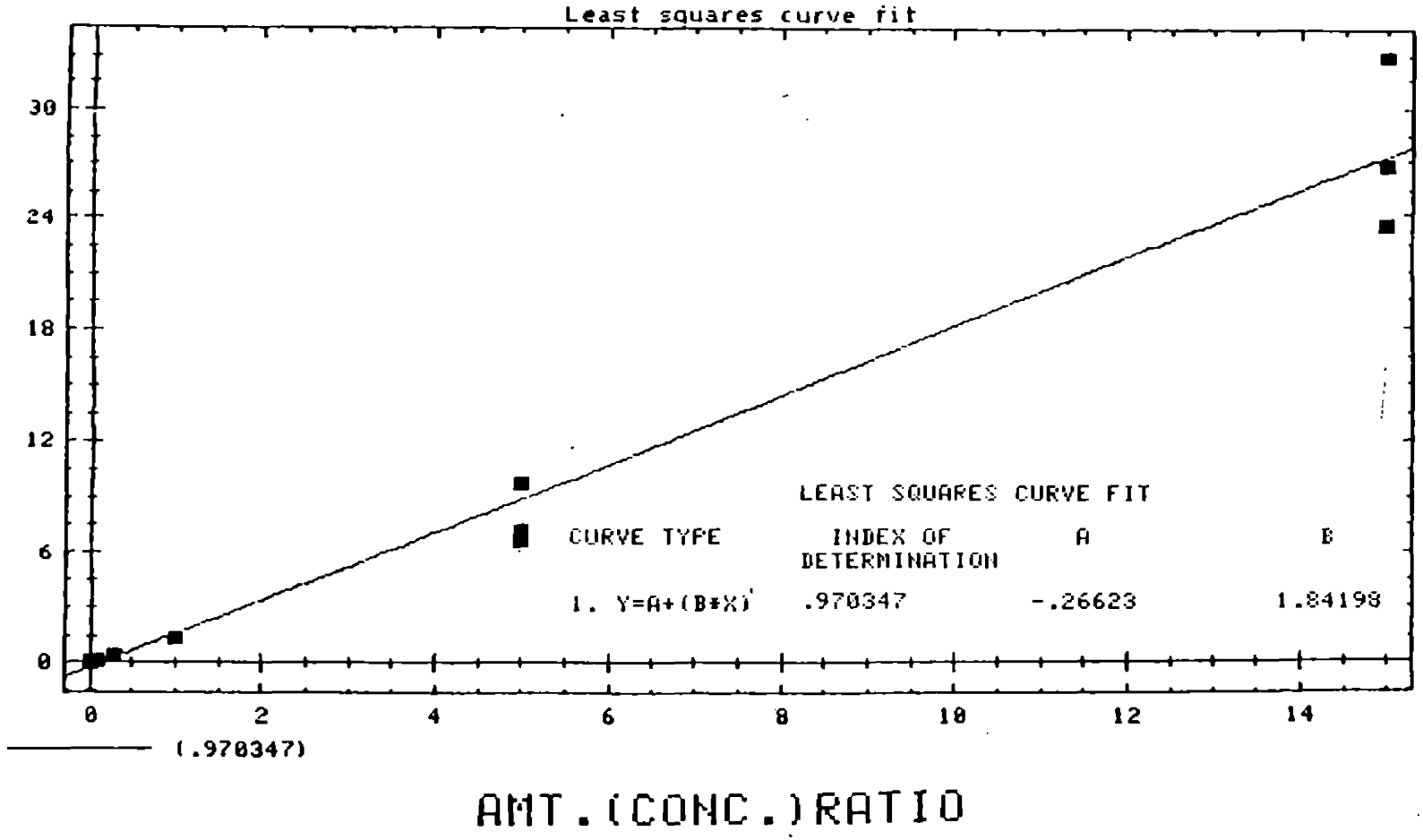


Figure B-12:

CALIBRATION CURVE FOR 1,2,3,4,7,8-HxCDF

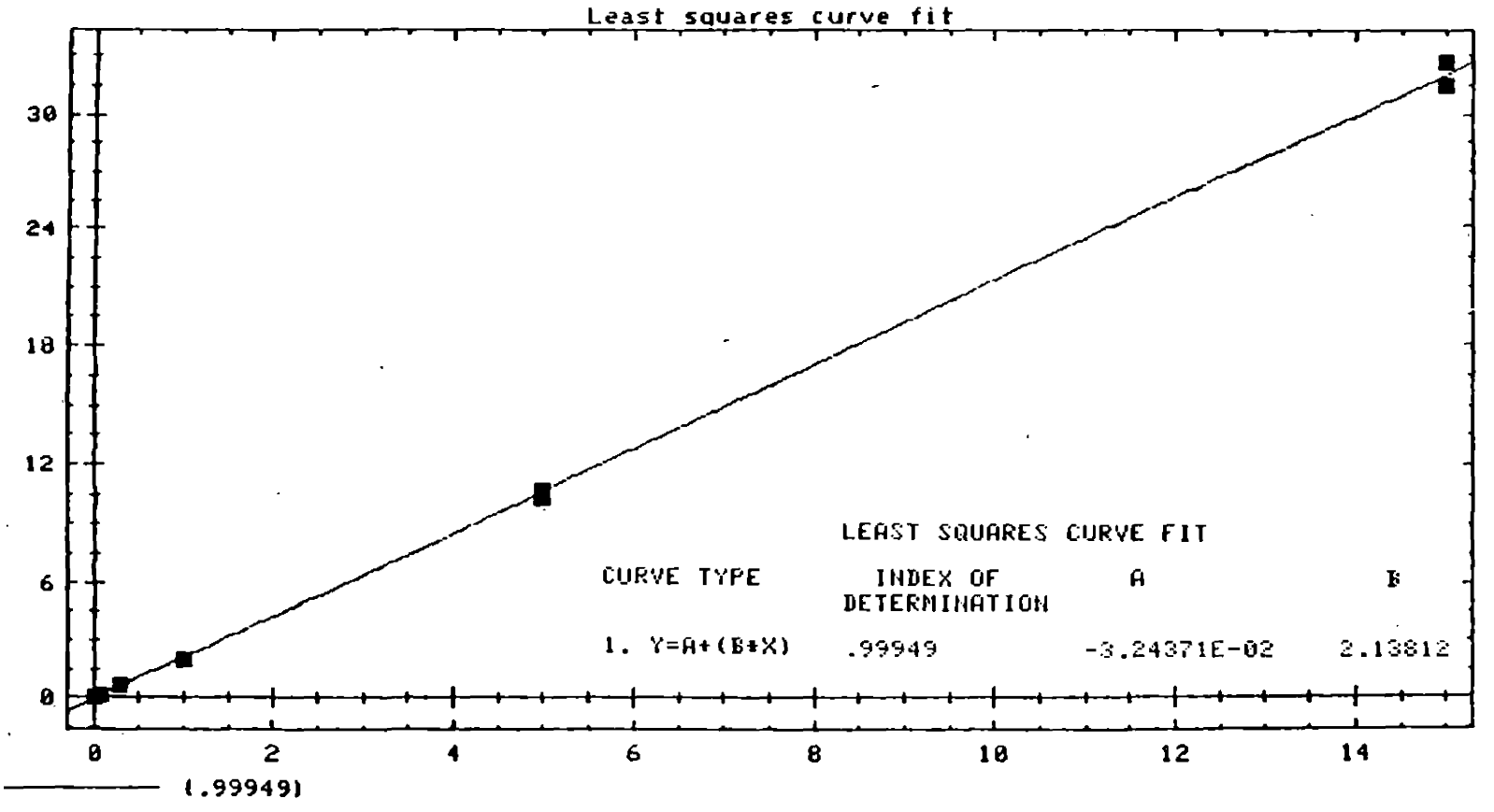
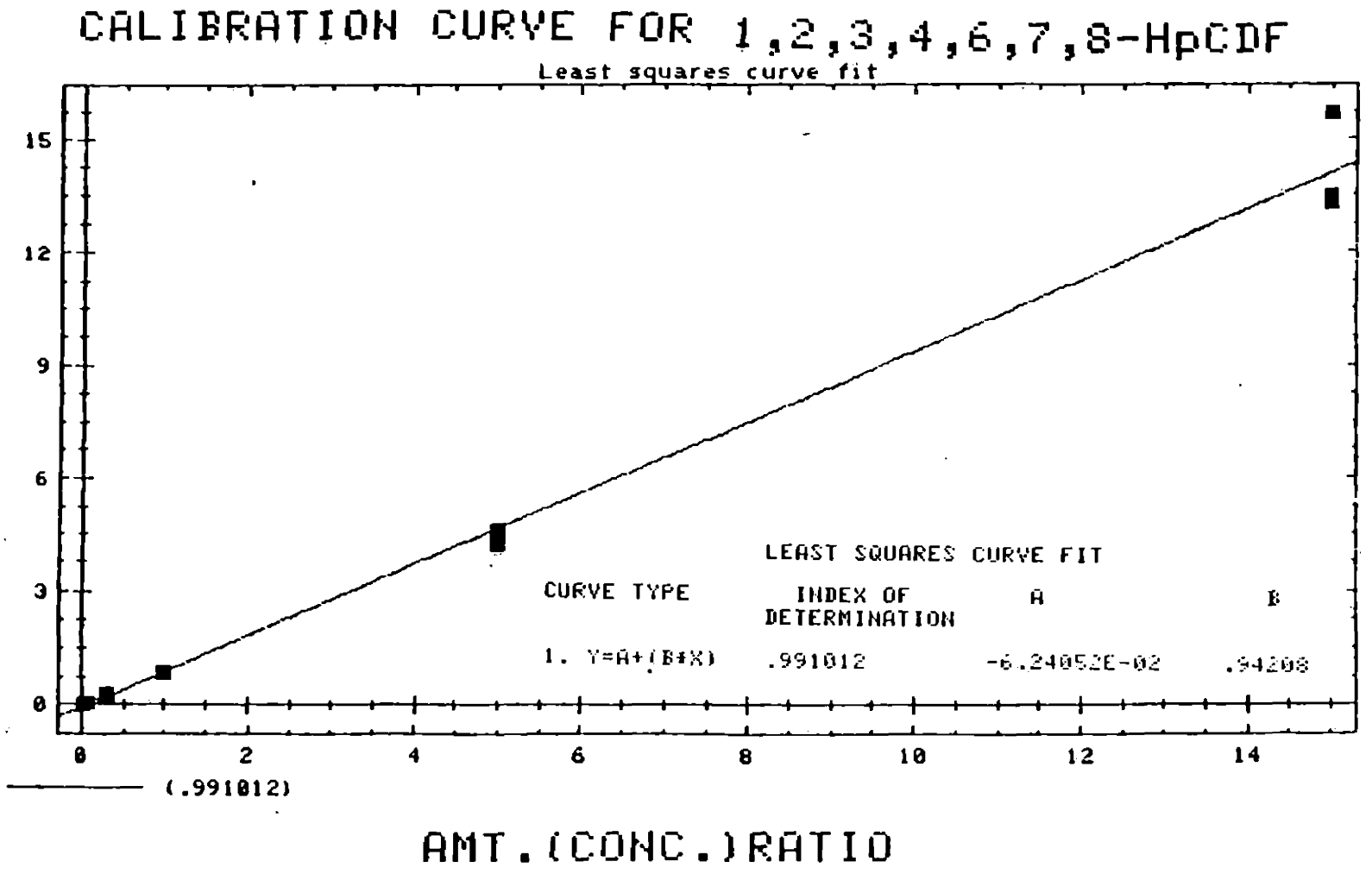


Figure B-13:


CALIBRATION CURVE FOR 1,2,3,4,6,7,8-HpCDF
Least squares curve fit
RESPONSE RATIO
15
12
9
6
3
0
0
2
4
6
8
10
12
14
(.991012)
AMT.(CONC.)RATIO
LEAST SQUARES CURVE FIT
CURVE TYPE
INDEX OF DETERMINATION
A
B
1. Y=A+(B*X)
.991012
-6.24052E-02
.94208

Appendix C

ANALYTE RESPONSE FACTOR CURVES

Figures C-1 through C-8. Area ratios from daily response factor determinations plotted onto the calibration curves.

FIGURE C-1:EPRI BASELINE PROJECT

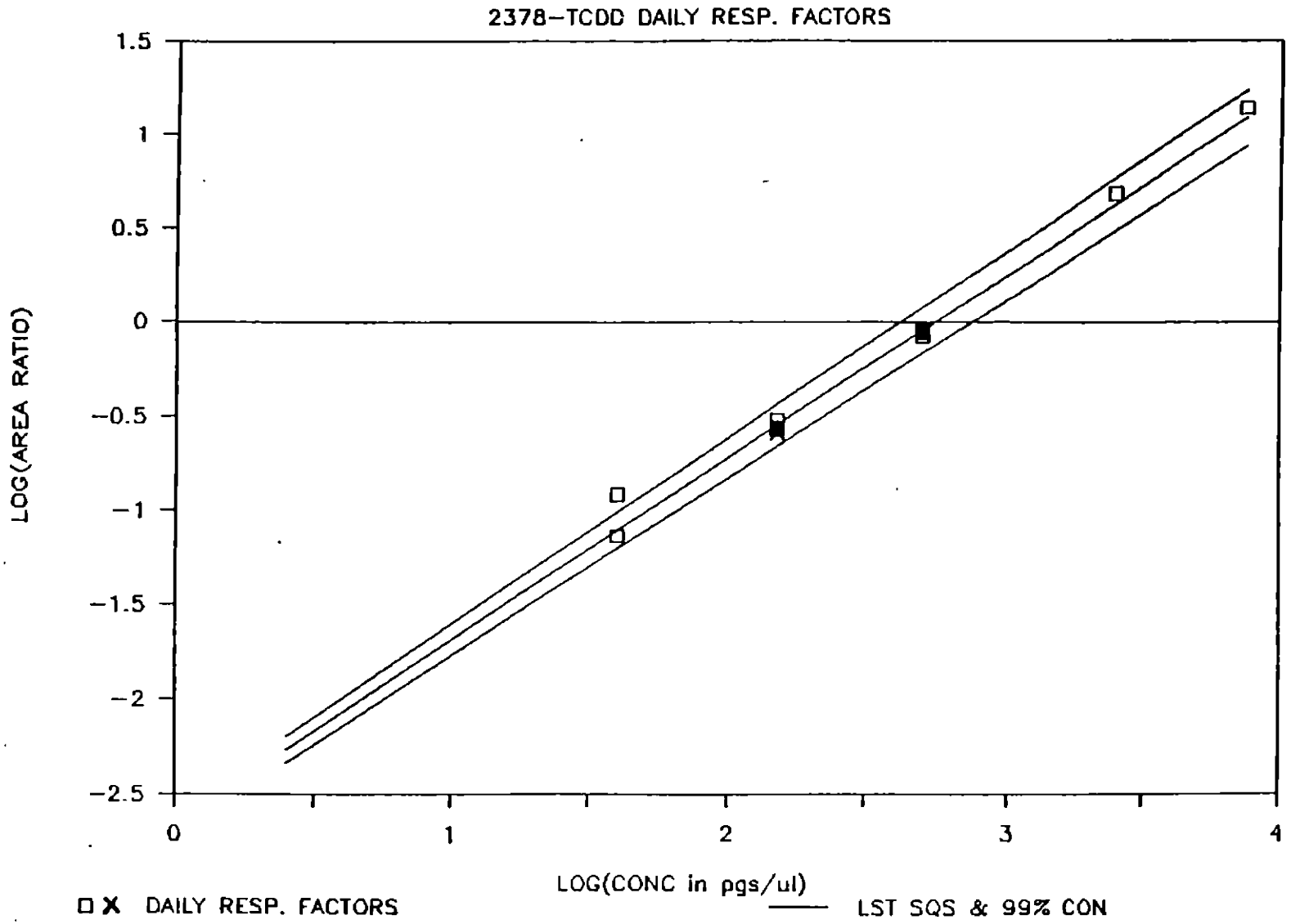


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FIGURE C-2: EPRI BASELINE PROJECT

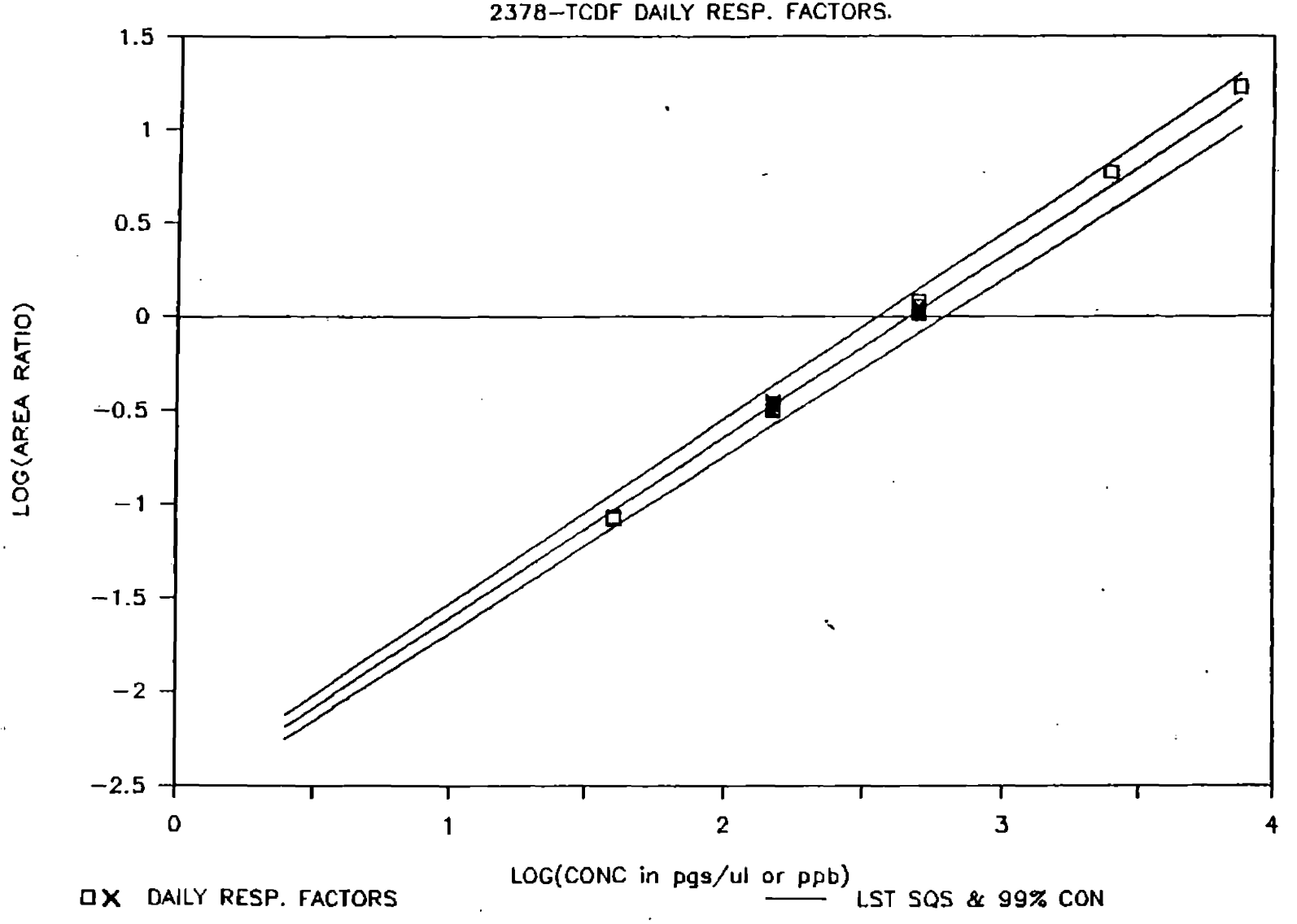

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FIGURE C-3:EPRI BASELINE PROJECT

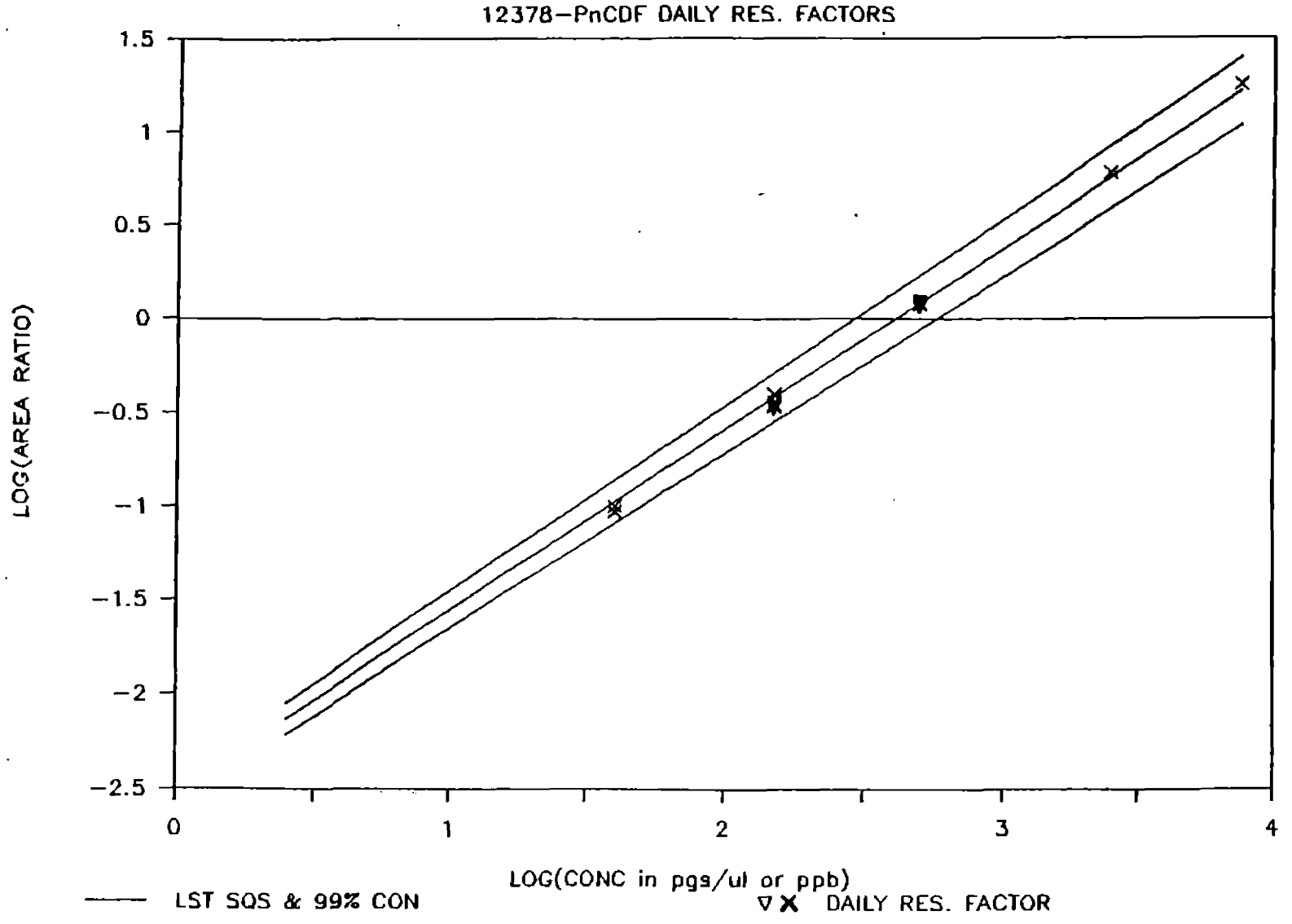

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FIGURE C-4: EPRI BASELINE PROJECT

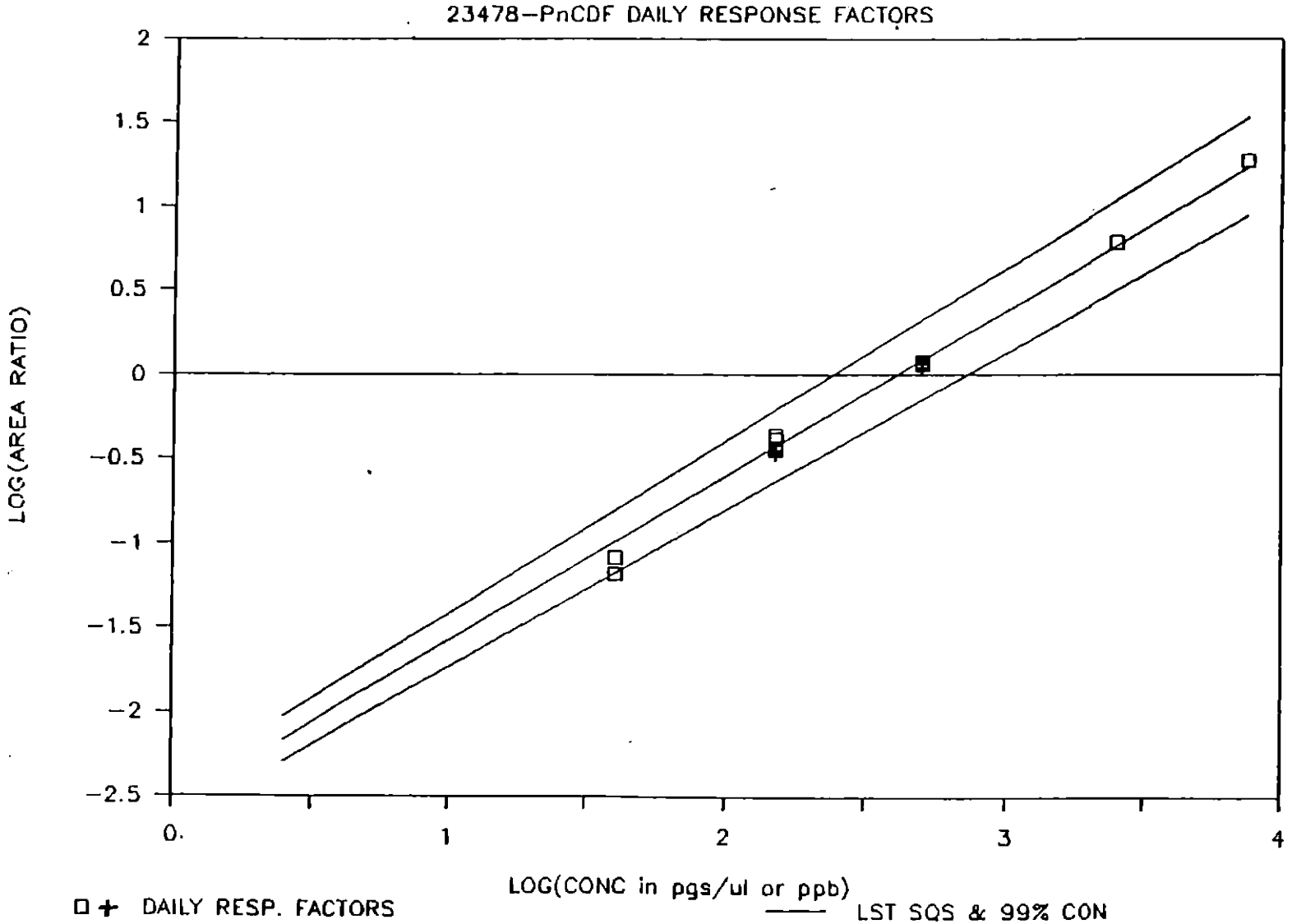
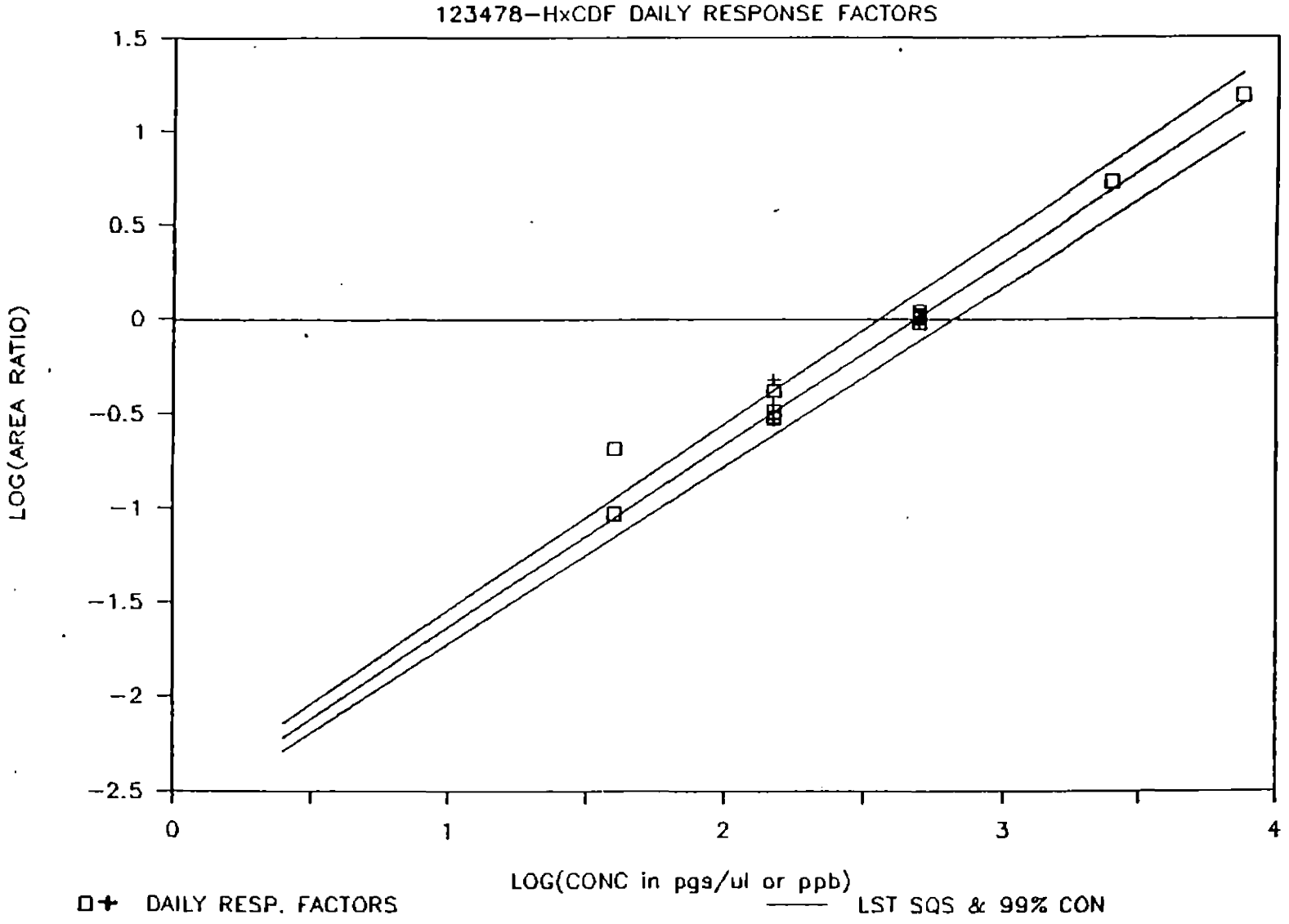


FIGURE C-5: EPRI BASELINE PROJECT



GENP 011008

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FIGURE C-6: EPRI BASELINE PROJECT

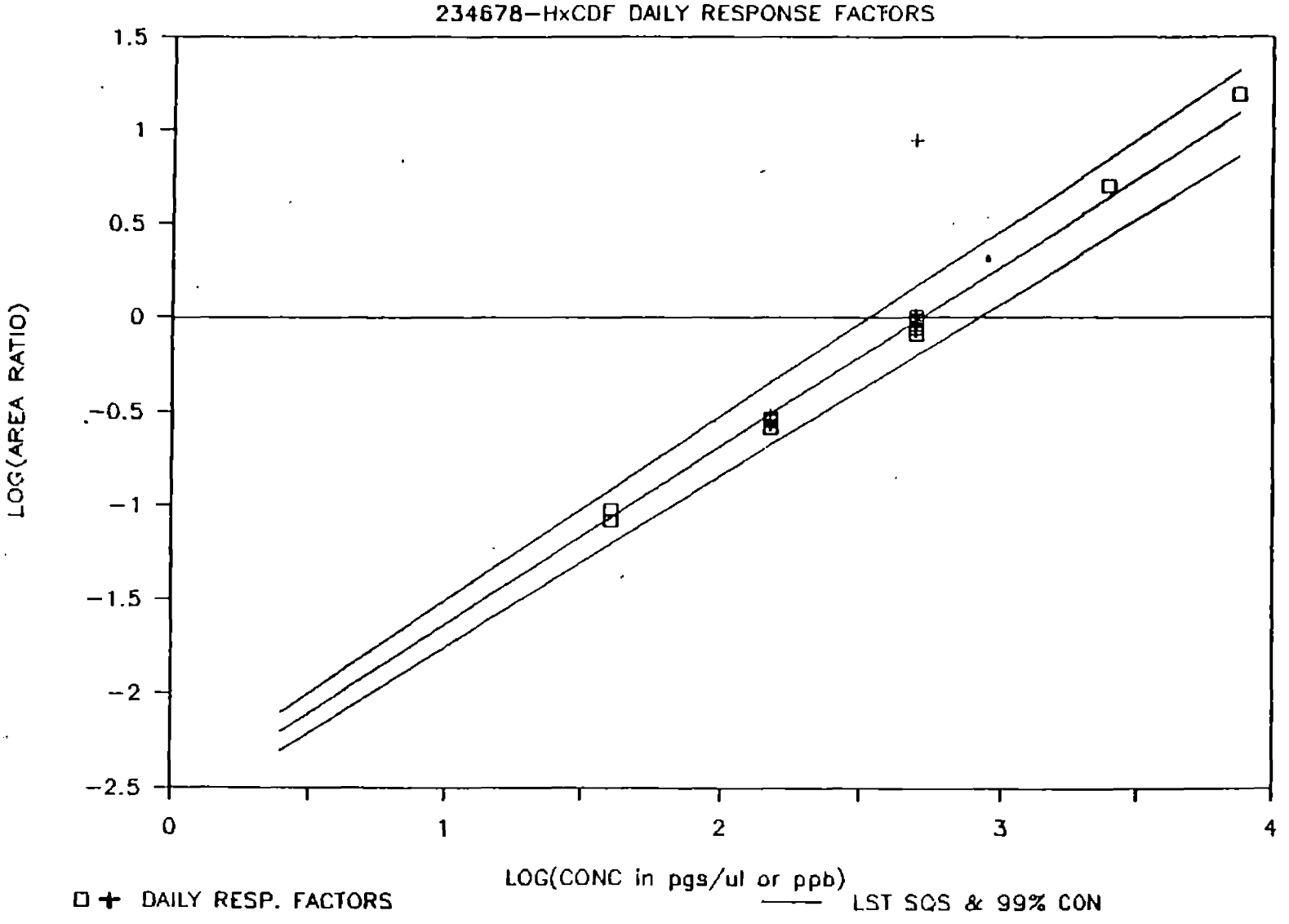

FIGURE C-7: EPRI BASELINE PROJECT

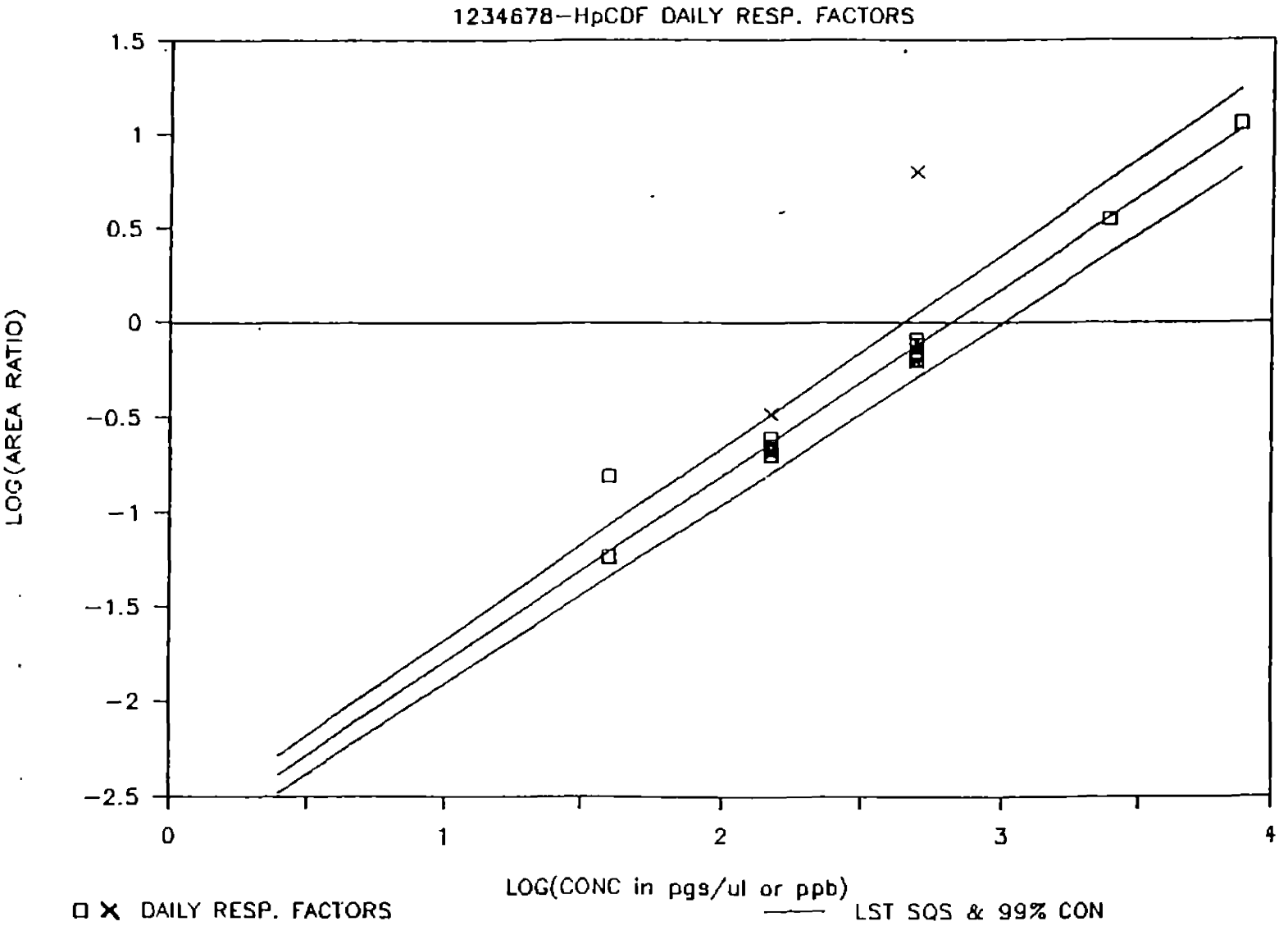

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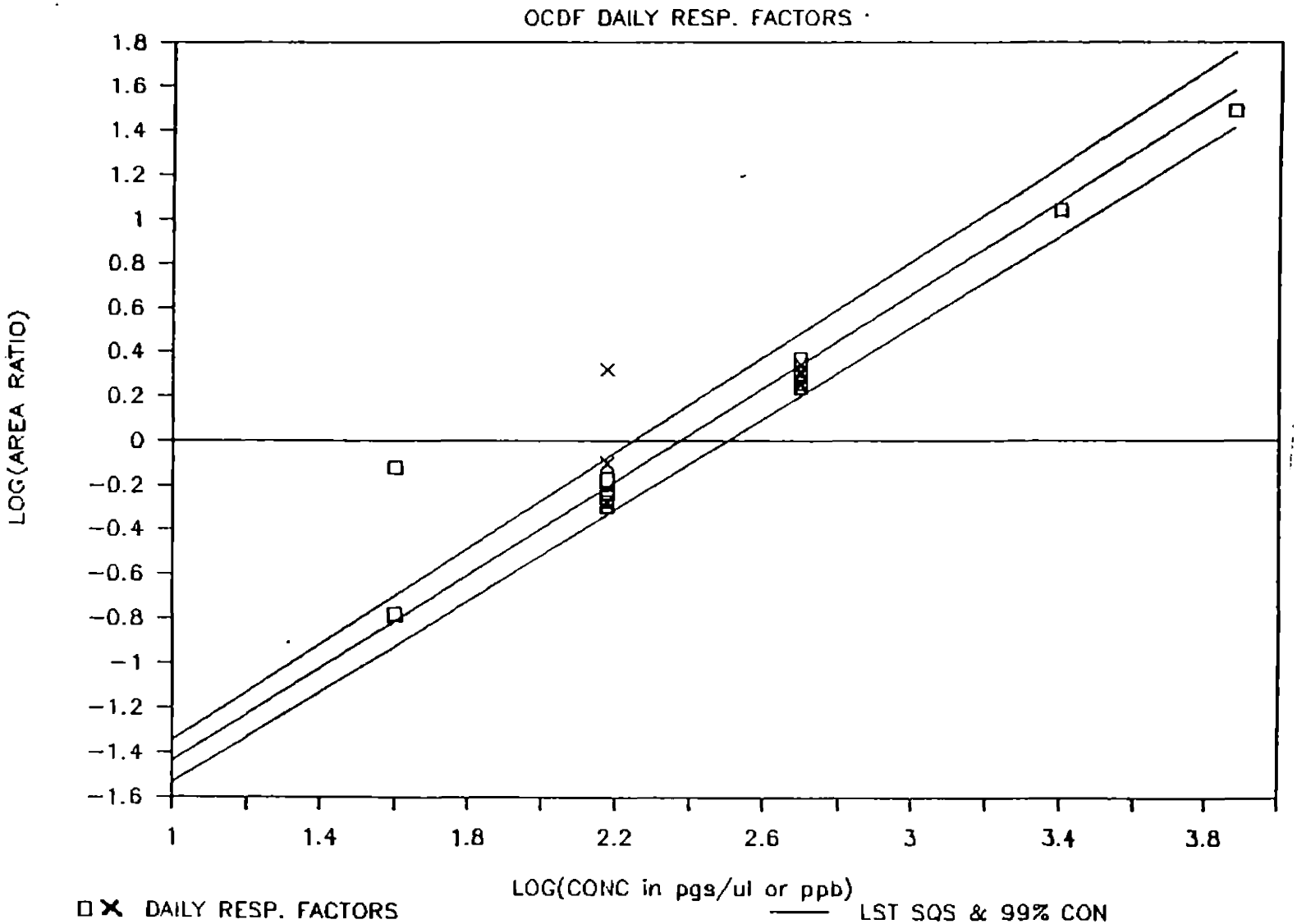
FIGURE C-8: EPRI BASELINE PROJECT



Appendix D

TABLES OF RESULTS

TABLE D-1

BASELINE SAMPLES
PRELIMINARY RESULTS OF SPECIFIC COMPOUND ANALYSIS

SAMPLES	CONC. 2378-TCDD ppb (D.L.)	RET. TIME (min.)	RATIO (%)	CONC. 2378-TCDF ppb (D.L.)	RET. TIME (min.)	CONC. 12378-PCDF ppb (D.L.)	RET. TIME (min.)
Aroclor 1016 E-729-09-01	--			0.14(0.12)	22.34	0.40(0.08)	23.41
Aroclor 1016 E729-09-01	0.51(0.19)	17.63	77	0.34(0.08) ND[1]	22.48	0.32(0.24) ND[1]	23.48
Aroclor 1242 E729-10-01	0.45(0.03)		106	150(1.6)	22.34	1000(25) 62(1.4)[2]	23.03
Aroclor 1260 E729-11-0	7.3(0.72)	17.35	79	190(1.7)	22.15	220(5)	23.20
Tri/Tetra Cl Benz E729-12-01	0.46(0.13)	17.29	44	0.43(0.05)	22.27	0.63(0.09)	23.24
Aged Mineral Oil E729-08-01	43(0.01)	17.55	80	34(0.27)	22.44	140*	23.52
Blind Spike Levels	40			20		40	
Blank	0.93(0.06)	17.58	83	0.14(0.05)	22.53	N.D.(0.41)	23.55
Nujol Spike	10(0.08)	17.58	91	12(0.58)	22.45	24(0.34)	23.48

*Sum of 1,2,3,7,8-PnCDF and 1,2,3,4,8-PnCDF
1.) ND = Not Detected in a second analysis
2.) The results of an alternative interpretation of the high resolution mass data.

TABLE D-2

EPRI BASELINE STUDY
PRELIMINARY TOTAL CONGENER GROUP RESULTS

SAMPLE	TOTAL TCDD ppb (D.L.)	TOTAL TCDF ppb (D.L.)	TOTAL PENTA CDF ppb (D.L.)	TOTAL HEXA CDF ppb (D.L.)	TOTAL HEPTA ppb (D.L.)	OCDF ppb (D.L.)
Aroclor 1016 E729-09-01	N.D.(0.6)	0.42	7.3	350	92	N.D.(12)
Aroclor 1242 E729-10-01	N.D.(0.2)	1900	584	140	130	2300
Aroclor 1260 E729-11-0	N.D.(1.0)	940	932	5500	2970	NA
Chlorobenzenes E729-12-01	N.D.(0.2)	3.0	12.5	1000	91	NA
Aged Mineral Oil E729-08-01	42	95	--	1100	--	NA
Blind Spike Levels	40	20	40	120	0	0
Total Spiked	40	120	240	220	0	160
Blank	N.D.(0.4)	0.75	--	28	--	NA
Nujol	11 (10)*	22 (10)*	--	420 (10)*	19 (10)*	NA

* Spike Level
NA - Not Available

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GENP 011013

TABLE D-3

EPRI BASELINE PROJECT: ANALYSIS OF AGED MINERAL OIL

	BLANK AGED MINERAL OIL (in ppb)	SPIKED MIN OIL (in ppb)	SPIKED LEVEL (in ppb)	%ERROR
TOTAL TCDF	(0.61)	178	200	11.0
TOTAL PnCDF	(1.03)	282	300	6.0
TOTAL HxCDF	(102.3)	400	475	15.8
TOTAL HpCDF	(164)	356	400	11.0
OCDF	(58.3)	3.37	NS	NA
TOTAL TCDD	(0.93)	18.8	25	24.8
OCDD	(58.3)	(2.1)	NS	NA
2,3,7,8-TCDD	(0.93)	18.8	25	24.8
2,3,7,8-TCDF	(0.61)	178	200	11.0
2,3,4,8-TCDF	(0.61)	91.3	100	8.7
1,2,3,7,8-PnCDF	(1.03)	135	150	10.0
1,2,3,4,8-PnCDF	(1.03)	(0.06)	NS	NA
2,3,4,7,8-PnCDF	(1.03)	141	150	6.0
1,2,3,4,7,8-HxCDF	(102)	227	250	9.2
1,2,3,4,7,9-HxCDF	(102)	(0.22)	NS	NA
1,2,3,7,8,9-HxCDF	(102)	(0.22)	NS	NA
1,2,3,6,7,8-HxCDF	(102)	41.5	50	17.0
2,3,4,6,7,8-HxCDF	(120)	160	175	8.6
1,2,3,4,6,7,8-HpCDF	(164)	354	400	11.5
			AVE. % ERR	12.5

1.) Numbers in parentheses indicate detection limits in ppb
2.) Average detection limit of three determinations
3.) NS = not spiked; NA = not applicable
4.) 2,3,7,8-TCDF value is the sum of 2,3,7,8- and 2,3,4,8-TCDF concentrations.

GENP 011014

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TABLE D-4

EPRI BASELINE PROJECT: ANALYSIS OF AROCLOR 1016

	BL AROCLOR 1016 (in ppb)	SPIKED AROCL 1016 (in ppb)	SPIKED LEVEL (in ppb)	% ERROR
TOTAL TCDF	(0.41)[1]	63	53	-18.9
TOTAL PnCDF	(0.50)	312	316	1.3
TOTAL HxCDF	(22.2)	212	185	-14.6
TOTAL HpCDF	(36)	627	526	-19.2
OCDF	(7.9)	120	132	9.1
TOTAL TCDD	(0.58)	48.2	53	9.1
OCDD	(16)	(2.1)[2]	NS[3]	NA[3]
2,3,7,8-TCDD	(0.58)	48.2	53	9.1
2,3,7,8-TCDF	(0.41)	62.3	53	-17.5
2,3,4,8-TCDF	(0.41)	(0.71)[2]	NS	NA
1,2,3,7,8-PnCDF	(0.50)	159	158	-0.6
1,2,3,4,8-PnCDF	(0.50)	(0.38)[2]	NS	NA
2,3,4,7,8-PnCDF	(0.52)	148	158	6.3
1,2,3,4,7,8-HxCDF	(22)	3.98	NS	NA
1,2,3,4,7,9-HxCDF	(22)	(0.26)[2]	NS	NA
1,2,3,7,8,9-HxCDF	(22)	(0.26)[2]	NS	NA
1,2,3,6,7,8-HxCDF	(22)	56.5	53	-6.6
2,3,4,6,7,8-HxCDF	(26)	151	132	-14.4
1,2,3,4,6,7,8-HpCDF	(36)	625	526	-18.8
			AVE % ERR	11

1.) Numbers in parentheses indicate detection limits
2.) Average detection limit of three determinations
3.) NS = not spiked; NA = not applicable

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GENP 011015

TABLE D-5

EPRI BASELINE PROJECT SAMPLE: ANALYSIS OF AROCLOR 1260

	SPIKED AROCL 1260 (in ppb)	SPIKED-BLANK (in ppb)	SPIKED LEVEL (in ppb)	% ERROR
TOTAL TCDF	1613	1093	1137	3.9
TOTAL PnCDF	1320	412	222	NA[3]
TOTAL HxCDF	2473	1253	834	NA[3]
TOTAL HpCDF	2390	732	111	NA[3]
OCDF	5037	403	222	-81.5[4]
TOTAL TCDD	64	64	111	42.3
OCDD	(4.78)[1,2]	NA[3]	NS[3]	NA[3]
2,3,7,8-TCDD	64	64	111	42.3
2,3,7,8-TCDF	1210	1033	1027	-0.6
2,3,4,8-TCDF	436	NA	194	NA
1,2,3,7,8-PnCDF	205	127	111	-14.4
1,2,3,4,8-PnCDF	140	61	NS	NA
2,3,4,7,8-PnCDF	294	119	111	-7.2
1,2,3,4,7,8-HxCDF	1240	647	500	-29.4
1,2,3,4,7,9-HxCDF	(0.5)[1,2]	NA	NS	NA
1,2,3,7,8,9-HxCDF	38	38	NS	NA
1,2,3,6,7,8-HxCDF	336	336	278	-20.9
2,3,4,6,7,8-HxCDF	297	260	56	-364.3[4]
1,2,3,4,6,7,8-HpCDF	845	358	111	-222.5[4]
			AVE % ERR	75

1.) Numbers in parentheses indicate detection limits
2.) Average detection limit of three determinations
3.) NS = not spiked; NA = not applicable
4.) These values are discussed in the text

GENP 011016

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TABLE D-6

EPRI BASELINE PROJECT SAMPLE
1953 LOAD CENTER NETWORK TRANSFORMER

	DET. 1	DET. 2	DET.3	AVE.	SD	CV
TOTAL TCDF	232	121	337	230	108.1	47
TOTAL PnCDF	1930	1690	1760	1793	123.4	6.9
TOTAL HxCDF	6840	6570	6520	6643	172.1	2.6
TOTAL HpCDF	10100	8610	8880	9196	793.9	8.5
OCDF	39000	37700	42100	39600	2234	5.6
TOTAL TCDD	(1.32)	(62.2)	(2.76)			
OCDD	11.8	9	14.3	12	3	25
	10	10.4	17			
2,3,7,8-TCDD	(1.32)	(62.2)	(2.76)			
2,3,7,8-TCDF	48.9	42.9	67.7	53.17	12.94	24.3
2,3,4,8-TCDF	16.5					
1,2,3,7,8-PnCDF	19.2	29.4	15.6	21.4	7.16	33
1,2,3,4,8-PnCDF	919	705	772	798.7	109.5	13.7
2,3,4,7,8-PnCDF	101	107	105	104.3	3.06	2.9
1,2,3,4,7,8-HxCDF	4790	4610	4620	4673	101.2	2.2
1,2,3,4,7,9-HxCDF	(2.34)	(4.14)	(5)			
1,2,3,7,8,9-HxCDF	(2.34)	(4.14)	(5)			
1,2,3,6,7,8-HxCDF	360	367	362	363	3.61	1
2,3,4,6,7,8-HxCDF	51.2	42.4	40.6	44.7	5.67	12.7
1,2,3,4,6,7,8-HpCDF	3890	3280	3990	3720	384.3	10.3

1.) Average of six (6) results

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GENP 011017

TABLE D-7

EPRI BASELINE PROJECT SAMPLE:
LOAD CENTER NETWORK TRANSFORMER

	DET. 1	DET. 2	DET.3	AVE.	SD	CV
TOTAL TCDF	228	182	518	309.3	182.2	58.9
TOTAL PnCDF	430	303	435	389.3	74.8	19.2
TOTAL HxCDF	983	897	938	939.3	43	4.6
TOTAL HpCDF	2400	1940	1850	2063	295	14.3
OCDF	6800	8000	7010	7270	640.9	8.8
TOTAL TCDD	(0.35)	(0.43)	(7.1)			
OCDD	(27.4)	(26.6)	(27.1)			
2,3,7,8-TCDD	(0.05)	(0.12)	(2)			
2,3,7,8-TCDF	68.6	61.1	90.9	73.5	15.5	21.1
2,3,4,8-TCDF		6.95				
1,2,3,7,8-PnCDF	29.4	30.7	28.9	29.7	0.93	3.13
1,2,3,4,8-PnCDF	88.8	38.1	72.3	66.4	25.9	39
2,3,4,7,8-PnCDF	60.6	39.6	59.4	53.2	11.8	22.2
2,3,4,7,8-HxCDF	619	596	610	608.3	11.6	1.9
1,2,3,4,7,9-HxCDF	1.5	1.54	1.8	1.6	0.16	10
1,2,3,7,8,9-HxCDF	37.9	17.7	36.1	29.9	7.	25
	33.9	22.2	31.5			
1,2,3,6,7,8-HxCDF	43	44.6	42.3	43.3	1.18	
2,3,4,6,7,8-HxCDF	15.2	9.1	12.3	12.2	3.05	2.7
1,2,3,4,6,7,8-HpCDF	662	545	631	612.7	60.6	25

TABLE D-8

EPRI BASELINE PROJECT SAMPLE:
CAPACITOR

	DET. 1	DET. 2	DET.3	AVE.	SD	CV
TOTAL TCDF	686	700	942	776	144	15
TOTAL PnCDF	368	351	346	355	11.5	3.3
TOTAL HxCDF	39.7	51.9	42	44.5	6.48	15
TOTAL HpCDF	8.3	12.5	9.51	10.1	2.16	21
OCDF	13.4	15.3	16.6	15.1	1.60	11
TOTAL TCDD	(0.16)	(0.39)	(0.83)			
OCDD	(1.1)	(0.38)	(0.29)			
2,3,7,8-TCDD	(0.16)	(0.39)	(0.83)			
2,3,7,8-TCDF	197	226	273	232	38.40	7
2,3,4,8-TCDF		44.1				
1,2,3,7,8-PnCDF	8.3	9.6	8.37	8.757	0.73	8.4
1,2,3,4,8-PnCDF	11.5	7.7	8.7	9.3	1.97	21
2,3,4,7,8-PnCDF	61.3	61.5	71.1	64.63	5.60	8.7
1,2,3,4,7,8-HxCDF	16.5	21.5	15.8	13.53	4.55	34
1,2,3,4,7,9-HxCDF	0.3	0.36	0.38	0.35	0.04	12
1,2,3,7,8,9-HxCDF	0.19	0.36	0.24	0.263	0.09	33
1,2,3,6,7,8-HxCDF	3.4	4.36	3.94	3.9	0.48	12
2,3,4,6,7,8-HxCDF	1.7	2	1.75	1.817	0.16	8.9
1,2,3,4,6,7,8-HpCDF	3.4	4.8	3.38	3.86	0.81	21

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TABLE D-9

EPRI BASELINE PROJECT SAMPLE: PRECIPITATOR TRANSFORMER

	DET. 1	DET. 2	DET.3	AVE.	SD	CV
TOTAL TCDF	1500	953	1100	1184	283.1	23.9
TOTAL PnCDF	10500	10200	10800	10500	300	2.9
TOTAL HxCDF	10500	10900	10700	10700	200	1.9
TOTAL HpCDF	2000	2020	2100	2040	52.9	2.6
OCDF	190	187	181	186	4.6	2.5
TOTAL TCDD	(0.05)	0.42	0.98			
OCDD	1.04	0.98	1.01	1.01	0.03	3
2,3,7,8-TCDD	(0.05)	(0.02)	(0.09)			
2,3,7,8-TCDF	480	349	395	408	66.5	16.3
2,3,4,8-TCDF		101				
1,2,3,7,8-PnCDF	799	796	818	804.3	11.9	1.5
1,2,3,4,8-PnCDF	307	256	317	293.3	32.7	11.2
2,3,4,7,8-PnCDF	1930	1920	1970	1940	26.5	1.4
1,2,3,4,7,8-HxCDF	4390	4410	4360	4387	25.2	0.6
1,2,3,4,7,9-HxCDF	22.5	21.6	22	22	0.45	2.1
1,2,3,7,8,9-HxCDF	162	177	175	171.3	8.2	4.8
1,2,3,6,7,8-HxCDF	1030	1030	1000	1020	17.3	1.7
2,3,4,6,7,8-HxCDF	366	404	398	389.3	20.4	5.2
1,2,3,4,6,7,8-HpCDF	690	678	700	689.3	11	1.6

TABLE D-10

EPRI BASELINE PROJECT SAMPLE:
1976 ARC FURNACE TRANSFORMER

	DET. 1	DET. 2	DET.3	AVE.	SD	CV
TOTAL TCDF	0.53	0.38	0.31	0.41	0.11	28
TOTAL PnCDF	1.72	1.94	2.14	1.93	0.21	11
TOTAL HxCDF	1.78	1.14	2.43	1.78	0.64	36
TOTAL HpCDF	0.51	0.23	0.19	0.31	0.17	56
OCDF	0.46	0.3	0.24	0.33	0.11	34
TOTAL TCDD	(0.09)	(0.08)	(20)			
OCDD	3.02	3.38	3.8	3.4	0.39	11
2,3,7,8-TCDD	(0.09)	(0.08)	(20)			
2,3,7,8-TCDF	0.2	0.24	0.23	0.223	0.02	9.3
2,3,4,8-TCDF		(0.54)				
1,2,3,7,8-PnCDF	0.21	0.26	0.23	0.23	0.03	11
1,2,3,4,8-PnCDF	(0.16)	(0.13)	(0.08)			
2,3,4,7,8-PnCDF	0.45	0.51	0.41	0.46	0.05	11
1,2,3,4,7,8-HxCDF	1.08	0.54	1.13	0.917	0.33	36
1,2,3,4,7,9-HxCDF	(0.2)	(0.06)	(0.03)			
1,2,3,7,8,9-HxCDF	0.22	0.11	0.28	0.203	0.09	42
1,2,3,6,7,8-HxCDF	0.25	0.13	0.27	0.217	0.08	35
2,3,4,6,7,8-HxCDF	(0.21)	(0.14)	(0.07)			
1,2,3,4,6,7,8-HpCDF	(0.28)	(0.08)	(0.09)			

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GENP 011021

TABLE D-11

EPRI BASELINE PROJECT SAMPLE: SUBSTATION DISTRIBUTION TRANSFORMER

	DET. 1	DET. 2	DET.3	AVE.	SD	CV
TOTAL TCDF	0.15	0.18	0.23	0.19	0.04	22
TOTAL PnCDF	(0.34)	(0.6)	(0.53)			
TOTAL HxCDF	0.56	0.52	0.71	0.6	0.1	16.7
TOTAL HpCDF	(0.65)	(0.23)	(0.21)			
OCDF	(0.63)	(2.4)	(3.8)			
TOTAL TCDD	(0.61)	0.19	0.1			
OCDD	20.8	16.8	17.1	18.2	2.23	12
2,3,7,8-TCDD	(0.6)	(0.12)	(0.09)			
2,3,7,8-TCDF	0.15	0.18	0.13	0.15	0.025	16
2,3,4,8-TCDF			(1.5)			
1,2,3,7,8-PnCDF	(0.34)	(0.09)	(0.07)			
1,2,3,4,8-PnCDF	(0.34)	(0.09)	(0.07)			
2,3,4,7,8-PnCDF	(0.34)	0.09	0.11			
1,2,3,4,7,8-HxCDF	0.56	0.52	0.53	0.54	0.02	3.7
1,2,3,4,7,9-HxCDF	(0.21)	(0.19)	(0.16)			
1,2,3,7,8,9-HxCDF	(0.21)	(0.19)	(0.16)			
1,2,3,6,7,8-HxCDF	(0.21)	(0.19)	(0.16)			
2,3,4,6,7,8-HxCDF	(0.32)	(0.2)	(0.18)			
1,2,3,4,6,7,8-HpCDF	0.65	(0.23)	(0.21)			

TABLE D-12

EPRI BASELINE PROJECT SAMPLE:
1962 LOAD CENTER NETWORK TRANSFORMER

	DET. 1	DET. 2	DET.3	AVE.	SD	CV
TOTAL TCDF	553	552	512	539	23.4	4.3
TOTAL PnCDF	1170	1130	1100	1133	35.1	3.1
TOTAL HxCDF	1760	1780	1820	1790	30.5	1.7
TOTAL HpCDF	1780	1580	1610	1657	107.8	6.5
OCDF	3960	4410	4730	4366	387.7	8.9
TOTAL TCDD	(1.16)	(0.82)	(0.71)			
OCDD	18.7	24.2	28.4	23.8	4.9	20.6
2,3,7,8-TCDD	(1.16)	(0.82)	(0.71)			
2,3,7,8-TCDF	195	184	182	187	7	3.7
2,3,4,8-TCDF		42.4				
1,2,3,7,8-PnCDF	59.4	60.8	61.4	60.5	1	1.7
1,2,3,4,8-PnCDF	230	216	206	217	12	5.5
2,3,4,7,8-PnCDF	170	151	173	164.6	11.9	7.2
1,2,3,4,7,8-HxCDF	1000	1030	1110	1047	56.9	5.4
1,2,3,4,7,9-HxCDF	(0.89)	(0.86)	(0.59)			
1,2,3,7,8,9-HxCDF	(0.89)	(0.86)	(0.59)			
1,2,3,6,7,8-HxCDF	64.9	65.2	61.7	63.9	1.9	3
2,3,4,6,7,8-HxCDF	70	58	56	61	7.6	12
1,2,3,4,6,7,8-HpCDF	815	761	784	787	27	3.4

783834

GENP 011023

TABLE D-13

EPRI BASELINE PROJECT SAMPLE: SPIKED AGED MINERAL OIL

	DET. 1	DET. 2	DET.3	AVE.	SD	CV
TOTAL TCDF	145	195	194	178	28.6	16.1
TOTAL PnCDF	251	312	283	282	30.5	10.8
TOTAL HxCDF	396	455	350	400	52.6	13.2
TOTAL HpCDF	354	341	372	356	15.6	4.4
OCDF	3.49	3.55	3.06	3.37	0.27	7.9
TOTAL TCDD	17	21.3	18.2	18.8	2.219	12
OCDD	(5.09)	(0.49)	(0.67)			
2,3,7,8-TCDD	17	21.3	18.2	18.8	2.219	12
2,3,7,8-TCDF	146	195	192	177.7	27.5	15.5
2,3,4,8-TCDF		91.3				
1,2,3,7,8-PnCDF	121	153	132	135	16.3	12.1
1,2,3,4,8-PnCDF	(0.04)	(0.07)	(0.07)			
2,3,4,7,8-PnCDF	127	158	138	141	15.7	11.1
1,2,3,4,7,8-HxCDF	224	259	199	227	30.1	13.3
1,2,3,4,7,9-HxCDF	(0.53)	(0.07)	(0.05)			
1,2,3,7,8,9-HxCDF	(0.53)	(0.07)	(0.05)			
1,2,3,6,7,8-HxCDF	42.1	47.4	35.1	41.5	6.2	14.9
2,3,4,6,7,8-HxCDF	150	161	170	160	10	6.3
1,2,3,4,6,7,8-HpCDF	351	341	369	353.7	14.2	4

TABLE D-14

EPRI BASELINE PROJECT SAMPLE: SPIKED AROCLOR 1016

	DET. 1	DET. 2	DET.3	AVE.	SD	CV
TOTAL TCDF	60.8	62.7	65.5	63	2.4	3.8
TOTAL PnCDF	276	336	324	312	31.7	10.1
TOTAL HxCDF	201	209	225	212	12.2	5.8
TOTAL HpCDF	578	712	591	627	73.9	11.8
OCDF	133	107	121	120.3	13	10.8
TOTAL TCDD	39.6	50.4	54.5	48.2	7.7	15.9
OCDD	(1.44)	(0.41)	(4.52)			
2,3,7,8-TCDD	39.6	50.4	54.5	48.2	7.7	15.9
2,3,7,8-TCDF	60.8	60.5	65.5	62.3	2.8	4.5
2,3,4,8-TCDF		(0.72)				
1,2,3,7,8-PnCDF	138	171	168	159	18.2	11.4
1,2,3,4,8-PnCDF	(0.30)	(0.09)	(0.75)			
2,3,4,7,8-PnCDF	122	159	163	148	22.6	15.3
1,2,3,4,7,8-HxCDF	2.56	3.8	5.6	3.98	1.52	38
1,2,3,4,7,9-HxCDF	(0.21)	(0.05)	(0.52)			
1,2,3,7,8,9-HxCDF	(0.21)	(0.05)	(0.52)			
1,2,3,6,7,8-HxCDF	58.5	54.6	56.5	6.5	2	3.5
2,3,4,6,7,8-HxCDF	140	150	162	150.7	11	7.3
1,2,3,4,6,7,8-HpCDF	575	709	592	625	73	11

GENP 011025

TABLE D-15

EPRI BASELINE PROJECT SAMPLE: SPIKED AROCLOR 1260

	DET. 1	DET. 2	DET.3	AVE.	SD	CV
TOTAL TCDF	1630	1620	1590	1613	20.82	1.3
TOTAL PnCDF	1330	1290	1340	1320	26.46	2
TOTAL HxCDF	2490	2380	2550	2473	86.2	3.5
TOTAL HpCDF	2470	2300	2400	2390	85.4	3.6
OCDF	4850	5090	5170	5037	166.5	3.3
TOTAL TCDD	65.1	65.5	106	79.9	23	30
OCDD	(3.73)	(0.72)	(9.89)			
2,3,7,8-TCDD	65.1	65.5	106[1]	79.9	23	30
2,3,7,8-TCDF	1210	1220	1200	1210	10	0.83
2,3,4,8-TCDF		436				
1,2,3,7,8-PnCDF	207	203	206	205	2.08	1
1,2,3,4,8-PnCDF	139	139	142	140	1.73	1.2
2,3,4,7,8-PnCDF	304	288	291	294.3	8.5	2.9
1,2,3,4,7,8-HxCDF	1228	1209	1284	1240	38.99	3.1
1,2,3,4,7,9-HxCDF	(0.41)	(0.09)	(0.99)			
1,2,3,7,8,9-HxCDF	37.8	35.8	39.9	37.8	2.05	5.5
1,2,3,6,7,8-HxCDF	339	321	347	336	13.32	4
2,3,4,6,7,8-HxCDF	313	286	291	297	14.36	4.9
1,2,3,4,6,7,8-HpCDF	865	805	865	845	34.64	4.1

1.) Analyzed by high resolution m.s.

Appendix E

BAR GRAPHS OF RESULTS

Figures E-1 through E-10. Bar plots of the results. The numbers listed along the analyte axis in each of these figures represent the following isomers or groups of isomers:

1. Total TCDF
2. Total PnCDF
3. Total HxCDF
4. Total HpCDF
5. OCDF
6. Total TCDD
7. OCDD
8. 2,3,7,8-TCDD
9. 2,3,7,8-TCDF
10. 2,3,4,8-TCDF
11. 1,2,3,7,8-PnCDF
12. 1,2,3,4,8-PnCDF
13. 2,3,4,7,8-PnCDF
14. 1,2,3,4,7,8-HxCDF
15. 1,2,3,4,7,9-HxCDF
16. 1,2,3,7,8,9-HxCDF
17. 1,2,3,6,7,8-HxCDF
18. 2,3,4,6,7,8-HxCDF
19. 1,2,3,4,6,7,8-HpCDF

In the Figures a bracket is used to indicate that most of the individual isomer determinations were multiplied by a factor of 10 before they were plotted. Thus, for example the concentration of 12347 8-HxCDF in the first figure is 4.7 x 10^{3} ppb.

GENP 011027

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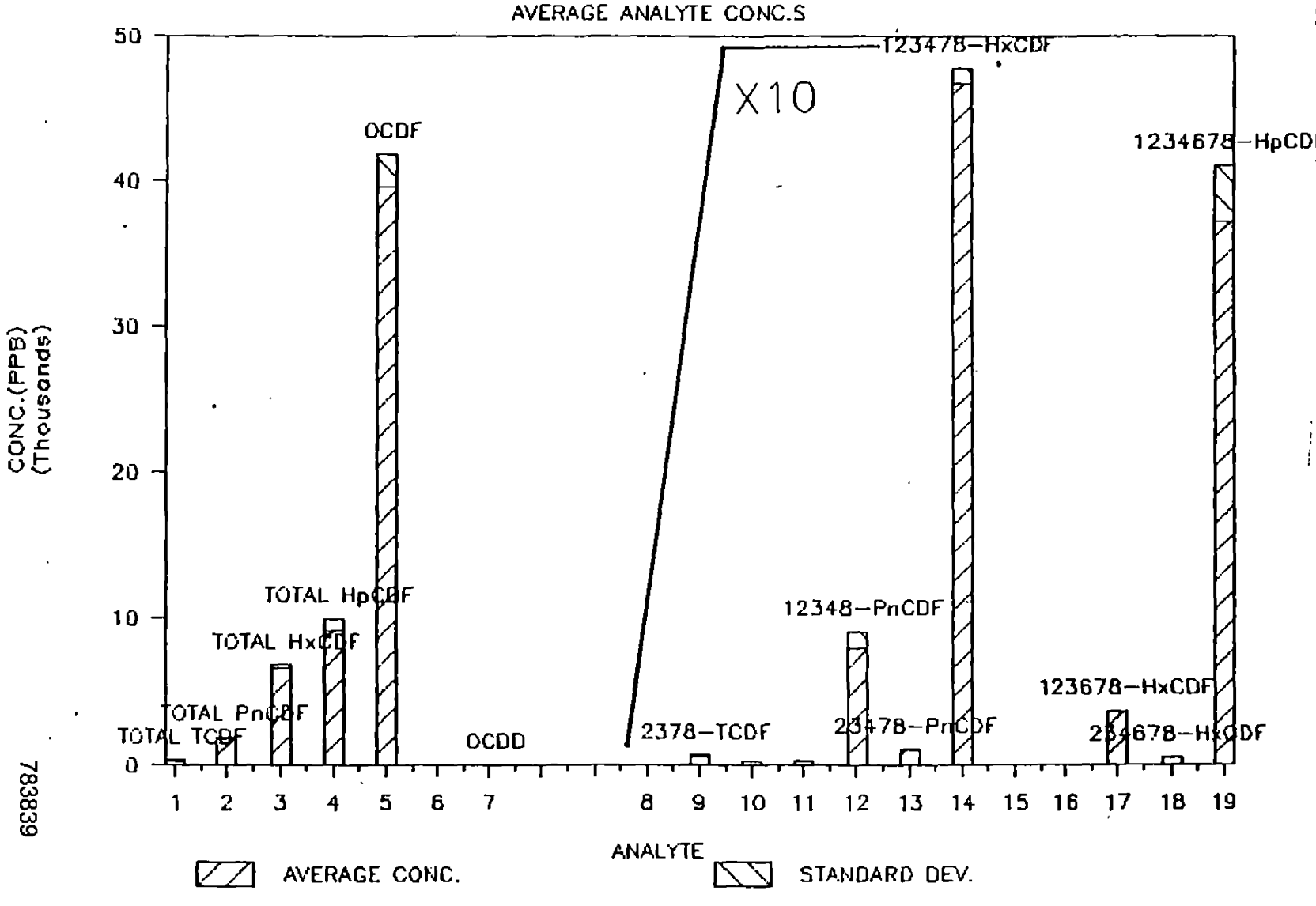
FIG E-1: LOAD CENTER NETW. TRANSFORMER



GENP 011028

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FIG E-2: LOAD CNTR NETWORK TRANSFORMER

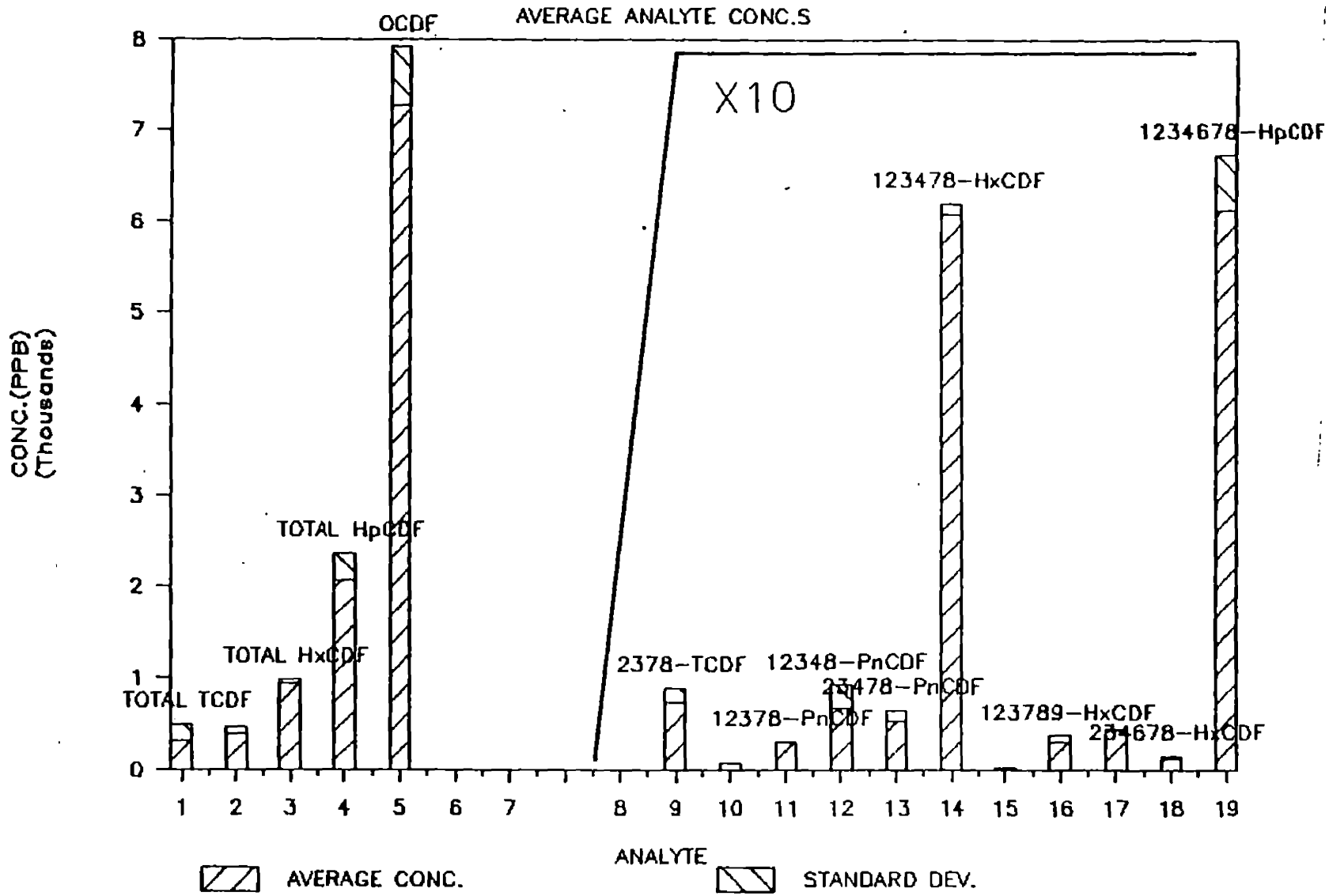
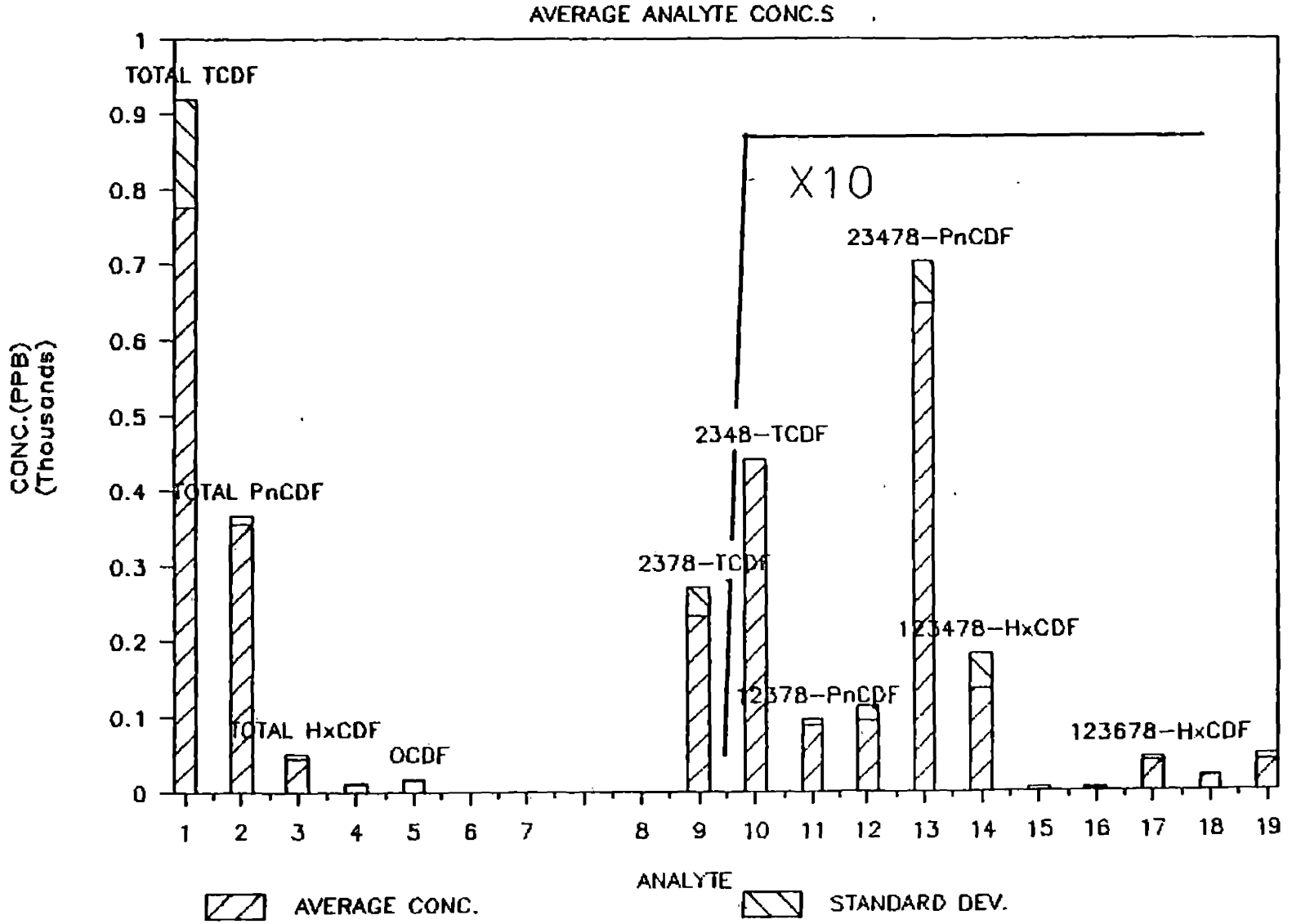


FIG E-3: CAPACITOR



GENP 011030

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FIG E-4: PRECIPITATOR TRANSFORMER

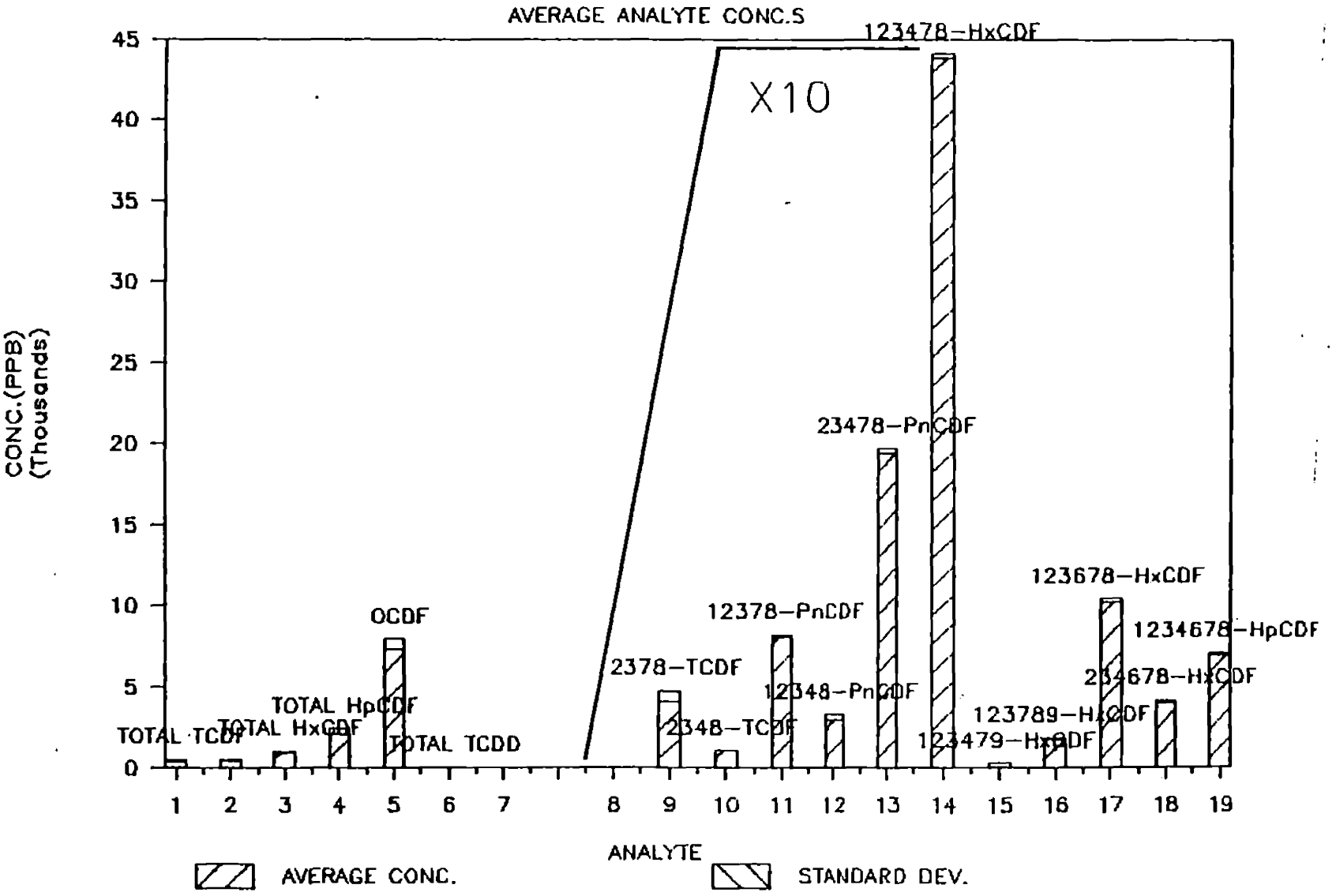

FIG E-5: ARC FURNACE TRANSFORMER

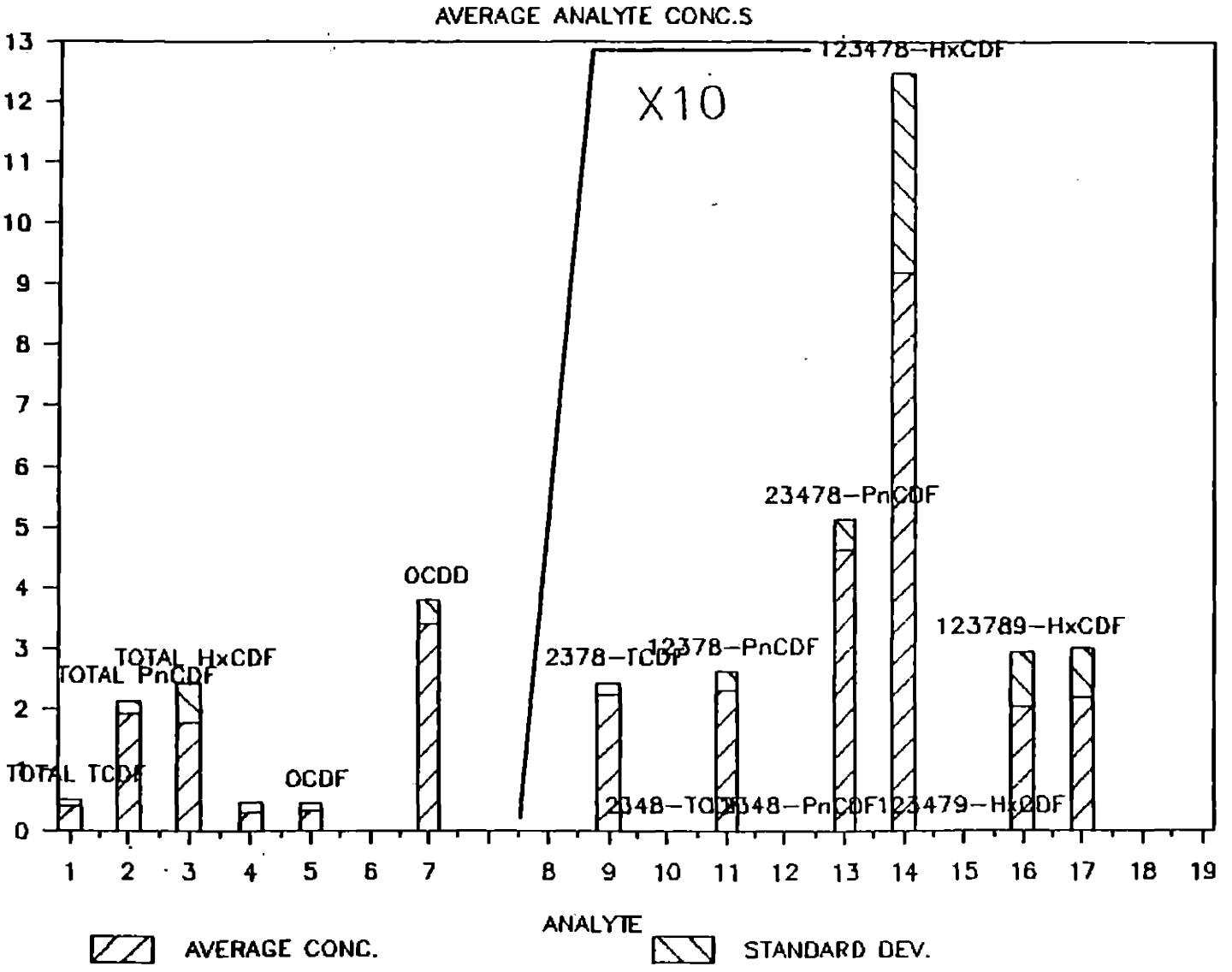

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FIG E-6: SUBSTATION DISTRIBUTION TRANSF

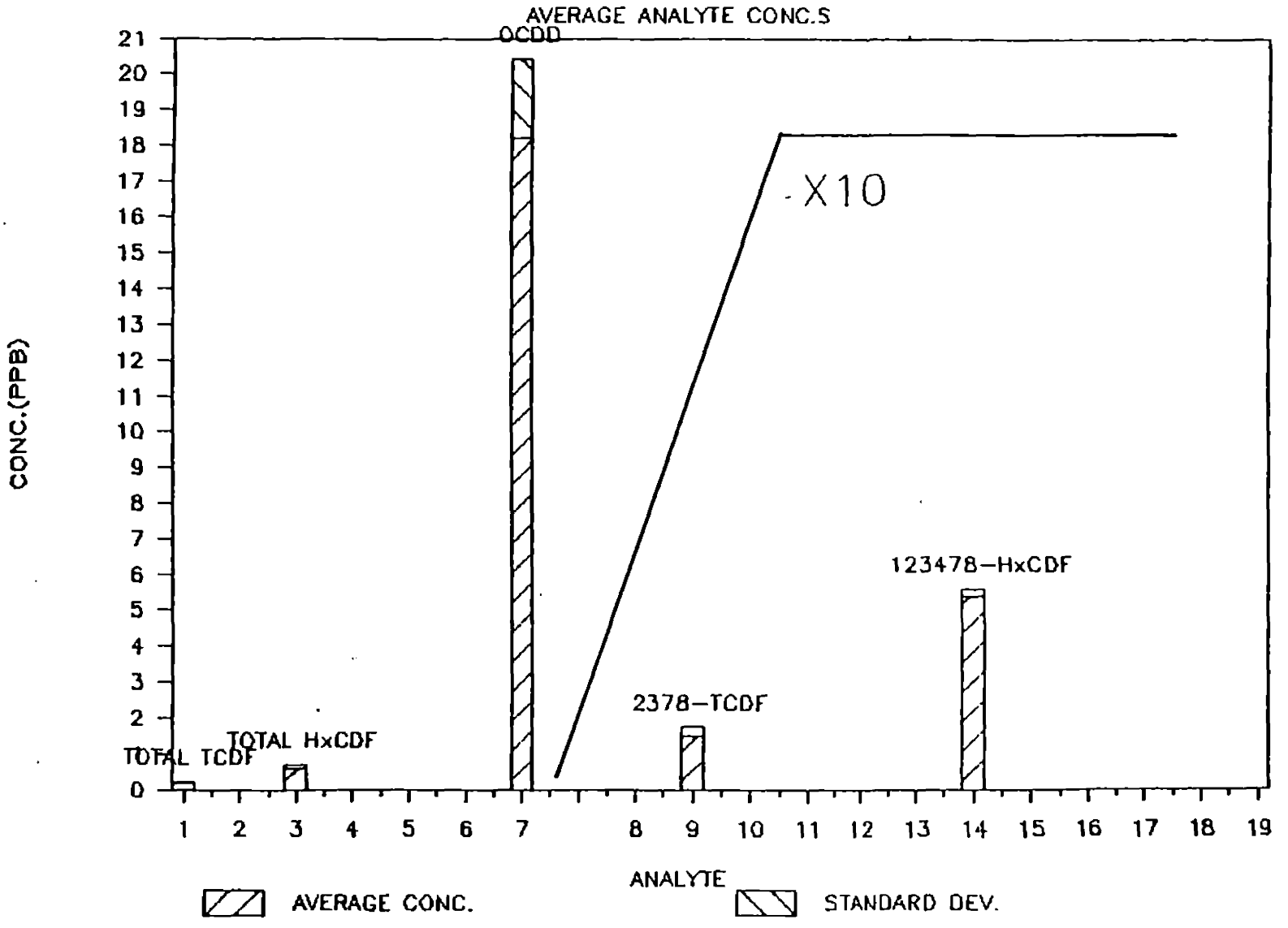
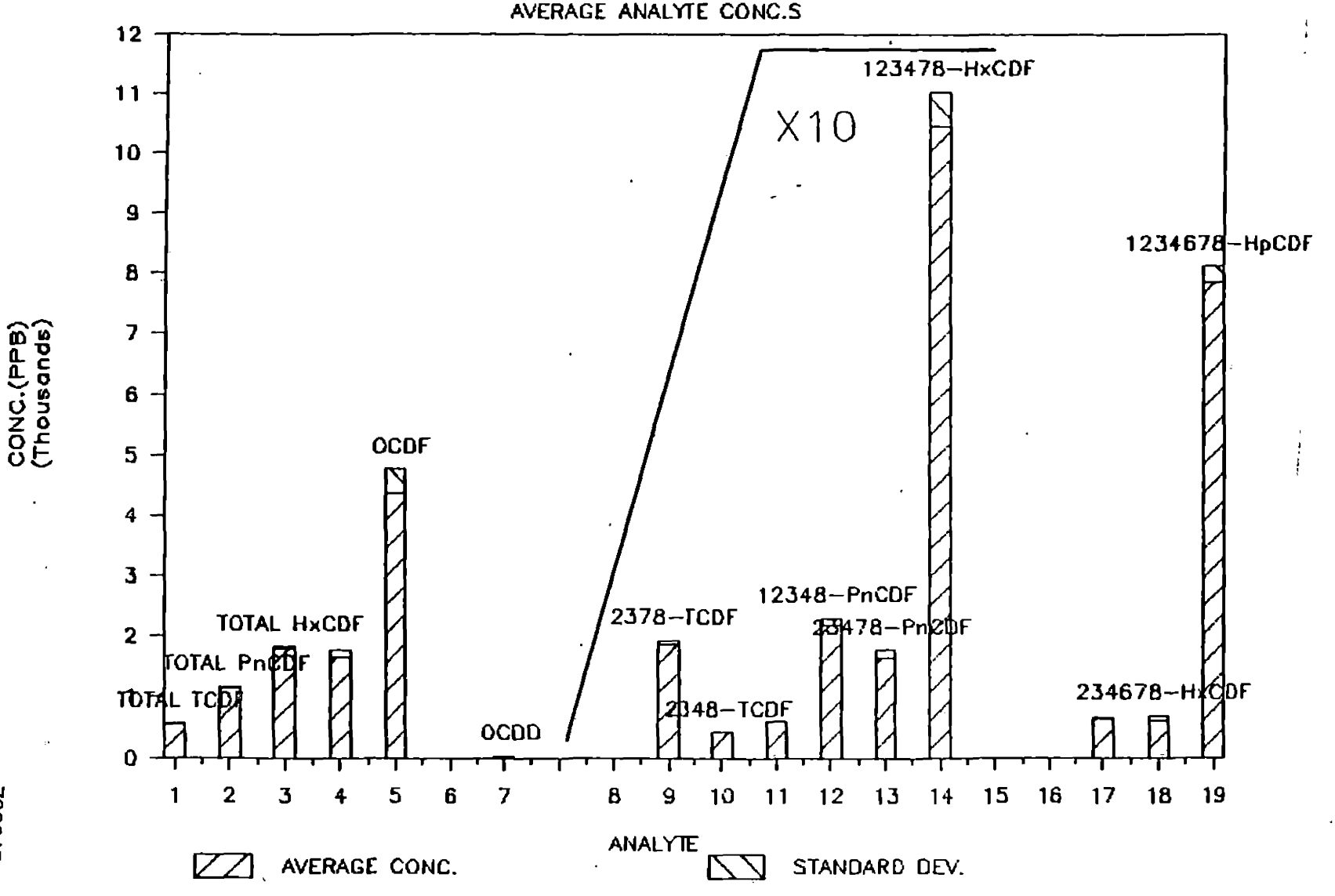


FIG E-7: LOAD CNTR NETWORK TRANSFORMER



GENP 011034

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FIGURE E-8: SPIKED MINERAL OIL

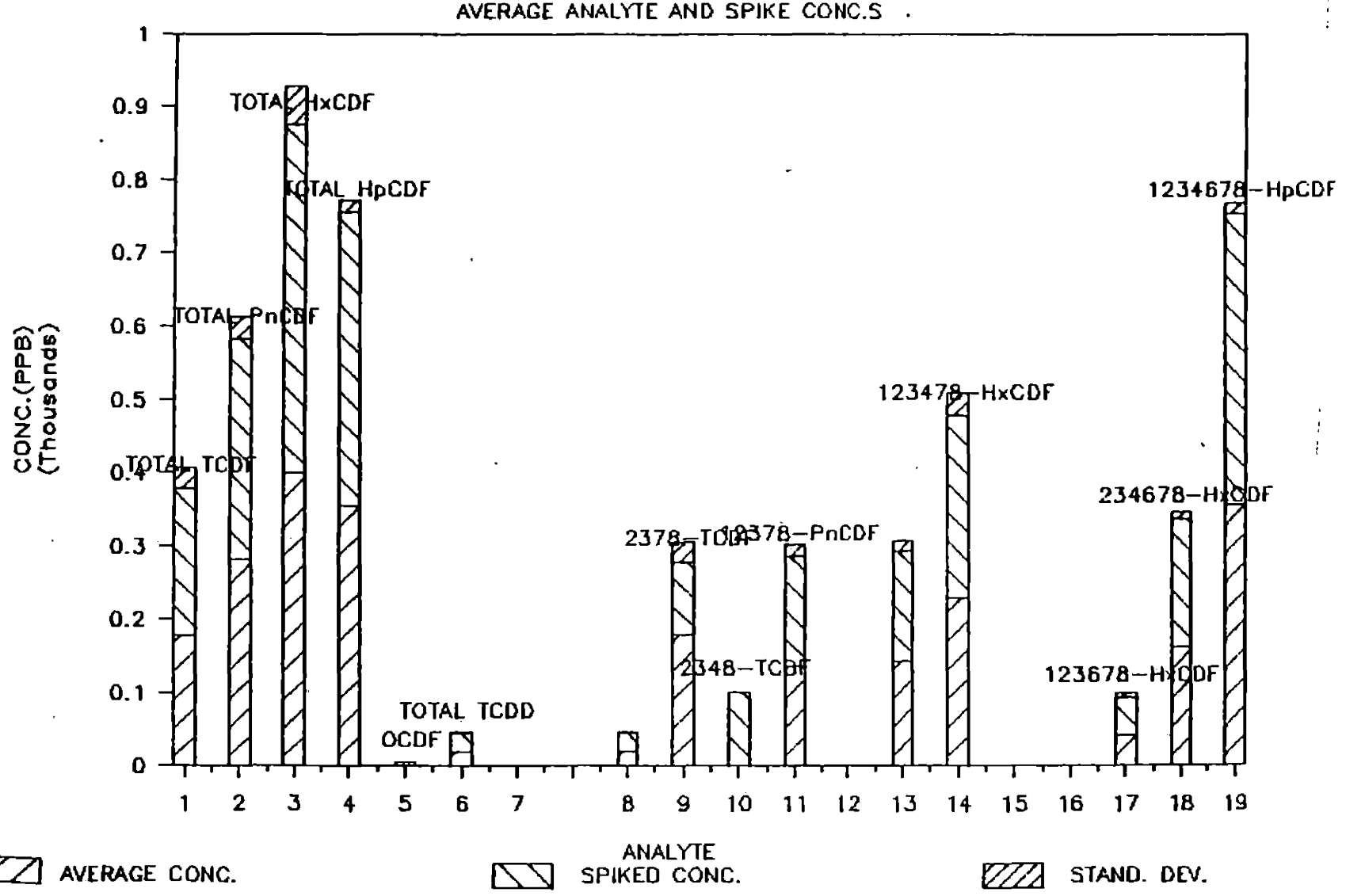
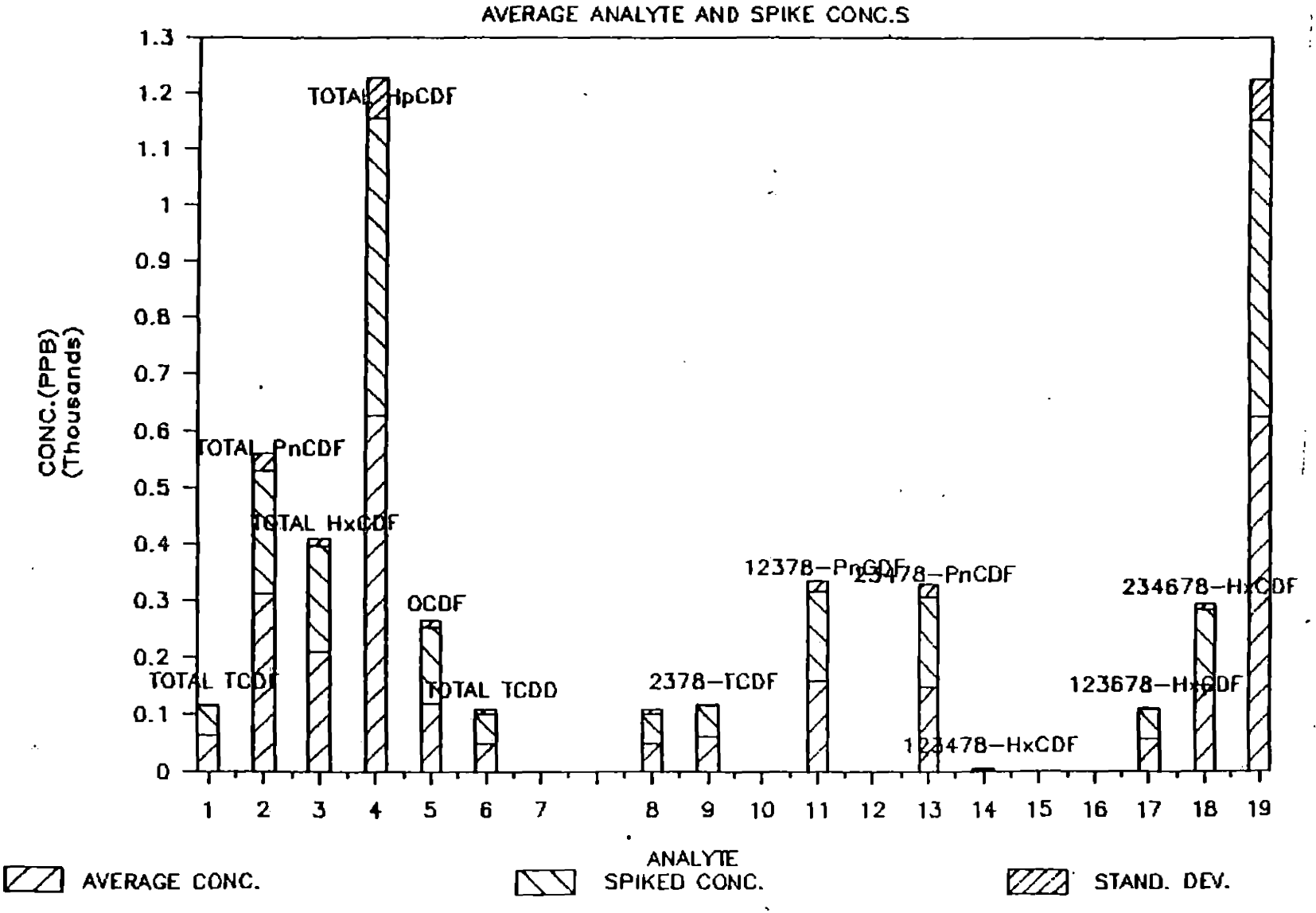


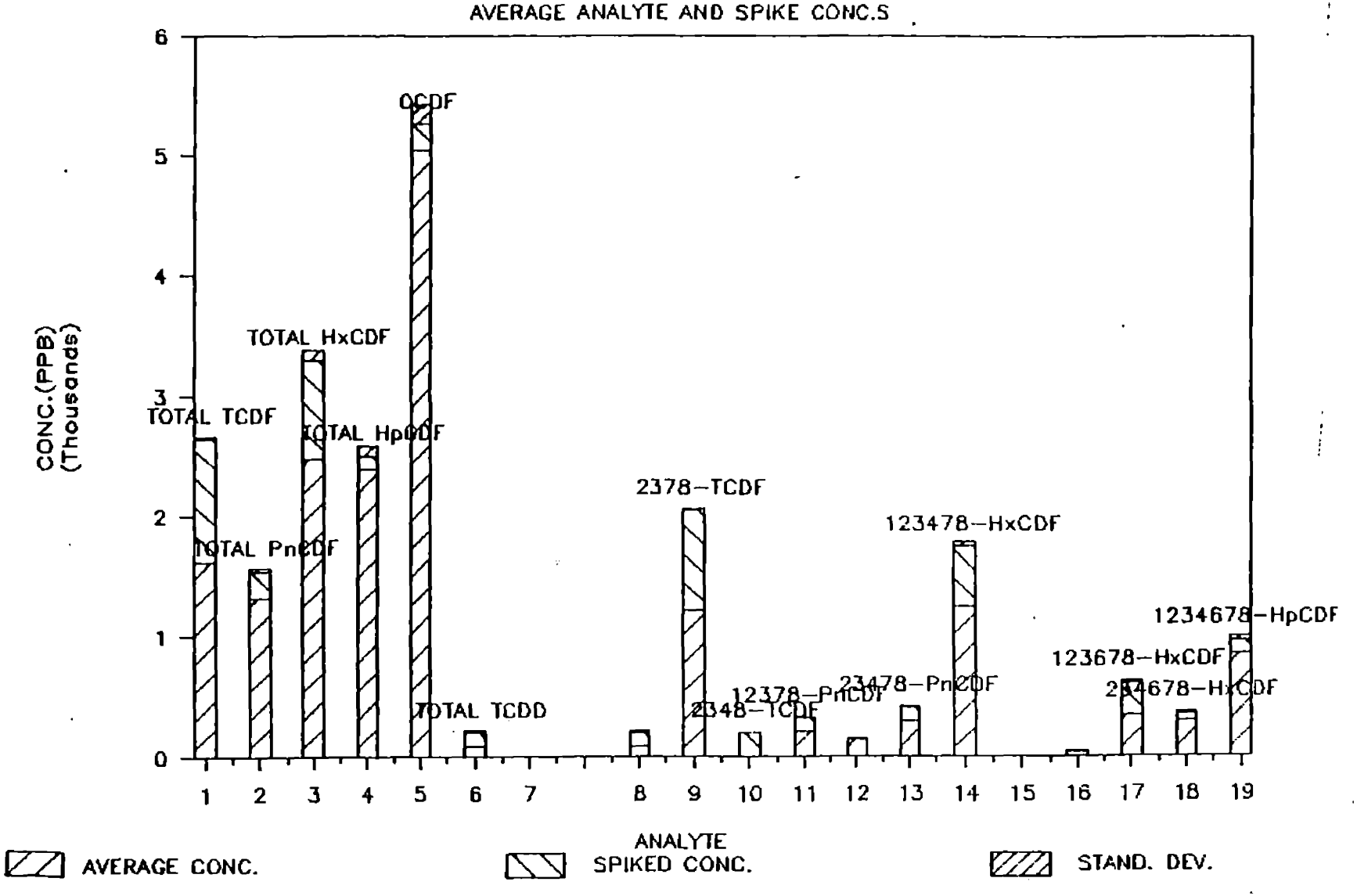
FIGURE E-9: SPIKED AROCLOR 1016



783847

GENP 011036

FIGURE E-10: SPIKED AROCLOR 1260



Appendix F

TABLES OF RECOVERIES, RELATIVE RECOVERIES AND DETECTION LIMITS

TABLE 1

RECOVERIES AND DETECTION LIMITS
EPRI BASELINE PROJECT SAMPLE:
1953 LOAD CENTER NETWORK TRANSFORMER

	DET. 1		DET. 2		DET. 3	
	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)
TOTAL TCDF	43	2.1	16	4.3	3	15.1
TOTAL PnCDF	47	2.1	19	4.0	10	5.2
TOTAL HxCDF	55	2.1	24	3.7	14	4.5
TOTAL HpCDF	55	2.9	24	3.7	14	6.1
OCDF	24	10.2	8	23.5	7	19.0
TOTAL TCDD	43	3.0	16	6.3	1	47.4
OCDD	24	20.3	8	46.9	7	38.1
2,3,7,8-TCDD	43	3.0	16	6.3	1	47.4
2,3,7,8-TCDF	43	2.1	16	4.3	3	15.1
2,3,4,8-TCDF	NA	NA	16	0.1	NA	NA
1,2,3,7,8-PnCDF	47	2.1	19	4.0	10	5.2
1,2,3,4,8-PnCDF	47	2.1	19	4.0	10	5.2
2,3,4,7,8-PnCDF	47	2.2	19	4.2	10	5.5
1,2,3,4,7,8-HxCDF	55	2.1	24	3.7	14	4.5
1,2,3,4,7,9-HxCDF	55	2.1	24	3.7	14	4.5
1,2,3,7,8,9-HxCDF	55	2.1	24	3.7	14	4.5
1,2,3,6,7,8-HxCDF	55	2.1	24	3.7	14	4.5
2,3,4,6,7,8-HxCDF	55	2.5	24	4.4	14	5.3
1,2,3,4,6,7,8-HpCDF	55	2.9	24	5.1	14	6.1

1.) NA = not applicable

783850

GENP 011039

TABLE 2

RECOVERIES AND DETECTION LIMITS
EPRI BASELINE PROJECT SAMPLE:
1946 LOAD CENTER NETWORK TRANSFORMER

	DET. 1		DET. 2		DET. 3	
	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)
TOTAL TCDF	29	1.2	18	2.1	10	2.9
TOTAL PnCDF	28	1.5	17	2.5	27	1.3
TOTAL HxCDF	31	1.5	18	2.7	30	1.3
TOTAL HpCDF	31	2.0	18	3.5	30	1.7
OCDF	22	5.6	7	17.9	18	5.9
TOTAL TCDD	35	1.6	18	3.2	4	19.0
OCDD	22	10.9	7	34.6	18	11.4
2,3,7,8-TCDD	35	1.6	18	3.2	4	19.0
2,3,7,8-TCDF	29	1.2	18	2.1	10	2.9
2,3,4,8-TCDF	NA	NA	18	0.2	NA	NA
1,2,3,7,8-PnCDF	28	1.5	17	2.5	27	1.3
1,2,3,4,8-PnCDF	28	1.5	17	2.5	27	1.3
2,3,4,7,8-PnCDF	28	1.5	17	2.5	27	1.3
1,2,3,4,7,8-HxCDF	31	1.5	18	2.7	30	1.3
1,2,3,4,7,9-HxCDF	31	1.5	18	2.7	30	1.3
1,2,3,7,8,9-HxCDF	31	1.5	18	2.7	30	1.3
1,2,3,6,7,8-HxCDF	31	1.5	18	2.7	30	1.3
2,3,4,6,7,8-HxCDF	31	1.7	18	3.0	30	1.5
1,2,3,4,6,7,8-HpCDF	31	2.0	18	3.5	30	1.7

1.) NA = not applicable

783851

GENP 011040

TABLE 3

RECOVERIES AND DETECTION LIMITS
EPRI BASELINE PROJECT SAMPLE:
CAPACITOR

	DET. 1		DET. 2		DET. 3	
	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)
TOTAL TCDF	35	0.1	17	0.2	2	1.3
TOTAL PnCDF	34	0.2	16	0.2	55	0.1
TOTAL HxCDF	38	0.2	19	0.2	63	0.1
TOTAL HpCDF	38	0.2	19	0.2	63	0.1
OCDF	35	0.7	23	0.3	59	0.1
TOTAL TCDD	61	0.1	18	0.2	5	0.9
OCDD	35	1.4	23	0.6	59	0.3
2,3,7,8-TCDD	61	0.1	18	0.2	5	0.9
2,3,7,8-TCDF	35	0.1	17	0.2	2	1.3
2,3,4,8-TCDF	NA	NA	17	0.3	NA	NA
1,2,3,7,8-PnCDF	34	0.2	16	0.2	55	0.1
1,2,3,4,8-PnCDF	34	0.2	16	0.2	55	0.1
2,3,4,7,8-PnCDF	34	0.2	16	0.2	55	0.1
1,2,3,4,7,8-HxCDF	38	0.2	19	0.2	63	0.1
1,2,3,4,7,9-HxCDF	38	0.2	19	0.2	63	0.1
1,2,3,7,8,9-HxCDF	38	0.2	19	0.2	63	0.1
1,2,3,6,7,8-HxCDF	38	0.2	19	0.2	63	0.1
2,3,4,6,7,8-HxCDF	38	0.2	19	0.2	63	0.1
1,2,3,4,6,7,8-HpCDF	38	0.2	19	0.2	63	0.1

1.) NA = not applicable

783852

GENP 011041

TABLE 4

RECOVERIES AND DETECTION LIMITS
EPRI BASELINE PROJECT SAMPLE: PRECIPITATOR TRANSFORMER

	DET. 1		DET. 2		DET. 3	
	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)
TOTAL TCDF	16	4.2	24	1.5	21	2.2
TOTAL PnCDF	17	4.3	28	1.5	32	1.6
TOTAL HxCDF	19	4.4	29	1.6	34	1.7
TOTAL HpCDF	19	5.7	29	2.1	34	2.3
OCDF	14	15.0	17	7.1	24	6.2
TOTAL TCDD	16	6.5	26	2.3	56	3.3
OCDD	14	30.6	17	14.5	24	12.7
2,3,7,8-TCDD	16	6.5	26	2.3	56	3.3
2,3,7,8-TCDF	16	4.2	24	1.5	21	2.2
2,3,4,8-TCDF	NA	NA	24	0.2	NA	NA
1,2,3,7,8-PnCDF	17	4.3	28	1.5	32	1.6
1,2,3,4,8-PnCDF	17	4.3	28	1.5	32	1.6
2,3,4,7,8-PnCDF	17	4.3	28	1.5	32	1.6
1,2,3,4,7,8-HxCDF	19	4.4	29	1.6	34	1.7
1,2,3,4,7,9-HxCDF	19	4.4	29	1.6	34	1.7
1,2,3,7,8,9-HxCDF	19	4.4	29	1.6	34	1.7
1,2,3,6,7,8-HxCDF	19	4.4	29	1.6	34	1.7
2,3,4,6,7,8-HxCDF	19	4.7	29	1.7	34	1.9
1,2,3,4,6,7,8-HpCDF	19	5.7	29	2.1	34	2.3

1.) NA = not applicable

TABLE 5

RECOVERIES AND DETECTION LIMITS
EPRI BASELINE PROJECT SAMPLE:
1976 ARC FURNACE TRANSFORMER

	DET. 1		DET. 2		DET. 3	
	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)
TOTAL TCDF	67	0.1	37	0.1	78	0.05
TOTAL PnCDF	81	0.1	51	0.1	128	0.03
TOTAL HxCDF	82	0.1	51	0.0	123	0.04
TOTAL HpCDF	82	0.2	51	0.1	123	0.05
OCDF	63	0.5	24	0.4	119	0.11
TOTAL TCDD	74	0.1	45	0.1	0.4	28.12
OCDD	63	1.0	24	0.8	119	0.19
2,3,7,8-TCDD	74	0.1	45	0.1	0.4	28.12
2,3,7,8-TCDF	67	0.1	37	0.1	78	0.05
2,3,4,8-TCDF	NA	NA	37	0.7	NA	NA
1,2,3,7,8-PnCDF	81	0.1	51	0.1	128	0.03
1,2,3,4,8-PnCDF	81	0.1	51	0.1	128	0.03
2,3,4,7,8-PnCDF	81	0.1	51	0.1	128	0.04
1,2,3,4,7,8-HxCDF	82	0.1	51	0.0	123	0.04
1,2,3,4,7,9-HxCDF	82	0.1	51	0.0	123	0.04
1,2,3,7,8,9-HxCDF	82	0.1	51	0.0	123	0.04
1,2,3,6,7,8-HxCDF	82	0.1	51	0.0	123	0.04
2,3,4,6,7,8-HxCDF	82	0.1	51	0.1	123	0.04
1,2,3,4,6,7,8-HpCDF	82	0.2	51	0.1	123	0.05

1.) NA = not applicable

783854

GENP 011043

TABLE 6

RECOVERIES AND DETECTION LIMITS
EPRI BASELINE PROJECT SAMPLE:
SUBSTATION DISTRIBUTION TRANSFORMER

	DET. 1		DET. 2		DET.3	
	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)
TOTAL TCDF	36	0.2	69	0.04	76	0.04
TOTAL PnCDF	48	0.2	80	0.04	66	0.05
TOTAL HxCDF	60	0.1	36	0.09	33	0.11
TOTAL HpCDF	60	0.2	36	0.12	33	0.13
OCDF	66	0.1	4	2.14	7	1.33
TOTAL TCDD	38	0.3	80	0.05	73	0.07
OCDD	66	1.0	4	21.61	7	2.54
2,3,7,8-TCDD	38	0.3	80	0.05	73	0.07
2,3,7,8-TCDF	36	0.2	69	0.04	76	0.04
2,3,4,8-TCDF	NA	NA	69	0.04	NA	NA
1,2,3,7,8-PnCDF	48	0.2	80	0.04	66	0.05
1,2,3,4,8-PnCDF	48	0.2	80	0.04	66	0.05
2,3,4,7,8-PnCDF	48	0.2	80	0.04	66	0.05
1,2,3,4,7,8-HxCDF	60	0.1	36	0.09	33	0.11
1,2,3,4,7,9-HxCDF	60	0.1	36	0.09	33	0.11
1,2,3,7,8,9-HxCDF	60	0.1	36	0.09	33	0.11
1,2,3,6,7,8-HxCDF	60	0.1	36	0.09	33	0.11
2,3,4,6,7,8-HxCDF	60	0.2	36	0.10	33	0.11
1,2,3,4,6,7,8-HpCDF	60	0.2	36	0.12	33	0.13

1.) NA = not applicable

TABLE 7

RECOVERIES AND DETECTION LIMITS
EPRI BASELINE PROJECT SAMPLE:
1962 LOAD CENTER NETWORK TRANSFORMER

	DET. 1		DET. 2		DET. 3	
	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)
TOTAL TCDF	42	2.0	45	1.4	55	1.2
TOTAL PnCDF	51	2.0	47	1.6	62	1.3
TOTAL HxCDF	51	2.3	40	2.2	60	1.5
TOTAL HpCDF	51	3.3	40	3.1	60	2.2
OCDF	30	8.6	29	26.7	26	7.7
TOTAL TCDD	46	2.8	50	2.0	59	1.7
OCDD	30	19.4	29	124.7	26	17.3
2,3,7,8-TCDD	46	2.8	50	2.0	59	1.7
2,3,7,8-TCDF	42	2.0	45	1.4	55	1.2
2,3,4,8-TCDF	NA	NA	45	0.2	NA	NA
1,2,3,7,8-PnCDF	51	2.0	47	1.6	62	1.3
1,2,3,4,8-PnCDF	51	2.0	47	1.6	62	1.3
2,3,4,7,8-PnCDF	51	1.8	47	1.5	62	1.1
1,2,3,4,7,8-HxCDF	51	2.3	40	2.2	60	1.5
1,2,3,4,7,9-HxCDF	51	2.3	40	2.2	60	1.5
1,2,3,7,8,9-HxCDF	51	2.3	40	2.2	60	1.5
1,2,3,6,7,8-HxCDF	51	2.3	40	2.2	60	1.5
2,3,4,6,7,8-HxCDF	51	2.6	40	2.5	60	1.7
1,2,3,4,6,7,8-HpCDF	51	3.3	40	3.1	60	2.2

1.) NA = not applicable

783856

GENP 011045

TABLE 8

RECOVERIES AND DETECTION LIMITS
EPRI BASELINE PROJECT SAMPLE:
SPIKED AGED MINERAL OIL

	DET. 1		DET. 2		DET. 3	
	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)
TOTAL TCDF	50	0.5	45	2.7	39	0.1
TOTAL PnCDF	55	0.6	44	3.2	43	0.1
TOTAL HxCDF	55	0.7	43	3.7	40	0.1
TOTAL HpCDF	55	1.1	43	4.8	40	0.1
OCDF	49	3.6	33	13.1	27	0.5
TOTAL TCDD	54	0.8	47	4.0	40	0.1
OCDD	49	6.3	33	123.6	27	0.9
2,3,7,8-TCDD	54	0.8	47	4.0	40	0.1
2,3,7,8-TCDF	50	0.5	45	2.7	39	0.1
2,3,4,8-TCDF	NA	NA	45	0.2	NA	NA
1,2,3,7,8-PnCDF	55	0.6	44	3.2	43	0.1
1,2,3,4,8-PnCDF	55	0.6	44	3.2	43	0.1
2,3,4,7,8-PnCDF	55	0.6	44	3.2	43	0.1
1,2,3,4,7,8-HxCDF	55	0.7	43	3.7	40	0.1
1,2,3,4,7,9-HxCDF	55	0.7	43	3.7	40	0.1
1,2,3,7,8,9-HxCDF	55	0.7	43	3.7	40	0.1
1,2,3,6,7,8-HxCDF	55	0.7	43	3.7	40	0.1
2,3,4,6,7,8-HxCDF	55	0.8	43	4.1	40	0.1
1,2,3,4,6,7,8-HpCDF	55	1.1	43	4.8	40	0.1

1.) NA = not applicable

TABLE 9

RECOVERIES AND DETECTION LIMITS
EPRI BASELINE PROJECT SAMPLE:
SPIKED AROCLOR 1016

	DET. 1		DET. 2		DET. 3	
	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)
TOTAL TCDF	52	0.3	32	2.2	30	0.9
TOTAL PnCDF	68	0.2	37	2.0	44	0.8
TOTAL HxCDF	77	0.2	36	2.4	42	1.0
TOTAL HpCDF	77	0.4	36	4.0	42	1.4
OCDF	103	0.5	37	7.0	40	2.2
TOTAL TCDD	57	0.4	34	3.1	42	1.1
OCDD	103	1.4	37	100.7	40	5.0
2,3,7,8-TCDD	57	0.4	34	3.1	42	1.1
2,3,7,8-TCDF	52	0.3	32	2.2	30	0.9
2,3,4,8-TCDF	NA	NA	32	2.3	NA	NA
1,2,3,7,8-PnCDF	68	0.2	37	2.0	44	0.8
1,2,3,4,8-PnCDF	68	0.2	37	2.0	44	0.8
2,3,4,7,8-PnCDF	68	0.2	37	2.1	44	0.7
1,2,3,4,7,8-HxCDF	77	0.2	36	2.4	42	1.0
1,2,3,4,7,9-HxCDF	77	0.2	36	2.4	42	1.0
1,2,3,7,8,9-HxCDF	77	0.2	36	2.4	42	1.0
1,2,3,6,7,8-HxCDF	77	0.2	36	2.4	42	1.0
2,3,4,6,7,8-HxCDF	77	0.3	36	3.2	42	1.1
1,2,3,4,6,7,8-HpCDF	77	0.4	36	4.0	42	1.4

1.) NA = not applicable

783858

GENP 011047

TABLE 10

RECOVERIES AND DETECTION LIMITS
EPRI BASELINE PROJECT SAMPLE:
SPIKED AROCLOR 1260

	DET. 1		DET. 2		DET. 3	
	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)	%REC	DET LIM. (in ppb)
TOTAL TCDF	52	0.5	75	2.4	63	4.6
TOTAL PnCDF	58	0.6	80	2.7	60	6.0
TOTAL HxCDF	64	0.6	91	2.8	61	6.8
TOTAL HpCDF	64	0.8	91	3.8	61	9.4
OCDF	57	1.8	72	9.4	45	24.8
TOTAL TCDD	161	0.3	112	2.5	98	4.8
OCDD	57	3.1	72	74.6	45	42.3
2,3,7,8-TCDD	161	0.3	112	2.5	98	4.8
2,3,7,8-TCDF	52	0.5	75	2.4	63	4.6
2,3,4,8-TCDF	NA	NA	75	1.0	NA	NA
1,2,3,7,8-PnCDF	58	0.6	80	2.7	60	6.0
1,2,3,4,8-PnCDF	58	0.6	80	2.7	60	6.0
2,3,4,7,8-PnCDF	58	0.6	80	2.7	60	5.8
1,2,3,4,7,8-HxCDF	64	0.6	91	2.8	61	6.8
1,2,3,4,7,9-HxCDF	64	0.6	91	2.8	61	6.8
1,2,3,7,8,9-HxCDF	64	0.6	91	2.8	61	6.8
1,2,3,6,7,8-HxCDF	64	0.6	91	2.8	61	6.8
2,3,4,6,7,8-HxCDF	64	0.6	91	2.9	61	7.1
1,2,3,4,6,7,8-HpCDF	64	0.8	91	3.8	61	9.4

1.) NA = not applicable

TABLE 11

RECOVERIES RELATIVE TO 2378-TCDD-13C12
EPRI BASELINE PROJECT SAMPLE:
1953 LOAD CENTER NETWORK TRANSFORMER

	DET. 1		DET. 2		DET. 3	
	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD
TOTAL TCDF	43	100	16	100	3	300
TOTAL PnCDF	47	109	19	119	10	1000
TOTAL HxCDF	55	128	24	150	14	1400
TOTAL HpCDF	55	128	24	150	14	1400
OCDF	24	56	8	50	7	700
TOTAL TCDD	43	100	16	100	1	100
OCDD	24	56	8	50	7	700
2,3,7,8-TCDD	43	100	16	100	1	100
2,3,7,8-TCDF	43	100	16	100	3	300
2,3,4,8-TCDF	NA	NA	16	100	NA	NA
1,2,3,7,8-PnCDF	47	109	19	119	10	1000
1,2,3,4,8-PnCDF	47	109	19	119	10	1000
2,3,4,7,8-PnCDF	47	109	19	119	10	1000
1,2,3,4,7,8-HxCDF	55	128	24	150	14	1400
1,2,3,4,7,9-HxCDF	55	128	24	150	14	1400
1,2,3,7,8,9-HxCDF	55	128	24	150	14	1400
1,2,3,6,7,8-HxCDF	55	128	24	150	14	1400
2,3,4,6,7,8-HxCDF	55	128	24	150	14	1400
1,2,3,4,6,7,8-HpCDF	55	128	24	150	14	1400

1.) NA = not applicable

783860

GENP 011049

TABLE 12

RECOVERIES RELATIVE TO 2378-TCDD-13C12
EPRI BASELINE PROJECT SAMPLE:
1946 LOAD CENTER NETWORK TRANSFORMER

	DET. 1		DET. 2		DET. 3	
	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD
TOTAL TCDF	29	83	18	100	10	1000
TOTAL PnCDF	28	80	17	94	27	2700
TOTAL HxCDF	31	89	18	100	30	3000
TOTAL HpCDF	31	89	18	100	30	3000
OCDF	22	63	7	39	18	1800
TOTAL TCDD	35	100	18	100	1	100
OCDD	22	63	7	39	18	1800
2,3,7,8-TCDD	35	100	18	100	1	100
2,3,7,8-TCDF	29	83	18	100	10	1000
2,3,4,8-TCDF	NA	NA	18	100	NA	NA
1,2,3,7,8-PnCDF	28	80	17	94	27	2700
1,2,3,4,8-PnCDF	28	80	17	94	27	2700
2,3,4,7,8-PnCDF	28	80	17	94	27	2700
1,2,3,4,7,8-HxCDF	31	89	18	100	30	3000
1,2,3,4,7,9-HxCDF	31	89	18	100	30	3000
1,2,3,7,8,9-HxCDF	31	89	18	100	30	3000
1,2,3,6,7,8-HxCDF	31	89	18	100	30	3000
2,3,4,6,7,8-HxCDF	31	89	18	100	30	3000
1,2,3,4,6,7,8-HpCDF	31	89	18	100	30	3000

1.) NA = not applicable

TABLE 13

RECOVERIES RELATIVE TO 2378-TCDD-13C12
EPRI BASELINE PROJECT SAMPLE:
CAPACITOR

	DET. 1		DET. 2		DET. 3	
	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD
TOTAL TCDF	35	57	17	94	2	40
TOTAL PnCDF	34	56	16	89	55	1100
TOTAL HxCDF	38	62	19	106	63	1260
TOTAL HpCDF	38	62	19	106	63	1260
OCDF	35	57	23	128	59	1180
TOTAL TCDD	61	100	18	100	5	100
OCDD	35	57	23	128	59	1180
2,3,7,8-TCDD	61	100	18	100	5	100
2,3,7,8-TCDF	35	57	17	94	2	40
2,3,4,8-TCDF	NA	NA	17	94	NA	NA
1,2,3,7,8-PnCDF	34	56	16	89	55	1100
1,2,3,4,8-PnCDF	34	56	16	89	55	1100
2,3,4,7,8-PnCDF	34	56	16	89	55	1100
1,2,3,4,7,8-HxCDF	38	62	19	106	63	1260
1,2,3,4,7,9-HxCDF	38	62	19	106	63	1260
1,2,3,7,8,9-HxCDF	38	62	19	106	63	1260
1,2,3,6,7,8-HxCDF	38	62	19	106	63	1260
2,3,4,6,7,8-HxCDF	38	62	19	106	63	1260
1,2,3,4,6,7,8-HpCDF	38	62	19	106	63	1260

1.) NA = not applicable

783862

GENP 011051

TABLE 14

RECOVERIES RELATIVE TO 2378-TCDD-13C12
EPRI BASELINE PROJECT SAMPLE:
PRECIPITATOR TRANSFORMER

	DET. 1		DET. 2		DET. 3	
	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD
TOTAL TCDF	16	100	24	92	21	38
TOTAL PnCDF	17	106	28	108	32	57
TOTAL HxCDF	19	119	29	112	34	61
TOTAL HpCDF	19	119	29	112	34	61
OCDF	14	88	17	65	24	43
TOTAL TCDD	16	100	26	100	56	100
OCDD	14	88	17	65	24	4
2,3,7,8-TCDD	16	100	26	100	56	100
2,3,7,8-TCDF	16	100	24	92	21	38
2,3,4,8-TCDF	NA	NA	24	92	NA	NA
1,2,3,7,8-PnCDF	17	106	28	108	32	57
1,2,3,4,8-PnCDF	17	106	28	108	32	57
2,3,4,7,8-PnCDF	17	106	28	108	32	57
1,2,3,4,7,8-HxCDF	19	119	29	112	34	61
1,2,3,4,7,9-HxCDF	19	119	29	112	34	61
1,2,3,7,8,9-HxCDF	19	119	29	112	34	61
1,2,3,6,7,8-HxCDF	19	119	29	112	34	61
2,3,4,6,7,8-HxCDF	19	119	29	112	34	61
1,2,3,4,6,7,8-HpCDF	19	119	29	112	34	61

1.) NA = not applicable

TABLE 15

RECOVERIES RELATIVE TO 2378-TCDD-13C12
EPRI BASELINE PROJECT SAMPLE:
1976 ARC FURNACE TRANSFORMER

	DET. 1		DET. 2		DET. 3	
	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD
TOTAL TCDF	67	91	37	82	78	19500
TOTAL PnCDF	81	109	51	113	128	32000
TOTAL HxCDF	82	111	51	113	123	30750
TOTAL HpCDF	82	111	51	113	123	30750
OCDF	63	85	24	53	119	29750
TOTAL TCDD	74	100	45	100	0.4	100
OCDD	63	85	24	53	119	29750
2,3,7,8-TCDD	74	100	45	100	0.4	100
2,3,7,8-TCDF	67	91	37	82	78	19500
2,3,4,8-TCDF	NA	NA	37	82	NA	NA
1,2,3,7,8-PnCDF	81	109	51	113	128	32000
1,2,3,4,8-PnCDF	81	109	51	113	128	32000
2,3,4,7,8-PnCDF	81	109	51	113	128	32000
1,2,3,4,7,8-HxCDF	82	111	51	113	123	30750
1,2,3,4,7,9-HxCDF	82	111	51	113	123	30750
1,2,3,7,8,9-HxCDF	82	111	51	113	123	30750
1,2,3,6,7,8-HxCDF	82	111	51	113	123	30750
2,3,4,6,7,8-HxCDF	82	111	51	113	123	30750
1,2,3,4,6,7,8-HpCDF	82	111	51	113	123	30750

1.) NA = not applicable

GENP 011053

783864

TABLE 16

RECOVERIES RELATIVE TO 2378-TCDD-13C12
EPRI BASELINE PROJECT SAMPLE:
SUBSTATION DISTRIBUTION TRANSFORMER

	DET. 1		DET. 2		DET. 3	
	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD
TOTAL TCDF	36	95	69	86	76	104
TOTAL PnCDF	48	126	80	100	66	90
TOTAL HxCDF	60	158	36	45	33	45
TOTAL HpCDF	60	158	36	45	33	45
OCDF	66	174	4	5	7	10
TOTAL TCDD	38	100	80	100	73	100
OCDD	66	174	4	5	7	10
2,3,7,8-TCDD	38	100	80	100	73	100
2,3,7,8-TCDF	36	95	69	86	76	104
2,3,4,8-TCDF	NA	NA	69	86	NA	NA
1,2,3,7,8-PnCDF	48	126	80	100	66	90
1,2,3,4,8-PnCDF	48	126	80	100	66	90
2,3,4,7,8-PnCDF	48	126	80	100	66	90
1,2,3,4,7,8-HxCDF	60	158	36	45	33	45
1,2,3,4,7,9-HxCDF	60	158	36	45	33	45
1,2,3,7,8,9-HxCDF	60	158	36	45	33	45
1,2,3,6,7,8-HxCDF	60	158	36	45	33	45
2,3,4,6,7,8-HxCDF	60	158	36	45	33	45
1,2,3,4,6,7,8-HpCDF	60	158	36	45	33	45

1.) NA = not applicable

GENP 011054

783865

TABLE 17

RECOVERIES RELATIVE TO 2378-TCDD-13C12
EPRI BASELINE PROJECT SAMPLE:
1962 LOAD CENTER NETWORK TRANSFORMER

	DET. 1		DET. 2		DET. 3	
	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD
TOTAL TCDF	42	91	45	90	55	93
TOTAL PnCDF	51	111	47	94	62	105
TOTAL HxCDF	51	111	40	80	60	102
TOTAL HpCDF	51	111	40	80	60	102
OCDF	30	65	29	59	26	44
TOTAL TCDD	46	100	50	100	59	100
OCDD	30	65	29	59	26	44
2,3,7,8-TCDD	46	100	50	100	59	100
2,3,7,8-TCDF	42	91	45	90	55	93
2,3,4,8-TCDF	NA	NA	45	90	NA	NA
1,2,3,7,8-PnCDF	51	111	47	94	62	105
1,2,3,4,8-PnCDF	51	111	47	94	62	105
2,3,4,7,8-PnCDF	51	111	47	94	62	105
1,2,3,4,7,8-HxCDF	51	111	40	80	60	102
1,2,3,4,7,9-HxCDF	51	111	40	80	60	102
1,2,3,7,8,9-HxCDF	51	111	40	80	60	102
1,2,3,6,7,8-HxCDF	51	111	40	80	60	102
2,3,4,6,7,8-HxCDF	51	111	40	80	60	102
1,2,3,4,6,7,8-HpCDF	51	111	40	80	60	102

1.) NA = not applicable

783866

GENP 011055

TABLE 18

RECOVERIES RELATIVE TO 2378-TCDD-13C12
EPRI BASELINE PROJECT SAMPLE:
SPIKED AGED MINERAL OIL

	DET. 1		DET. 2		DET. 3	
	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD
TOTAL TCDF	50	93	45	96	39	98
TOTAL PnCDF	55	102	44	94	43	108
TOTAL HxCDF	55	102	43	91	40	100
TOTAL HpCDF	55	102	43	91	40	100
OCDF	49	91	33	70	27	68
TOTAL TCDD	54	100	47	100	40	100
OCDD	49	91	33	70	27	68
2,3,7,8-TCDD	54	100	47	100	40	100
2,3,7,8-TCDF	50	93	45	96	39	98
2,3,4,8-TCDF	NA	NA	45	96	NA	NA
1,2,3,7,8-PnCDF	55	102	44	94	43	108
1,2,3,4,8-PnCDF	55	102	44	94	43	108
2,3,4,7,8-PnCDF	55	102	44	94	43	108
1,2,3,4,7,8-HxCDF	55	102	43	91	40	100
1,2,3,4,7,9-HxCDF	55	102	43	91	40	100
1,2,3,7,8,9-HxCDF	55	102	43	91	40	100
1,2,3,6,7,8-HxCDF	55	102	43	91	40	100
2,3,4,6,7,8-HxCDF	55	102	43	91	40	100
1,2,3,4,6,7,8-HpCDF	55	102	43	91	40	100

1.) NA = not applicable

TABLE 19

RECOVERIES RELATIVE TO 2378-TCDD-13C12
EPRI BASELINE PROJECT SAMPLE:
SPIKED AROCLOR 1016

	DET. 1		DET. 2		DET. 3	
	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD
TOTAL TCDF	52	91	32	94	30	71
TOTAL PnCDF	68	119	37	109	44	105
TOTAL HxCDF	77	135	36	106	42	100
TOTAL HpCDF	77	135	36	106	42	100
OCDF	103	181	37	109	40	95
TOTAL TCDD	57	100	34	100	42	100
OCDD	103	181	37	109	40	95
2,3,7,8-TCDD	57	100	34	100	42	100
2,3,7.8-TCDF	52	91	32	94	30	71
2,3,4,8-TCDF	NA	NA	32	94	NA	NA
1,2,3,7,8-PnCDF	68	119	37	109	44	105
1,2,3,4,8-PnCDF	68	119	37	109	44	105
2,3,4,7,8-PnCDF	68	119	37	109	44	105
1,2,3,4,7,8-HxCDF	77	135	36	106	42	100
1,2,3,4,7,9-HxCDF	77	135	36	106	42	100
1,2,3,7,8,9-HxCDF	77	135	36	106	42	100
1,2,3,6,7.8-HxCDF	77	135	36	106	42	100
2,3,4,6,7,8-HxCDF	77	135	36	106	42	100
1,2,3,4,6,7,8-HpCDF	77	135	36	106	42	100

1.) NA = not applicable

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TABLE 20

RECOVERIES RELATIVE TO 2378-TCDD-13C12
EPRI BASELINE PROJECT SAMPLE:
SPIKED AROCLOR 1260

	DET. 1		DET. 2		DET. 3	
	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD	%REC	%REC vs 13C-TCDD
TOTAL TCDF	52	32	75	67	63	64
TOTAL PnCDF	58	36	80	71	60	61
TOTAL HxCDF	64	40	91	81	61	62
TOTAL HpCDF	64	40	91	81	61	62
OCDF	57	35	72	64	45	46
TOTAL TCDD	161	100	112	100	98	100
OCDD	57	35	72	64	45	46
2,3,7,8-TCDD	161	100	112	100	98	100
2,3,7,8-TCDF	52	32	75	67	63	64
2,3,4,8-TCDF	NA	NA	75	67	NA	NA
1,2,3,7,8-PnCDF	58	36	80	71	60	61
1,2,3,4,8-PnCDF	58	36	80	71	60	61
2,3,4,7,8-PnCDF	58	36	80	71	60	61
1,2,3,4,7,8-HxCDF	64	40	91	81	61	62
1,2,3,4,7,9-HxCDF	64	40	91	81	61	62
1,2,3,7,8,9-HxCDF	64	40	91	81	61	62
1,2,3,6,7,8-HxCDF	64	40	91	81	61	62
2,3,4,6,7,8-HxCDF	64	40	91	81	61	62
1,2,3,4,6,7,8-HpCDF	64	40	91	81	61	62

1.) NA = not applicable

EPRI 443

REPORT SUMMARY

SUBJECTS: Hazardous and toxic waste management / Transmission substation design and operation / Distribution substations

TOPICS: PCB; Capacitors; Chemical analysis; PCDF–PCDD; Transformers; Insulating oil

AUDIENCE: Environmental managers / Distribution engineers

Analysis of Polychlorinated Dibenzofurans and Polychlorinated Dibenzo-*p*-Dioxins in Transformers and Capacitors

Volumes 1–3

PCDF and PCDD—by-products of PCB pyrolysis and combustion—apparently cause many of the health effects originally attributed to PCB. In parallel and round-robin studies, independent laboratories found that normal equipment operation and arcing failure cause little if any oxidation and do not significantly modify PCB fluids.

BACKGROUND: Partial oxidation of askarels—mixtures of polychlorinated biphenyls (PCB) and tri- and tetrachlorobenzenes—produces polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-*p*-dioxins (PCDD). However, analytic techniques for separating the active components in these mixtures had not matured prior to this study. Information had also been insufficient on the quantity and types of PCDF and PCDD in as-manufactured PCB and on the formation of these by-products during normal operation or arcing failure in utility equipment. Because of these uncertainties, utilities and regulators have assumed the worst-case PCDF–PCDD formation when there is a PCB-related accident, thereby overstating the potential toxicity. EPRI report EL/EA-4858 describes portions of the research performed in these projects.

OBJECTIVE: To improve techniques for measuring PCDF/PCDD in the presence of PCB.

APPROACH: Five laboratories (including two whose studies are discussed in report EL/EA-4858) performed round-robin gas chromatography–mass spectrometry analyses of PCDF–PCDD samples using specially prepared carbon-13 (^{13}C) PCDF spiking compounds. After the first set of analyses, each laboratory modified its techniques and analyzed a larger group of samples from utility equipment. Another organization statistically analyzed all the laboratory results.

RESULTS: These three volumes report the second group of PCDF–PCDD analyses. Results from all participating laboratories correlated remarkably well. Some of the results follow.

GENP 011059

- Use of the ^{13}C compounds allowed successful separation of the components in several closely eluting pairs of physiologically active PCDF and less-active compounds.
- Analyses of samples from utility equipment suggested that the PCDF–PCDD present did not result from high-load, high-temperature operation.

Volumes 1 and 2 describe several analytic techniques, with the appendix of Volume 2 including a report on the analysis by the University of Umeå in Sweden, as well. Volume 3 summarizes all the laboratory techniques, presents comprehensive statistical analyses, and provides an executive summary.

EPRI PERSPECTIVE

This research is a landmark in PCDF–PCDD analysis. One important result is the synthesis of new PCDFs for use as spiking compounds in gas chromatography–mass spectrometry analysis. The ability to separate many of the active PCDF compounds from more innocuous materials is also of great value. The high correlation of results from several laboratories indicates that researchers can exercise great flexibility in selecting techniques and developing facilities.

PROJECTS

RP2028-7, RP2028-6, RP2028-10
EPRI Project Managers: Gil Addis; Jacques Guertin
Electrical Systems Division; Environment Division
Contractors: New York State Department of Health; Battelle Columbus Laboratories; Research Triangle Institute

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GENP 011060

Analysis of Polychlorinated Dibenzofurans and Polychlorinated Dibenzo-*p*-Dioxins in Transformers and Capacitors

Volume 2: Formation of PCDF and PCDD in Askarel and Contaminated Mineral Oil Equipment

EL/EA-5443, Volume 2
Research Project 2028-6

Final Report, March 1988

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ABSTRACT

A study was conducted by five independent laboratories to evaluate methods for the measurement of trace levels of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-p-dioxins (PCDDs) in utility dielectric liquids. Each laboratory evaluated a different analytical method. The method evaluated by Battelle involved spiking with labelled internal standards, analyte enrichment by column chromatography, and analysis by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS).

Baseline analyses of five dielectric fluids were conducted by each laboratory as an initial test of their methods. The variability of results between the five methods was within expectations, and the five methods were deemed suitable for round robin evaluation.

In the round robin evaluation, the five methods were used to analyze 10 liquid samples, including seven utility dielectric fluids. The liquid samples were spiked with native PCDD/PCDF to estimate accuracy. The Battelle results were generally within 70 percent of the spike value except for HxCDF which exhibited a strong positive bias. This result was considered an outlier. The Battelle results were consistently lower than the average results obtained for the five laboratories. Overall, the methods proved to be both rugged and reliable when used with labelled quantification and recovery standards.

The in-service liquids used in this study were found to have relatively low levels of PCDDs and trace amounts of PCDF. Liquids from transformers with more than 25 years of service had higher PCDF levels than liquids from newer transformers. Liquids from utility appliances exposed to thermal and electrical stresses did not have elevated PCDD or PCDF levels. PCDF levels increased as the Aroclor number (and relative amount of chlorination) increased.

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The authors are grateful for the technical assistance provided by Battelle scientists who developed the methods, and performed the analyses reported in this document. Sample preparation was coordinated by Joe Hatchel, data computation was conducted by Susan Watson, and mass spectral analyses were performed by Dan Aichele, Charles Sueper, and Michael Zimmerman.

We would also like to thank our colleagues who contributed the parallel analyses to this interlaboratory collaborative study. They are Dr. Sydney Gordon (Illinois Institute of Technology Research Institute), Drs. George Eadon and David Hilker (New York State Department of Health), Ms. Maureen Kilpatrick (Radian Corporation), Dr. Edo Pellizzari (Research Triangle Institute), and Dr. Christoffer Rappe (University of Umea).

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SUMMARY

SYNOPSIS

This report describes a study to develop reliable methods for the chemical analysis of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) in utility dielectric liquids. Prior to this study a great deal of data was available on levels of PCDD and PCDF in utility fluids; but since the methods used to obtain this data had never been rigorously challenged in a statistically valid study, the reliability of the existing data bases was suspect. Current analytical methods are affected by high levels of polychlorinated biphenyl (PCB). Since PCB is an interferent in most methods proposed for the analysis of PCDD/PCDF, and many existing utility test appliances contain PCB as the major dielectric fluid or as a contaminant of other fluids (due to residual PCB), investigation of reliable analytical methods was warranted.

Five independent laboratories participated in this program. Each laboratory employed a different analytical procedure to measure trace levels of PCDF and PCDD in dielectric liquids. The method used by Battelle involved spiking with labelled internal standards, analyte enrichment by multiple column chromatography, and analysis by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS).

BASELINE ANALYSES

The first phase of this study, baseline analyses, involved comparing methods for analyzing difficult matrices; e.g., Aroclor. Baseline analyses of five dielectric fluids were conducted by each laboratory as an initial test of their methods. The variability of results among the five methods was within expected levels. The five analytical procedures proved to be rugged and reliable when used with isotopically labelled, quantification and recovery standards. Use of labelled standards is especially important in the Askarel and mineral oil matrices since the high organic burden found in these materials requires secondary partitioning to remove interfering PCB and background hydrocarbons.

The relative average deviation is the absolute difference between individual results and the mean (or true value), divided by the number of determinations. For samples such as Aroclor 1242, Aroclor 1016, and mineral oil, the average deviation for baseline samples was often less than 50 percent. This result was found in individual laboratory replicate analyses and in grouped data from different laboratories. All five methods were deemed suitable for use in the second phase of the program.

ROUND ROBIN STATISTICAL ANALYSIS

The second phase of the study involved a more severe test, a round robin statistical evaluation that employed field samples collected from in-service utility applications. In the round robin evaluation, the five independent laboratories used different methods to analyze 10 liquid samples, including seven utility dielectric fluids. The in-service dielectric liquid samples were collected from four Askarel-filled network transformers, one arc furnace transformer, one electrostatic precipitator (ESP) transformer, and one capacitor.

The Battelle results were generally within 70 percent of the spike value except for hexachlorodibenzofurans, which exhibited a strong positive bias. This result was considered an outlier. The Battelle results were consistently lower than the average of results obtained for data from all five laboratories.

All samples of the in-service liquids tested had relatively low amounts of PCDD. This result supports the hypothesis that dioxins are not a major constituent of Askarel and PCB-containing fluids. Except for the mineral oils, trace amounts of polychlorinated dibenzofurans were found in all of the Aroclor samples tested. The mineral oil samples had extremely low amounts, if any, of PCDF. Even the in-service liquid from Transformer 4, a network distribution transformer that was involved in a high-energy excursion, had minimal amounts of PCDD and PCDF. Transformer 4 had visible carbon, and major signs of scorching on the case and in the area of the electrodes.

The Askarel transformers with more than 25 years of service tended to have higher levels of PCDF than the appliances put into service for a shorter time. It is possible that manufacturing controls improved as the production of Askarel matured in the 1960s such that Askarel was produced with lower levels of PCDF in the as-delivered fluids.

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Two utility appliances were selected by the EPRI Technical Advisory Committee for special attention: an Askarel-filled ESP transformer and an oil-filled arc furnace transformer. These two samples were the "worst case" for testing service stress on the dielectric fluids. The arc furnace had essentially no PCDF or PCDD, and the PCDF levels in the ESP transformer appeared to be dictated by the age of the unit (27 years of service) and the fluid composition (Aroclor 1260). This extended period of stress did not appear to elevate the PCDF levels above typical Aroclor 1260 levels.

One capacitor was tested in the in-service liquid screening program. It was found to have relatively low levels of PCDF compared to the other Askarel-filled units.

CONCLUSIONS

The in-service liquids used in this study were found to have minimal levels of PCDD and only trace amounts of PCDF. Liquids from transformers with more than 25 years of service had higher PCDF levels than liquids from newer transformers. Liquids from utility appliances exposed to thermal and electrical stresses did not show elevated PCDD or PCDF levels, indicating that high-energy events (electrical and thermal excursions) do not appear to generate PCDD or PCDF in utility electrical appliances that employ PCB. PCDF levels increased as the Aroclor number (and relative amount of chlorination) increased.

This study was successful in developing a set of methodologies that reliably determine trace levels of PCDF and PCDD in a very difficult matrix -- chlorinated organic oil. The development of commercially available reference standards, and the statistical validation of reference methods that can be used to analyze utility fluids was a major contribution to the quality and defensibility of data. The method verification program showed that a range of instrumentation can be successfully used to measure trace levels of PCDF and PCDD in utility fluids. In general, the methods employing low-resolution mass spectrometry (LRMS) require additional column chromatography to isolate PCDF/PCDD-enriched fractions for final HRGC/LRMS analysis. The precision of blind, spiked samples was superior to that expected by experienced analysts who had previously performed these analyses. The availability of labelled quantification standards helped improve method performance and lowered data scatter.

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Section 1

INTRODUCTION

The Electric Power Research Institute initiated this program to investigate the chemistry and analysis of polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-p-dioxins (PCDD) as they relate to the utility use of Askarel, a dielectric liquid which contains polychlorinated biphenyl (PCB), frequently diluted with tri/tetra chlorinated benzene. Askarel is sometimes contaminated with traces of PCDF/PCDD. The program had three goals; (1) to develop reliable, well characterized analytical methods to measure PCDF/PCDD, (2) to measure PCDF/PCDD levels in typical dielectric liquids used in utility operations, and (3) to investigate possible PCDF/PCDD production in unusual transformer events such as high energy arcing.

This report describes analytical methodology and results of chemical analyses conducted by Battelle Columbus Division in support of EPRI Research Project 2028-6, "Formation of PCDD and PCDF in Askarel and Contaminated Mineral Oil Equipment". Chemical laboratories of four additional organizations conducted parallel measurements during this program: the Illinois Institute of Technology Research Institute, the New York State Department of Health, Radian Corporation, and the University of UMEA (Sweden). The data presented in this report were replicated by these four laboratories and separate reports are available from each of the study participants. Combined data from the replicate analyses conducted by the five collaborating laboratories (including Battelle), are presented in a summary report prepared by the Research Triangle Institute. The summary report examines pooled data and gives a rigorous statistical analysis, including variability and reliability of analytical results reported by laboratories routinely conducting PCDF/PCDD analyses in Askarel, mineral oil, and related sample matrices.

Reliable analytical methods are especially difficult to develop for Askarel since PCB constitutes the major interference in most PCDF/PCDD analytical methods. In Askarel, the normal PCB concentration is 60-70 percent by weight. Thus, Askarel presents perhaps the most difficult matrix for these analyses. Parts-per-billion (ppb) levels of PCDF, and even lower concentrations of PCDD must be measured. When this program was initiated in early 1984, the accuracy of PCDF analyses was hampered by a lack of accurate quantification standards. A major product of this

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study was the synthesis of a series of ^{13}C-labelled PCDF standards that allowed use of sensitive isotope dilution techniques to measure PCDF. Quantification and recovery measurements based on labelled standards permit the chemist to measure accurate mass spectral response ratios for naturally occurring PCDF (and PCDD) compared to the response factors of labelled standards spiked into the sample matrix.

The method development phase involved testing methods proposed by the five participating laboratories; Battelle, the Illinois Institute of Technology, Research Institute (IITRI), the New York State Department of Health (NYS), Radian Corporation, and the University of Umea. These five methods were challenged by analyzing PCDF/PCDD in baseline liquids, a series of five typical sample matrices: Aroclor 1260, Aroclor 1242, Aroclor 1016, chlorobenzene, and mineral oil containing approximately 50 ppm of Aroclor 1260. The baseline Askarel liquids contained native PCDF/PCDD, and the mineral oil was spiked with labelled PCDF.

The presence of PCDF/PCDD in typical utility liquids was investigated by measuring characteristic compounds and congener groups in samples provided by EPRI member utilities. A report was generated by the General Electric Company which outlined the main types and distribution of PCB containing liquids (1). A repository was established at Battelle where samples of transformer oils provided by several EPRI members were stored. A questionnaire was developed, and a data base was accumulated on the repository samples. Seven of these liquids were selected by the EPRI Technical Advisory Committee. The seven samples, along with three control liquids, were analyzed by the participating laboratories. The seven "in-service" liquids selected for analysis represented either high energy operation, or utility applications that typify PCB usage. The data collected in the in-service liquid study were analyzed by chemists and statisticians at the Research Triangle Institute (RTI). A separate report will be generated by RTI that summarizes the results of all five laboratories. In summary, the independent methods worked well, indicating that good results can be expected when appropriate quality control procedures are applied.

Arcing experiments were conducted by General Electric. Transformer test units were fabricated out of the normal materials of transformer construction. These test units were exposed to high electric energy such as might be expected in catastrophic transformer failure. In the engineering tests, final PCDF/PCDD levels were either the same or lowered from initial levels, indicating that short term

electrical discharges are not a primary source of production for PCDF/PCDD in transformer liquids.

Section 2

EXPERIMENTAL

ACQUISITION OF IN-SERVICE SAMPLES

The selection of in-service dielectric fluids for this study was coordinated by the EPRI Technical Advisory Committee with the assistance of Dr. Thomas Rouse of the General Electric Company. It was important to select a series of in-service liquids that represented a range of utility applications. In the early planning for this program, Dr. Rouse prepared a criteria document that discussed the distribution of Askarel among different types of electrical devices, the relative chemical stresses expected from these devices in normal usage, the dispersion of Askarels, heating processes in transformers, and situations that represent the worst case for Askarel usage. He estimated that the United States has 140,000 Askarel-filled transformers in service, 30 million oil-filled transformers, and 5-10 million power capacitors. The oil-filled transformers constitute a large number; however, only about 50,000 are considered PCB-containing (>500 ppm (w/w)), and another approximately 1 million are PCB-contaminated (50-500 ppm). Since PCDF and PCDD are minor constituents of PCB, PCB-containing and PCB-contaminated transformers are expected to contain very low concentrations of PCDF/PCDD (perhaps not measurable). Dr. Rouse published an EPRI report (1) in which he recommended establishing a repository of 30-40 in-service samples that would be reviewed by the technical advisory group to select a final set of in-service liquids for exhaustive testing by the methods developed in this program.

The samples recommended by Dr. Rouse for inclusion in the final test group are given in Table 2-1.

In order to acquire suitable samples for this study, several participating utility representatives were asked to survey their records on available samples, searching for dielectric materials that met the criteria established by Dr. Rouse. To prescreen the available samples, a questionnaire was developed and a data base of questionnaire information maintained. A copy of the questionnaire is given in Appendix A.

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TABLE 2.1

EQUIPMENT RECOMMENDED FOR SAMPLING FOR PCDF AND PCDD ANALYSES

ASKAREL-FILLED	
Load Center/Network Transformers	8-10
Rectifier Transformers	1-2
Precipitator Transformers	2
Capacitors	4
OIL-FILLED WITH PCB	
Load Center/Network Transformers	4

After gathering completed questionnaires on a large number of candidate fluids, a repository was established, that contained the samples listed in Table 2.1. As shown in Table 2.2, suitable dielectric liquids were collected from each of the categories of interest. A final selection was made using information in the repository data base, and the materials listed in the last column of Table 2.2 were selected for inclusion in the in-service testing program.

ANALYTICAL TECHNIQUES

The following method description gives the procedures that were used by Battelle to analyze all Askarel and mineral oil samples in this program. The samples preparation procedures, the spiking protocols, the instrumental conditions, and the equations used to calculate final results are given here. The oil samples (five "baseline" samples) were spiked with labelled internal standards prior to distribution to the participating laboratories. The sample preparation and spiking procedures for the baseline liquids are given in a separate report in this series prepared by Radian Corporation (Austin, TX).

SAMPLE PREPARATION AND SPIKING

The seven in-service liquids were diluted to a concentration of approximately 2 µg/mL with hexane prior to spiking with internal standards. Aliquots of each liquid were weighed by difference into 100 mL volumetric flasks which were then filled to the mark with Distilled-in-Glass hexane. The flasks were placed on a

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Utility Appliance	Service Life (Years)
CAPACITORS	
Capacitor 1	?
Capacitor 2	?
Capacitor 3	?
TRANSFORMERS	
Arc Furnace 1	8
Arc Furnace 2	8
CIRCUIT BREAKER	
Circuit Breaker	30(Est.)
LOAD CENTER NETWORK TRANSFORMERS	
Network Load Center 1	?
Network Load Center 2	30(Est.)
Network Load Center 3	21
Network Load Center 4	28
Network Load Center 5	20
Network Load Center 6	19
Network Load Center 7	20
Network Load Center 8	19
Network Load Center 9	31
Network Load Center 10	30(Est.)
Network Load Center 11	30(Est.)
Network Load Center 12	20(Est.)
Network Load Center 13	15(Est.)
Network Load Center 14	10(Est.)
Network Load Center 15	20(Est.)
Network Load Center 16	20(Est.)
Network Load Center 17	20(Est.)
Network Load Center 18	10(Est.)
Network Load Center 19	20(Est.)
Network Load Center 20	?
PRECIPITATORS	
Precipitator 1	20(Est.)
Precipitator 2	20(Est.)
Precipitator 3	27

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TABLE 2.2

AVAILABLE FOR THE IN-SERVICE TRANSFORMER LIQUIDS STUDY

Rating	Dielectric	PCB (ppm)	Volume (Gal.)	Field Code	Laboratory Code
7200 KVAR	?	--	?	ISL-28	--
7200 KVAR	?	--	?	ISL-27	41159-11-13
?	Aroclor 1242	--	?	ISL-01-A	--
12470/280 KVA	Mineral Oil	161	4,500	ISL-24-0	--
12470/280 KVA	Mineral Oil	150	4,500	ISL-23-0	41159-11-15
34.5 KV	Mineral Oil	403	360	ISL-29-0	--
450 KVA	Mineral Oil	248	?	ISL-05-0	--
1500 KVA	Aroclor 1242	--	500	ISL-06-A	--
1000 KVA	Inerteen	--	3780 (lb)	ISL-07-A	--
500 KVA	Aroclor 1260	--	3175 (lb)	ISL-08-A	41159-11-12
750 KVA	Aroclor 1260	--	2650 (lb)	ISL-02-A	41159-11-17
500 KVA	Aroclor 1260	--	?	ISL-09-A	--
750 KVA	Aroclor 1260	--	240	ISL-10-A	--
500 KVA	Aroclor 1260	--	3200 (lb)	ISL-11-A	--
500 KVA	Aroclor 1260	--	?	ISL-03-A	41159-11-11
243 KVA	Pyranol	--	195	ISL-12-A	--
559 KVA	Pyranol	--	370	ISL-13-A	--
313 KVA	?	--	195	ISL-14	--
339 KVA	Pyranol	--	360	ISL-15-A	--
126 KVA	Pyranol	--	490	ISL-16-A	--
11 KVA	Inerteen	--	235	ISL-18-A	--
11 KVA	Chlorestol	--	272	ISL-19-A	--
11.5 KVA	Inerteen	--	282	ISL-20-A	--
11.5 KVA	Inerteen	--	184	ISL-21-A	--
11.5 KVA	Chlorextol	--	277	ISL-22-A	--
?	Mineral Oil	100	200	ISL-04-0	41159-11-16
440/53.5 KVA	?	--	94	ISL-26	--
440/53.5 KVA	?	--	94	ISL-25	--
440/53.5 KVA	?	--	143	ISL-17-A	41159-11-14

mechanical shaker and mixed overnight. After approximately 12 hours of mixing, the flasks were removed from the shaker and spiked with the five isotopically labelled internal standards. The internal standards were: 2,3,7,8-TCDD-$^{13}C_{12}$, 2,3,7,8-TCDF-$^{13}C_{12}$, 1,2,3,7,8-Penta-CDF-$^{13}C_{12}$, 1,2,3,4,7,8-Hexa-CDF-$^{13}C_{12}$, and Octa-CDD-$^{13}C_{12}$.

ANALYTE ENRICHMENT

One-milliliter aliquots (containing ~250 mg of the original fluid) of each sample were transferred to separatory funnels and diluted with 49 mL of hexane. The hexane solutions were washed with 10-mL portions of concentrated sulfuric acid until the washes were only slightly colored. A maximum of five acid washes were used. Following the acid washes, each oil was washed with 20 mL of distilled water to remove any residual acid. The hexane solutions were transferred to glass collection tubes and concentrated to approximately 2 mL using gentle streams of nitrogen gas.

The hexane solutions were transferred to multilayered silica gel columns containing activated silica gel, 44 percent concentrated sulfuric acid on silica gel, and 33 percent 1M sodium hydroxide on silica gel. The purpose of these columns was to remove acidic and basic compounds from the solutions as well as materials that were easily oxidized. The silica gel support provided a large surface area for contact with the sample solutions thus improving the cleanup efficiency. The PCDD/PCDF isomers were eluted from the columns using 70 mL of hexane and the entire eluents, including the original sample volume, were collected.

The eluates from the multilayered silica gel columns were concentrated to approximately 1 mL using gentle streams of nitrogen and transferred to the top of columns containing 5 g of activated basic alumina. These columns were eluted using hexane, hexane/methylene chloride (97:3, v/v) and hexane/methylene chloride (1:1, v/v) as elution solvents. The 1:1 hexane/methylene chloride eluates were collected, solvent exchanged into hexane, and transferred to the top of alumina columns containing approximately 2 g of activated basic alumina. These columns were eluted with hexane, hexane/methylene chloride (97:3, v/v), and hexane/methylene chloride (1:1, v/v). The 1:1 hexane/methylene chloride eluates were collected, concentrated to near dryness with nitrogen gas, and spiked with 10 ng of 1,2,3,4-TCDD-$^{13}C_{12}$. These solutions were stored at 0°C until they were analyzed.

Several of the sample solutions were still too contaminated with interferences to be analyzed. These solutions (41159-11-12,13,14,17 and E729-53-02,03) were

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chromatographed through florisil columns to provide additional analyte enrichment. Each of the florisil columns was prepared using 5 g of activated florisil (60-100 mesh) which was slurry packed with hexane. The sample solutions were diluted with approximately 5 mL of hexane and transferred to the florisil columns. The columns were eluted with hexane, hexane/ethyl ether (94:6, v/v), and hexane/methylene chloride (25:75, v/v). The 25:75 hexane/methylene chloride fraction was collected and concentrated to approximately 20 µL. These solutions were stored at 0°C until they were analyzed.

ANALYSIS

The sample solutions were analyzed and quantified for PCDD/PCDF using combined capillary column gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The HRGC/HRMS consisted of a Carlo Erba Model 4160 Gas Chromatograph interfaced directly into the ion source of a VG Model 7070 high resolution mass spectrometer. The chromatographic column was a 60 M DB-5 fused silica column using helium carrier gas at a flow velocity of 30 cm/sec. The mass spectrometer was operated in the electron impact (EI) ionization mode at a mass resolution of 9000-12000 (M/ M, 10 percent valley definition). All HRGC/HRMS data were acquired by multiple-ion-detection (MID) using a VG Model 2035 Data System.

QUALITY ASSURANCE

The operation of the HRGC/HRMS was evaluated each day by analyzing standard mixtures of selected PCDD/PCDF compounds. These were used to define the chromatographic elution windows, the compound resolution of the chromatographic column, and to verify the quantitative accuracy of the analyses. The mass focus accuracy of the MID unit was evaluated at least every four hours by observing selected ion masses from perfluorokerosene (PFK) which was introduced continually into the ion source as a reference compound. Mass focus stability was assured by using a reference PFK "lock mass" to correct for any mass focus drift.

Native spike and laboratory method blank samples were also processed during the extraction and cleanup of the samples. The native spike samples were used to evaluate the accuracy of the quantification while the laboratory method blank sample was used to demonstrate freedom from laboratory contamination.

RECOVERY OF INTERNAL STANDARDS

The recoveries of the internal standards were calculated by comparison to an external standard, 1,2,3,4-TCDD-$^{13}C_{12}$ which was added following the column cleanup steps. Relative response factors were determined from triplicate analyses of a

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standard mixture containing the labelled internal standards and the 1,2,3,4-TCDD-$^{13}C_{12}$. The equation used to calculate the recoveries was:

$$\text{Recovery (\%)} = \frac{Ais \times Qrs \times 100}{Ars \times Qis \times Rf}$$

Where:

Ais = Sum of integrated areas for internal standard;
Qrs = Quantity of recovery standard in ng;
Qis = Quantity of internal standard in ng;
Ars = Sum of integrated areas for recovery standard; and
Rf = Response factor.

QUANTIFICATION

The PCDD/PCDF isomers and congener class concentrations were determined by comparing the sum of the two ion masses monitored for each class to the sum of the two ion masses monitored for the corresponding internal standard. It was assumed that the response factors for each of the individual isomers in a class were the same as the response factors calculated for the specific isomers present in the standard solutions. The response factors were calculated from six replicate analyses of standard solutions at four concentrations. The sum of the two most intense ion masses in the molecular ion region of each isomer were used to generate the response factors.

The formula used for quantifying the PCDD/PCDF isomers was:

$$\text{Quantity/Sample (ng/g)} = \frac{Ac \times Qis}{Ais \times Rf \times W}$$

Where:

Quantity = Total quantity in ng of target isomer or congener class
Ac = Sum of integrated areas for the target isomer or congener class
Qis = Quantity of internal standard in ng
Ais = Total integrated areas for the internal standard
Rf = Response factor.

Each pair of resolved peaks in the selected-ion-current chromatograms was evaluated manually to determine if it met the criteria for a PCDD or PCDF isomer. By examining each pair of peaks separately, quantitative accuracy was improved over what is obtained when all of the peaks in a selected chromatographic window are averaged. When averaged data are used, it is possible for pairs of peaks with high and low chlorine isotope ratios to produce averaged data that meet the chlorine isotope ratio criterion. For example, two pairs of peaks having chlorine isotope ratios of 0.56 and 0.96, both outside of the acceptable range, would have an

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average ratio of 0.76. By checking the isotope ratio of these peaks separately, they would be eliminated from the class total.

The criteria that were used to identify PCDD and PCDF isomers were:

1. Simultaneous responses at both ion masses.
2. Chlorine isotope ratio within +/-15% of the theoretical value.
3. Chromatographic retention times within windows determined from analyses of standard mixtures.
4. Signal-to-noise ratio equal to or greater than 2.5 to 1.0.

The individual isomers for which an isotopically labelled analog was available included the additional criterion that they eluted within 2 seconds of the internal standard.

The limit of detection (LOD) was calculated for samples in which isomers of a particular chlorine congener class were not detected. The formula used for calculating the LOD was:

$$\text{LOD/g (ng)} = \frac{Hc \times Qis \times 2.5}{His \times Rf \times W}$$

Where:

LOD = Single isomer limits of detection for a congener class in ng;
Hc = Height of congener class isomer;
Qis = Quantity of internal standard in ng;
His = Peak height of internal standard;
Rf = Response factor; and
W = Weight in grams of sample.

CALIBRATION CURVE

Calibration curves were generated for each of the native/isotopically labelled isomer pairs. The calibration standards were analyzed at least five times at each of four concentration levels between 0.04 and 2.5 parts-per-million (ppm). The response factors were calculated by dividing the sum of the areas for the two most abundant ion masses in the molecular ion cluster of each native PCDD/PCDF isomer by the sum of the areas for the corresponding ion masses from the isotopically labelled standard. The isomer pairs for which response factors were calculated included:

2,3,7,8-TCDD vs 2,3,7,8-TCDD-$^{13}C_{12}$
2,3,7,8-TCDF vs 2,3,7,8-TCDF-$^{13}C_{12}$
OCDD vs OCDD-$^{13}C_{12}$
OCDF vs OCDD-$^{13}C_{12}$
1,2,3,7,8-Penta-CDF vs 1,2,3,7,8-Penta-CDF-$^{13}C_{12}$
2,3,4,7,8-Penta-CDF vs 1,2,3,7,8-Penta-CDF-$^{13}C_{12}$
1,2,3,4,7,8-Hexa-CDF vs 1,2,3,4,7,8-Hexa-CDF-$^{13}C_{12}$
2,3,4,6,7,8-Hexa-CDF vs 1,2,3,4,7,8-Hexa-CDF-$^{13}C_{12}$
1,2,3,4,6,7,8-Hepta-CDF vs OCDD-$^{13}C_{12}$

It was assumed that each of the isomers of a particular chlorine congener class had the same response factor.

DETECTION LIMITS

Detection limits were calculated for samples that did not contain PCDD/PCDF isomers in a particular class. These detection limits were calculated using the quantification equation with the addition of a factor of 2.5 to account for the signal-to-noise criterion.

The noise level was measured as the average of the peak to peak noise that occurred in the baseline of the particular MID channel. If at all possible, the noise was measured at the retention time of the isotopically labelled internal standard. The height of the internal standard peak was measured to the average of the noise that occurred on the peak top. The reported limits of detection are for single PCDD/PCDF isomers and have been corrected for recovery losses during sample workup.

BASELINE ANALYSES

The three baseline samples, Aroclor 1260, Aroclor 1254, and Aroclor 1016, were analyzed using the methodology described above. The Aroclor 1016 presented the greatest analytical problem due to the relatively high amount of the sample that passed through the column cleanup steps. Even after the four column cleanup steps, a noticeable PCB background was evident in the single ion current chromatograms.

IN-SERVICE ANALYSES

The seven in-service samples were also analyzed using the methodology described above.

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Section 3

BASELINE ANALYSES

This section describes the baseline analyses performed by all five laboratories participating in this study. The baseline analyses were designed as a rigorous blind analysis of authentic Aroclor and mineral oil samples. If the proposed analytical methods performed well in the baseline tests, the additional steps of method validation, and screening of in-service utility fluids could be performed with confidence in the reported results. Consequently, the baseline analyses were critical to the success of this program.

BACKGROUND RESEARCH

Before the baseline analyses could be performed, five test liquids were obtained by Radian Corporation and a combination of native and labelled (^{13}C) PCDF/PCDD were spiked into the test liquids to ensure a rugged test of the proposed analytical techniques. The test liquids selected for the baseline study are listed in Table 3.1.

TABLE 3.1

BASELINE TEST LIQUIDS

Dielectric Fluid	Composition
Aroclor 1016	Narrow boiling point fraction of Aroclor 1242.
Aroclor 1242	42% Cl (w/w)
Aroclor 1260	60% Cl (w/w)
Chlorobenzenes	-Synthetic Mixture 1,2,4-Trichlorobenzene (51.2% (w/w)) 1,2,3-Trichlorobenzene (13.4% (w/w)) 1,2,4,5-Tetrachlorobenzene (2.6% (w/w)) 1,2,3,4-Tetrachlorobenzene (28.4% (w/w)) Pentachlorobenzene (4.4% (w/w))
Aged Mineral Oil	-Spiked Aroclor 1260 (500 ppm (w/w)) Tri/Tetrachlorobenzene Mix Listed Above (500 ppm (w/w))

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RESULTS

Chemical Analyses

The results of chemical analyses performed by Battelle on the baseline samples are given in Table 3.3. All results are given as parts-per-billion by weight in the original oil (ng/g).

These data are interesting because they show the expected trends in the Aroclor PCDF/PCDD concentrations. PCDD are not found at the detection limit of the high resolution mass spectrometer used for this analysis, approximately 10 ppb (w/w) in the mineral oil samples. Trace levels of PCDF are observed with the concentrations increasing as the level of chlorination increases in the supporting Aroclor. The PCDF levels increase as the Aroclor number (and relative amount of chlorination increase), Aroclor 1260 >Aroclor 1242 >Aroclor 1016. Even though Aroclor 1016 is similar in composition to Aroclor 1242, the wider distribution of PCB congener groups in Aroclor 1242 would produce higher quantities of the high chlorination number PCB, 6-10 chlorine atoms per molecule, which are believed to be the precursors of PCDF when HCl elimination is the route for PCDF formation.

An increase in PCDF concentrations is shown in Table 3.3 as the total high chlorine PCB concentration increases. Total PCDF levels increase from 280 ppb for Aroclor 1016, to 1,710 ppb for Aroclor 1242, to 10.4 ppm for Aroclor 1260. Data for three of the participating laboratories are given in Tables 3.4 through 3.6. These data show the same trends observed in the Battelle data. The measured levels of spiked PCDF/PCDF were also similar among the reporting laboratories.

Baseline Recovery Data

The recovery of labelled standards is used to determine how much of the native PCDF/PCDD present in the sample is lost during the sample preparation steps of candidate methods. Since the internal quantification standard corrects for method losses, the reported value should be accurate within the measured precision of the method; however, excessive loss of analytes during sample preparation will have a profound affect on both method sensitivity, and precision. Recoveries for six labelled PCDF/PCDD standards spiked into the baseline samples are given in Table 3.7.

These data are interesting because they show the outliers that can affect individual data points.

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TABLE 3.3

BATTELLE FURAN AND DIOXIN DATA FOR THE BASELINE ANALYSES STUDY (ppb (w/w))

Compound	Aroclor 1016	Aroclor 1242	Aroclor 1260	Tri and Tetra Chlorobenzene	Mineral Oil Run No. 1	Mineral Oil Run No. 2
Polychlorinated Dibenzofurans						
Total TCDF	0	990	640	0	119	115
Total PnCDF	5	400	2100	2	270	228
Total HxCDF	190	84	3100	82	278	246
Total HpCDF	85	184	2100	100	128	113
OCDF	NR*	52	2500	NR	152	157
Total PCDF	280	1710	10440	184	947	859
Polychlorinated Dibenzodioxins						
Total TCDD	0	0	0	0	32	34
OCDD	NR	NR	NR	NR	NR	NR
Specific Molecules and Unresolved Molecular Clusters						
2,3,7,8-TCDD	0	0	0	0	32	34
2,3,7,8-/2,3,4,8-TCDF	0	84	150	0	119	115
1,2,3,7,8-/1,2,3,4,8-PnCDF	0	54	480	0	170	143
2,3,4,7,8-PnCDF	0	0	0	0	98	95
1,2,3,4,7,8-HxDF	190	84	1700	82	246	262
1,2,3,7,8,9-HxCDF	NR	NR	NR	NR	NR	NR
2,3,4,6,7,8-HxCDF	NR	NR	NR	NR	NR	NR
1,2,3,4,6,7,8-HpCDF	85	147	680	100	113	120

* NR=Not Reported.

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TABLE 3.4

IITRI FURAN AND DIOXIN DATA FOR THE BASELINE ANALYSES STUDY (ppb (w/w))

Compound	Aroclor 1016	Aroclor 1242	Aroclor 1260	Tri and Tetra Chlorobenzene	Mineral Oil Aged
Total TCDF	30	866	612	0	70
Total PnCDF	42	353	1037	0	165
Total HxCDF	92	105	1433	103	207
Total HpCDF	81	109	1985	98	40
OCDF	20	127	4154	0	393
Total TCDD	0	0	0	0	42
OCDD	0	0	1	1	0
2,3,7,8-TCDD	0	0	0	0	42
2,3,7,8-/2,3,4,8-TCDF	8	227	193	0	70
1,2,3,7,8-/1,2,3,4,8-PnCDF	11	42	231	0	112
2,3,4,7,8-PnCDF	0	51	88	0	52
1,2,3,4,7,8-HxCDF	76	63	601	103	206
1,2,3,7,8,9-HxCDF	0	0	0	0	0
2,3,4,6,7,8-HxCDF	0	0	0	0	0
1,2,3,4,6,7,8-HpCDF	58	77	684	98	68

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TABLE 3.5

NYSDOH FURAN AND DIOXIN DATA FOR THE BASELINE ANALYSES STUDY (ppb (w/w))

Compound	Aroclor 1016	Aroclor 1242	Aroclor 1260	Tri and Tetra Chlorobenzene	Mineral Oil Aged
Total TCDF	1	1900	940	3	95
Total PnCDF	7	584	996	13	0
Total HxCDF	350	140	5500	1000	1100
Total HpCDF	92	130	2430	91	0
OCDF	0	2300	NA	0	0
Total TCDD	0	0	0	0	42
OCDD	0	0	0	0	0
2,3,7,8-TCDD	0	0	7	0	43
2,3,7,8-/2,3,4,8-TCDF	0	150	190	0	34
1,2,3,7,8-/1,2,3,4,8-PnCDF	0	1000	220	1	140
2,3,4,7,8-PnCDF	0	0	0	0	0
1,2,3,4,7,8-HxCDF	0	0	0	0	0
1,2,3,7,8,9-HxCDF	0	0	0	0	0
2,3,4,6,7,8-HxCDF	0	0	0	0	0
1,2,3,4,6,7,8-HpCDF	0	0	0	0	0

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TABLE 3.6

RADIAN FURAN AND DIOXIN DATA FOR THE BASELINE ANALYSES STUDY (ppb (w/w))

Compound	Aroclor 1016	Aroclor 1242	Aroclor 1260	Tri and Tetra Chlorobenzene	Mineral Oil Aged
Total TCDF	0	0	0	0	125
Total PnCDF	0	0	477	0	226
Total HxCDF	97	60	872	161	178
Total HpCDF	80	46	807	170	36
OCDF	0	24	1786	0	126
Total TCDD	0	0	0	0	0
OCDD	0	0	0	386	0
2,3,7,8-TCDD	0	0	0	0	0
2,3,7,8-/2,3,4,8-TCDF	0	0	0	0	125
1,2,3,7,8-/1,2,3,4,8-PnCDF	0	0	477	0	159
2,3,4,7,8-PnCDF	0	0	0	0	66
1,2,3,4,7,8-HxCDF	97	60	872	161	177
1,2,3,7,8,9-HxCDF	0	0	0	0	0
2,3,4,6,7,8-HxCDF	0	0	0	0	0
1,2,3,4,6,7,8-HpCDF	80	46	807	170	36

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2,3,7,8-TCDF-$^{13}C_{12}$

1,2,3,7,8-PCDF-$^{12}C_{12}$

2,3,7,8-TCDD-$^{13}C_{12}$

OCDD-$^{13}C_{12}$

1,2,3,4,7,8-HxCDF-$^{13}C_{12}$

1,2,3,4,6,7,8-HpCDF

*Outliers

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TABLE 3.7

ABSOLUTE RECOVERIES OF LABELLED STANDARDS
IN THE BASELINE STUDY (UNIVERSITY OF UMEA)

Spike Level (ppb)	Aroclor 1016	Aroclor 1242	Aroclor 1260	Chloro-benzenes	Mineral Oil
100	93	95	95	97	116
100	111	134	140	97	114
100	67	86	86	101	118
100	102	89	157	86	121
100	83	90	317	110	NA
100	57	45	1224*	68	NA

One effect is evident in these data, the recoveries from chlorobenzene and mineral oil appear to be superior to recoveries from Aroclors. The Aroclors contain the major interferents in the analysis of PCDF and PCDD. It appears that there may be matrix dependent difficulties in the analytical preparation of complex samples.

Method Reliability

The baseline data are grouped to show the relative performance of the four laboratories reported here. A compilation of data for aged mineral oil, baseline sample results is given in Table 3.8.

Method reliability is compared by computing the Student's-t variability for four independent determinations around the mean value, the known spiked amount of standard PCDF/PCDD.

In the four determinations shown the Student's-t variability of the mean varies from 20 to 167 percent. This is the degree of expected scatter in a group of four PCDD/PCDF determinations conducted by independent laboratories using the Student's-t distribution to approximate variance at the 95 percent confidence level.

TABLE 3.8

FOUR-LABORATORY RESULTS OF ANALYSIS OF AGED MINERAL OIL (ng/g (w/w))

Compound	Battelle	ITIRI	NYSDOH	Radian	Group Mean	Native Spike Level	Confidence Interval (95%)
Total TCDF	115	70	95	125	101		
Total PnCDF	228	165	0	226	155	200	109%
Total HxCDF	246	207	1100	178	433		
Total HpCDF	113	40	0	36	47		
OCDF	157	393	0	126	169	160	167%
Total TCDD	34	42	42	0	30		
2,3,7,8-TCDD	34	42	43	0	30		
2,3,7,8-/2,3,4,8-TCDF	115	70	34	125	86		
1,2,3,7,8-/1,2,3,4,8-PnCDF	143	112	140	159	139	100	20%
2,3,4,7,8-PnCDF	95	52	0	66	53	100	41%
1,2,3,4,7,8-HxCDF	262	206	0	177	161		
1,2,3,4,6,7,8-HpCDF	120	68	0	36	56		

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Section 4

SUPPLEMENTAL ANALYSES

CROSS CHECK ANALYSES

To validate the analytical curve for each method proposed by the participating laboratories, a series of calibration runs were performed to establish the variability, linear dynamic range, and the limit of detection for each instrument. The calibration range is different from the method range. The validation of the calibration curve range only deals with standard solutions eluted through a tuned gas chromatograph or mass spectrometer (GC/MS) system. The quantities used to develop the calibration curve were 40 pg, 150 pg, 500 pg, and 2.5 ng. Each concentration level was analyzed with five replicates. The analytes used for the calibration curve validation were 2,3,7,8-TCDD, OCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PCDF, 2,3,4,7,8-PCDF, 1,2,3,4,7,8-HxCDF, 2,3,4,6,7,8-HxCDF, and 1,2,3,4,5,7,8-HpCDF.

An illustration of the type of calibration plot generated in this study is given in Figure 1, which shows the standard curve for 2,3,7,8-TCDF.

The complete set of calibration curve plots, along with statistical parameters describing calibration precision are given in Appendix B. The standard deviation and the relative standard deviation at each point show the variability of response which increases at higher concentrations. The calibration curves were linear over the range of concentrations measured.

ANALYSES FROM THE UNIVERSITY OF UMEA

Under a subcontract to Battelle, the University of Umea participated in the baseline and round robin analyses. A letter report of the data from Umea is included as Appendix C.

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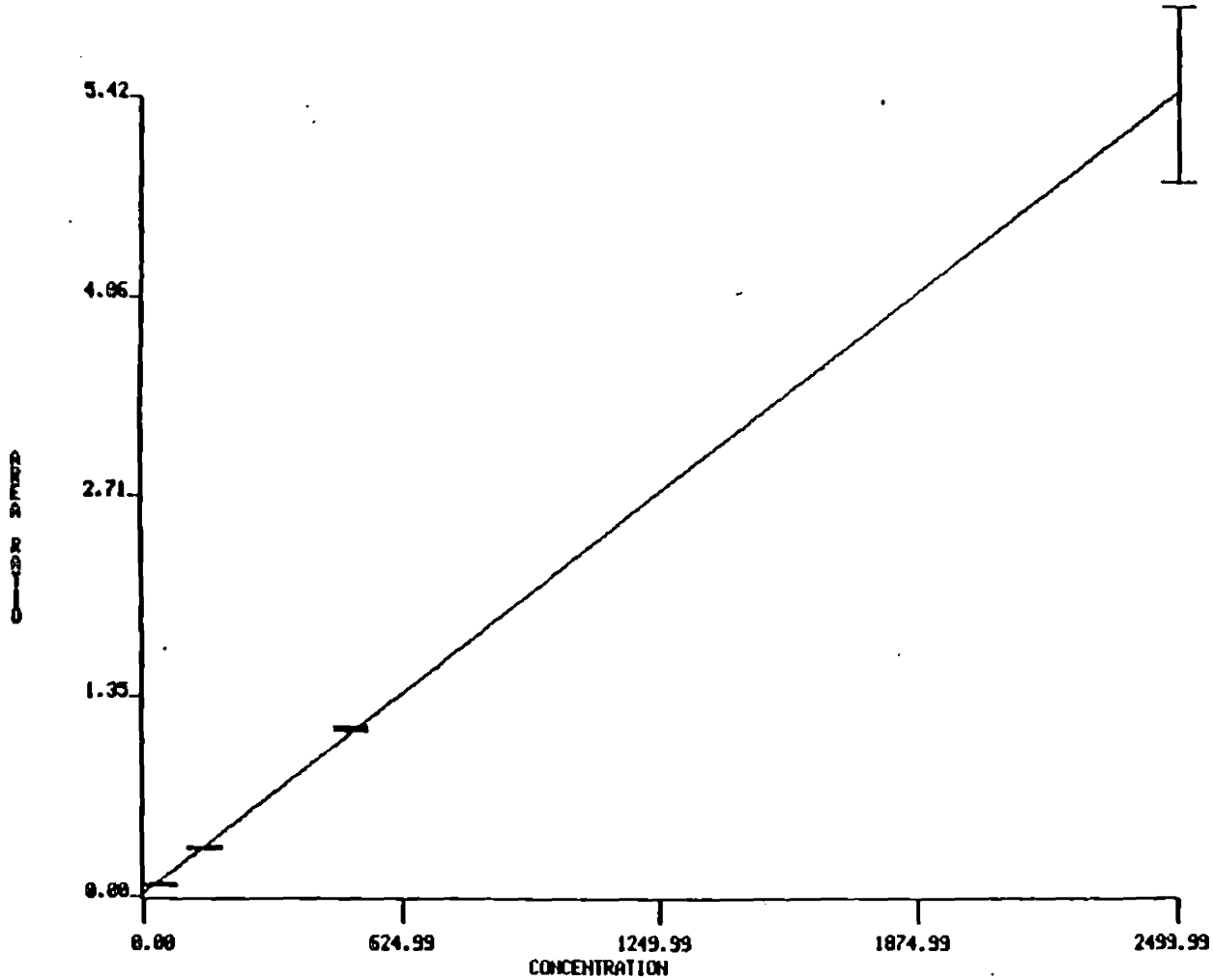

Figure 4-1. Calibration curve for 2,3,7,8-tetrachlorodibenzofuran over the range 40 pg to 2.5 ng

Section 5

ROUND ROBIN ANALYSES OF IN-SERVICE LIQUIDS

The main thrust of this study was to evaluate a series of congeners and individual molecular species in typical in-service utility transformer and capacitor liquids. Ten (10) liquid samples, including the seven utility dielectric fluids, were analyzed for PCDD/PCDF using the methods developed in this program. All five participating laboratories contributed independent data sets, which will be statistically analyzed by an independent contractor, the Research Triangle Institute. The results of the Battelle analyses are given in Table 5.1 to 5.10.

Accuracy Estimate

The in-service analyses were designed with a quality control check on accuracy of reported PCDD/PCDF concentrations. The "Baseline" liquids included with actual utility operating fluids were spiked with known amounts of several PCDD/PCDF standards. These known spike concentrations allowed an estimate of accuracy using the mean of three determinations performed by each participating laboratory compared with the "true" or spiked concentration. The "Baseline" analyses revealed that the aged mineral oil sample and the Aroclor 1016 had minimal concentrations of the PCDD/PCDF standards present as contaminants. Thus, native PCDD/PCDF were suitable spike materials.

The Battelle results for in-service liquids are presented in Table 5.11 for the mineral oil and Aroclor 1016 samples. Table 5.11 gives the spike levels and compares the percent difference in spiked levels with measured levels of PCDD/PCDF.

The Battelle results are generally within ±70 percent of the spike value except for one notable exception. HxCDF exhibited a strong positive bias. Most of the elevated HxCDF levels were 2,3,4,6,7,8-HxCDF. The HxCDF measured levels were 438 percent higher than the HxCDF spike levels in mineral oil, and 1325 percent higher in Aroclor 1016, even after subtraction of the native HxCDF found in the earlier baseline investigations. These HxCDF data are deemed to be outliers.

TABLE 5.1

CHLORINATED FURAN AND DIOXIN RESULTS (ppb (w/w), ng/g)
SAMPLE ISL-02-A

Congener	Determination Det 1	Det 2	Det 3	Mean	Standard Deviation	Relative Standard Deviation
PCDF Congeners						
Total TCDF	360	230	290	293	53	18%
Total P_nCDF	69	72	71	71	1	2%
Total H_xCDF	8,100	19,000	20,000	15,700	5,389	34%
Total H_pCDF	1,600	1,400	2,000	1,667	249	15%
OCDF	4,300	4,800	5,100	4,733	330	7%
Total PCDF	14,429	25,502	27,461	22,464	5,738	26%
Furan Compounds						
2,3,7,8-/2,3,4,8-TCDF	190	140	180	170	22	13%
2,3,4,7,8-P_nCDF	300	260	290	283	17	6%
1,2,3,7,8-/1,2,3,4,8-P_nCDF	69	72	71	71	1	2%
1,2,3,4,7,8-/1,2,3,4,7,9-H_xCDF	2,700	6,400	6,400	5,167	1,744	34%
1,2,3,6,7,8-H_xCDF	760	2,300	2,700	1,920	836	44%
2,3,4,6,7,8-H_xCDF	1,100	3,400	3,800	2,767	1,190	43%
1,2,3,4,6,7,8-H_pCDF	180	230	320	243	58	24%
1,2,3,4,7,8,9-H_pCDF	280	250	320	283	29	10%
Dioxin Congeners						
Total TCDD	NA	NA	NA	NA	NA	NA
OCDD	18	18	10	15	4	25%
PCDD Compounds						
2,3,7,8-TCDD	NA	NA	NA	NA	NA	NA

GENP 011095

TABLE 5.2

CHLORINATED FURAN AND DIOXIN RESULTS (ppb (w/w), ng/g)
SAMPLE ISL-03-A

Congener	Determination Det 1	Det 2	Det 3	Mean	Standard Deviation	Relative Standard Deviation
PCDF Congeners						
Total TCDF	170	110	40	170	53	50%
Total P_nCDF	2,300	2,100	720	1,707	702	41%
Total H_xCDF	52,000	11,000	9,700	24,233	19,641	81%
Total H_pCDF	3,800	13,000	11,000	9,267	3,951	43%
OCDF	22,000	28,000	22,000	24,000	2,828	12%
Total PCDF	80,270	54,210	43,460	59,313	15,455	26%
Furan Compounds						
2,3,7,8-/2,3,4,8-TCDF	31	30	40	34	4	13%
2,3,4,7,8-P_nCDF	130	130	110	123	9	8%
1,2,3,7,8-/1,2,3,4,8-P_nCDF	35	49	26	37	9	26%
1,2,3,4,7,8-/1,2,3,4,7,9-H_xCDF	29,000	6,100	5,100	13,400	11,038	82%
1,2,3,6,7,8-H_xCDF	6,000	1,100	1,200	2,767	2,287	83%
2,3,4,6,7,8-H_xCDF	1,800	240	210	750	743	99%
1,2,3,4,6,7,8-H_pCDF	1,100	4,500	3,100	2,900	1,395	48%
1,2,3,4,7,8,9-H_pCDF	1,200	2,600	2,600	2,133	660	31%
Dioxin Congeners						
Total TCDD	NA	NA	NA	NA	NA	NA
OCDD	18	12	NA	10	7	75%
PCDD Compounds						
2,3,7,8-TCDD	NA	NA	NA	NA	NA	NA

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GENP 011096

TABLE 5.3

CHLORINATED FURAN AND DIOXIN RESULTS (ppb (w/w), ng/g)
SAMPLE ISL-04-0

Congener	Determination Det 1	Det 2	Det 3	Mean	Standard Deviation	Relative Standard Deviation
PCDF Congeners						
Total TCDF	NA	NA	NA	NA	NA	NA%
Total P_nCDF	NA	NA	NA	NA	NA	NA%
Total H_xCDF	NA	NA	NA	NA	NA	NA%
Total H_pCDF	NA	NA	NA	NA	NA	NA%
OCDF	NA	NA	NA	NA	NA	NA%
Total PCDF	NA	NA	NA	NA	NA	NA%
Furan Compounds						
2,3,7,8-/2,3,4,8-TCDF	NA	NA	NA	NA	NA	NA%
2,3,4,7,8-P_nCDF	NA	NA	NA	NA	NA	NA%
1,2,3,7,8-/1,2,3,4,8-P_nCDF	NA	NA	NA	NA	NA	NA%
1,2,3,4,7,8-/1,2,3,4,7,9-H_xCDF	NA	NA	NA	NA	NA	NA%
1,2,3,6,7,8-H_xCDF	NA	NA	NA	NA	NA	NA%
2,3,4,6,7,8-H_xCDF	NA	NA	NA	NA	NA	NA%
1,2,3,4,6,7,8-H_pCDF	NA	NA	NA	NA	NA	NA%
1,2,3,4,7,8,9-H_pCDF	NA	NA	NA	NA	NA	NA%
Dioxin Congeners						
Total TCDD	NA	NA	NA	NA	NA	NA%
OCDD	NA	13	11	8	6	71%
PCDD Compounds						
2,3,7,8-TCDD	NA	NA	NA	NA	NA	NA%

TABLE 5.4

CHLORINATED FURAN AND DIOXIN RESULTS (ppb (w/w), ng/g)
SAMPLE ISL-08-A

Congener	Determination Det 1	Det 2	Det 3	Mean	Standard Deviation	Relative Standard Deviation
PCDF Congeners						
Total TCDF	62	64	99	75	17	23%
Total P_nCDF	79	230	310	206	96	46%
Total H_xCDF	1,500	1,300	990	1,263	210	17%
Total H_pCDF	1,500	2,100	2,700	2,100	490	23%
OCDF	4,000	4,800	4,900	4,567	403	9%
Total PCDF	7,141	8,494	8,999	8,211	784	10%
Furan Compounds						
2,3,7,8-/2,3,4,8-TCDF	20	20	30	23	5	20%
2,3,4,7,8-P_nCDF	39	41	37	39	2	4%
1,2,3,7,8-/1,2,3,4,8-P_nCDF	22	25	34	27	5	19%
1,2,3,4,7,8-/1,2,3,4,7,9-H_xCDF	880	740	640	753	98	13%
1,2,3,6,7,8-H_xCDF	54	50	36	47	8	17%
2,3,4,6,7,8-H_xCDF	47	25	43	38	10	25%
1,2,3,4,6,7,8-H_pCDF	350	530	750	543	164	30%
1,2,3,4,7,8,9-H_pCDF	340	450	580	457	98	21%
Dioxin Congeners						
Total TCDD	NA	NA	NA	NA	NA	NA%
OCDD	NA	NA	NA	NA	NA	NA%
PCDD Compounds						
2,3,7,8-TCDD	NA	NA	NA	NA	NA	NA%

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GENP 011098

TABLE 5.5

CHLORINATED FURAN AND DIOXIN RESULTS (ppb (w/w), ng/g)
SAMPLE ISL-17-A

Congener	Determination Det 1	Det 2	Det 3	Mean	Standard Deviation	Relative Standard Deviation
PCDF Congeners						
Total TCDF	970	580	610	720	177	25%
Total P_nCDF	11,000	6,700	6,500	8,067	2,076	26%
Total H_xCDF	14,000	36,000	16,000	22,000	9,933	45%
Total H_pCDF	3,100	2,800	4,200	3,367	602	18%
OCDF	150	170	220	180	29	16%
Total PCDF	29,220	46,250	27,530	34,333	8,455	25%
Furan Compounds						
2,3,7,8-/2,3,4,8-TCDF	400	380	350	377	21	5%
2,3,4,7,8-P_nCDF	2,300	2,400	1,800	2,167	262	12%
1,2,3,7,8-/1,2,3,4,8-P_nCDF	870	900	780	850	51	6%
1,2,3,4,7,8-/1,2,3,4,7,9-H_xCDF	4,700	7,200	4,100	5,333	1,342	25%
1,2,3,6,7,8-H_xCDF	1,800	3,200	2,300	2,433	579	24%
2,3,4,6,7,8-H_xCDF	620	6,800	930	2,783	2,832	102%
1,2,3,4,6,7,8-H_pCDF	920	560	1,300	927	302	33%
1,2,3,4,7,8,9-H_pCDF	740	860	990	863	102	12%
Dioxin Congeners						
Total TCDD	NA	NA	NA	NA	NA	NA%
OCDD	NA	18	NA	NA	8	141%
PCDD Compounds						
2,3,7,8-TCDD	NA	NA	NA	NA	NA	NA%

TABLE 5.6

CHLORINATED FURAN AND DIOXIN RESULTS (ppb (w/w), ng/g)
SAMPLE ISL-23-0

Congener	Determination Det 1	Det 2	Det 3	Mean	Standard Deviation	Relative Standard Deviation
PCDF Congeners						
Total TCDF	NA	NA	NA	NA	NA	NA%
Total P_nCDF	NA	NA	NA	NA	NA	NA%
Total H_xCDF	NA	NA	NA	NA	NA	NA%
Total H_pCDF	NA	NA	NA	NA	NA	NA%
OCDF	NA	NA	NA	NA	NA	NA%
Total PCDF	NA	NA	NA	NA	NA	NA%
Furan Compounds						
2,3,7,8-/2,3,4,8-TCDF	NA	NA	NA	NA	NA	NA%
2,3,4,7,8-P_nCDF	NA	NA	NA	NA	NA	NA%
1,2,3,7,8-/1,2,3,4,8-P_nCDF	NA	NA	NA	NA	NA	NA%
1,2,3,4,7,8-/1,2,3,4,7,9-H_xCDF	NA	NA	NA	NA	NA	NA%
1,2,3,6,7,8-H_xCDF	NA	NA	NA	NA	NA	NA%
2,3,4,6,7,8-H_xCDF	NA	NA	NA	NA	NA	NA%
1,2,3,4,6,7,8-H_pCDF	NA	NA	NA	NA	NA	NA%
1,2,3,4,7,8,9-H_pCDF	NA	NA	NA	NA	NA	NA%
Dioxin Congeners						
Total TCDD	NA	NA	NA	NA	NA	NA%
OCDD	NA	12	NA	4	6	141%
PCDD Compounds						
2,3,7,8-TCDD	NA	NA	NA	NA	NA	NA%

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TABLE 5.7

CHLORINATED FURAN AND DIOXIN RESULTS (ppb (w/w), ng/g)
SAMPLE ISL-27

Congener	Determination Det 1	Det 2	Det 3	Mean	Standard Deviation	Relative Standard Deviation
PCDF Congeners						
Total TCDF	580	570	350	500	106	21%
Total P_nCDF	250	270	220	247	21	8%
Total H_xCDF	140	130	160	143	21	9%
Total H_pCDF	26	28	6	20	10	51%
OCDF	46	44	10	33	17	50%
Total PCDF	1,042	1,042	746	943	140	15%
Furan Compounds						
2,3,7,8-/2,3,4,8-TCDF	300	280	270	283	12	4%
2,3,4,7,8-P_nCDF	71	74	53	66	9	14%
1,2,3,7,8-/1,2,3,4,8-P_nCDF	580	570	350	500	106	21%
1,2,3,4,7,8-/1,2,3,4,7,9-H_xCDF	37	16	31	28	9	32%
1,2,3,6,7,8-H_xCDF	15	15	27	19	6	30%
2,3,4,6,7,8-H_xCDF	25	17	39	27	9	34%
1,2,3,4,6,7,8-H_pCDF	7	9	2	6	3	50%
1,2,3,4,7,8,9-H_pCDF	5	5	1	4	2	45%
Dioxin Congeners						
Total TCDD	NA	NA	NA	NA	NA	NA%
OCDD	NA	NA	NA	NA	NA	NA%
PCDD Compounds						
2,3,7,8-TCDD	NA	NA	NA	NA	NA	NA%

TABLE 5.8

CHLORINATED FURAN AND DIOXIN RESULTS (ppb (w/w), ng/g)
SAMPLE EZ29-53-01 (Spiked Mineral Oil)

Congener	Determination Det 1	Det 2	Det 3	Mean	Standard Deviation	Relative Standard Deviation
PCDF Congeners						
Total TCDF	220	160	190	190	24	13%
Total P_nCDF	310	550	260	373	127	34%
Total H_xCDF	680	6,600	390	2,557	2,862	112%
Total H_pCDF	530	33	220	261	205	79%
OCDF	NA	NA	3	1	1	141%
Total PCDF	1,740	7,343	1,063	3,382	2,815	83%
Furan Compounds						
2,3,7,8-/2,3,4,8-TCDF	220	160	190	190	24	13%
2,3,4,7,8-P_nCDF	170	370	120	220	108	49%
1,2,3,7,8-/1,2,3,4,8-P_nCDF	140	170	140	150	14	9%
1,2,3,4,7,8-/1,2,3;4,7,9-H_xCDF	310	550	260	373	127	34%
1,2,3,6,7,8-H_xCDF	140	120	76	112	27	24%
2,3,4,6,7,8-H_xCDF	200	5,900	140	2,080	2,701	130%
1,2,3,4,6,7,8-H_pCDF	530	33	220	261	205	79%
1,2,3,4,7,8,9-H_pCDF	NA	NA	NA	NA	NA	NA%
Dioxin Congeners						
Total TCDD	19	19	23	20	2	9%
OCDD	NA	NA	1	NA	NA	141%
PCDD Compounds						
2,3,7,8-TCDD	19	19	23	20	2	9%

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TABLE 5.9

CHLORINATED FURAN AND DIOXIN RESULTS (ppb (w/w), ng/g)
SAMPLE E729-53-02 (Spiked Aroclor 1016)

Congener	Determination Det 1	Det 2	Det 3	Mean	Standard Deviation	Relative Standard Deviation
PCDF Congeners						
Total TCDF	57	67	66	63	4	7%
Total P_nCDF	600	500	460	520	59	11%
Total H_xCDF	5,400	2,300	780	2,827	1,923	68%
Total H_pCDF	65	150	340	185	115	62%
OCDF	110	120	120	117	5	4
Total PCDF	6,232	3,137	1,766	3,712	1,868	50%
Furan Compounds						
2,3,7,8-/2,3,4,8-TCDF	57	67	66	63	4	7%
2,3,4,7,8-P_nCDF	410	310	260	327	62	19%
1,2,3,7,8-/1,2,3,4,8-P_nCDF	180	180	190	183	5	3%
1,2,3,4,7,8-/1,2,3,4,7,9-H_xCDF	NA	NA	NA	NA	NA	NA%
1,2,3,6,7,8-H_xCDF	100	84	81	88	8	9%
2,3,4,6,7,8-H_xCDF	5,200	2,100	680	2,660	1,887	77%
1,2,3,4,6,7,8-H_pCDF	65	150	180	132	49	37%
1,2,3,4,7,8,9-H_pCDF	NA	NA	NA	NA	NA	NA%
Dioxin Congeners						
Total TCDD	46	46	44	45	1	2%
OCDD	NA	NA	NA	NA	NA	NA%
PCDD Compounds						
2,3,7,8-TCDD	46	46	45	46	NA	1%

TABLE 5.10

CHLORINATED FURAN AND DIOXIN RESULTS (ppb (w/w), ng/g)
SAMPLE EZ29-53-03 (Spiked Aroclor 1260)

Congener	Determination Det 1	Det 2	Det 3	Mean	Standard Deviation	Relative Standard Deviation
PCDF Congeners						
Total TCDF	1,400	1,700	970	1,357	300	22%
Total P_nCDF	1,100	1,300	1,200	1,200	82	7%
Total H_xCDF	20,000	4,700	11,000	11,900	6,279	53%
Total H_pCDF	1,700	3,000	660	1,787	957	54%
OCDF	5,000	4,900	2,600	4,167	1,109	27%
Total PCDF	29,200	15,600	16,430	20,410	6,225	30%
Furan Compounds						
2,3,7,8-/2,3,4,8-TCDF	1,200	1,300	880	1,127	179	16%
2,3,4,7,8-P_nCDF	590	410	800	627	194	31%
1,2,3,7,8-/1,2,3,4,8-P_nCDF	1,400	1,170	970	1,357	300	22%
1,2,3,4,7,8-/1,2,3,4,7,9-H_xCDF	1,900	1,800	1,600	1,767	125	7%
1,2,3,6,7,8-H_xCDF	1,200	93	780	691	456	66%
2,3,4,6,7,8-H_xCDF	10,000	770	6,500	5,757	3,805	66%
1,2,3,4,6,7,8-H_pCDF	55	850	49	318	376	118%
1,2,3,4,7,8,9-H_pCDF	300	360	180	280	75	27%
Dioxin Congeners						
Total TCDD	110	100	80	97	12	13%
OCDD	NA	12	NA	4	6	141%
PCDD Compounds						
2,3,7,8-TCDD	110	100	80	97	12	13%

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TABLE 5.11

ACCURACY DETERMINATION USING SPIKED BASELINE LIQUID ANALYSES

	Mineral Oil E729-53-01			Aroclor 106 E729-53-02			Corrected[2] Aroclor 1016 E729-53-02	Corrected Percent Difference
	Spike Level	Amount Found	Percent[1] Difference	Spike Level	Amount Found	Percent Difference		
Total TCDF	200	190	-5	53	63	19	63	19
Total PnCDF	300	373	24	316	520	65	515	63
Total HxCDF	475	2557	438	185	2827	1428	2637	1325
Total HpCDF	400	261	-35	526	185		100	-81
OCDF	0	1		132	117		117	-11
Total PCDF	1375	3382	146	1212	3712	206	3432	183
Total TCDD	25	2	-92	53	45	-15	45	-15
OCDD	0	0		0	0		0	
2,3,7,8-TCDD	25	2	-92	53	46	-13	46	-13
2,3,7,8-/2,3,4,8-TCDF	200	190	-5	53	63	19	63	19
1,2,3,7,8-/1,2,3,4,8-PnCDF	150	150	0	158	183	16	183	16
2,3,4,7,8-PnCDF	150	220	47	158	327	107	327	107
1,2,3,4,7,8-/ 1,2,3,4,7,9-HxCDF	250	373	49	0	0	--	-190	--
1,2,3,6,7,8-HxCDF	50	0	-100	53	88	66	88	66
2,3,4,6,7,8-HxCDF	175	2080	1089	132	2660	1915	2660	1915
1,2,3,4,6,7,8-HpCDF	400	261	-35	526	132	-75	132	-75
1,2,3,4,7,8,9-HpCDF	--	0	--	--	0	--	-85	--

(1) Percent Difference = 100 (Measured Value - True Spike)/True Spike

(2) Corrected by subtracting the Aroclor 1016 "Baseline" PCDD/PCDF concentrations from the measured values.

Comparison with Parallel Analyses

The Battelle results are compared with the results reported by the other four laboratories participating in this program in Table 5.12. The data presented in this table for the other laboratories are taken from a draft statistical report (2) presented by Edo Pellizari (Research Triangle Institute, Research Triangle Park, N.C.) at a meeting of the five participating laboratories held in Washington, D.C. on November 19, 1986.

The Battelle results averaged over three replicate determinations are presented in Table 5.12 where the average reported values by the five collaborating laboratories conducting parallel analyses are also given.

The two sets of data compare favorably; except for hexachlorinated furans, the Battelle results are consistently lower overall than the data contributed by other laboratories. The general trends in the data such as very low values for PCDD/PCDF in mineral oil samples was apparent in all the reported data. The 30-year-old network transformer containing Askarel (Sample Number 3 in Table 5.12) had the highest reported PCDF level of all the in-service samples tested, approximately 32 ppm (w/w) using the average of all reported determinations.

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TABLE 5.12

MEAN FURAN RESULTS FOR SEVEN UTILITY DIELECTRIC FLUIDS

	Network Transformer No. 1	Network Transformer No. 2	Network Transformer No. 3	Network Transformer No. 4	Arc Furnace Transformer	Precipitator Transformer	Capacitor
Sample Identifier							
Fluid	Askarel	Askarel	Askarel	Mineral Oil	Mineral Oil	Askarel	Askarel
Years Service	20	28	30	UNK	UNK	27	UNK
Field Code	ISL-02-A	ISL-08-A	ISL-03-A	ISL-04-O	ISL-23 O	ISL-17-A	ISL-27
Laboratory Code	17	12	11	16	15	14	13
Battelle Furan Results							
Total TCDF	293	17	53	0	0	177	106
Total PnCDF	71	96	702	0	0	2076	21
Total HxCDF	15700	1263	24233	0	0	22000	143
Total HpCDF	249	490	3951	0	0	602	10
OCDF	330	403	2828	0	0	29	17
Total PCDF	16643	2269	31767	0	0	24884	297
Combined Furan Results (1)							
Total TCDF	415	128	158	1	1	782	586
Total PnCDF	991	279	1672	45	4	7746	221
Total HxCDF	4886	1012	10824	80	2	12931	67
Total HpCDF	1277	1822	6587	15	8	1767	12
OCDF	3346	6244	21830	14	18	188	21
Total PCDF	10914	9486	41071	154	34	23413	906
Percent Difference in Furan Congener Results (2)							
	52%	-76%	-23%	(3)	(3)	6%	-67%

(1) Combined Mean = Average reported values from the five participating laboratories.
(2) Percent Difference = (Combined Mean - Battelle Value)/Combined Mean X 100%
(3) Zero value division not possible.

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Section 6

DISCUSSION

This study produced a great amount of insight into the variability of analytical methods applied to trace analysis in a complex dielectric fluid matrix. PCB constitute the principal class of interferents for modern mass spectrometric methods when used to analyze polychlorinated aromatic species such PCDF and PCDD. Several conclusions can be drawn concerning performance of the methodology and the accurate screening of in-service dielectric fluids.

METHODS

The analytical procedures proved to be both rugged and reliable when used with labelled quantification and recovery standards. The quantification standards are used to calculate errors from extraction and sorbent partitioning losses. This is especially important in the Askarel and mineral oil matrix since the high organic burden found with this matrix requires secondary partitioning to remove interfering PCB and the background hydrocarbons from mineral oil.

Random outliers were observed in this study. One example was the elevated levels of hexachlorodibenzofuran found in the in-service analyses. This kind of acute error can arise from a mistake in the gravimetric preparation and dilution of the labelled recovery standard. Another possible cause for this random error is selective partitioning losses during sample clean up. Observing this type of spurious result indicates that the methods should be used with enough quality control checks to "flag" this type of error during the analysis. Using a second labeled internal standard to quantify the mass recovery of the quantification standards would identify this problem if it is related to quantification standard losses.

The observed data variability in this program was smaller than the participants had anticipated before the program began. In this very difficult matrix, a chlorinated oil, most analysts would predict a variability of one to two orders of magnitude (10x to 100x scatter around the true value). In most cases, the relative standard devia-tions were found to be less than 100 percent. The relative average deviation is the absolute difference between individual results and the mean (or true value), divided by the number of determinations. For samples such as the Aroclor 1242,

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Aroclor 1016, and mineral oil, the average deviation was most often less than 50 percent.-- This result was seen in individual laboratory replicate analyses and in grouped data from different laboratories.

IN-SERVICE LIQUID ANALYSES

The in-service liquids tested in this program were found to have relatively low amounts of PCDD in all samples tested. This result supports the hypothesis held by many chemists who have investigated Askarel and PCB-containing fluids that dioxins are not a major constituent of these mixtures. Except for the mineral oils, polychlorinated dibenzofurans, however, were found in trace amounts in all of the samples tested. The mineral oil samples were found to have extremely low amounts of PCDD/PCDF if any were found at all. Even the in-service liquid from Transformer 4, a network distribution transformer that was involved in a high energy excursion, was found to have minimal amounts of PCDD and PCDF. Transformer 4 had visible carbon, and major signs of scorching on the case and the area of the electrodes.

The Askarel transformers with more than 25 years of service tended to have higher levels of PCDF than the appliances put into service for a shorter time. It is possible that manufacturing controls improved as the production of Askarel matured in the 1960s such that Askarel was produced with lower levels of PCDF in the as-delivered fluids. It would be interesting to obtain replacement Askarel with a known lot number which matched a sample with extended service life. Analyzing samples of an unused Askarel and comparing the PCDF levels in a sample subjected to heavy load and environmental stress for an extended period would challenge the theory that utility operation contributed to elevated PCDF levels in Askarel fluids used in transformer and capacitor applications.

Two utility appliances were selected by the EPRI Technical Advisory Committee for special attention. An Askarel-filled electrostatic precipitator (ESP) transformer, and an oil-filled arc furnace transformer. Both of these applications involve extended thermal and electrical excursions during daily operation. These two samples were the "worst case" for testing service stress on the dielectric fluids. The arc furnace had essentially no PCDF or PCDD, and the PCDF levels in the ESP transformer appeared to be dictated by the age of the unit (27 years of service) and the fluid composition (Aroclor 1260). This extended period of stress did not appear to elevate the PCDF levels above typical Aroclor 1260 contamination levels.

One capacitor was tested in the in-service liquid screening program. It was found to have relatively low levels of PCDF compared to the other Askarel-filled units.

The observed distribution of PCDF seemed to follow a trend of higher concentrations of PCDF arising with the highest degree of chlorination Aroclor. The Aroclor 1260 with 60 percent chlorine by weight tended to have higher levels of PCDF in every comparable sample than Aroclor 1242, or Aroclor 1016, both of which have approximately 42 percent chlorine in the base Aroclor.

A second effect was observed in these data, the relative concentrations of PCDF congeners in any sample tends to favor the analogs with higher chlorination as the chlorination number of the underlying Aroclor increases. For instance the proportion of hexa, hepta, and octachlorodibenzofurans are higher in Aroclor 1260 than Aroclor 1242. If PCDF are formed by direct internal condensation with the addition of oxygen, HCl elimination can occur. This reaction would preferentially produce higher levels of chlorination in the product PCDF when high degrees of chlorination occur in the reactant PCB.

SUMMARY

This study was successful in developing a set of methodologies that reliably determine trace levels of PCDF and PCDD in a very difficult matrix -- chlorinated organic oil. The development of commercially available reference standards and the statistical validation of reference methods that can be used to analyze utility fluid was a major contribution to the quality and defensibility of data in this field. The method verification program showed that a range of instrumentation can be successfully used to measure trace levels of PCDF and PCDD in utility fluids. In general, the methods employing low resolution mass spectrometry require additional column chromatography to isolate PCDF/PCDD-enriched fraction for final gas chromatography. The precision of blind spiked samples was superior to the variance expected by experienced analysts who had previously performed this analysis. The availability of labelled quantification standards helped improve method performance and lower data scatter.

Although the in-service liquids tested in this program were a very limited set, there were interesting findings about the type and distribution of polychlorinated dioxin and furan species in the systems tested. Dioxin levels were very low, or not measurable in the liquids analyzed. The mineral-oil-filled equipment had levels that were found only at the quantification limits of the methods employed. Aroclor 1260 appears to have higher levels of PCDF with enrichment of the higher chlorine-number analogs, when compared to Aroclor 1242, 1016, or Aroclors with fewer chlorine atoms per molecule than 1260. This data base can not support the

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premise that the age of Askarel transformers tends to favor higher PCDF levels in older transformers since only four units were investigated.

Appendix A

IN-SERVICE LIQUID QUESTIONNAIRE

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GENERAL INFORMATION QUESTIONNAIRE

EPRI IN SERVICE LIQUIDS STUDY (RP 2028)

Respondant: _____

Samples provided	Number Shipped
Askarel	
Load Center/Network Transformers	_____
Rectifier Transformer	_____
Precipitator Transformer	_____
Capacitor	_____
Oil Filled	
Load Center/Network Transformer	_____

DATA SHEET FOR EACH SAMPLE

EPRI IN SERVICE LIQUIDS STUDY (RP 2028)

General Information

Voltage Rating: _____

Liquid Volume: _____

Manufacturer and Year: _____

PCB/Askarel Manufacturer: (if available)

_____

_____

PCB/Askarel Type:

_____

_____

PCB Levels: (for oil filled, if known)

_____

_____

Additional Equipment Identification: (name plate data)

_____

_____

Equipment Maintenance History

Length of Actual Service: _____

Major Maintenance:

_____

_____

Recorded Failure in Service:

_____

_____

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GENP 011114

DATA SHEET FOR EACH SAMPLE

Was Fluid Changed at Failure:

Any Record of Temperature Excursions:

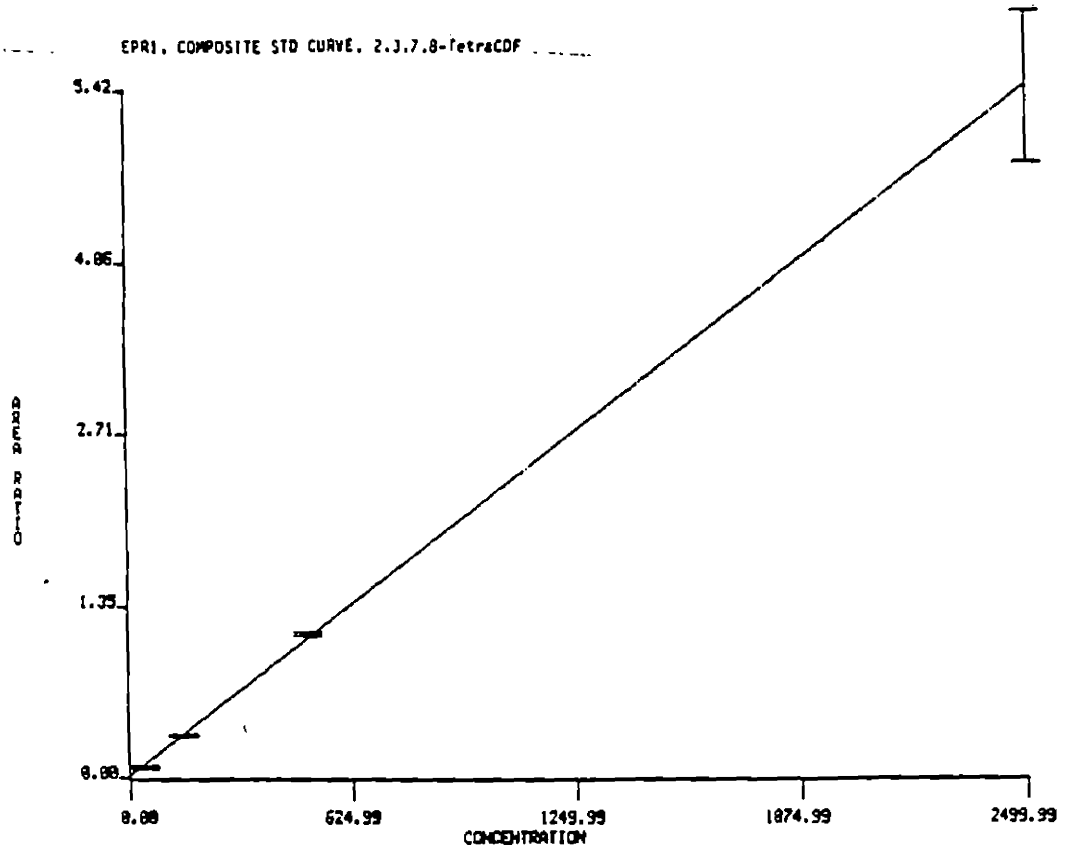
Are Maintenance Records Available:

Any Other Pertinent Information About This Unit or the Sample Liquid:

Appendix B

ANALYTICAL CURVE DATA FOR THE CROSS CHECK ANALYSES

GENP 011116

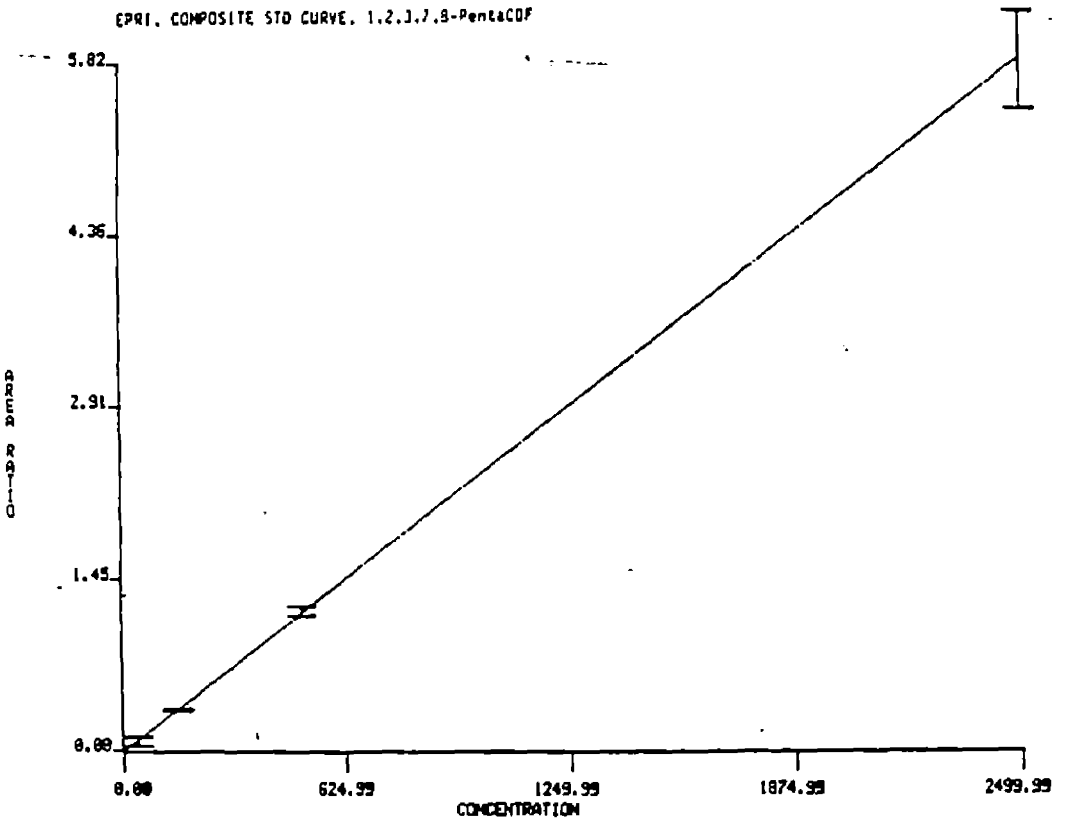


EPRI, COMPOSITE STD CURVE, 2.1.7.8-TetraCDF

X-VALUES	Y-VALUES				
39.9999	0.0794	0.0802	0.0766	0.0910	0.0868
149.9990	0.3249	0.3249	0.3394	0.3430	0.3441
499.9990	1.1476	1.1605	1.1407	1.1278	1.1336
2499.9900	5.8409	5.9660	5.7829	4.9650	4.4329

X-VALUE	Y-VALUE	STD DEV	RSD%	RES. E.	R.R.E.
DATA WITH RESPECT TO THE Y-VALUES					
40.0001	0.0833	0.0035	4.46	0.0086	0.0050
150.0000	0.3366	0.0093	2.70	0.0146	0.0084
500.0000	1.1431	0.0117	1.01	0.0185	0.0106
2500.0000	5.4247	0.6015	11.08	0.9510	0.5449
DATA WITH RESPECT TO THE REGRESSION LINE					
40.0001	0.0833	0.0271	32.36	0.0427	0.0245
150.0000	0.3366	0.0135	3.95	0.0212	0.0122
500.0000	1.1431	0.0435	3.96	0.0719	0.0412
2500.0000	5.4247	0.6016	11.08	0.9511	0.5445

A SLOPE OF 0.00216 GIVES AN INTERCEPT OF 0.02068
A SLOPE OF 442.02200 GIVES AN INTERCEPT OF -9.55865
THE DATA HAS A CORRELATION COEFFICIENT OF 0.99993



EPRI, COMPOSITE STD CURVE, 1,2,3,7,8-PentaCDF

X-VALUES	Y-VALUES				
40.0000	0.0989	0.1021	0.0961	0.0929	0.0926
150.0000	0.3347	0.3332	0.3345	0.3321	0.3603
499.9990	1.2183	1.2171	1.2201	1.1445	1.1469
2499.9900	6.2080	6.3210	5.9500	5.9030	5.2400

X-VALUE	Y-VALUE	STD DEV	RSD%	RES. E.	R R. E.
40.0000	0.0809	0.0396	49.19	0.0626	0.0337
150.0000	0.3441	0.0115	3.33	0.0182	0.0098
500.0000	1.1823	0.0398	3.35	0.0626	0.0339
2500.0000	5.8222	0.4165	7.15	0.6585	0.3546

DATA WITH RESPECT TO THE Y-VALUES

40.0000	0.0809	0.0396	49.19	0.0626	0.0337
150.0000	0.3441	0.0115	3.33	0.0182	0.0098
500.0000	1.1823	0.0398	3.35	0.0626	0.0339
2500.0000	5.8222	0.4165	7.15	0.6585	0.3546

DATA WITH RESPECT TO THE REGRESSION LINE

40.0000	0.0809	0.0419	51.91	0.0636	0.0353
150.0000	0.3441	0.0123	3.56	0.0194	0.0105
500.0000	1.1823	0.0446	3.76	0.0709	0.0380
2500.0000	5.8222	0.4165	7.15	0.6586	0.3546

A SLOPE OF 0.00233 GIVES AN INTERCEPT OF -0.00197
A SLOPE OF 429.03700 GIVES AN INTERCEPT OF 0.67517
THE DATA HAS A CORRELATION COEFFICIENT OF 0.99998

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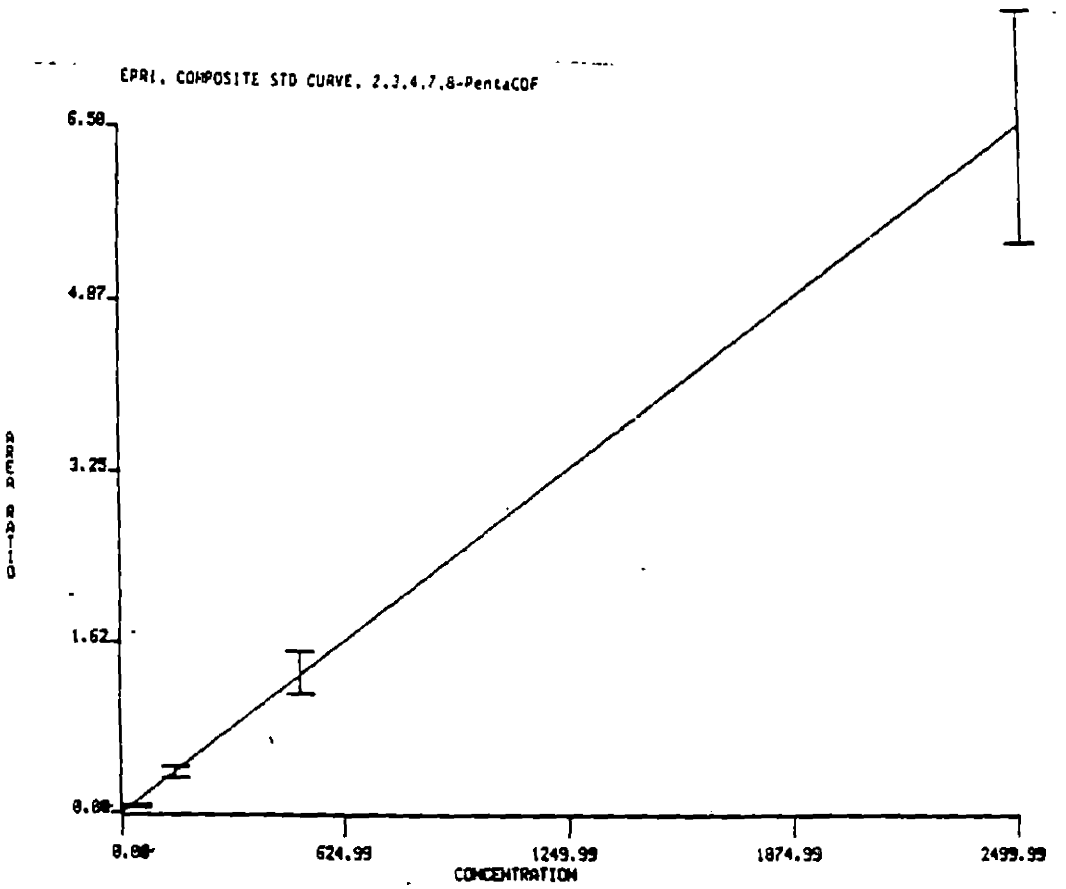


EPRI. COMPOSITE STD CURVE. 2,3,4,7,8-PentaCDF

X-VALUES	Y-VALUES				
40.0000	0.0703	0.0743	0.0714	0.0899	0.0810
150.0000	0.4369	0.4409	0.4472	0.3384	0.3394
499.9990	1.5357	1.4388	1.4633	1.1333	1.1114
2499.9900	7.4239	7.4420	7.0960	5.1620	5.3965

X-VALUE	Y-VALUE	STD DEV	RSDX	RES. E.	A R E.

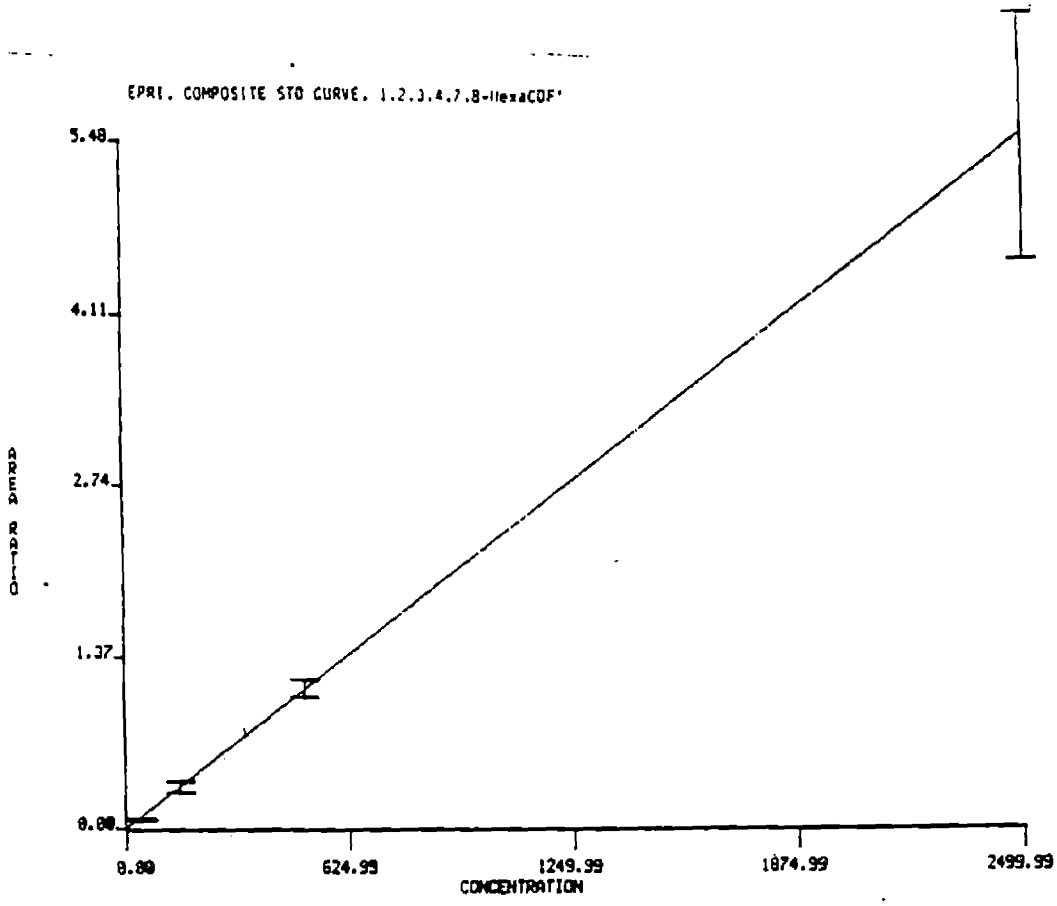
DATA WITH RESPECT TO THE Y-VALUES

40.0000	0.0773	0.0080	10.33	0.0113	0.0055
150.0000	0.4005	0.0564	14.06	0.0797	0.0383
500.0000	1.3403	0.2015	15.03	0.2850	0.1370
2500.0000	6.5040	1.1296	17.36	1.5974	0.7678

DATA WITH RESPECT TO THE REGRESSION LINE

40.0000	0.0772	0.0069	47.62	0.0921	0.0251
150.0000	0.4005	0.0566	14.13	0.0801	0.0385
500.0000	1.3403	0.2050	15.29	0.2900	0.1394
2500.0000	6.5040	1.1296	17.36	1.5975	0.7679

A SLOPE OF 0.00260 GIVES AN INTERCEPT OF 0.00536
A SLOPE OF 384.30900 GIVES AN INTERCEPT OF -2.06203
THE DATA HAS A CORRELATION COEFFICIENT OF 0.99999



EPRI. COMPOSITE STD CURVE. 1.2.3.4.7.8-HexaCDF

X-VALUES	Y-VALUES				
40.0000	0.0702	0.0777	0.0708	0.0864	0.0723
150.0000	0.3139	0.3101	0.3073	0.4198	0.3709
499.9990	1.1786	1.0725	1.2032	1.0597	1.0391
2499.9900	5.6339	5.7745	5.4700	4.6040	4.3120

X-VALUE	Y-VALUE	STD DEV	RSD%	RES. E.	R R E.
DATA WITH RESPECT TO THE Y-VALUES					
40.0000	0.0764	0.0069	8.39	0.0102	0.0058
150.0000	0.3425	0.0447	13.04	0.0707	0.0403
500.0000	1.1145	0.0674	6.05	0.1068	0.0609
2500.0000	5.4872	0.9961	18.15	1.5750	0.8974
DATA WITH RESPECT TO THE REGRESSION LINE					
40.0000	0.0764	0.0199	25.94	0.0314	0.0179
150.0000	0.3425	0.0455	13.28	0.0720	0.0410
500.0000	1.1145	0.0688	6.17	0.1088	0.0620
2500.0000	5.4872	0.9961	18.15	1.5750	0.8974

A SLOPE OF 0.00219 GIVES AN INTERCEPT OF 0.00367
A SLOPE OF 455.84400 GIVES AN INTERCEPT OF -2.38863
THE DATA HAS A CORRELATION COEFFICIENT OF 0.99998

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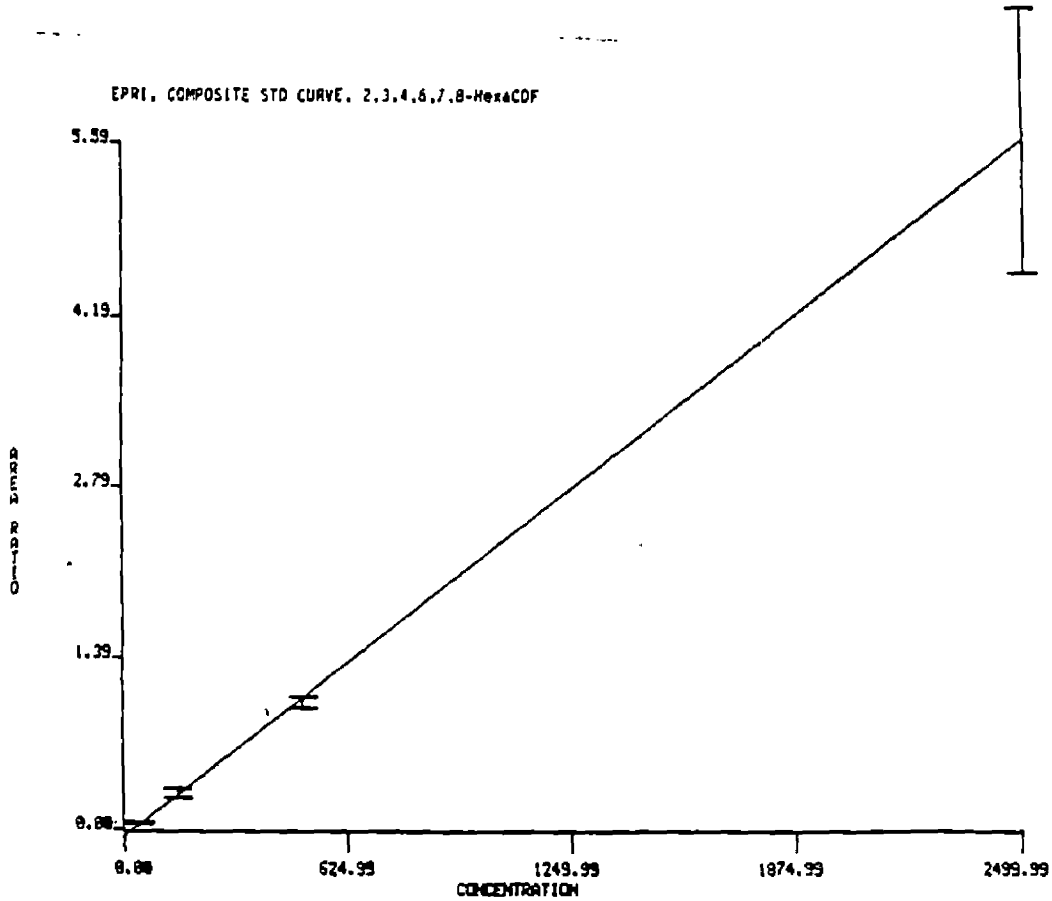
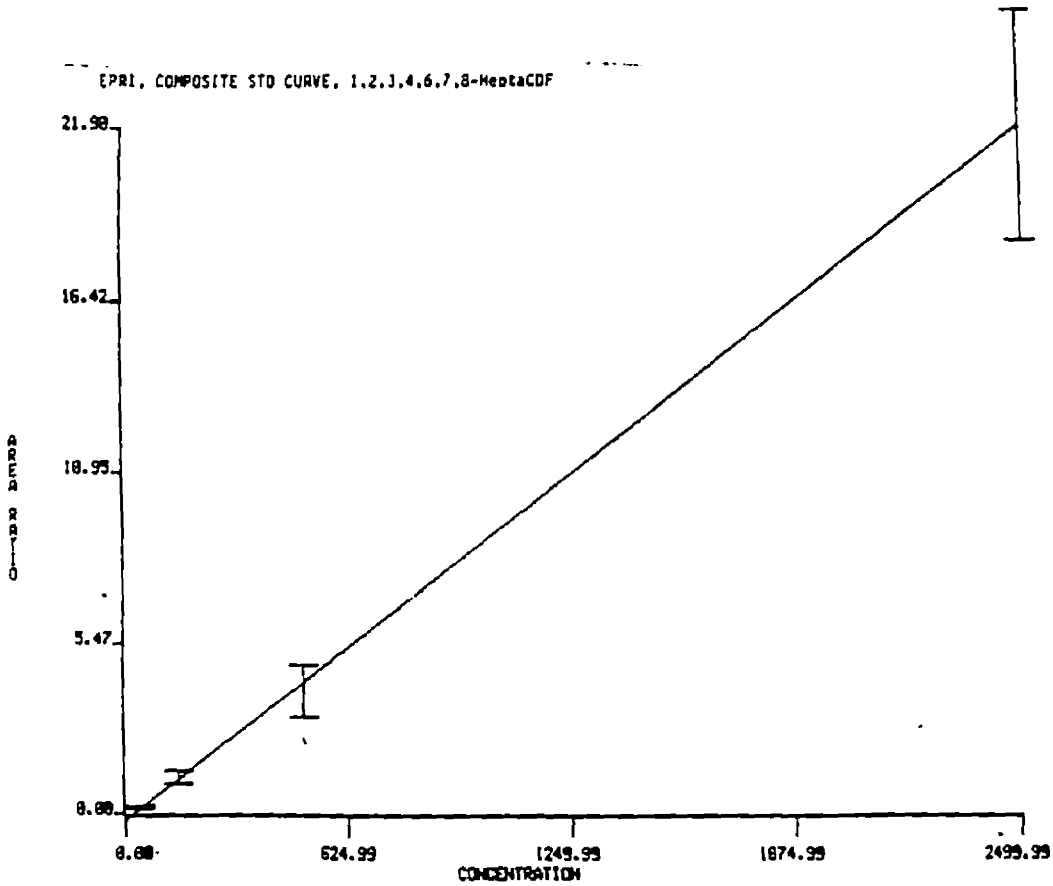


EPRI, COMPOSITE STD CURVE, 2,3,4,6,7,8-HexaCDF

X-VALUES	Y-VALUES				
40.0000	0.0598	0.0437	0.0573	0.0710	0.0715
150.0000	0.2834	0.2798	0.2854	0.3753	0.2986
499.9990	1.0389	1.0862	0.9732	1.0334	1.0598
2499.9900	5.4259	5.5679	5.3115	4.3340	7.3400

X-VALUE	Y-VALUE	STD DEV	RSD%	RES. E.	R.A.E.
DATA WITH RESPECT TO THE Y-VALUES					
40.0000	0.0647	0.0065	9.95	0.0092	0.0052
150.0000	0.3046	0.0402	13.19	0.0569	0.0325
500.0000	1.0383	0.0419	4.03	0.0593	0.0339
2500.0000	5.5957	1.0892	19.46	1.5403	0.8798
DATA WITH RESPECT TO THE REGRESSION LINE					
40.0000	0.0647	0.0248	38.25	0.0350	0.0200
150.0000	0.3046	0.0429	14.06	0.0606	0.0347
500.0000	1.0383	0.0429	6.05	0.0889	0.0508
2500.0000	5.5957	1.0892	19.46	1.5404	0.8798

A SLOPE OF 0.00225 GIVES AN INTERCEPT OF -0.04686
A SLOPE OF 443.63000 GIVES AN INTERCEPT OF 20.79250
THE DATA HAS A CORRELATION COEFFICIENT OF 0.99993



EPRI, COMPOSITE STD CURVE, 1,2,3,4,6,7,8-HeptaCDF

X-VALUES	Y-VALUES				
40.0000	0.2673	0.2037	0.2485	0.2504	0.3065
150.0000	1.3476	1.1855	1.4126	0.6935	1.1430
499.9990	3.2079	4.2144	3.9305	4.0030	3.7310
2499.9900	23.2270	22.5794	26.0165	23.0300	21.7000

X-VALUE	Y-VALUE	STD DEV	RSD%	RES. E.	R.R.E.
DATA WITH RESPECT TO THE Y-VALUES					
40.0000	0.2519	0.0340	13.46	0.0937	0.0079
150.0000	1.1927	0.1814	15.21	0.2871	0.0421
500.0000	3.9437	0.8478	21.49	1.3404	0.1765
2500.0000	21.9055	3.7442	17.09	5.9200	0.8674
DATA WITH RESPECT TO THE REGRESSION LINE					
40.0000	0.2519	0.1355	53.80	0.2143	0.0314
150.0000	1.1927	0.2041	17.27	0.3258	0.0478
500.0000	3.9437	0.8915	22.60	1.4096	0.2066
2500.0000	21.9055	3.7445	17.09	5.9209	0.8677

A SLOPE OF 0.00883 GIVES AN INTERCEPT OF -0.22126
A SLOPE OF 113.30400 GIVES AN INTERCEPT OF 23.04810
THE DATA HAS A CORRELATION COEFFICIENT OF 0.99985

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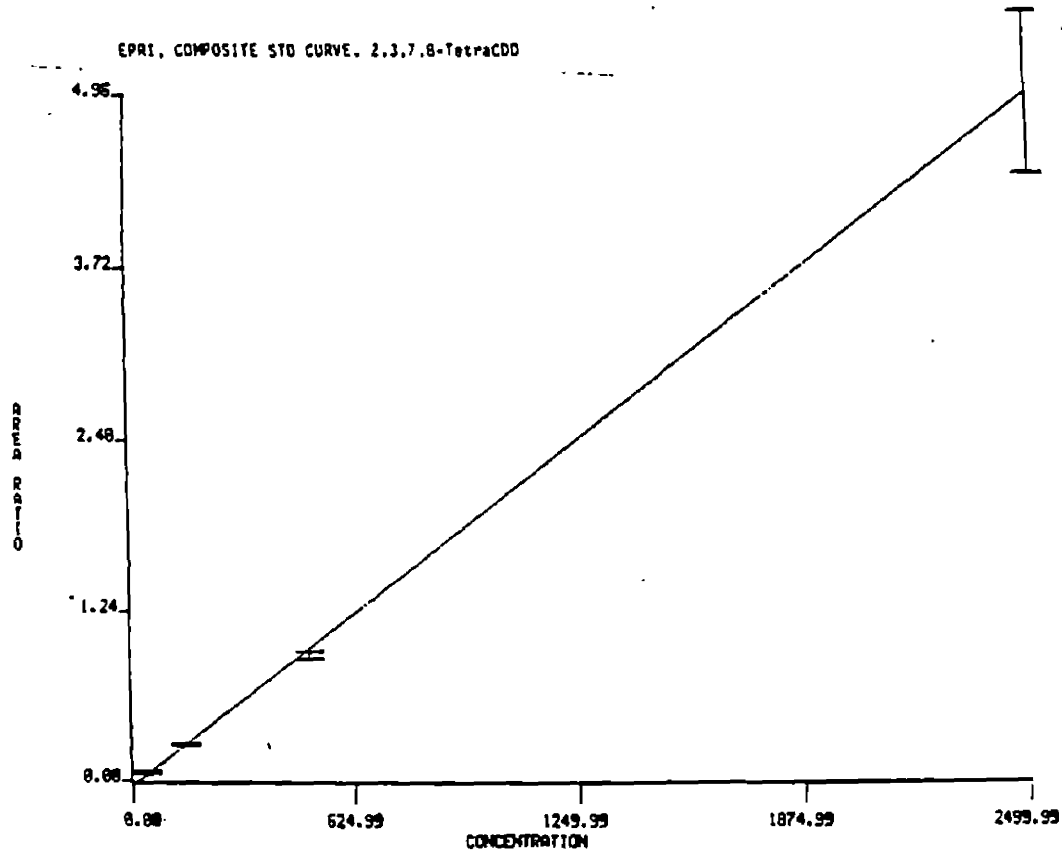

EPRI, COMPOSITE STD CURVE, 2,3,7,8-TetraCDD

X-VALUES	Y-VALUES				
40.0000	0.0602	0.0605	0.0619	0.0765	0.0677
150.0000	0.2680	0.2833	0.2703	0.2758	0.2617
499.9990	0.9703	0.9534	0.9117	0.8976	0.9212
2499.9900	4.8330	4.8235	4.7193	4.9290	4.1390

X-VALUE	Y-VALUE	STD DEV	RSD%	RES. E.	R.R.E.
DATA WITH RESPECT TO THE Y-VALUES					
40.0000	0.0663	0.0066	9.87	0.0104	0.0067
150.0000	0.2760	0.0061	2.19	0.0096	0.0062
500.0000	0.9282	0.0278	2.98	0.0439	0.0282
2500.0000	4.9490	0.6066	12.20	0.9592	0.6149
DATA WITH RESPECT TO THE REGRESSION LINE					
40.0000	0.0663	0.0233	35.10	0.0368	0.0236
150.0000	0.2760	0.0128	4.62	0.0202	0.0130
500.0000	0.9282	0.0493	5.30	0.0779	0.0499
2500.0000	4.9490	0.6067	12.20	0.9592	0.6150

A SLOPE OF 0.00199 GIVES AN INTERCEPT OF -0.03408
A SLOPE OF 500.33900 GIVES AN INTERCEPT OF 17.03420
THE DATA HAS A CORRELATION COEFFICIENT OF 0.99993

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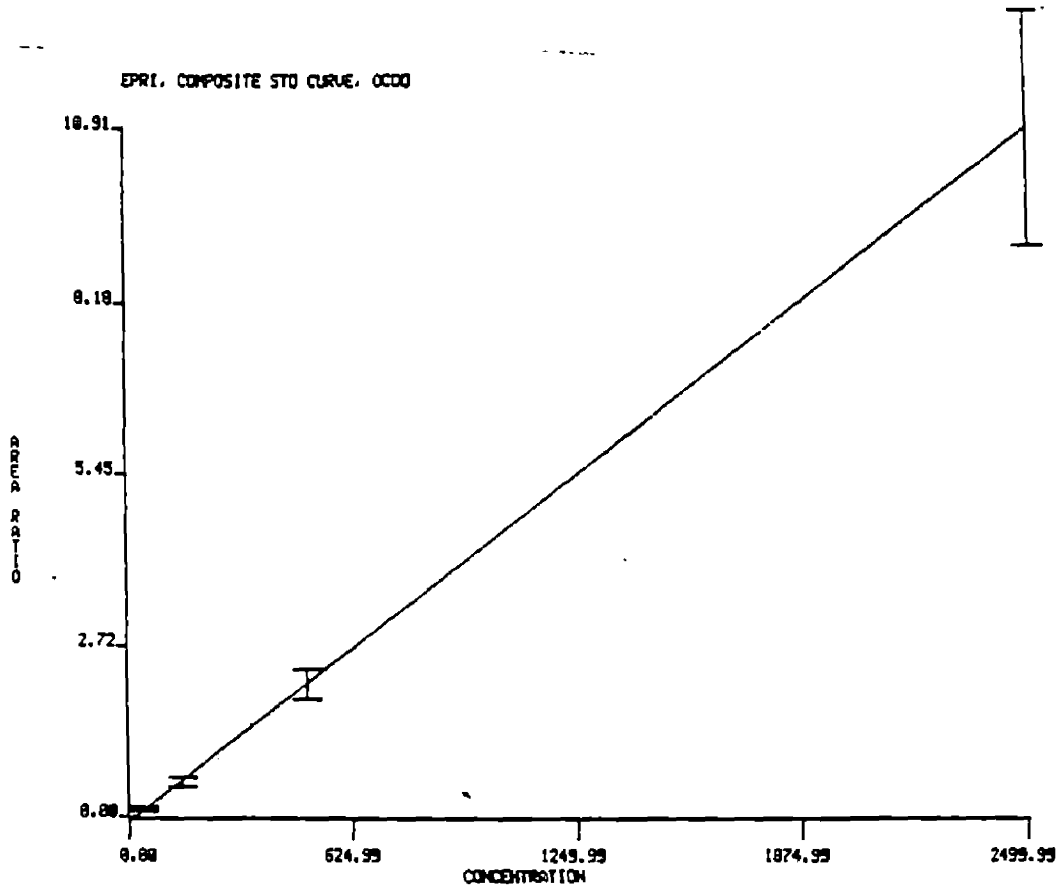

EPRI. COMPOSITE STD CURVE. OCDD

X-VALUES	Y-VALUES				
40.0000	0.0978	0.1027	0.1092	0.1533	0.1589
150.0000	0.5264	0.5040	0.5418	0.4943	0.5581
499.9990	2.2410	2.0311	2.1435	2.4200	2.1320
2499.9900	12.3695	12.5445	12.8465	9.9800	9.1020

X-VALUE	Y-VALUE	STD DEV	RSD%	RES. E.	R R E.
DATA WITH RESPECT TO THE Y-VALUES					
40.0000	0.1247	0.0268	21.46	0.0423	0.0124
150.0000	0.5544	0.0713	12.85	0.1127	0.0329
500.0000	2.1209	0.2292	10.80	0.3623	0.1057
2500.0000	10.9141	1.8884	17.30	2.9858	0.8709
DATA WITH RESPECT TO THE REGRESSION LINE					
40.0000	0.1247	0.0394	31.56	0.0623	0.0182
150.0000	0.5544	0.0774	13.96	0.1224	0.0357
500.0000	2.1209	0.2292	10.80	0.3623	0.1057
2500.0000	10.9141	1.8884	17.30	2.9858	0.8709

A SLOPE OF 0.00439 GIVES AN INTERCEPT OF -0.07755
A SLOPE OF 227.47100 GIVES AN INTERCEPT OF 17.64260
THE DATA HAS A CORRELATION COEFFICIENT OF 0.99999

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Appendix C

LETTER REPORT FROM UMEA

Umeå Universitet
901 87 Umeå

86-06-23

Analys results EPRI-pro jekt 2028

Formation of PCDD and PCDF in askarel and contaminated mineral oil equipment.

Contents.

Normalized raw counts for regression lines.

Levels in baseline liqids

Levels in inservice liqids:
TCDF/TCDDs
PnCDF/PnCDDs and HxCDF/HxCDDs
HpCDF/HpCDDs and OCDF/OCDDs

GC/MS Conditions.

Clean-up

By
Lars-Owe Kjeller
Umeå Universitet
901 87 umeå

Sweden.

783937

GENP 011126

LARS-OWE KJELLER
Umeå Universitet
901 87 UMEÅ Sweden

Umeå 85-06-18

EPRI-prodjekt 2028

Calibration curve, raw counts normalized for ^{13}C-internalstandard.

Concentration injected pg	10	10	10
2.3.7.8-TCDF	9.5	12.4	10.8
2.3.7.8-TCDD	3.4	7.9	7.0
1.2.3.7.8-PnCDF	6.0	9.5	8.1
2.3.4.7.8-PnCDF	4.9	8.5	7.5
1.2.3.4.7.8-HxCDF	3.9	7.8	7.3
2.3.4.6.7.8-HxCDF	1.7	4.6	4.5
1.2.3.4.6.7.8-HpCDF	1.4	3.9	3.8
OCDF	ND	ND	ND

Concentration injected pg	40	40	40
2.3.7.8-TCDF	38.5	42.3	42.6
2.3.7.8-TCDD	18.0	27.6	27.8
1.2.3.7.8-PnCDF	24.8	35.0	37.8
2.3.4.7.8-PnCDF	17.8	29.0	29.0
1.2.3.4.7.8-HxCDF	19.1	27.3	27.3
2.3.4.6.7.8-HxCDF	8.8	18.2	18.2
1.2.3.4.6.7.8-HpCDF	5.8	13.0	13.0
OCDF	0.9	2.0	1.5

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Concentration injected pg	150	150	150
2.3.7.8-TCDF	160.9	155.1	160.1
2.3.7.8-TCDD	100.1	94.2	105.7
1.2.3.7.8-PnCDF	133.4	124.5	137.9
2.3.4.7.8-PnCDF	100.7	98.2	118.1
1.2.3.4.7.8-HxCDF	105.3	82.2	103.3
2.3.4.6.7.8-HxCDF	51.7	43.1	68.9
1.2.3.4.6.7.8-HpCDF	39.3	33.7	53.4
OCDF	5.6	2.1	6.7

Concentration injected pg	500	500	500
2.3.7.8-TCDF	545.3	570.0	550.9
2.3.7.8-TCDD	370.9	371.0	359.9
1.2.3.7.8-PnCDF	470.0	471.0	467.7
2.3.4.7.8-PnCDF	403.5	401.0	402.1
1.2.3.4.7.8-HxCDF	339.8	338.0	341.9
2.3.4.6.7.8-HxCDF	214.3	203.0	216.2
1.2.3.4.6.7.8-HpCDF	182.4	177.0	181.9
OCDF	23.5	17.0	17.4

Concentration injected pg	2500	2500	2500
2.3.7.8-TCDF	2791.7	2769.4	2767.9
2.3.7.8-TCDD	1925.0	1806.1	1924.3
1.2.3.7.8-PnCDF	2412.8	2384.7	2413.1
2.3.4.7.8-PnCDF	2012.5	1979.0	2061.7
1.2.3.4.7.8-HxCDF	1780.1	1760.3	1793.3
2.3.4.6.7.8-HxCDF	1118.9	1145.8	1100.5
1.2.3.4.6.7.8-HpCDF	973.4	889.8	952.1
OCDF	166.4	125.8	169.1

783939

GENP 011128

Concentration injected pg	7500	7500	7500
2.3.7.8-TCDF	8731.9	8593.7	9037.3
2.3.7.8-TCDD	5966.9	5480.0	5735.6
1.2.3.7.8-PnCDF	7401.8	7264.2	6989.2
2.3.4.7.8-PnCDF	6219.0	5897.8	5980.2
1.2.3.4.7.8-HxCDF	5565.6	5502.2	5494.7
2.3.4.6.7.8-HxCDF	3532.6	3577.6	3638.1
1.2.3.4.6.7.8-HpCDF	3067.2	2790.8	2768.4
OCDF	513.6	419.3	433.1

Characterization for the regression line $y = a*x + b$
y = amont of Dioxin/furans. x = normalized counts.

	a	b	Residual standard deviation.
2.3.7.8-TCDF	0.853	29.89	135.7
2.3.7.8-TCDD	1.306	18.28	117.5
1.2.3.7.8-PnCDF	1.037	9.24	77.5
2.3.4.7.8-PnCDF	1.240	6.76	76.1
1.2.3.4.7.8-HxCDF	1.357	24.50	38.0
2.3.4.6.7.8-HxCDF	0.487	-21.26	34.1
1.2.3.4.6.7.8-HpCDF	2.595	31.83	159.6
OCDF[1]	0.061	-4.44	22.6

[1] 10 pg conc. not included.

783940

GENP 011129

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EPRI-prodject 2028

Levels of dioxins and dibensofurans in inservice liquid (ng/g)[1 2]

Att nr. 41159-11-	11	12	13	14	15	16	17
TCDF							
1368	<1.1	0.46	11	1.2	<0.04	<0.05	<0.34
1379/1378	5.4	2.0	5.0	8.9	<0.04	<0.05	<0.34
1347	4.0	1.4	130	6.1	<0.04	<0.05	3.0
1468	5.4	1.9	Int	23	<0.04	<0.05	3.0
1247/1367	8.5	2.1	Int	27	<0.04	<0.05	14
1348	2.3	2.2	2.5	4.8	<0.04	<0.05	5.4
1346/1248	85	11	130	170	<0.04	<0.05	75
1246/1237/1268/1478/1369	14	6.7	95	61	<0.04	<0.05	31
1234/2349	5.4	1.4	32	9.7	<0.04	<0.05	5.4
1238/1467/2468/1236	2.3	4.2	9.1	15	<0.04	<0.05	8.4
1349	<1.1	<0.14	<0.03	<0.32	<0.04	<0.05	<0.34
1278	9.9	8.4	29	110	<0.04	<0.05	56
1267/1279	4.0	2.5	7.6	16	<0.04	<0.05	13
1469	<1.1	<0.14	<0.03	<0.32	<0.04	<0.05	<0.34
1249	<1.1	<0.14	<0.03	<0.32	<0.04	<0.05	<0.34
2368	8.5	4.6	Int	41	<0.04	<0.05	16
2467	4.5	0.81	37	31	<0.04	<0.05	2.3
1239/2347	9.0	3.9	140	68	<0.04	<0.05	13
1269	<1.1	<0.14	<0.03	<0.32	<0.04	<0.05	<0.34
2378	18	14	8.8	170	<0.04	<0.05	120
2348	20	7.0	86	120	<0.04	<0.05	40
2346	<1.1	<0.14	<0.03	<0.32	<0.04	<0.05	<0.34
2367	24	9.5	8.8	77	<0.04	<0.05	64
3467	<1.1	<0.14	<0.03	<0.32	<0.04	<0.05	<0.34
1289	<1.1	<0.14	<0.03	<0.32	<0.04	<0.05	<0.34
TOT TCDF	240	86	740	960	ND	ND	470
REC ^{13}C-2378-TCDF	45%	50%	47%	54%	65%	67%	81%
TCDD	No TCDD isomers detected. Detection limit 0.2 ng/g						
REC ^{13}C-2378-TCDD	41%	48%	49%	50%	56%	44%	26%

GENP 011130

783941

Att nr. 41159-11-

PnCDF

13468
12468
23479
13478
13479/12368
12478
12479/13467
12467
23469/12347
13469
12348/12378
12346
12379
12367
12469/23489
13489
12369
23468
12349
12489
23478
12389
23467
TOT PnCDF

REC ^{13}C-12378-PnCDF

PnCDD

HxCDF

123468
124678/134679
134678
124679
123479/123478
123678
124689
123467
123679
123469/123689
123789
123489
234678
TOT HxCDF

REC ^{13}C-123478-HxCDF

HxCDD

GENP 011131

783942

11	12	13	14	15	16	17
<1.1	2.5	2.0	33	<0.05	<0.06	<0.36
27	5.6	7.0	200	<0.05	<0.06	30
<1.1	1.9	3.0	65	<0.05	<0.06	<0.36
<1.1	<0.17	<0.04	<0.35	<0.05	<0.06	<0.36
23	24	<0.04	490	<0.05	<0.06	54
300	57	Int	2500	<0.05	<0.06	280
<1.1	3.1	2.0	81	<0.05	<0.06	16
76	11	15	360	<0.05	<0.06	58
150	14	15	340	<0.05	<0.06	60
<1.1	<0.17	<0.04	<0.35	<0.05	<0.06	<0.36
1100	100	13	1000	<0.05	<0.06	320
35	4.3	1.5	<0.35	<0.05	<0.06	6.0
<1.1	4.3	0.98	110	<0.05	<0.06	18
200	22	29	1200	<0.05	<0.06	130
<1.1	<0.17	<0.04	49	<0.05	<0.06	<0.36
<1.1	<0.17	<0.04	49	<0.05	<0.06	<0.36
<1.1	<0.17	<0.04	<0.35	<0.05	<0.06	2.0
<1.1	1.9	3.5	66	<0.05	<0.06	10
<1.1	<0.17	<0.04	<0.35	<0.05	<0.06	<0.36
21	4.3	21	1100.	<0.05	<0.06	4.0
110	37	40	1900	<0.05	<0.06	560
<1.1	<0.17	1.5	81	<0.05	<0.06	<0.36
7.0	6.5	6.0	200	<0.05	<0.06	50
2100	310	160	10000	ND	ND	1600
55%.	47%	52%	57%	73%	63%	86%

No PnCDD isomers detected. Detection limit 0.2 ng/g

410	23	0.35	100	<0.06	<0.07	110
490	43	0.86	380	<0.06	<0.07	72
800	86	4.8	1500	<0.06	<0.07	490
<1.7	11	0.35	160	<0.06	<0.07	15
5300	570	14	4200	<0.06	<0.07	1300
530	47	2.3	1000	<0.06	<0.07	83
310	93	2.5	1100	<0.06	<0.07	68
1100	70	1.6	460	<0.06	<0.07	230
30	10	1.6	130	<0.06	<0.07	15
190	18	<0.05	670	<0.06	<0.07	52
100	17	0.19	230	<0.10	<0.12	36
330	34	1.5	1400	<0.10	<0.12	49
120	18	0.75	470	<0.10	<0.12	73
9700	1000	31	12000	ND	ND	2600.
49%	50%	51%	56%	78%	69%	95%

No HxCDD isomers detected. Detection limit 0.2 ng/g

Act nr. 41159-11-	11	12	13	14	15	16	17
HpCDF[3]							
1234678	150	1000	1.7	880	<0.06	<0.07	22
1234679	16	40	<0.07	170	<0.06	<0.07	1.8
1234689	150	1000	1.2	610	<0.06	<0.07	15
1234789	80	460	0.27	470	<0.06	<0.07	3.8
TOT HpCDF	400	2500	3.2	2100	ND	ND	41
HpCDD	No HpCDD isomers detected. Detection limit 0.5 ng/g						
OCDF							
12346789	20000[4]	10000	5.4	100	<3.3	<3.8	5400
OCDD							
12346789	<10	7.7	<0.73	<4.7	<1.2	8.5	<5.2
REC ^{13}C-OCDD	ND	45%	41%	69%	43%	41%	100%

[1] All quantifications are made with the ^{12}C-compunds in RADIAN calibration standards set.

[2] Corection for recovery is made.

[3] Corection for recovery made with ^{13}C-123478-HxCDF spike.

[4] The values ar not corected for recovery.

783943

GENP 011132

High Resolution Gas Chromatography and Mass Spectrometry Conditions

Column

SUPELCO SP-2330, Fused Silica

Length	60	m
Diameter	0.25	mm
Film Thickness	0.20	um

GC-Conditions

HP 5890 with Helium as carrier gas

Column pressure	40 psi
Temperatur program:	100°C for 2 min splittless, to 180°C with 20°C/min, to 250°C with 3°C/min, hold at 250°C for 30 min.
Injections volym	1-3 ul

MS-Conditions

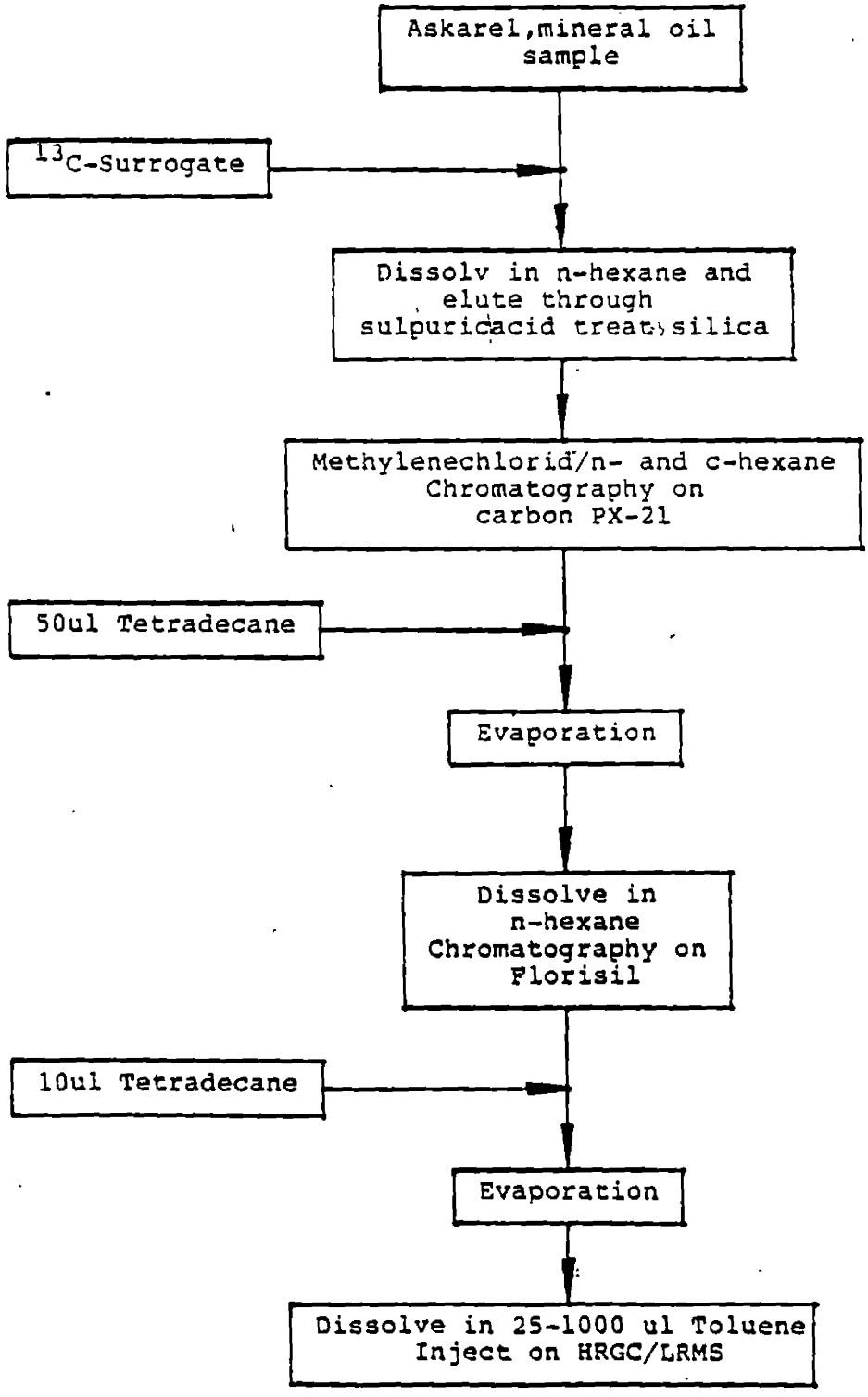
Instrument: VG 12-250 Low Resolution Quadrupole in EI mode

Electrone Energi	37	eV
Source Potential	9	V
Repeller Voltage	9	V
Amplifier Gain	Low sens SIR	
Multiplier Voltage	2000	V
Tuning Calibrant	PFK	
Source Temp.	200	°C
Transfer Line Temp.	250	°C

783944

GENP 011133

Clean-up technique for the determination of PCDF in askarel and miniral oil



783945

GENP 011134

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EPRI-prodject 2028

Levels of dioxins and dibensofurans in baseline liquids

Att nr. E729-53-	01	02	03
2.3.7.8-TCDF	89	62	1300
2,3,4,8-TCDF	100	<0.8	ND[1]
Tot. TCDF's	190	62	2900
REC ^{13}C-2378-TCDF	60%	60%	67%
2.3.7.8-TCDD	14	38	92
Tot. TCDD's	14	38	92
REC ^{13}C-2378-TCDD	67%	58%	62%
1.2.3.4.8-/ 1.2.3.7.8-PnCDF	160	190	400
2.3.4.7.8-PnCDF	160	180	370
Tot. PnCDF's	320	370	1800
REC ^{13}C-12378-PnCDF	59%	63%	58%
1.2.3.7.8-PnCDD	<1.5	<1.3	<1.6
Tot. PnCDD's	ND	ND	ND
1.2.3.4.7.9-/ 1.2.3.4.7.8-HxCDF	290	<1.1	1400
1.2.3.6.7.8-HxCDF	31	40	370
1.2.3.7.8.9-HxCDF	<2.1	<1.1	<1.8
2.3.4.6.7.8-HxCDF	170	110	350
Tot. HxCDF's	490	150	2800
REC ^{13}C-123478-HxCDF	59%	66%	69%
1.2.3.4.7.8-HxCDD	<2.7	<2.2	<2.4
1.2.3.6.7.8-HxCDD	<2.7	<2.2	<2.4
1.2.3.7.8.9-HxCDD	<2.7	<2.2	<2.4
Tot. HxCDD's	ND	ND	ND
1,2,3,4,6,7,8-HpCDF	450	630	1000
Tot. HpCDF's	450	630	2300
Tot. HpCDD's	ND	ND	ND
OCDF	ND[2]	56	3800
OCDD	ND[2]	<10	<8.1
REC ^{13}C-OCDD	ND[2]	76%	110%

783946

GENP 011135

The values are given in ng/g

ND = Not Detected.

ND^1 = Not detected due to interferens from the big 2,3,7,8-TCDF peak.

ND^2 = Not detected , no singnal for 13C-OCDDn.

GENP 011136

783947

0026-895X/88/020229-09$02.00/0
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MOLECULAR PHARMACOLOGY, 34:229-237

Kinetic and Equilibrium Studies of *Ah* Receptor-Ligand Binding: Use of [^{125}I]2-Iodo-7,8-dibromodibenzo-*p*-dioxin

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SUMMARY

In this report, we have used the radioligand [^{125}I]2-iodo-7,8-dibromo-dibenzo-*p*-dioxin to describe the kinetics of ligand binding to the *Ah* receptor prepared from C57BL/6J mouse liver. The higher specific activity of this radioligand (2176 Ci/mmol), compared with the usual tritiated ligand [1,6-^{3}H]2,3,7,8-tetrachlorodibenzo-*p*-dioxin (58 Ci/mmol) permitted the study of ligand-receptor interactions at much lower component concentrations. For this radioiodinated ligand, Scatchard analysis of saturation binding curves, determined at six different protein concentrations, indicated that the apparent equilibrium dissociation constant, K_D, was directly related to the dilution of the receptor preparation; for example, at 1160 μg of protein/ml, $K_D = 1.6 \times 10^{-10}$ M; at 36 μg of protein/ml, $K_D = 1.2 \times 10^{-11}$ M. Extrapolation of this function to infinite receptor dilution yielded $K_D = 6 \times 10^{-12}$ M. The addition of 70 μg/ml of bovine serum albumin to a receptor preparation of 30 μg of protein/ml produced a 10-fold decrease in the slope of the Scatchard plot (i.e., 10-fold increase in the apparent K_D). Conversely, enrichment of the receptor by high performance liquid chromatography led to an increased slope and thus decreased estimate of K_D. The association rate constant (k_1), calculated from the integrated second-order rate equation, was 2.8×10^{10} M^{-1} hr^{-1} and, from the initial velocity equation, had a value of 5.25×10^{10} M^{-1} hr^{-1}. The dissociation rate constant was biphasic, consisting of a predominant fast component with a rate constant of 0.36 hr^{-1} (k_{-1}) and a slower component with a rate constant of 4.2–9.4 $\times 10^{-3}$ hr^{-1} (k_{-2}). Higher protein concentrations produced a decrease in estimates of k_1 but not k_{-1} or k_{-2}. The K_D determined from the ratio of the kinetic rate constant, $k_{-1}/k_1 = 6.9 \times 10^{-12}$ M, is in excellent agreement with that derived from the results of equilibrium binding experiments extrapolated to infinite dilution, $K_D = 6 \times 10^{-12}$ M. The decrease in K_D, observed in equilibrium binding studies upon dilution of the receptor preparation, is best explained by a more accurate classification of "free" radioligand at lower protein concentrations. Finally, ligand binding to the *Ah* receptor is best described by a two-step process, the formation of an initial complex, characterized by rapid ligand dissociation, which undergoes transformation to a second distinct complex displaying a much slower ligand dissociation rate.

783948

Planar halogenated aromatic hydrocarbons elicit a variety of biological effects, including induction of cytochrome P-450 isozymes, epidermal hyperplasia and metaplasia, thymic involution, teratogenesis, tumor promotion, and lethality (1). These diverse biological responses are believed to arise from the altered gene expression that is consequent to the high affinity, stereospecific binding of planar halogenated aromatic hydrocarbons to a soluble protein known as the *Ah* receptor (1, 2).

Like the steroid hormone receptors, the *Ah* receptor is routinely found in the high speed supernatant fraction ("cytosol") of tissue homogenates. After ligand binding and a poorly defined "transformation"[1] step, the ligand-receptor complex displays an increased affinity for polyanions, such as DNA (3–6), and functions as a transcriptional activator (7, 8). The *Ah* receptor also bears many physicochemical similarities to steroid hormone receptors, including a high molecular weight "aggregate" form in low salt buffers (M_r 2.5–2.8 $\times 10^5$) that dissociates to a low molecular weight "monomeric subunit" form in high salt buffers (M_r approximately 1×10^5) (9). For the *Ah* receptor present in the hepatic cytosol of the C57BL/6 mouse and the Sprague-Dawley rat estimates of sedimentation coefficients,

This paper is dedicated to Dr. Elizabeth Miller.

This work was supported in part by the National Institute of Environmental Health Science Grant ES-01884, National Cancer Institute Core Grant-07175, and National Cancer Institute Postdoctoral Training Grant T32-CA09020.

[1] Receptor transformation is defined in this report as an alteration in the physiochemical nature of the ligand-receptor complex, which results in an increased affinity for DNA or other polyanionic matrices.

ABBREVIATIONS: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; MOPS, 3-(*N*-morpholino)propanesulfonic acid; K_D, equilibrium dissociation constant; [*L*], concentration of unbound ligand; [*R*], concentration of unliganded receptor; [*LR*], concentration of receptor-ligand complex; [*LR*]$_e$, receptor-ligand complex at equilibrium; [*LR'*], concentration of activated RL complex; [*L*]$_T$, total radioligand concentration; [*R*]$_T$, total receptor concentration; B_{max}, receptor concentration per mg; k_1, second-order rate constant of association; k_{-1}, fast component dissociation rate constant; k_{-2}, slow component dissociation rate constant; HPLC, high performance liquid chromatography; TEA, triethylamine; $t_{1/2}$, half-life of dissociation; ASPPT, 40–55% ammonium sulfate precipitate; BCA, bicinchoninic acid; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide.

GENP 011137

Stokes radii, molecular weights, and frictional and axial ratios (9) are all within the ranges described for steroid hormone receptors (e.g., for the low salt aggregate form of steroid hormone receptors the ranges reported are: sedimentation coefficient, 8–10 S; Stokes radius, 7–10 nm; molecular weight, 2–3.5 × 10^5; frictional ratio, 1.45–2; and axial ratio 8–20; for a complete listing see Ref. 10).

We recently reported the synthesis and use of an ^{125}I-labeled azidodibenzo-*p*-dioxin as a photoaffinity ligand for the *Ah* receptor (11). The use of ^{125}I-labeled dioxin congeners is appealing because of their higher specific activity and counting efficiency compared with tritiated ligands. We now report the synthesis of a reversible radioligand, [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin, and its use to characterize the ligand binding kinetics of the murine hepatic *Ah* receptor.

Materials and Methods

Reagents

TCDD was a gift of Dow Chemical Co. (Midland, MI); 2,3,7,8-tetrachlorodibenzofuran was a gift from Dr. David Firestone (Food and Drug Administration, Washington, D.C.). Activated charcoal, grade PX-21, was a gift from Amoco Research Corp. (Chicago, IL). Bacto-Gelatin was from Difco Laboratories (Detroit, MI). Magnesium sulfate was from Fisher Scientific Co. (Fair Lawn, NJ). Ammonium sulfate (ultra-pure) was from Schwarz/Mann (Cambridge, MA). Methanol (HPLC grade) was from Burdick and Jackson Laboratories, Inc. (Muskegan, MI). Glycerol was from J. T. Baker (Phillipsburg, NJ). EDTA was from EM Scientific (Cherry Hill, NJ). Carrier-free Na^{125}I (NE2-033L) was from New England Nuclear (North Billerica, MA). BSA, dithiothreitol, β-mercaptoethanol, sodium azide, chloramine T, MOPS (free acid and sodium salt), TEA, and TEA hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). Dichloromethane (reagent grade), tetrahydrofuran (anhydrous, 99.9% pure), *n*-butyl nitrite (97% pure), *p*-dioxane (anhydrous 99+% pure), and dimethyl sulfoxide (anhydrous, 99% pure, stored under N_2 gas) were purchased from Aldrich Chemical Co. (Milwaukee, WI). BCA Protein Assay Reagent was purchased from Pierce Chemical Co. (Rockford, IL).

Buffers

MN represents the stock buffer which contains 25 mM MOPS and 0.02% sodium azide, pH 7.5 (at 4°). MEN is the stock buffer plus 1 mM EDTA. MβENG and MDENG represent MEN with the addition of 10% (w/v) glycerol, plus the addition of either 10 mM β-mercaptoethanol or 1 mM dithiothreitol.

Radiosynthesis of [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin

The synthesis was performed in a room dedicated to radiosynthesis, equipped with a high flow chemical hood, with the use of sealed vials and airtight syringes to minimize escape of radioiodine. The entire procedure was monitored with a γ-radiation detector.

Synthesis of [^{125}I]2-amino-3-iodo-7,8-dibromodibenzo-*p*-dioxin (Fig. 1). To 5 mCi of carrier-free Na^{125}I (≥350 mCi/ml in 6 mM NaOH, ≤15 μl of water) in a septum-sealed conical vial was added 2.5 nmol of 2-amino-7,8-dibromodibenzo-*p*-dioxin (11) in 25 μl of methanol, 1.13 μmol of sulfuric acid in 10 μl of methanol/water (9:1), and 25 nmol of chloramine T in 5 μl of methanol (12). The iodination reaction was complete in 30 min and terminated by the addition of 500 μg of sodium metabisulfite and 5 μmol of sodium hydroxide in 35 μl of water. The reaction product, [^{125}I]2-amino-3-iodo-7,8-dibromodibenzo-*p*-dioxin, was extracted with 250 μl of dichloromethane with the use of a vortex mixer (30 sec). The organic phase was aspirated into a syringe (gas syringe, pressure-locked plunger tip, P010032; Pierce Chemical Co., Rockford, IL) transferred to a 1-ml sealed conical vial (Reacti-vial 13221, Pierce Chemical Co.) containing 10 mg of magnesium sulfate, 75 μl of dichloromethane, and a microstirring bar, and the mixture was stirred for 45 min to dry the organic phase.

[^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin was formed from [^{125}I]2-amino-3-iodo-7,8,dibromodibenzo-*p*-dioxin by generation of the aryldiazonium salt, which produces the aryl radical with subsequent abstraction of hydrogen from tetrahydrofuran to form the deaminated product (13). This was accomplished as follows. The dried dichloromethane solution was aspirated and transferred to a 1-ml conical vial containing 250 μl of anhydrous tetrahydrofuran and 67 μmol of *n*-butyl nitrite. The reaction mix was then heated at 55° with stirring. After 15 min, the vial was opened and the solvent evaporated, with heating, under a stream of nitrogen gas, venting the vial into a funnel (2 cm diameter) attached to two serially connected charcoal filters in line with negative airflow. This system trapped most of the volatile radioiodine in the first charcoal filter and minimized release into the laboratory hood ventilation system. The reaction mix solute was dissolved in 50 μl of methanol/water (92:8) and the ^{125}I-labeled product purified by HPLC as detailed below.

Analysis and Purification of the Radioligand

Unlabeled 2-iodo-7,8-dibromodibenzo-*p*-dioxin, synthesized as above on a milligram scale, was used as an analytical standard. The unlabeled compound was characterized by 1) low resolution mass spectrometry: *m/e* (more than 40% of the base peak) 467.9 (100% M^+), 469.9 (46%), 468 (50%), 71 (55%), and 43.1 (60%); and 2) reverse phase HPLC: using a C-18 column, 4.6 × 250 mm, 5 μm particle (Altex Scientific Inc., Berkeley, CA), and an isocratic solvent system (methanol/water, 92:8) with a flow rate at 1 ml/min. The compound was detected by its UV absorbance at 254 nm and eluted with a retention time of 24 min.

The radiolabeled reaction mix was subjected to HPLC separation as above. Approximately 60% of the radioactivity injected eluted with a UV absorption peak that had a retention time identical to that of the unlabeled compound (i.e., 24 min). In this methanol/water HPLC system, the potentially significant reaction product contaminants, 2-amino-7,8-dibromodibenzo-*p*-dioxin, 2-iodo-3-amino-7,8-dibromodibenzo-*p*-dioxin, and 2,3-dibromodibenzo-*p*-dioxin, elute with retention times of 6.8, 11.6, and 13.0 min, respectively. Reinjection of the 24-min eluate (from above) also cochromatographs with authentic 2-iodo-7,8-dibromodibenzo-*p*-dioxin using an 9:1 acetonitrile/water isocratic solvent system (retention time, 22 min; 98% radiochemical purity). The overall yield of radioligand, judged by incorporation of ^{125}I into the purified product, ranges from 40 to 50%. The specific activity was assumed to be equivalent to that of carrier-free Na^{125}I, (2176 Ci/mmol, 4,831 dpm/fmol). The radioligand was stored at 6 × 10^8 dpm/ml in *p*-dioxane in a capped test tube protected from light. For use in binding experiments, an aliquot of the stock solution was dried under a stream of nitrogen gas and redissolved in DMSO at the appropriate concentration.

Receptor Preparation

Cytosol. C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred in our laboratory. Adult male and female mice were killed by cervical dislocation and their livers were removed, rinsed with ice-cold KCl (150 mM), homogenized in 9 volumes of MβENG buffer, and centrifuged at 10,000 × *g* for 20 min at 4°. The postmitochondrial supernatant was carefully removed to avoid contamination by the surface lipid layer and subjected to centrifugation at 105,000 × *g*. The supernatant (approximately 10 mg of protein/ml) was

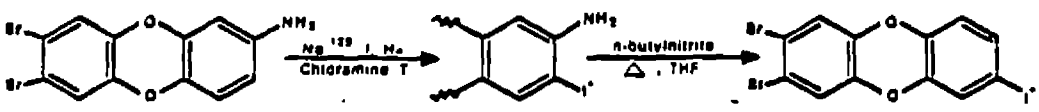

Fig. 1. Radiosynthesis of [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin. *THF*, tetrahydrofuran.

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separated from surface lipids and the microsomal pellet and stored at −80° for use in binding experiments or until processed further.

ASPPT. The frozen cytosolic fraction was thawed and placed in an ice bath with slow stirring. A saturated solution of ammonium sulfate in MEN buffer was added to the cytosolic preparation, to a final concentration of 40%, over a period of 30 min and stirred for an additional 30 min. The solution was centrifuged at 10,000 × *g* for 20 min, and the supernatant fraction was removed, slowly brought to an ammonium sulfate concentration of 55%, stirred for an additional 30 min, and centrifuged at 10,000 × *g*, and the supernatant was discarded. The ASPPT was resuspended in 55% ammonium sulfate in MβEN, and aliquots equivalent to 0.5 g wet weight of liver (15 mg of protein) were placed in 12 × 75 mm borosilicate tubes (VWR Scientific, San Francisco, CA) and spun at 5000 × *g*. The supernatant was removed by careful aspiration, and the pellets were stored in stoppered tubes at −80° until use.

HPLC-anion exchange-enriched receptor preparation. HPLC-anion exchange chromatography was performed on a Mono-Q HR 5/5 column (5 × 50 mm; Pharmacia, Uppsala, Sweden). All column parts, external tubing, injection loop, and buffers were placed on ice during the entire run. The HPLC instrumentation was identical to that outlined in the purification of the radioligand (above). The sample (ASPPT fraction, 200–900 μg of protein) was dissolved in 1–2 ml of 20 mM TEA buffer pH 7.5 (4°) and injected into a 2-ml sample loop. The elution buffers used were the following: buffer A, 20 mM TEA, pH 7.5; and buffer B, 20 mM TEA, pH 7.5, plus 500 mM NaCl. The linear gradient program ran from 100:0 (A:B) to 40:60 over 8 min, then to 10:90 over 10 min, followed by isocratic elution (10:90) for 5 min. The 16–18-min fractions were collected and pooled for use in binding studies. These fractions appear at the beginning of a large UV-absorbing peak and thus some variation in purification was observed between runs. Protein concentrations were determined by the method of Warburg and Christian (14) or by the use of BCA Protein Assay using BSA as standard.

Binding Experiments

Binding experiments were carried out at 4°. The frozen ASPPT pellets or cytosolic fractions were dissolved and diluted in ice-cold MDENG buffer to the appropriate protein concentration. The radioligand and the competing unlabeled ligand (2,3,7,8-tetrachlorodibenzofuran) were dissolved in DMSO and added to the buffer/receptor solution so that the final concentration of solvent was 6 μl of DMSO per ml of buffer. Binding reactions were performed in 1.0-ml volumes (12 × 75 mm borosilicate test tubes), or in 50-ml volumes (Ehrlenmeyer flasks), from which 1.0-ml samples were transferred to the smaller tubes. The binding reaction was terminated by the addition of a 0.5-ml volume of an ice-cold charcoal/gelatin suspension (3%/0.3% in MN buffer). The suspension was then stirred vigorously on a vortex mixer (2 sec), followed by incubation for 10 min at 4°. The charcoal/gelatin was then sedimented at 2000 × *g* for 10 min at 4°. A 1.0-ml aliquot of the supernatant fraction of each tube was transferred to 12 × 75 mm polypropylene tubes (for higher counting efficiency, 75%), and the bound radioligand was quantified in a MINAXI Series-5000 γ-counter (United Technologies/Packard Instrument Co., Downers Grove, IL). Sample counting times were adjusted to achieve a counting error of 3%.

Total radioligand, $[L]_T$, was defined as the concentration of radioligand in solution after the 16-hr incubation. Total radioligand bound was defined as the radioligand in solution after charcoal adsorption. Nonspecific binding was defined as the amount of radioligand bound in the presence of a 200-fold molar excess of 2,3,7,8-tetrachlorodibenzofuran. Specific binding was defined as the difference between total radioligand bound and radioligand nonspecifically bound. Unbound ("free") radioligand was defined as the difference between total radioligand and total bound radioligand.

Software

Weighted nonlinear curve-fitting estimates of binding parameters were determined as described (15, 16) using a commercially available software package for the IBM-PC (Kinetic, EBDA, Ligand, Lowry; Elsevier Biosoft, Cambridge, England).

Results

Equilibrium binding analysis. Equilibrium saturation binding of [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin to the hepatic *Ah* receptor of C57BL/6J mice, determined at three different protein concentrations, is shown in Fig. 2. Analysis of saturation binding curves by the method of Scatchard (17) is presented in Figure 3 and Table 1. With progressive 2-fold dilutions of the receptor preparation, the estimate of maximal binding sites per ml, B_{max} (*x* axis intercept), shows approximately 2-fold decreases, i.e., B_{max}/mg of protein remains constant (Table 1; Fig. 3). Unexpectedly, the slopes of the Scatchard plots ($-1/K_D$) increased from 2.6- to 1.2-fold with each 2-fold dilution (i.e., the estimate of K_D decreased from 1.6×10^{-10} to 1.2×10^{-11} M, as the protein concentration decreased from 1160 to 36 μg/ml).

Hill coefficients were 1.0 at all receptor dilutions, indicating that site-site cooperatively did not contribute to the increase in the slope of Scatchard plots observed with dilution (18). Scatchard and Hill analyses of saturation binding data were reproducible and of good linear fit, with coefficients of variation below 30% and with correlation coefficients of at least 0.96 (Table 1).

Regression analysis of the relationship between the protein concentration of the ASPPT and the estimate of K_D as determined by Scatchard analysis suggested a simple linear function (Fig. 4). Extrapolation of the least squares fit of the line to the *y* intercept (infinite dilution of protein) yielded an estimate of K_D of $6 \pm 3 \times 10^{-12}$ M.

Two experiments were conducted to describe more clearly the relationship between protein concentration and the increase in apparent binding affinity that was observed with dilution. First, when the *Ah* receptor concentration (B_{max} mg of protein) in the ASPPT fraction was enriched 5- to 9-fold by HPLC-anion exchange chromatography, the estimated K_D was approximately 4-fold lower than the K_D determined on the corresponding ASPPT fraction (Table 1; compare 20 mg/ml wet weight values). Second, saturation binding was described on an ASPPT preparation (30 μg/ml) in the absence or presence of 70 μg/ml of a highly purified protein, BSA (Fig. 5). Scatchard analysis of the binding data described a 10-fold increase in the apparent K_D when BSA was present. Despite the large effect of BSA on the apparent K_D, the estimate of B_{max} was only slightly increased in the presence of the additional protein, approximately 1.3-fold. It should be noted that this ASPPT preparation was from a different batch than that used to generate the data in Table 1 and Figs. 2, 3, and 4. This preparation yielded a slightly greater receptor concentration per milligram of protein.

Binding kinetics. For a simple bimolecular reaction governed by the law of mass action, the K_D of ligand binding is equal to the rate constant of ligand-receptor dissociation divided by the second-order rate constant of ligand-receptor association:

$$R + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RL \qquad (1)$$

and

$$K_D = \frac{k_{-1}}{k_1} \qquad (2)$$

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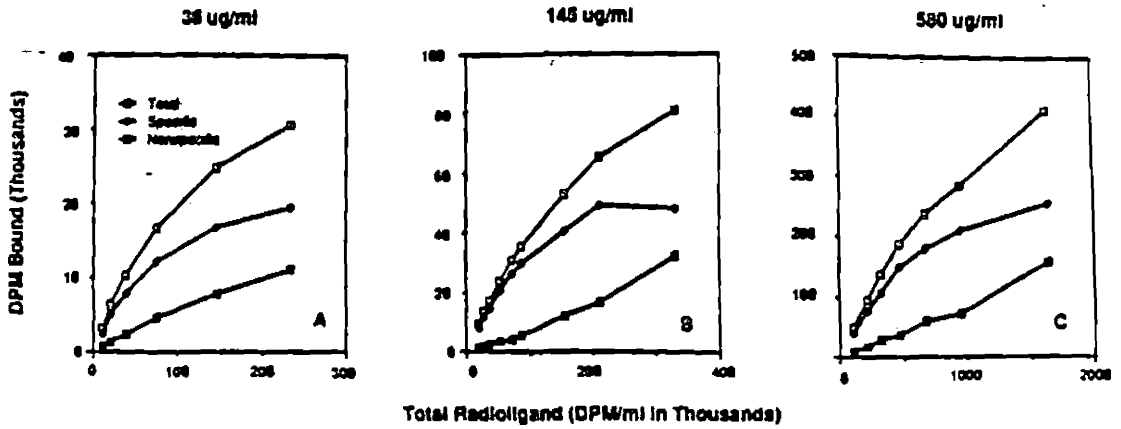

Fig. 2. Equilibrium binding of [^{125}I]2-iodo-7,8-dibromodibenzo-p-dioxin. The receptor preparation (ASPPT, 36 (A), 145 (B), or 580 (C) μg of protein/ml) was incubated at 4° for 16 hr, with concentrations of radioligand ranging from 2.5 to 250 fmol/ml. Total, specifically bound, and nonspecifically bound radioligand were determined as outlined in Materials and Methods. Each point represents the average of duplicate determinations.

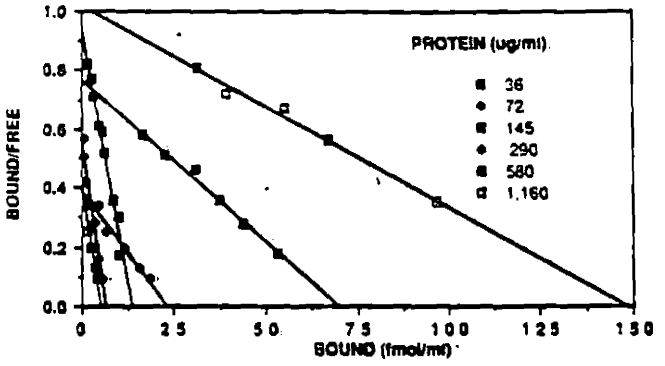

Fig. 3. Scatchard analysis of equilibrium binding data generated at various dilutions of receptor. Equilibrium saturation binding curves of [^{125}I]2-iodo-7,8-dibromodibenzo-p-dioxin were determined at various dilutions of the ASPPT fraction (as described in Fig. 2). The binding parameters K_D (−1/slope of the line) and B_{max} (receptor concentration/ml; the x intercept) were calculated from a weighted least squares fit of a plot of specifically bound radioligand/free radioligand versus specifically bound radioligand (16, 17). When more than two plots were generated at a given protein concentration, the plot that yielded the median K_D value was chosen for presentation.

Eq. 2 provides an estimate of the K_D, independent of that calculated from equilibrium binding studies.

Association rate constant. The rate constant of association was calculated from the time course of ligand-receptor binding, via the integrated second-order rate equation, as described by Weiland and Molinoff (19):

$$\ln\left[\frac{[LR]_e\,[[L]_T - [LR]\,[LR]_e/[R]_T]}{[L]_T([LR]_e - [LR])}\right] = k_1 t\left[\frac{[L]_T[R]_T}{[LR]_e} - [LR]_e\right] \quad (3)$$

Substitution of $\ln Y$ for the left side of the equation and rearrangement yields the equation of a straight line, with slope $\ln Y$/time. Thus, k_1 can be calculated from the relationship:

$$k_1 = \frac{\text{slope}}{\frac{[L]_T[R]_T}{[LR]_e} - [LR]_e} \quad (4)$$

This method allows determination of k_1 from estimates of the concentrations of total ligand, $[L]_T$, total receptor, $[R]_T$ (obtained from Scatchard analysis), ligand-receptor complex at equilibrium, $[LR]_e$, and ligand-receptor complex, $[LR]$, at various time intervals, t. The advantage of this method is that no constraints are placed upon the concentrations of ligand or receptor used in the binding studies. By this method, dilution of the protein concentration by 18-fold resulted in a 37-fold increase in the calculated rate constant of association (k_1 = 7.6 × 10^8 to 3.0 × 10^{10} M^{-1} hr^{-1}; Fig. 6, A and B, and Table 2). In contrast, at a fixed protein concentration a 13-fold change in total radioligand concentration did not alter the association rate constant (i.e., k_1 = 3.0 and 2.6 × 10^{10} M^{-1} hr^{-1}; Fig. 6, B and C, and Table 2).

The association rate constant was also estimated from the initial binding velocities (v_0) under conditions in which $[L]_T$ and $[R]_T \gg [LR]$, so the free radioligand and free receptor concentrations are approximated by $[L]_T$ and $[R]_T$, respectively.

TABLE 1

Summary of saturation binding experiments performed on the *Ah* receptor at various protein concentrations

The saturation binding isotherms for the radioligand and *Ah* receptor in hepatic cytosol, the ASPPT fraction of cytosol, and the HPLC-enriched preparation of the ASPPT fraction were determined over a range of protein concentrations. The binding parameters K_D and B_{max} were calculated by Scatchard analysis of binding data as described in the legend to Fig. 2 or by Hill analysis of binding data as described (18). Values represent the mean ± the standard deviation, except in the case of a single data point or duplicate data points, for which only the mean is given. Correlation coefficients were at least 0.96 for both Scatchard and Hill plots. The standard deviation of the Hill coefficient did not exceed 0.02.

Equivalent wet weight	Protein	K_D	B_{max}	Hill coefficient	Number of experiments
mg/ml	*μg/ml*	*pM*	*fmol/mg of protein*		
ASPPT					
1.25	36	12	141	1.0	1
2.5	72	14 ± 4	114 ± 21	1.00	5
5	145	19 ± 3	110 ± 14	0.97	4
10	290	49 ± 9	121 ± 7	0.98	3
20	580	92	121	0.98	1
40	1160	160	131	0.98	2
HPLC-Enriched					
6.7	8	11	780	1.00	1
14	9	12	611	0.99	1
20	25	22	684	0.98	1
30	48	22	1100	0.98	1
Cytosol					
0.62	42	7	95	1.00	1
1.25	85	13	113	1.00	2
2.5	170	24	80	0.99	1

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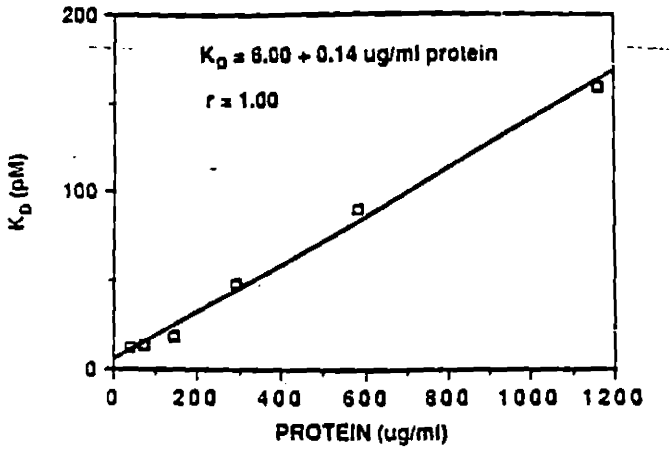

Fig. 4. The apparent K_d as a function of protein concentration. The apparent K_d, calculated from Scatchard analysis of radioligand binding to the ASPPT fraction, plotted as a function of the protein concentration (μg/ml). The data were fit to the equation of a straight line by the method of least squares. The equation of the line is inset. *r*, correlation coefficient.

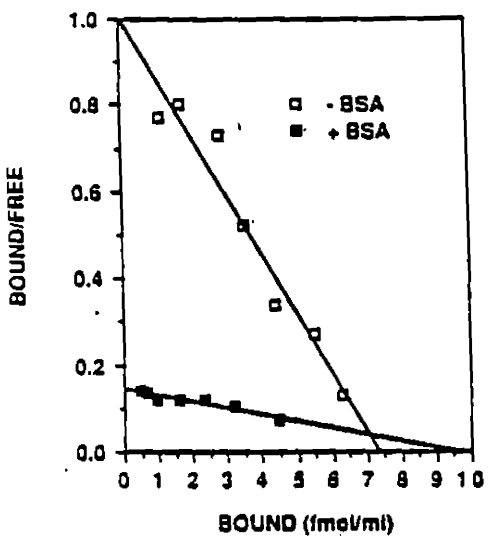

Fig. 5. The effect of BSA on the slope of the Scatchard plot. Equilibrium saturation binding curves were generated, as described in Fig. 3, for an ASPPT sample of 30 μg of protein/ml with and without the addition of 70 μg/ml BSA. Calculated binding parameters: plus BSA, K_D = 8.3 × 10^{-11} M, B_{max} = 9.2 fmol/ml; without BSA, K_D = 7.3 × 10^{-12} M, B_{max} = 7.4 fmol/ml.

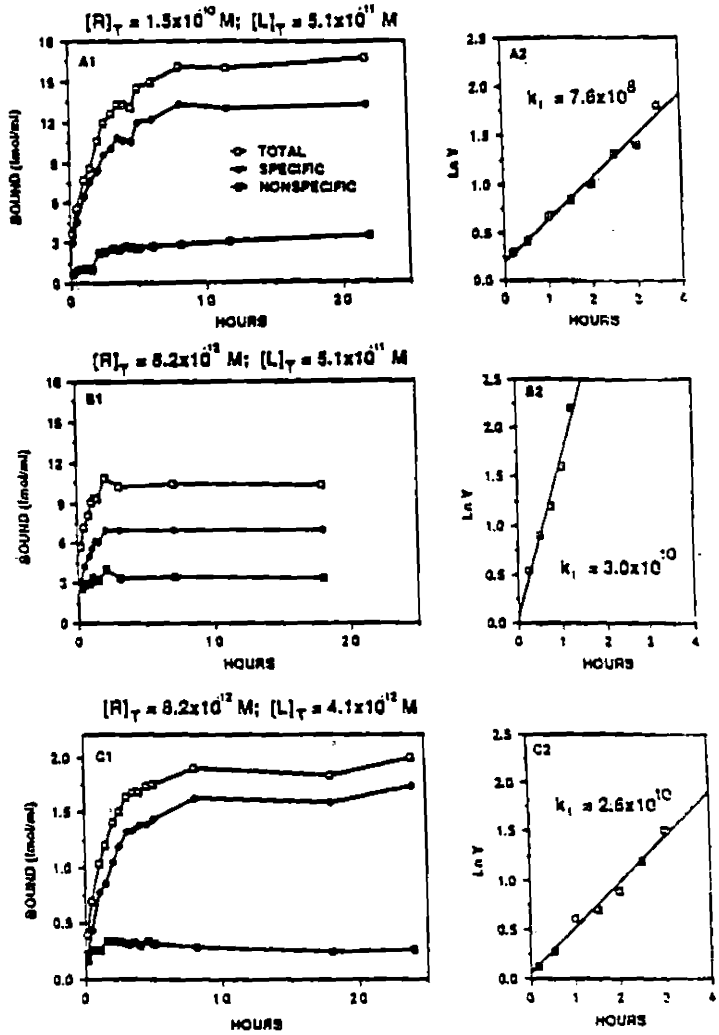

Fig. 6. Time course of association of [^{125}I]2-iodo-7,8dibromodibenzo-*p*-dioxin to the hepatic *Ah* receptor at 4°: estimate of the association rate constant. The time course of ligand receptor association was monitored at receptor concentrations of 1.5 × 10^{-10} M and 8.2 × 10^{-12} M (ASPPT fraction equal to protein concentrations of 1160 and 72 μg/ml) and radioligand concentrations of 5.1 × 10^{-11} M and 4.1 × 10^{-12} M (250,000 and 20,000 dpm/ml). Each *point* represents the average of two determinations. The data are presented as a plot of bound radioligand as a function of time (A1, B1, and C1), and as a plot of lnY (left side of Eq. 3) as a function of time (A2, B2, and C2). The rate constant of association was determined from the slope of lnY versus time plots as described in Eq. 4 and is inset (units of M^{-1} hr^{-1}).

where:

$$v_0 = k_1 [R]_T[L_T] \quad (5)$$

rearranging gives:

$$k_1 = v_0/[R]_T[L]_T \quad (6)$$

Plotting initial binding velocity as a function of receptor or radioligand concentration, with the other variable fixed, allows the determination of k_1.

A plot of initial binding velocity versus receptor concentration yielded a hyperbolic plot (Fig. 7A). The correlation coefficient of the plot (for a linear fit) using all data points presented is 0.94. By elimination of the values obtained above a receptor concentration of 1 × 10^{-11} M and inclusion of the theoretical *y* intercept of (0,0), a correlation coefficient of 0.99 is obtained and an association rate constant of 5.5 × 10^{10} M^{-1} hr^{-1} is calculated (i.e., calculation of k_1 from the asymptote of the hyperbola). The initial binding velocity as a function of radioligand concentration, at a fixed receptor concentration of 6.8 × 10^{-12} M, is shown in Fig. 7B. This plot yielded a good linear fit and an estimate of the second order rate constant of association equal to 5 × 10^{10} M^{-1} hr^{-1}.

Dissociation rate constants. As shown in Figs. 8 and 9, the dissociation of the ligand-receptor complex does not follow a simple first-order process. The dissociation profile is independent of the receptor concentration or the type of preparation. The data is best fit by a two-component exponential curve ($y = Ae^{k_1 t} + Be^{k_2 t}$).[2] The initial rapidly dissociating component comprises approximately 75% of the total complex and has a

[2] Our results indicate that a statistically better fit was obtained by using a biexponential model over a monoexponential one ($p < 0.001$). We used the partial *F* test as described by Munson and Rodbard (15). No further improvement was obtained when attempts were made to fit the data to a triexponential model ($p < 0.98$), although this and higher order exponential models cannot be ruled out by the data presented.

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TABLE 2

Summary of kinetic data

Rate constants were calculated as described in the text and expressed as the mean and, where possible, the standard error. The equilibrium dissociation constant, K_D, was determined by 1) Scatchard analysis of the saturation binding isotherm at infinite dilution (Fig. 3) or 2) as a ratio of the dissociation rate constant, k_{-1} (0.36 hr^{-1}) and the association rate constant from either the integrated rate equation or the initial binding velocity method. The dissociation rate constants presented above have not been corrected for receptor-ligand complex destruction rates, thus they are the sum of dissociation and destruction rates (see Results).

	L_T	R_T	k_1
	M	M	$M^{-1}\ hr^{-1}$
Association rate constant			
Integrated 2nd-order	4.1×10^{-12}	8.2×10^{-12}	2.8×10^{10}
	5.1×10^{-11}	8.2×10^{-12}	3.0×10^{10}
	5.1×10^{-11}	1.5×10^{-10}	7.8×10^{9}
Initial velocity method			
Ligand	2.1×10^{-11}	Varied	5.5×10^{10}
Receptor	Varied	6.8×10^{-12}	5.0×10^{10}

	k_{-1}	Percent	k_{-2}	Percent
	hr^{-1}		$hr^{-1} \times 10^3$	
Dissociation rate constant				
ASPPT	0.37 ± 0.02	78 ± 4	4.3 ± 0.4	22 ± 1
Cytosol	0.36 ± 0.05	77 ± 7	9.4 ± 0.8	23 ± 2
Warmed cytosol	0.36 ± 0.04	72 ± 5	7.2 ± 0.5	28 ± 1

	K_D
	M
Equilibrium dissociation constant	
Scatchard (infinite dilution)	$6.0 \pm 3 \times 10^{-12}$
Kinetic (initial velocity)	6.9×10^{-12}
Kinetic (integrated 2nd order)	13.0×10^{-12}

dissociation rate of 0.36 hr^{-1} ($t_{1/2}$ = 1.9 hr). The second slower dissociating component (25%) has a rate constant of 4.3 to 9.4 $\times 10^{-3}$ hr^{-1} ($t_{1/2}$ = 74–161 hr) (Table 2). The rate constant for "degradation"[1] of the receptor-ligand complex at 4° is ~2 $\times$ 10^{-3} hr^{-1}, monoexponential for over 120 hr, and not significantly affected by protein concentration. Thus, the second component of "dissociation" is a composite, representing both ligand-receptor dissociation and degradation. The rate constant for degradation of the unoccupied receptor is ~4 $\times$ 10^{-3} hr^{-1} and is monoexponential for over 100 hr.

As shown in Table 2, K_D values calculated as the ratio of kinetic constants, k_{-1}/k_1 (where k_{-1} is the dissociation rate constant of the major rapid dissociating pool and k_1 is estimated from the integrated second-order equation or initial velocity method), are 6.9×10^{-12} and 13.0×10^{-12} M, which agree very well with the K_D determined by equilibrium saturation data extrapolated to infinite dilution, 6.0×10^{-12} M.

Discussion

Due to its high receptor affinity, high biological potency, commercial availability, and environmental importance, TCDD has become the most studied *Ah* receptor ligand. Despite extensive characterization of the *Ah* receptor using [^{3}H]TCDD or other ligands, no detailed kinetic analysis of ligand-receptor binding has appeared. The kinetic and equilibrium studies in this report challenge the following two assumptions implicit in past binding studies: 1) that both specifically bound radioligand and free radioligand concentrations can be determined accurately at high protein concentrations and 2) that ligand-*Ah* receptor binding can be described by a simple reversible bimolecular model (Eq. 1).

The most common method of analyzing equilibrium saturation binding data is the linear transformation of Scatchard (17):

$$\frac{[LR]}{[L]} = \frac{[R]_T - [LR]}{K_D} \tag{7}$$

or

$$\frac{[\text{Bound}]}{[\text{Free}]} = -\frac{1}{K_D}[\text{Bound}] + \frac{B_{max}}{K_D} \tag{8}$$

A plot of the ratio of specifically bound ligand/free ligand as a function of specifically bound permits one to estimate K_D and total binding sites, $[R]_T$. Analysis of equilibrium binding data by the Scatchard equation requires that a number of criteria be met as follows: 1) the concentration of specifically bound and free ligand are measured accurately at equilibrium; 2) both ligand and receptor are homogenous species; 3) binding obeys the law of mass action as described in Eq. 1 and 2; and 4) no cooperativity exists.

Criterion 1: Measurement of free and specifically bound ligand is accurate. The accuracy of K_D and B_{max} values generated from Eq. 8 is dependent upon the accuracy of the determination of bound and free. Estimates of bound radioligand are fairly straightforward and can be supported by physicochemical characterization of the ligand-receptor complex (e.g., density gradient centrifugation, size exclusion chromatography, anion exchange chromatography, etc.). Conversely, the concentration of free radioligand is most often determined indirectly as the difference between total radioligand in solution and total bound radioligand. In *Ah* receptor-ligand binding studies, free is defined operationally as the amount of radioactivity that can be adsorbed to charcoal (20), that can be removed from hydroxyapatite by extensive detergent washing (21), or that does not sediment in a sucrose gradient (22). By these methods, radioligand, bound to low affinity nonspecific binding sites, that dissociates during charcoal adsorption, hydroxyapatite washing, or long-term centrifugation will be misclassified as free radioligand. This systematic misclassification will decrease the ratio of bound/free ligand and hence decrease the slope of the Scatchard plot (i.e., $-1/K_D$), although it may not significantly compromise estimates of B_{max} (18, 23, 24). One would expect the misclassification of nonspecifically bound radioligand as free radioligand to be greatest, and thus the overestimation of K_D to be greatest, at higher protein concentrations. We believe this is the most plausible explanation of the data presented in Figs. 3–5 and Table 1.

Using [^{3}H]TCDD, Farrel and Safe (25) have noted a relationship between protein concentration and K_D estimates generated from Scatchard analysis of saturation binding data. Characterization of *Ah* receptor ligand binding with a radioligand of modest specific activity, such as [^{3}H]TCDD, necessitates the use of protein concentrations greater than 1 mg/ml (20–22, 25). Thus, K_D values generated previously for [^{3}H]

[1] The degradation rate of the ligand-receptor complex was determined by incubation of the ASPPT fraction (72, 145, and 290 μg of protein/ml) with radioligand with or without 200-fold excess 2,3,7,8-tetrachlorodibenzofuran at 4° for 16 hr (as described in the legend to Fig. 5). Specific binding was determined in aliquots removed at time intervals from 16 to 136 hr. The degradation rate of unoccupied receptor was estimated by sampling a receptor preparation stored at 4° after various time intervals. The receptor sample was incubated with radioligand with or without 200-fold excess 2,3,7,8-tetrachlorodibenzofuran at 4° for 16 hr, and specifically bound radioligand was determined. The degradation rates were estimated from the plots of the log of specific binding versus time.

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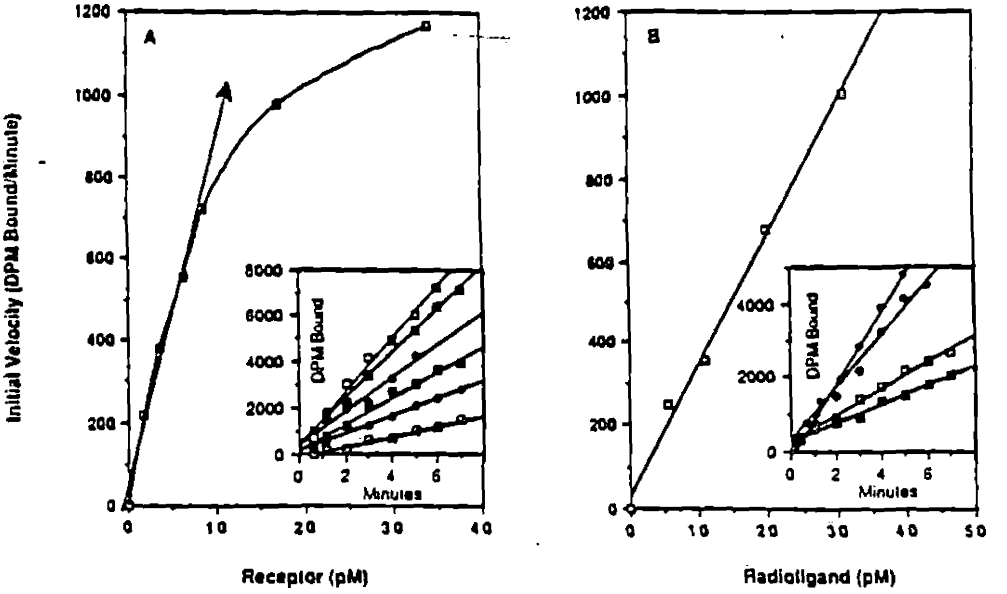

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Fig. 7. Initial binding velocity of [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin to the hepatic *Ah* receptor: estimate of the association rate constant. The ASPPT fraction in MDENG buffer was incubated with radioligand in 50-ml Erlenmeyer flasks. At the indicated times intervals, 1.0-ml aliquots were transferred to borosilicate tubes containing 1000-fold molar excess of 2,3,7,8-tetrachlorodibenzofuran (800-fold for nonspecific binding) to prevent further binding of the radioligand and total, specifically, and nonspecifically bound radioligand was determined. Receptor concentrations were determined by duplicate Scatchard analyses. A, Initial binding velocity as a function of *Ah* receptor concentration. Receptor concentrations of 1.7×10^{-12} M (□), 3.4×10^{-12} M (◆), 6.1×10^{-12} M (■), 8.5×10^{-12} M (◇), 1.7×10^{-11} M (■), and 2.4×10^{-11} M (□) were incubated with a fixed radioligand concentration, 2.7×10^{-11} M. The initial binding velocity was determined for each receptor concentration from the slope of the plot of specifically bound radioligand as a function of time (*inset*). The initial binding velocities were then plotted as a function of receptor concentration. The rate constant of association was calculated from the slope of the linear portion of the line (asymptote of the hyperbola) divided by the ligand concentration (see Materials and Methods). B, Initial binding velocity as a function of radioligand concentration. A fixed concentration of receptor, 6.8×10^{-12} M, was incubated with radioligand concentrations of 5.6×10^{-12} M (■), 1.1×10^{-11} M (□), 2.0×10^{-11} M (◆), and 3.1×10^{-11} M (·). The initial binding velocity was calculated from the slope of specifically bound radioligand as a function of time (*inset*). The initial velocity was plotted as a function of radioligand concentration. The rate constant of association was calculated from the slope of this line divided by the receptor concentration.

TCDD are probably overestimates. Using a competitive binding assay with [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin as the radioligand and low protein concentration, we estimate the K_D for TCDD to be approximately 4×10^{-12} M.[4]

Misclassification of nonspecifically bound radioligand as free radioligand has been described in a number of other receptor systems that bind hydrophobic ligands: estrogen (23), progesterone (24), and thyroxine (26). In these systems, misclassification errors have been minimized by the use of methods that better estimate free and bound radioligand, e.g., equilibrium dialysis (23, 24) or centrifugal ultrafiltration-dialysis (27). Because our radioligand adsorbs strongly to dialysis tubing and filter membranes,[5] we provided support for radioligand misclassification by the following: 1) demonstrating that addition of a purified protein (BSA) decreases, whereas receptor enrichment increases, the slope of the Scatchard plot (Fig. 5 and Table 1), and 2) eliminating the possibility that the rate constants of association or dissociation were altered by dilution.

We observed that, like K_D, the apparent association rate constant also varies as a function of the concentration of receptor, or more likely, protein (Figs. 6 and 7A). Association rate constant estimates are also dependent upon accurate determination of free radioligand. From the initial velocity rate equation (Eq. 6), it follows that for a given ligand concentration a plot of v_o as a function of receptor concentration will yield a linear plot, with a slope equal to $k_1[L]_T$. The hyperbolic shape of the curve generated (Fig. 6A) indicates that $k_1[L]_T$ is decreasing as the concentration of the receptor preparation increases and supports the idea that free is being overestimated at higher receptor concentrations. An alternative explanation is that k_1 is actually increasing with dilution of the receptor. Because k_1 is a function of diffusion rate and the energy of activation for the binding step (28), an increase in k_1 with dilution implies that a decrease in the energy of activation for ligand binding is also occurring with dilution. A decrease in the energy of activation for the forward reaction should also result in a decrease in the energy of activation for the dissociation reaction and lead to a increasing k_{-1} with dilution. Because k_{-1} is not affected by protein concentration (Fig. 7 and discussion below), the hyperbolic nature of the curve depicted in Fig. 6A is best explained by an overestimation of free radioligand at the higher protein concentrations.

Criterion 2: Both ligand and receptor are homogenous binding species. The time course of ligand-receptor dissociation is biphasic, suggesting two ligand-receptor species that have dissociation rate constants differing by a factor of at least 60-fold (Figs. 8 and 9 and Table 2). The dissociation of [^{3}H]TCDD from the rat *Ah* receptor has previously been described as "irreversible" (25). Our preliminary comparisons between the dissociation kinetics of the *Ah* receptor-radioligand complex in the Harlan-Sprague Dawley rat and C57BL/6J mouse indicate significant differences.[5]

Criterion 3: Binding obeys the law of mass action as described in Eq. 1. The linear functions generated from plots of initial binding velocity (v_o) versus either the ligand c-

[4] C. A. Bradfield and A. Poland, manuscript in preparation.
[5] C. A. Bradfield, and A. Poland, unpublished observations.

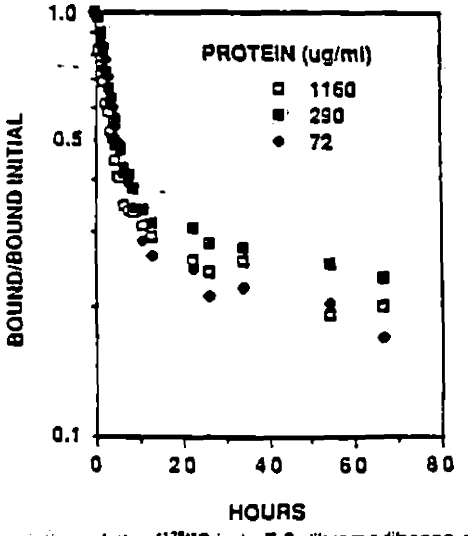

Fig. 8. Dissociation of the [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin *Ah* receptor complex at 4°: the effect of protein concentration. Radioligand-receptor complexes were preformed by incubation of radioligand and receptor for 16 hr at 4° in 50-ml Ehrlenmeyer flasks. Fractional receptor occupancy of radioligand was between 15 and 18% for all protein concentrations. This was achieved by the following protein (receptor) and radioligand concentrations: 1160 μg/ml (150 pM) and 62 pM; 290 μg/ml (35 pM) and 15 pM; and 72 μg/ml (8.2 pM) and 4 pM. After equilibrium binding had been obtained, a 1000-fold excess of 2,3,7,8-tetrachlorodibenzofuran was added to prevent rebinding of radioligand (time zero). Bound radioligand was determined, at the indicated times, on 1.0-ml aliquots. Nonspecific binding was determined by the addition of a 200-fold excess of 2,3,7,8-tetrachlorodibenzofuran during the pre-binding stage, followed by the addition of an 800-fold excess at time zero. All values were determined in duplicate. Results are presented as specifically bound radioligand/radioligand specifically bound at time zero, plotted as a function of time.

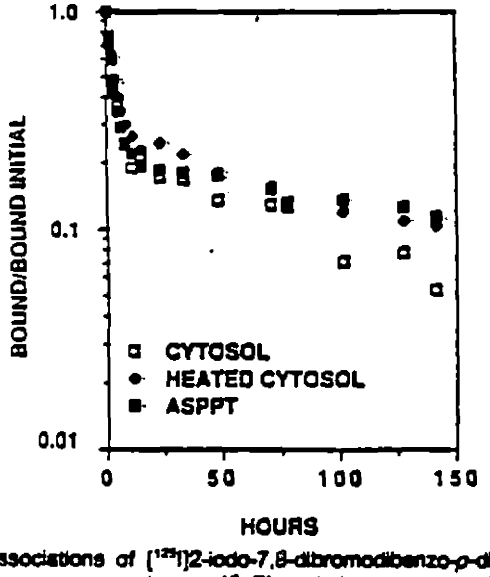

Fig. 9. Dissociations of [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin from different receptor preparations at 4°. Dissociation rates were determined as described in Fig. 8. *Cytosol* (85 μg of protein/ml) and ASPPT (72 μg/ml) were preincubated with 10 pM radioligand for 16 hr at 4° before dissociation rate determinations. *Heated cytosol* was preincubated for 18 hr at 4° as above, followed by a 30-min incubation at 30°, then recooled to 4° (30 min) before dissociation rate determination. Kinetic rate constants were calculated by the nonlinear curve fitting technique, constrained to a biexponential model, as described (16).

receptor concentrations (Fig. 7) indicate that the binding reaction is first order with respect to both radioligand and receptor and thus second order overall (formation of the ligand receptor complex) (28). This lends support to the assumption that one molecule of ligand binds to one molecule of receptor.

Biphasic ligand-receptor dissociation is not in agreement with the equilibrium binding scheme presented in Eq. 1. Biphasic ligand-receptor dissociation curves have been observed for ligands bound to the estrogen (29, 30), progesterone (31), glucocorticoid (32), and androgen (33) receptors. For these steroid hormone receptors, the slow dissociating species (believed to represent a "transformed" receptor species) has an increased affinity for polyanionic matrices such as DNA (33–35) and an altered molecular mass as compared with the fast dissociating species (36, 37). In these systems, in which the ratio of the fast to slow component can be altered by increasing the ionic strength, heating, etc., we did not observe a significant change in the ratio of the fast and slow components by heating a 30° for 30 min, dilution, or ammonium sulfate precipitation.

Ligand binding to the *Ah* receptor appears to conform to the model proposed for the steroid hormone receptor (32) in which:

$$L + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} LR \underset{k_{-12}}{\overset{k_{12}}{\rightleftharpoons}} LR' \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} L + R' \qquad (9)$$

and thus at equilibrium:

$$K_D = \frac{[L]\,[R]}{[LR] + [LR']} \qquad (10)$$

Criterion 4: No site-site cooperatively exists. Hill coefficients were 1.0 at all dilutions; there is no indication of cooperativity in this system. Hill coefficients of approximately 1.0 have previously been reported for a number of mammalian species (25, 38).

In this report, analysis of the *Ah* receptor-ligand binding, facilitated by a new high specific activity radioligand has revealed important findings. 1) Estimates of the apparent K_D, as determined by Scatchard analysis, are a function of protein concentration and attributable to a systematic overestimation of free ligand. The true K_D (from equilibrium saturation at infinite dilution and ratio of kinetic constants k_{-1}/k_1) is approximately 100 times lower than previous estimates. 2) The *Ah* receptor ligand complex can exist as two distinct species, which have different dissociation rates, and thus ligand binding does not conform to a simple reversible mass action model, as has been assumed previously.

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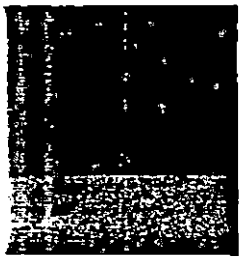

Analysis of Polychlorinated Dibenzofurans and Polychlorinated Dibenzo-*p*-Dioxins in Transformers and Capacitors

Volume 3: Summary and Statistical Analysis of the Round-Robin Test

Prepared by
Research Triangle Institute
Research Triangle Park, North Carolina

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REPORT SUMMARY

SUBJECTS: Hazardous and toxic waste management / Transmission substation design and operation / Distribution substations

TOPICS:
- PCB
- Chemical analysis
- Transformers
- Capacitors
- PCDF-PCDD
- Insulating oil

AUDIENCE: Environmental managers / Distribution engineers

Analysis of Polychlorinated Dibenzofurans and Polychlorinated Dibenzo-*p*-Dioxins in Transformers and Capacitors

Volumes 1–3

PCDF and PCDD—by-products of PCB pyrolysis and combustion—apparently cause many of the health effects originally attributed to PCB. In parallel and round-robin studies, independent laboratories found that normal equipment operation and arcing failure cause little if any oxidation and do not significantly modify PCB fluids.

BACKGROUND: Partial oxidation of askarels—mixtures of polychlorinated biphenyls (PCB) and tri- and tetrachlorobenzenes—produces polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-*p*-dioxins (PCDD). However, analytic techniques for separating the active components in these mixtures had not matured prior to this study. Information had also been insufficient on the quantity and types of PCDF and PCDD in as-manufactured PCB and on the formation of these by-products during normal operation or arcing failure in utility equipment. Because of these uncertainties, utilities and regulators have assumed the worst-case PCDF–PCDD formation when there is a PCB-related accident, thereby overstating the potential toxicity. EPRI report EL/EA-4858 describes portions of the research performed in these projects.

OBJECTIVE: To improve techniques for measuring PCDF/PCDD in the presence of PCB.

APPROACH: Five laboratories (including two whose studies are discussed in report EL/EA-4858) performed round-robin gas chromatography–mass spectrometry analyses of PCDF–PCDD samples using specially prepared carbon-13 (^{13}C) PCDF spiking compounds. After the first set of analyses, each laboratory modified its techniques and analyzed a larger group of samples from utility equipment. Another organization statistically analyzed all the laboratory results.

RESULTS: These three volumes report the second group of PCDF–PCDD analyses. Results from all participating laboratories correlated remarkably well. Some of the results follow.

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- Use of the ^{13}C compounds allowed successful separation of the components in several closely eluting pairs of physiologically active PCDF and less-active compounds.
- Analyses of samples from utility equipment suggested that the PCDF–PCDD present did not result from high-load, high-temperature operation.

Volumes 1 and 2 describe several analytic techniques, with the appendix of Volume 2 including a report on the analysis by the University of Umeå in Sweden, as well. Volume 3 summarizes all the laboratory techniques, presents comprehensive statistical analyses, and provides an executive summary.

EPRI PERSPECTIVE

This research is a landmark in PCDF–PCDD analysis. One important result is the synthesis of new PCDFs for use as spiking compounds in gas chromatography–mass spectrometry analysis. The ability to separate many of the active PCDF compounds from more innocuous materials is also of great value. The high correlation of results from several laboratories indicates that researchers can exercise great flexibility in selecting techniques and developing facilities. In addition, new work has started (EPRI project RP2028-21) in which the best features of each of the five analytic methods will be assembled to provide a single recommended method.

PROJECTS

RP2028-6, RP2028-7, RP2028-10
EPRI Project Managers: Gil Addis; Jacques Guertin
Electrical Systems Division; Environment Division
Contractors: New York State Department of Health; Battelle Columbus Laboratories; Research Triangle Institute

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Analysis of Polychlorinated Dibenzofurans and Polychlorinated Dibenzo-*p*-Dioxins in Transformers and Capacitors

Volume 3: Summary and Statistical Analysis of the Round-Robin Test

EL/EA-5443, Volume 3
Research Project 2028-10

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ABSTRACT

An interlaboratory study involving five independent laboratories was conducted to quantitatively assess accuracy and precision of polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congener analysis in three spiked and seven in-service utility fluids. Data was obtained using capillary gas chromatography/mass spectrometry for 16 different PCDD and PCDF compounds. Five different ^{13}C-labeled internal standards were added to the samples and the samples were purified prior to analysis. Each laboratory's generated calibrations for the individual compounds were linear for concentrations over four orders of magnitude. Analyses were performed in triplicate on an extract to provide insight to instrumental reproducibility.

The median and range of percent accuracy for four of the five laboratories was 102 and 56-149, respectively, across all chemicals measured in the two spiked samples. The fifth laboratory had a median and range of 107 and 8-3939, respectively.

The median and range of percent reproducibility (coefficients of variation) for three of the four laboratories was 8.2 and 0-46, respectively, across all 10 synthetic and in-service samples and all compounds measured. The fourth laboratory had a median and range of 30 and 2-159, respectively. The fifth laboratory did not report replicate analyses on each extract and thus reproducibility for instrumental analysis could not be calculated.

The largest variability (accuracy and precision) was attributed to the different types of samples (>90%) in three of the four laboratories while the fourth had as much as 63.1% variability from the sample type analyzed and as much as 36.9% due to instrumental measurement error.

At the approximate 95% prediction intervals, the analysis of these 10 samples by any one of the five laboratories would be expected to produce variability in the results for each PCDD or PCDF congener measured ranging from a factor of two to 300.

Of the five participating laboratories, the analytical method employed by Lab-D gave for the most part the best accuracy and precision while Lab-C was the poorest.

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We would like to also thank our research comrades who contributed the analytical data for this interlaboratory collaborative study. They are Dr. S. Gordon (Illinois Institute of Technology Research Institute), Drs. G. Eadon and Dr. Hilker (New York State Department of Health), Dr. M. Kilpatrick (Radian Corp.), Dr. M. Cooke (Battelle Columbus Div.), and Dr. C. Rappe (U. of Umeå).

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SUMMARY

EPRI has sponsored a four-year research program to evaluate the analysis of selected individual compounds of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in dielectric fluids. These are impurities often associated with askarel mixtures of polychlorinated biphenyls (PCBs) and tri/tetrachlorobenzene (TCB). They have been known to form either in the PCB manufacturing process or as a result of excessive heat (fires). This program included the synthesis of new natural (^{12}C) and isotopically labeled (^{13}C) PCDD and PCDF analytical standards. Standards and spiked test samples were prepared by one of the laboratories and distributed to the four other participating laboratories as part of a round robin study. The samples were then analyzed as unknowns. The impetus for the studies has been the wide range of toxicities (4-5 orders of magnitude) seen for the individual compounds in animal tests and the difficulty of quantitatively analyzing for some of these chemically similar compounds of dissimilar toxicity, often in the presence of a great excess of PCBs. PCBs themselves are a group of compounds with chemical behavior relatively similar to PCDF/PCDD during sample cleanup stages.

It should be remembered that the major object of the study was to evaluate and upgrade methods of analysis; analysis of field samples was an important secondary output. The study involving five participating laboratories, was conducted in two phases. Phase I was designed to located glaring discrepancies in the techniques, and Phase II to statistically improve results of the different laboratories on a series of unknown samples.

Phase I involved the analysis of PCDFs and PCDDs in five spiked basic samples: Aroclor-1016, Aroclor-1242, Aroclor-1260, a tri- and tetrachlorobenzene mixture, and aged mineral oil; and four in-service dielectric fluids. Each participating laboratory used its best available in-house extraction and analysis techniques (Gas Chromatography/Mass Spectrometry, GC/MS). Since validated analytical methods did not exist before this program was initiated, this phase of the program was necessary to assess any deficiencies and optimize the analytical methodology.

The isotopically labeled standards were needed to calibrate the efficiency of cleanup and to determine the sensitivity (response) of the MS for the various individual compounds of PCDF and PCDD being analyzed.

After evaluating the results of Phase I and implementing several improvements to the methodology, Phase II was initiated. Phase II was designed to provide quantitative data on PCDF and PCDD compounds in selected in-service dielectric fluids and to make a statistical interlaboratory comparison of these data. The specific aims were 1) to determine the accuracy of analysis by each participating laboratory for a selected list of PCDFs and PCDDs in spiked samples, 2) to determine the precision of analysis by each laboratory for PCDFs and PCDDs in spiked and in-service utility samples, 3) to perform an interlaboratory comparison and thus a comparison of methods employed for accuracy and precision, and finally 4) to measure the levels of PCDFs and PCDDs in a few selected in-service utility samples.

This study suggests that there is no strongly preferred analytical procedure (among those using a combination of extraction/chemical cleanup, gas chromatography, and mass spectrometry) for measuring compound specific concentrations of PCDF and PCDD. For spiked samples, originally free of PCDF and PCDD, five different methods (corresponding to five different participating laboratories) yielded concentrations accurate to within ±20% (except one laboratory measured 2,3,4,6,7,8-hexachlorinated dibenzofuran [2,3,4,6,7,8-H_6-CDF] with an order of magnitude error).

Since standards for all 150 PCDF and PCDD compounds were not available, it was not possible to know the accuracy of measurement on the unknown field samples. Only the precision of measurement could be ascertained.

Considerable variation in measurement precision was observed between laboratory procedures. For example, measurement of very low concentrations (1 to 10 ng/g range) showed an order of magnitude variation, whereas measurement in higher concentration range (100 to 10,000 ng/g) were considerably more precise. Again, a specific H_6CDF measurement was an exception, having as much as four orders of magnitude variation. For total individual congener classes, concentration measurements had only one order of magnitude variation.

What range of concentrations were found in the field samples? The maximum concentration for 2,3,7,8-Tetra-CDD was 32 ng/g and the maximum concentration of 2,3,7,8-tetra-CDF + 2,3,4,8-tetra-CDF was 459 ng/g. The highest concentration found was for one sample of the much less critical octachlorinated dibenzofuran at 65,000 ng/g. These concentrations are reported with a statistical 95% confidence level.

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SECTION 1
INTRODUCTION

Because of the concerns about the health effects of polychlorinated biphenyls (PCBs) their production was banned and regulation instituted in 1976 by the Toxic Substance Control Act (TSCA), PL 94-469. Certain totally enclosed electrical uses of PCBs are allowed for the remainder of the useful life of existing electrical equipment. Many of these adverse health effects appear to be attributable to trace contaminants such as polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-p-dioxins (PCDD) in the PCBs.

The Electric Power Research Institute (EPRI) inititated a program to investigate the chemistry of PCDF and PCDD as they may relate to use of Askarel, a dielectric liquid, which contains PCBs and potential trace contamination of PCDF/PCDD. This program had three primary objectives: (1) to develop and optimize analytical methods and quantitatively characterize these methods for measuring PCDF/PCDD in dielectric fluids, (2) to apply these methods to measurement of PCDF/PCDD levels in typical dielectric fluids used in utility operations, and (3) to investigate possible PCDF/PCDD formation in simulated transformer events.

As part of the objective to evaluate methodology, the program included the synthesis of native and isotopically labeled PCDD and PCDF analytical standards (1) to allow for analysis of selected individual congeners of PCDDS and PCDFs. Analytical standards and spiked matrix samples were prepared by a contract laboratory and then distributed to four other participating laboratories as part of a round robin study. These samples were analyzed blind by five independent contract laboratories - Illinois Institute of Technology Research Institute (IITRI), New York State Department of Health (NYSDOH), Radian Corp., Battelle, Columbus Division, and U. Umea.

The method evaluation study was conducted in two phases. Phase I involved the analysis of PCDF's and PCDD's in five spiked baseline samples; for example, Aroclor-1016, Aroclor-1242, Aroclor-1260, tri- and tetrachlorobenzene mixture, aged mineral oil, and four in-service dielectric fluids (1-4). Each participating laboratory used their best available in-house extraction and analysis techniques (1-4). Since validated methods for PCDD and PCDF analysis did not exist before this program was initiated, this phase was necessary to assess any deficiencies and to optimize the analytical methodology. After Phase I results were evaluated and

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several improvements were implemented, then Phase II was undertaken. This report covers the effort under Phase II.

Phase II was designed to yield quantitative data on PCDD and PCDF congeners in selected in-service dielectric fluids and to make a statistical interlaboratory comparison on these data. The specific aims were (1) to determine the accuracy of analysis by each participating laboratory for a selected list of PCDD's and PCDF's in spiked samples, (2) to determine the precision of analysis by each laboratory for PCDF's and PCDD's in spiked and in-service utility samples, (3) to perform an interlaboratory comparison of methods for accuracy and precision, and finally (4) to measure the levels of PCDF's and PCDD's in a few selected in-service utility samples.

Although this report primarily addresses the statistical analysis of PCDD and PCDF congener data from the five laboratories, a section on chemical methodology is included to allow the reader to examine the similarities and differences in the analytical procedures employed by each laboratory. These procedures are also described in more detail in the reports from the five individual laboratories (1-4).

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SECTION 2
CHEMICAL METHODOLOGY

SELECTION OF SAMPLES FROM ELECTRICAL EQUIPMENT FOR PCDF AND PCDD ANALYSIS

The rationale for the selection of transformers and capacitors to be sampled for PCDF and PCDD analysis was previously developed and reported elsewhere (5). Since the analysis is time consuming and difficult, the intention was to choose a small number of samples, which would yield the greatest amount of information about the large population of equipment in use. Also, the samples were chosen to provide a potentially wide spectrum of challenge to the analytical methodology which was being statistically evaluated in the Round Robin Study.

ACQUISITION OF IN-SERVICE SAMPLES

The acquisition of in-service samples was performed by one of the participating laboratories and the procedures employed have been reported (4).

The samples selected for use in the Round Robin Study are shown in Table 2. Two baseline samples were spiked with a selected number of congeners at known concentrations into mineral oil and Aroclor 1016, respectively. A third sample, Aroclor 1260, was spiked at known levels with these same congeners but it also contained endogenous PCBF's and PCDD's. Samples 11-17 (Table 2) were in-service utility samples that were selected to represent the various types of utility uses (5). In some cases the dielectric was Aroclor 1260, or mineral oil containing PCBs. Dielectric fluids from transformers, capacitors, or precipitators were selected for this Round Robin Study. Some of the devices had been in use for many years (Table 2).

SYNTHESIS OF STANDARDS

Because ^{13}C isotope is present in a natural abundance of 1.1%, synthetically enriched compounds can be prepared for use as analytical internal standards. Isotopically enriched compounds can be clearly distinguished from native substances by mass spectrometry. For this reason, Radian Corporation prepared ^{13}C-labeled and unlabeled (native) PCDD and PCDF congeners as analytical standards for use by all laboratories participating in the Round Robin Study (1). Subsequently, analytical standard solutions were also prepared of these authentic PCDD and PCDF congeners and distributed by Radian Corp.

Standard Materials

Radian synthesized the following materials (1):

1,2,3,7,8-Pentachlorodibenzofuran (PnCDF)
1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)
1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)
2,3,7,8-Tetrachlorodibenzofuran-$^{13}C_{12}$ (TCDF-^{13}C)
1,2,3,7,8-Pentachlorodibenzofuran-$^{13}C_{12}$ (PnCDF-^{13}C)
1,2,3,4,7,8-Hexachlorodibenzofuran-$^{13}C_{12}$ (HxCDF-^{13}C)

Several congeners in Radian's PCDF/PCDD Standards Inventory were also donated to the program. These congeners were:

2,3,7,8-Tetrachlorodibenzo-p-dioxin
2,3,7,8-Tetrachlorodibenzo-p-dioxin-$^{13}C_{12}$
2,3,7,8-Tetrachlorodibenzofuran
Octachlorodibenzo-p-dioxin-$^{13}C_{12}$
2,3,4,8-Tetrachlorodibenzofuran
2,3,4,7,8-Pentachlorodibenzofuran
1,2,3,6,7,8-Hexachlorodibenzofuran
2,3,4,6,7,8-Hexachlorodibenzofuran
Octachlorodibenzofuran

A mixture of congeners was obtained from Professor Christoffer Rappe of the University of Umea, Sweden. This semi-quantitative mixture, the "toxic cocktail", contained the following isomers:

1,3,4-Trichlorodibenzofuran
2,3,4,8-Tetrachlorodibenzofuran
2,3,4,7,8-Pentachlorodibenzofuran
1,2,3,4,8-Pentachlorodibenzofuran
1,2,3,4,7,9-Hexachlorodibenzofuran
1,2,3,4,6,7,8-Heptachlorodibenzofuran
Octachlorodibenzofuran

All the compounds prepared or obtained by Radian were made up as stock spiking solutions at 50 μg/mL or 100 μg/mL. These solutions were further diluted to produce standard solutions for distribution to the participating analytical laboratories. Table 15 lists the standards provided by Radian.

Baseline Liquids

To determine the accuracy and precision of PCDD/PCDF measurements, a selection of baseline liquids were re-analyzed in the Round Robin Analysis portion of the program (1). The Baseline liquids (Table 16) were dissolved in hexane at levels of 0.18 - 0.20 grams/mL and spiked with measured levels of ^{13}C-labeled congeners. In this instance, however, a selection of native congeners were spiked "blind" at levels known only to the person performing the spiking. Additionally, a set of samples were prepared with no native congener spikes in order to determine background PCDD/PCDF levels. Table 17 describes these samples and reveals the spiking levels. The seven "In-Service" Liquids were spiked with ^{13}C-labeled

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TABLE 15. STANDARD SOLUTIONS[a]

I.D. No.	Description	Concentration
E729-14-02	2,3,7,8-Tetrachlorodibenzofuran (TCDF)-^{13}C	100 ng/mL
E729-06-04	2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-^{13}C	100 ng/mL
E729-15-02	1,2,3,7,8-Pentachlorodibenzofuran (PnCDF)-^{13}C	100 ng/mL
E729-16-02	Octachlorodibenzo-p-dioxin (OCDD)-^{13}C	40 ng/mL
E729-05-04	2,3,7,8-Tetrachlorodibenzofuran	100 ng/mL
E729-06-02	2,3,7,8-Tetrachlorodibenzo-p-dioxin	100 ng/mL
E729-05-01	1,2,3,7,8-Pentachlorodibenzofuran	100 ng/mL
E729-05-02	1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)	100 ng/mL
E729-05-03	1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)	100 ng/mL
E729-06-01	Radian PCDF/TCDD Mixture	
	2,3,7,8-TCDF	100 ng/mL
	2,3,7,8-TCDD	100 ng/mL
	1,2,3,7,8-PnCDF	100 ng/mL
	1,2,3,4,7,8-HxCDF	100 ng/mL
	1,2,3,4,6,7,8-HpCDF	100 ng/mL
E729-07-02	Professor Rappe's "Toxic Cocktail"	
	1,2,4-TrCDF	~116 ng/mL
	2,3,4,8-TCDF	~100 ng/mL
	2,3,4,7,8-PnCDF	~100 ng/mL
	1,2,3,4,8-PnCDF	~100 ng/mL
	1,2,3,4,7,9-HxCDF	~100 ng/mL
	1,2,3,4,6,7,8-HpCDF	~100 ng/mL
	OCDF	~160 ng/mL
E729-55-01	Calibration Mixture I	
	Native Congeners	
	2,3,7,8-TCDD	7.5 µg/mL
	2,3,7,8-TCDF	7.5 µg/mL
	1,2,3,7,8-PnCDF	7.5 µg/mL
	2,3,4,7,8-PnCDF	7.5 µg/mL
	1,2,3,4,7,8-HxCDF	7.5 µg/mL
	2,3,4,6,7,8-HxCDF	7.5 µg/mL
	1,2,3,4,6,7,8-HpCDF	7.5 µg/mL
	OCDF	7.5 µg/mL
	Labeled Congeners	
	2,3,7,8-TCDD-$^{13}C_{12}$	500 ng/mL
	2,3,7,8-TCDF-$^{13}C_{12}$	500 ng/mL

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TABLE 15 (cont'd.)

I.D. No.	Description	Concentration
	1,2,3,7,8-PnCDF-$^{13}C_{12}$	500 ng/mL
	1,2,3,4,7,8-HxCDF-$^{13}C_{12}$	500 ng/mL
	OCDD-$^{13}C_{12}$	200 ng/mL
E729-55-03	Calibration Mixture II	
	Native Congeners	
	2,3,7,8-TCDD	2.5 µg/mL
	2,3,7,8-TCDF	2.5 µg/mL
	1,2,3,7,8-PnCDF	2.5 µg/mL
	2,3,4,7,8-PnCDF	2.5 µg/mL
	1,2,3,4,7,8-HxCDF	2.5 µg/mL
	2,3,4,6,7,8-HxCDF	2.5 µg/mL
	1,2,3,4,6,7,8-HpCDF	2.5 µg/mL
	OCDF	2.5 µg/mL
	Labeled Congeners	
	2,3,7,8-TCDD-$^{13}C_{12}$	500 ng/mL
	2,3,7,8-TCDF-$^{13}C_{12}$	500 ng/mL
	1,2,3,7,8-PnCDF-$^{13}C_{12}$	500 ng/mL
	1,2,3,4,7,8-HxCDF-$^{13}C_{12}$	500 ng/mL
	OCDD-$^{13}C_{12}$	200 ng/mL
E729-55-04	Calibration Mixture III	
	Native Congeners	
	2,3,7,8-TCDD	500 ng/mL
	2,3,7,8-TCDF	500 ng/mL
	1,2,3,7,8-PnCDF	500 ng/mL
	2,3,4,7,8-PnCDF	500 ng/mL
	1,2,3,4,7,8-HxCDF	500 ng/mL
	2,3,4,6,7,8-HxCDF	500 ng/mL
	1,2,3,4,6,7,8-HpCDF	500 ng/mL
	OCDF	500 ng/mL
	Labeled Congeners	
	2,3,7,8-TCDD-$^{13}C_{12}$	500 ng/mL
	2,3,7,8-TCDF-$^{13}C_{12}$	500 ng/mL
	1,2,3,7,8-PnCDF-$^{13}C_{12}$	500 ng/mL
	1,2,3,4,7,8-HxCDF-$^{13}C_{12}$	500 ng/mL
	OCDD-$^{13}C_{12}$	200 ng/mL

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TABLE 15 (cont'd.)

I.D. No.	Description	Concentration
E729-55-05	Calibration Mixture IV	
	Native Congeners	
	2,3,7,8-TCDD	150 ng/mL
	2,3,7,8-TCDF	150 ng/mL
	1,2,3,7,8-PnCDF	150 ng/mL
	2,3,4,7,8-PnCDF	150 ng/mL
	1,2,3,4,7,8-HxCDF	150 ng/mL
	2,3,4,6,7,8-HxCDF	150 ng/mL
	1,2,3,4,6,7,8-HpCDF	150 ng/mL
	OCDF	150 ng/mL
	Labeled Congeners	
	2,3,7,8-TCDD-$^{13}C_{12}$	500 ng/mL
	2,3,7,8-TCDF-$^{13}C_{12}$	500 ng/mL
	1,2,3,7,8-PnCDF-$^{13}C_{12}$	500 ng/mL
	1,2,3,4,7,8-HxCDF-$^{13}C_{12}$	500 ng/mL
	OCDD-$^{13}C_{12}$	200 ng/mL
E729-55-06	Calibration Mixture V	
	Native Congeners	
	2,3,7,8-TCDD	40 ng/mL
	2,3,7,8-TCDF	40 ng/mL
	1,2,3,7,8-PnCDF	40 ng/mL
	2,3,4,7,8-PnCDF	40 ng/mL
	1,2,3,4,7,8-HxCDF	40 ng/mL
	2,3,4,6,7,8-HxCDF	40 ng/mL
	1,2,3,4,6,7,8-HpCDF	40 ng/mL
	OCDF	40 ng/mL
	Labeled Congeners	
	2,3,7,8-TCDD-$^{13}C_{12}$	500 ng/mL
	2,3,7,8-TCDF-$^{13}C_{12}$	500 ng/mL
	1,2,3,7,8-PnCDF-$^{13}C_{12}$	500 ng/mL
	1,2,3,4,7,8-HxCDF-$^{13}C_{12}$	500 ng/mL
	OCDD-$^{13}C_{12}$	200 ng/mL
E729-56-01	Calibration Mixture VI	
	Native Congeners	
	2,3,7,8-TCDD	10 ng/mL
	2,3,7,8-TCDF	10 ng/mL

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TABLE 15 (cont'd.)

I.D. No.	Description	Concentration
	1,2,3,7,8-PnCDF	10 ng/mL
	2,3,4,7,8-PnCDF	10 ng/mL
	1,2,3,4,7,8-HxCDF	10 ng/mL
	2,3,4,6,7,8-HxCDF	10 ng/mL
	1,2,3,4,6,7,8-HpCDF	10 ng/mL
	OCDF	10 ng/mL
	Labeled Congeners	
	2,3,7,8-TCDD-$^{13}C_{12}$	500 ng/mL
	2,3,7,8-TCDF-$^{13}C_{12}$	500 ng/mL
	1,2,3,7,8-PnCDF-$^{13}C_{12}$	500 ng/mL
	1,2,3,4,7,8-HxCDF-$^{13}C_{12}$	500 ng/mL
	OCDD-$^{13}C_{12}$	200 ng/mL
E729-56-02	Calibration Mixture VII	
	Native Congeners	
	2,3,7,8-TCDD	2.5 ng/mL
	2,3,7,8-TCDF	2.5 ng/mL
	1,2,3,7,8-PnCDF	2.5 ng/mL
	2,3,4,7,8-PnCDF	2.5 ng/mL
	1,2,3,4,7,8-HxCDF	2.5 ng/mL
	2,3,4,6,7,8-HxCDF	2.5 ng/mL
	1,2,3,4,6,7,8-HpCDF	2.5 ng/mL
	OCDF	2.5 ng/mL
	Labeled Congeners	
	2,3,7,8-TCDD-$^{13}C_{12}$	500 ng/mL
	2,3,7,8-TCDF-$^{13}C_{12}$	500 ng/mL
	1,2,3,7,8-PnCDF-$^{13}C_{12}$	500 ng/mL
	1,2,3,4,7,8-HxCDF-$^{13}C_{12}$	500 ng/mL
	OCDD-$^{13}C_{12}$	200 ng/mL

[a]See Ref. 1. for details.

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TABLE 16. BASELINE FLUID SAMPLES - GC/MS CROSSCHECK[a]

ID No.	Description	Spike	Spiking Level
E729-09-01	Aroclor 1016	2,3,7,8-TCDF-^{13}C	100 ng/g
		1,2,3,7,8-PnCDF-^{13}C	100 ng/g
		2,3,7,8-TCDD-^{13}C	100 ng/g
		OCDD-^{13}C	40 ng/g
		1,2,3,4,7,8-HxCDF	100 ng/g
		1,2,3,4,6,7,8-HpCDF	100 ng/g
E729-10-0	Aroclor 1242	2,3,7,8-TCDF-^{13}C	100 ng/g
		1,2,3,7,8-PnCDF-^{13}C	100 ng/g
		2,3,7,8-TCDD-^{13}C	100 ng/g
		OCDD-^{13}C	40 ng/g
		1,2,3,4,7,8-HxCDF	100 ng/g
		1,2,3,4,6,7,8-HpCDF	100 ng/g
E729-11-01	Aroclor 1260	2,3,7,8-TCDF-^{13}C	100 ng/g
		1,2,3,7,8-PnCDF-^{13}C	100 ng/g
		2,3,7,8-TCDD-^{13}C	100 ng/g
		OCDD-^{13}C	40 ng/g
		1,2,3,4,7,8-HxCDF	100 ng/g
		1,2,3,4,6,7,8-HpCDF	100 ng/g
E729-12-01	Tri-Tetrachlorobenzenes	2,3,7,8-TCDF-^{13}C	100 ng/g
		1,2,3,7,8-PnCDF-^{13}C	100 ng/g
		2,3,7,8-TCDD-^{13}C	100 ng/g
		OCDD-^{13}C	40 ng/g
		1,2,3,4,7,8-HxCDF	100 ng/g
		1,2,3,4,6,7,8-HpCDF	100 ng/g
E729-08-01	Aged Mineral Oil	2,3,7,8-TCDF-^{13}C	100 ng/g
		1,2,3,7,8-PnCDF-^{13}C	100 ng/g
		2,3,7,8-TCDD-^{13}C	100 ng/g
		OCDD-^{13}C	40 ng/g
		2,3,7,8-TCDD	40 ng/g
		2,3,7,8-TCDF	20 ng/g
		1,2,3,7,8-PnCDF	40 ng/g
		1,2,3,4,7,8-HxCDF	120 ng/g
		1,2,3,4,6,7,8-HpCDF	0 ng/g
	Professor Rappe's "Toxic Cocktail"		
E729-08-01	Aged Mineral Oil	OCDF	~160 ng/g
		1,2,3,4,7,9-HxCDF	~100 ng/g
		2,3,4,7,8-PnCDF	~100 ng/g
		1,2,3,4,8-PnCDF	~100 ng/g
		2,3,4,8-TCDF	~100 ng/g
		1,3,4-TrCDF	~116 ng/g
		Aroclor 1260	510 µg/g
		Tri-Tetrachlorobenzenes	530 µg/g

[a]See Ref. 1 for details.

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TABLE 17. BASELINE FLUID SAMPLES - ROUND ROBIN ANALYSIS[a]

ID No.	Description	Spike	Spiking Level
E729-53-01	Mineral Oil	Aroclor 1260	640 µg/g
		Tri/Tetrachlorobenzenes	1250 µg/g
		2,3,7,8-TCDD-^{13}C	100 ng/g
		2,3,7,8-TCDF-^{13}C	100 ng/g
		1,2,3,7,8-PnCDF-^{13}C	100 ng/g
		1,2,3,4,7,8-HxCDF-^{13}C	100 ng/g
		OCDD-^{13}C	40 ng/g
		2,3,7,8-TCDD	25 ng/g
		2,3,7,8-TCDF	100 ng/g
		2,3,4,8-TCDF	100 ng/g
		1,2,3,7,8-PnCDF	150 ng/g
		2,3,4,7,8-PnCDF	150 ng/g
		1,2,3,4,7,8-HxCDF	250 ng/g
		1,2,3,6,7,8-HxCDF	50 ng/g
		2,3,4,6,7,8-HxCDF	175 ng/g
		1,2,3,4,6,7,8-HpCDF	400 ng/g
		OCDF	0 ng/g
E729-53-02	Aroclor 1016	2,3,7,8-TCDD-^{13}C	100 ng/g
		2,3,7,8-TCDF-^{13}C	100 ng/g
		1,2,3,7,8-PnCDF-^{13}C	100 ng/g
		1,2,3,4,7,8-HxCDF-^{13}C	100 ng/g
		OCDD-^{13}C	40 ng/g
		2,3,7,8-TCDD	53 ng/g
		2,3,7,8-TCDF	53 ng/g
		2,3,4,8-TCDF	0 ng/g
		1,2,3,7,8-PnCDF	158 ng/g
		2,3,4,7,8-PnCDF	158 ng/g
		1,2,3,4,7,8-HxCDF	0 ng/g
		1,2,3,6,7,8-HxCDF	53 ng/g
		2,3,4,6,7,8-HxCDF	132 ng/g
		1,2,3,4,6,7,8-HpCDF	526 ng/g
		OCDF	132 ng/g
E729-53-03	Aroclor 1260	2,3,7,8-TCDD-^{13}C	100 ng/g
		2,3,7,8-TCDF-^{13}C	100 ng/g
		1,2,3,7,8-PnCDF-^{13}C	100 ng/g
		1,2,3,4,7,8-HxCDF-^{13}C	100 ng/g
		OCDD-^{13}C	40 ng/g
		2,3,7,8-TCDD	111 ng/g
		2,3,7,8-TCDF	833 ng/g
		2,3,4,8-TCDF	194 ng/g
		1,2,3,7,8-PnCDF	111 ng/g
		2,3,4,7,8-PnCDF	111 ng/g
		1,2,3,4,7,8-HxCDF	500 ng/g
		1,2,3,6,7,8-HxCDF	278 ng/g
		2,3,4,6,7,8-HxCDF	56 ng/g
		1,2,3,4,6,7,8-HpCDF	111 ng/g
		OCDF	222 ng/g
		Aroclor 1260	680 µg/g
		Tri/Tetrachlorobenzenes	1320 µg/g

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TABLE 17 (cont'd.)

ID No.	Description	Spike	Spiking Level
E729-54-02	Mineral Oil	2,3,7,8-TCDD-^{13}C	100 ng/g
		2,3,7,8-TCDF-^{13}C	100 ng/g
		1,2,3,7,8-PnCDF-^{13}C	100 ng/g
		1,2,3,4,7,8-HxCDF-^{13}C	100 ng/g
		OCDD-^{13}C	40 ng/g
E729-54-03	Aroclor 1260	2,3,7,8-TCDD-^{13}C	100 ng/g
		2,3,7,8-TCDF-^{13}C	100 ng/g
		1,2,3,7,8-PnCDF-^{13}C	100 ng/g
		1,2,3,4,7,8-HxCDF-^{13}C	100 ng/g
		OCDD-^{13}C	40 ng/g
E729-54-04	Aroclor 1016	2,3,7,8-TCDD-^{13}C	100 ng/g
		2,3,7,8-TCDF-^{13}C	100 ng/g
		1,2,3,7,8-PnCDF-^{13}C	100 ng/g
		1,2,3,4,7,8-HxCDF-^{13}C	100 ng/g
		OCDD-^{13}C	40 ng/g

[a]See Ref. 1 for details.

congeners at Battelle Memorial Institute, Columbus, Ohio, using stock solutions provided by Radian Corporation (4).

PREPARATION OF PCDD AND PCDF STANDARDS

Calibration Standards)

PCDD and PCDF standard compounds including ^{13}C-labelled standard compounds, dissolved in solvent, were received at each laboratory sealed in glass vials. The seven vials each contained all of the following compounds:

2,3,7,8-TCDD	1,2,3,4,6,7,8,9-OCDF
2,3,7,8-TCDF	2,3,7,8-TCDD-$^{13}C_{12}$
1,2,3,7,8-PnCDF	2,3,7,8-TCDF-$^{13}C_{12}$
2,3,4,7,8-PnCDF	1,2,3,7,8-PnCDF-$^{13}C_{12}$
1,2,3,4,7,8-HxCDF	1,2,3,4,7,8-HxCDF-$^{13}C_{12}$
2,3,4,6,7,8-HxCDF	1,2,3,4,6,7,8,9-OCDD-$^{13}C_{12}$

Each vial contained all of the native compounds at one of the following concentration levels:

7,500 ng/mL
2,500 ng/mL
500 ng/mL
150 ng/mL
40 ng/mL
10 ng/mL
2.5 ng/mL

Each standard solution contained all of the ^{13}C compounds at 500 ng/mL each.

Baseline Samples

Three baseline samples (Aroclor 1016, Aroclor 1260, and aged mineral oil) were prepared at Radian Corporation as solutions in n-hexane at a concentration of about 0.2 g/mL. Each sample was spiked with 100 ng/g of each of the five 13C-compounds listed above. Each sample was also spiked with "blind" amounts of the following unlabeled compounds:

2,3,7,8-TCDD
2,3,7,8-TCDF
2,3,4,8-TCDF
1,2,3,7,8-PnCDF
2,3,4,7,8-PnCDF
1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-HxCDF
2,3,4,6,7,8-HxCDF
1,2,3,4,6,7,8-HpCDF
OCDF

In addition, the mineral oil sample was spiked with 625 µg/g Aroclor 1260 and 1250 µg/g tri-/tetrachlorobenzenes.

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In-Service Samples

Seven in-service samples were fluids consisting of five askarels and two mineral oils, all of which were prepared at a concentration of approximately 200 mg/mL in n-hexane. Each sample was spiked at Battelle with the five isotopically-labeled internal standards at concentrations of 100 ng per gram of oil.

SAMPLE EXTRACTION AND EXTRACT PREPARATIONS

For this Round Robin Study each participating laboratory employed their best available in-house extraction and analysis techniques which are shown in Table 1. Because the methods varied between the laboratories, a parameter being statistically tested in the Round Robin Study, a detailed description of each is presented here.

Procedures Employed by NYSDOH (3)

The volume of a portion of samples of electric insulating fluids and mineral oil was measured and extracted by adding 25 mL of acetone and mixing thoroughly. Hexane (25 mL) was added and the sample was stirred and extracted for a minimum of 1 h. To remove PCBs and nonplanar aromatics and to isolate one fraction containing all tetra- to OCDFs and OCDDs the sample extract was chromatographed on acid alumina, PX-21 carbon and neutral alumina. A preprogrammed Hamilton valve control system designed at NYSDOH was used to reproducibly select solvents and chromatographic columns (3). The recovery of all tetra- to OCDFs and OCDDs (as verified with available standards) from an individual chromatographic column was >95%.

The sample extract was applied to an activated acidic alumina column, followed by 30 mL of 3% CH_2Cl_2 in hexane. The CDF/CDD fraction was eluted with 70 mL of 50% CH_2Cl_2 in hexane onto a carbon column followed by 50 mL of 10% benzene in hexane. The CDF/CDD fraction was eluted in the reverse direction with 30 mL of 50% xylene in hexane onto a neutral alumina column (replaces xylene with a volatile solvent) followed by 30 mL of 3% CH_2Cl_2 in hexane. The final purified CDF/CDD fraction was eluted with 70 mL of easily volatilized 50% CH_2Cl_2 in hexane and concentrated with first boiling water and then vacuum to 4-10 μL (3). Extracts were stored in the dark for GC/MS in sealed 150 μL borosilicate capillary tubes.

Procedures Employed by IITRI (2)

The extraction and cleanup procedure which was employed was based on the general method described by Albro and coworkers (2) for the fractionation and class determination of complex mixtures of chlorinated aromatic compounds. An overview is shown in Figure 1. Briefly, the procedure consisted of the following major steps.

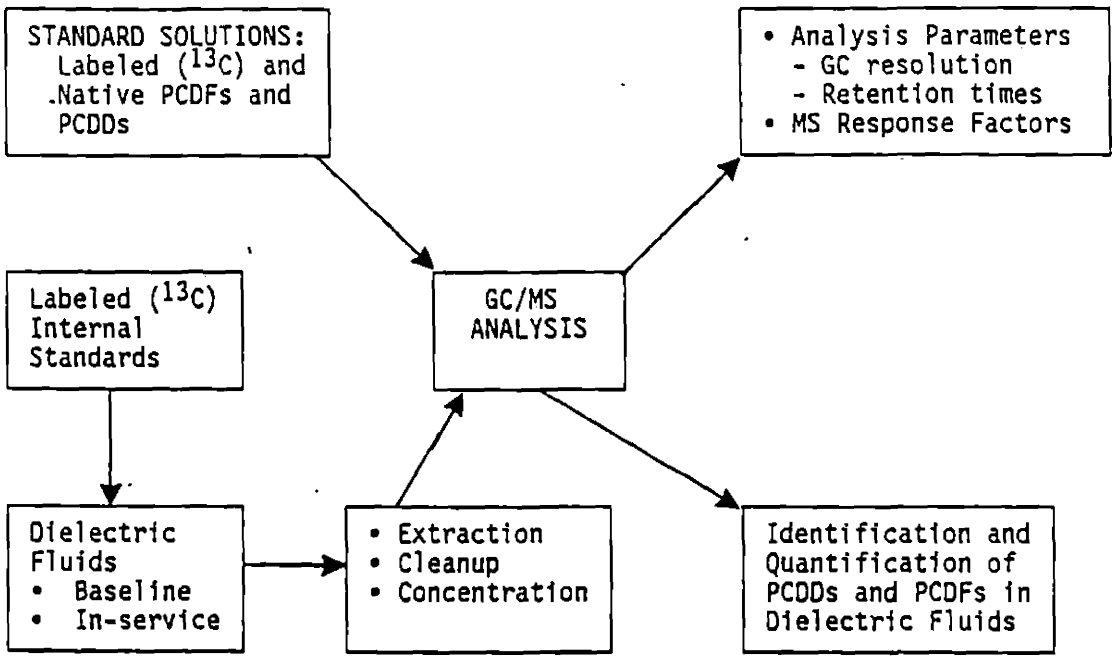

Figure 1. Overview of Analytical Protocol (Ref. 2)

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1. To the sample (ca. 0.1 g), spiked with a mixture of ^{13}C-labeled PCDF and/or PCDD internal standards, about 30 ng of 2,3,7-tri-CDD was added to serve as a "carrier" compound.
2. The sample was chromatographed on a gel permeation column (20 g Sephadex LH-20), eluting with 50% methylene chloride-in-methanol and collecting the 60-150 mL fraction, to separate halogenated aromatic compounds from any alphatics present.
3. About 10 µL of propylene glycol were added as a "keeper" compound, to prevent sample loss during roto-evaporation.
4. After roto-evaporation, the halogenated aromatic fraction was chromatographed on an A-540 basic alumina column (20 grams topped with a layer of anhydrous sodium sulfate), eluting with 180 mL of 2% methylene chloride-in-hexane, to elute the PCB fraction.
5. Congeners eluted with 20% methylene chloride-in-hexane (200-220 mL), to recover PCDFs, PCDDs, polychlorinated quadriphenyls, and less chlorinated PCBs.
6. About 10 µL of propylene glycol were added, then the fraction was concentrated to near dryness and loaded onto a 10 g EM-1078 acidic alumina column, eluting with 80 mL of 1% methylene chloride-in-hexane, to purify the PCDF/PCDD fraction. The eluate was discarded.
7. The purified PCDF/PCDD fraction was eluted with 20% methylene chloride-in-hexane (120 mL).
8. The extract was concentrated to 25 µL immediately before analysis using toluene.
9. When necessary, the extract was purified further by loading it in hexane onto a small (1 cm) column of Carbopack C mixed with Celite 545, eluting with 2 mL hexane, 1 mL of 50% methylene chloride-in-cyclohexane and 1 mL of methylene chloride:methanol:benzene (75:20:5). The column flow was reversed and 8 mL of toluene were collected.

Procedures Employed by Radian (1)

The extraction technique used by Radian for the Round Robin analysis phase of this study was a modified Smith-Stallings/EPA Region VII clean-up (1). Figure 2 describes the clean-up procedure used for these dielectric samples.

An aliquot of the sample (approximately 0.25 g) was transferred to a column consisting of 2.5 cm sodium sulfate (on bottom), 5 g silica gel type 60, (70-230 mesh), 6 g basic silica gel (35% potassium hydroxide), and 10 g acid silica gel (40% sulfuric acid). This column was eluted with 170 mL 1:1 methylene chloride:cyclohexane directly onto a 5.0 cm column of 1-9 µm carbon (PX-2) on glass fibers. The silica gel was discarded and the carbon column washed with 25 mL 1:1

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Part 1
Modified Smith-Stallings Clean-Up

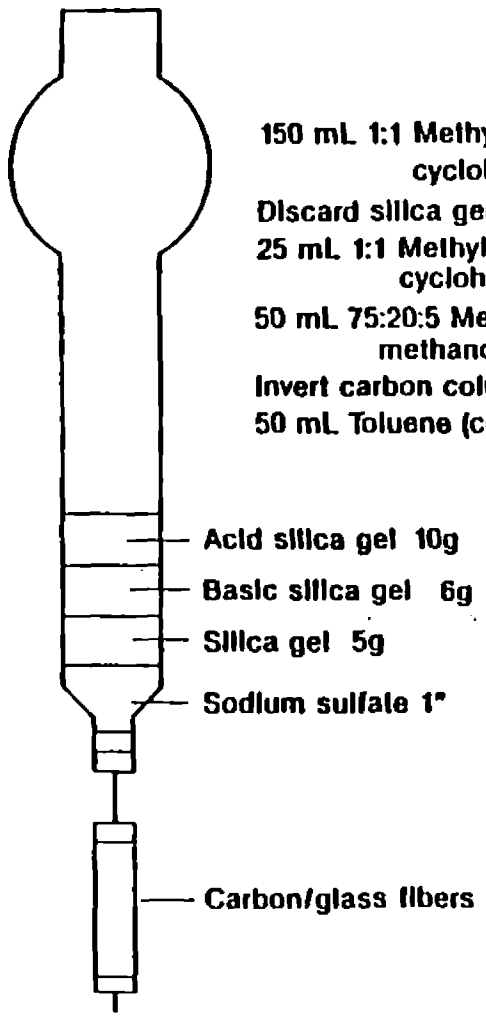

Figure 2. Sample Clean-up Procedure:

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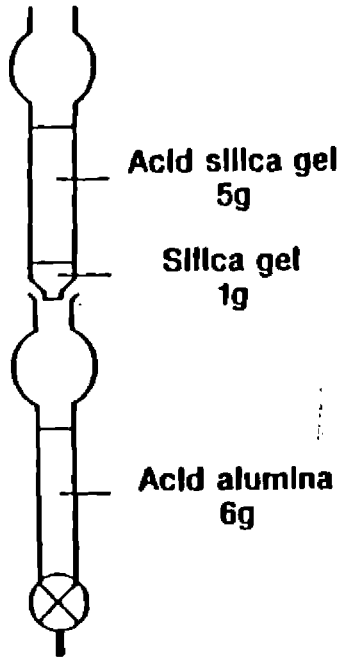
Part 2
"Region 7" Clean-Up



Solvent exchange the toluene to hexane
Transfer hexane extract to silica gel column
60 mL hexane
Discard silica gel
20 mL hexane
20 mL 20% methylene chloride: hexane (collect)
Concentrate

Round Robin Analysis (Ref. 1)

methylene chloride:cyclohexane and 50 mL 75:20:5 methylene chloride:methanol: benzene. The eluted solvents were discarded. The PCDDs and PCDFs were eluted from the carbon column with 50 mL toluene. The toluene was evaporated to dryness on a rotary evaporator. The dried extract was redissolved in hexane and transferred to a column of 5 g acid-impregnated silica gel and 1 g silica gel (lower layer). This column was eluted with 60 mL hexane directly onto a 6 g acid alumina (AG 4, 100-200 mesh) column. The silica gel was discarded and the acid alumina was washed with another 20 mL hexane. The eluted hexane was discarded. The PCDDs and PCDFs were eluted off the alumina with 20 mL 20% methylene chloride/hexane. The volume of this eluate was reduced to 1-2 mL with a stream of nitrogen. The extract was transferred to a 1 mL conical vial and taken to dryness. Each sample was processed in triplicate (this was the only lab which performed sample processing in replicate).

Procedures Employed by Battelle (4)

One milliliter aliquots (containing ~250 mg of the original fluid) of each sample was transferred to separatory funnels and diluted with 49 mL of hexane. The hexane solutions were washed with 10 mL portions of concentrated sulfuric acid until the washes were only slightly colored. A maximum of five acid washes were used. Following the acid washes, each oil was washed with 20 mL of distilled water to remove any residual acid. The hexane solutions were transferred to glass collection tubes and concentrated to approximately 2 mL using gentle streams of nitrogen gas.

The hexane solutions were transferred to multilayered silica gel columns containing activated silica gel, 44 percent concentrated sulfuric acid on silica gel, and 33 percent 1M sodium hydroxide on silica gel. The PCDD/PCDF isomers were eluted from the columns using 70 mL of hexane and the entire eluates, including the original sample volume, were collected.

The eluates from the multilayered silica gel columns were concentrated to approximately 1 mL using gentle streams of nitrogen and transferred to the top of columns containing 5 g of activated basic alumina. These columns were eluted using hexane, hexane/methylene chloride (97:3, v/v) and hexane/methylene chloride (1:1, v/v) as elution solvents. The 1:1 hexane/methylene chloride eluates were collected, solvent exchanged into hexane, and transferred to the top of alumina columnns containing approximately 2 g of activated basic alumina. These columns were eluted with hexane, hexane/methylene chloride (97:3, v/v), and hexane/methylene chloride (1:1, v/v). The 1:1 hexane/methylene chloride eluates were collected, concentrated to near dryness with nitrogen gas, and spiked with 10 ng of 1,2,3,4-TCDD-$^{13}C_{12}$. These solutions were stored at 0°C until they were analyzed.

For samples still too contaminated with interferences to be analyzed, they (41159-11-12,13,14,17 and E729-53-02,03) were chromatographed through florisil columns to provide additional analyte enrichment. Each of the florisil columns was prepared using 5 g of activated florisil (60-100 mesh) which was slurry packed with hexane. The sample solutions were diluted with approximately 5 mL of hexane and transferred to the florisil columns. The columns were eluted with hexane, hexane/ethyl ether (94:6, v/v), and hexane/methylene chloride (25:75, v/v). The 25:75 hexane/methylene chloride fraction was collected and concentrated to approximately 20 µL. These solutions were stored at 0°C until they were analyzed.

CAPILLARY GAS CHROMATOGRAPHY/MASS SPECTROMETRY ANALYSIS

Procedures Used by NYSDOH (3)

Low Resolution Mass Spectrometry--

All sample extracts were analyzed with a Hewlett-Packard 5970 Mass Selective Detector. A portion of each extract was injected into the split-splitless injection port (250°C) and eluted through a 50 m (.25 mm i.d.; .33 µm film thickness) Hewlett-Packard 5% phenyl methyl silicone fused silica capillary column. The following temperature program was used:

H-P 5890 Temperature Program

Level	Initial Temp.	Initial Time	Rate (°C/min)	Final Temp.	Final Time	Total Time
1	190	1.00	5.0	220	16.00	23.00
2			5.0	235	7.00	33.00
3			5.0	250	18.00	54.00
4			5.0	270	7.00	65.00
5			5.0	300	24.00	95.00

The gas chromatograph is interfaced directly to the source of the mass selective detector and the transfer line and ion source are heated to 275°C and 150°C respectively. The mass selective detector was operated in the selective ion monitoring (SIM) mode. The m/z values characteristic of native TCDD, TCDF, PnCDF, HxCDF, OCDF, and OCDD and TCDD-$^{13}C_{12}$, TCDF-$^{13}C_{12}$, PnCDF-$^{13}C_{12}$, HxCDF-$^{13}C_{12}$ and OCDD-$^{13}C_{12}$ were monitored at various times during the ninety-five (95) minute runs. The time window used for monitoring the different congener classes (TCDD, TCDF, etc.) were determined by the previous injection of either window standards (TCDD) or a mixture of combusted PCB. Raw data was collected into and processed by an H-P Quicksilver data system. The data system was used to generate electronic copies of

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reports of retention times and areas at each m/z value monitored. Additional custom software was used to identify GC peaks and quantitate analyte levels. This software was written by NYSDOH staff and processed on an IBM PC or XT. The H-P reports were input in ASCII and transferred by using a customized H-P Pascal operating system (sender) and the program called Kermit (receiver). Each sample extract was instrumentally analyzed in triplicate.

High Resolution Mass Spectrometry--

Some extracts were analyzed with a Kratos MS-50 High Resolution GC/MS/DS. In this system portions of the extracts were injected into the on-column injection port of a Carlo-Erba 4160 high resolution gas chromatograph and eluted through a 50 m (.20 mm i.d.; .33 μm film thickness) Hewlett-Packard 5% methyl phenol silicone fused capillary column. The oven was temperature programmed as follows:

H-P 5890 Temperature Program

Level	Initial Temp.	Initial Time	Rate (°C/min)	Final Temp.	Final Time	Total Time
1	190°C	1.0	5.0	220	16.00	23.00
2			5.0	235	7.00	33.00
3			5.0	300	50.00	86.00

The gas chromatograph is interfaced to the MS-50 through a direct line heated to 250°C. The ion source temperature was 250°C. The high resolution mass spectrometry was operated in either of two SIM type modes. In normal multiple peak monitoring peak (NMPM) the m/x values of a particular analyte and its corresponding internal standard were observed at 10,000 RP (10% valley) by integrating all the signal detected within 50 ppm of their exact mass values. In high resolution multiple peak monitoring mode (HRMPM) the mass spectrometry is operated at the same high resolving power but the analyte and internal standard exact masses are profiled. The data system adjusts the accelerating and electric sector voltages to focus a mass slightly below the mass of interest. The computer then triggers a 0.3 sec analog scan through a 300 ppm section of the mass range which includes the exact mass of the ion of interest (3). In both ion monitoring modes the raw data was acquired into and processed by the Kratos DS-55 data system. Quantitation was done manually by calculating concentrations using peak areas obtained from the DS-55 output.

Procedures Used by IITRI (2)

Samples and standards were analyzed using combined capillary column gas chromatography/low-resolution mass spectrometry (HRGC/LRMS or GC/MS). Figure 3 summarizes the major elements of their analysis protocol.

The gas chromatograph, a Varian 3700, was equipped with a 60 m x 0.25 mm i.d. fused-silica SP-2330 (Supelco) column. Samples were injected in the splitless mode, with the column programmed limit for 60 minutes. The SP-2330 column had been shown to separate the 2,3,7,8-TCDD from all other tetra-isomers, and to partially separate the 2,3,7,8-TCDF from the 2,3,4,8-TCDF (2). However, it was unable to resolve 1,2,3,7,8-PnCDF from 1,2,3,4,8-PnCDF and 1,2,3,4,7,8-HxCDF from 1,2,3,4,7,9-HxCDF. These isomers can be separated to some extent on less polar columns, such as OV-17 or DB-5 (2).

An example of the chromatographic resolution obtained using the SP-2330 column is shown in Figure 4 for 2,3,7,8-TCDD and several closely-eluting tetradioxin isomers. The plot clearly shows that this column was isomer-specific for 2,3,7,8-TCDD. Figure 5 shows the separation obtained between the 2,3,7,8- and 2,3,4,8-TCDF isomers. Here the resolution was not as good as in the previous case, but is nonetheless sufficient to permit quantification of the two isomers in most cases.

All data were acquired by software-controlled multiple ion detection using two ion masses from the molecular ion cluster for each of the PCDF and PCDD levels of chlorination. Two ions were also monitored for each internal standard which permitted the calculation and comparison of the isotope ratios with their theoretical values to verify their identity. Several additional ion masses were monitored to indicate possible interferences from chlorinated diphenyl ethers (2). The presence of these compounds could give rise to fragment ions with the same masses as those monitored for the PCFs. Therefore, the absence of a co-response for the diphenyl ether ion mass when a PCDF compound shows a positive response was taken to indicate that the signal for the PCDF was "real".

A listing of the masses that were monitored, along with the theoretical isotope ratios, is given in Table 18. In order to obtain maximum sensitivity and selectivity during an experiment, the run was divided into 4 adjoining time windows. Each window contained up to 16 ion masses (for specific native and labeled PCDFs and PCDDs, and chlorinated diphenyl ethers), and the sample dwell times and interchannel delay times were chosen to give the most sensitive and rapid cycle time. Since the GC column used in this work did not yield complete separation of each chlorination level of PCDFs/PCDDs from the other groups in a given time windows, some of the ion masses were included in more than one window to ensure that all isomers from a particular congener class were monitored.

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GC/MS ANALYSIS
(IITRI)

- HRGC/LRMS
  - UNIT MASS RESOLUTION ~1,000
  - SP-2330, OV-17 COLUMNS

- SELECTED ION MONITORING
  - TWO ION MASSES MONITORED PER PCDF/PCDD CONGENER CLASS

- IDENTIFICATION CRITERIA
  - COINCIDENT GC RETENTION TIMES
  - CORRECT INTENSITY RATIOS FOR MONITORED IONS

- QUANTIFICATION
  - DIRECT COMPARISON WITH ADDED INTERNAL STANDARDS
  - MS RESPONSE FACTORS FROM STANDARDS

Figure 3. Summary of Major Elements of IITRI's GC/MS Analysis Protocol (Ref. 2).

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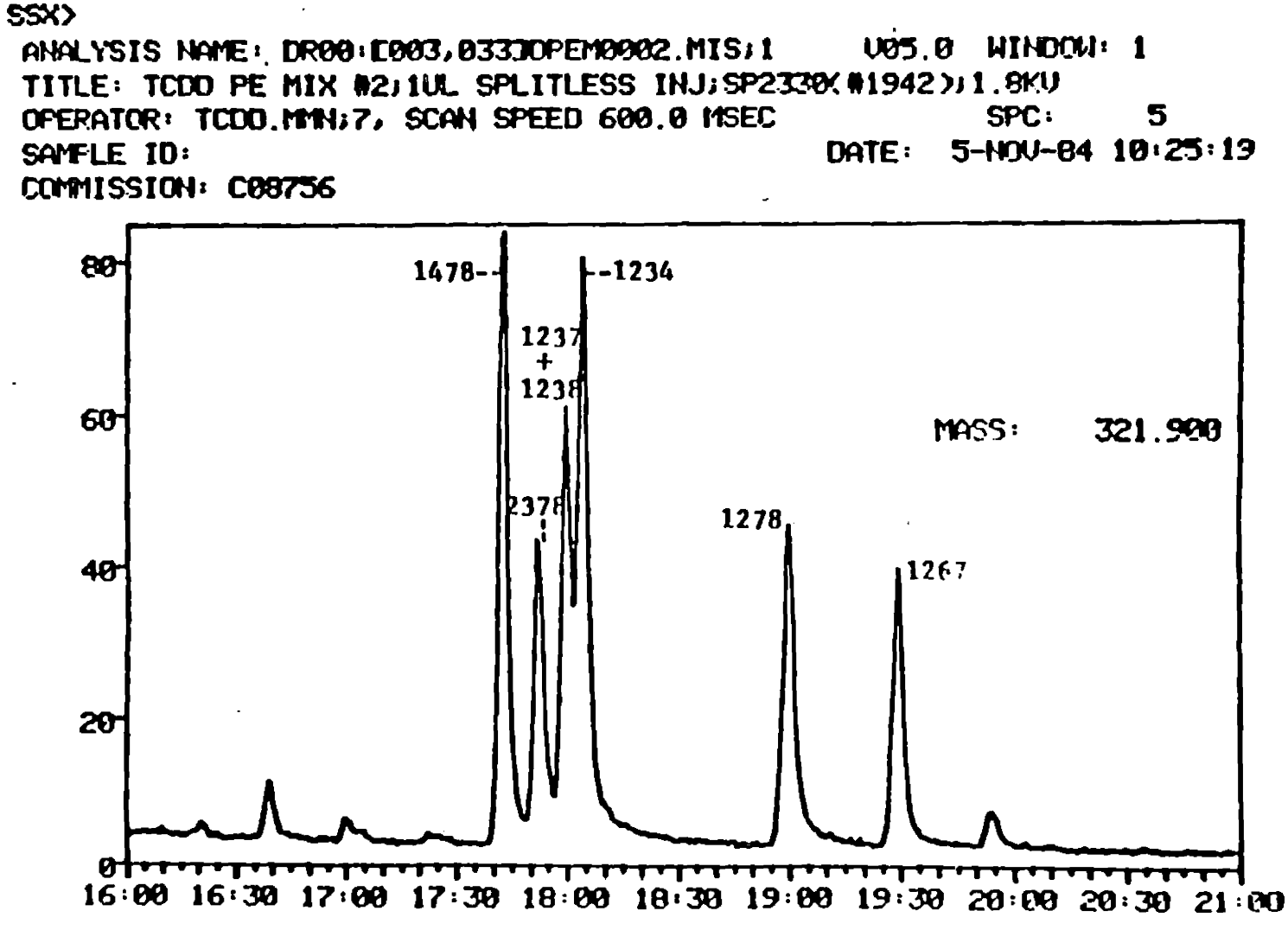

Figure 4. Selected ion current profile for mass 322 from GC/MS analysis of performance check solution containing TCDDs as noted in the plot, using a 60 m SP-2330 fused silica capillary column (Ref. 2).

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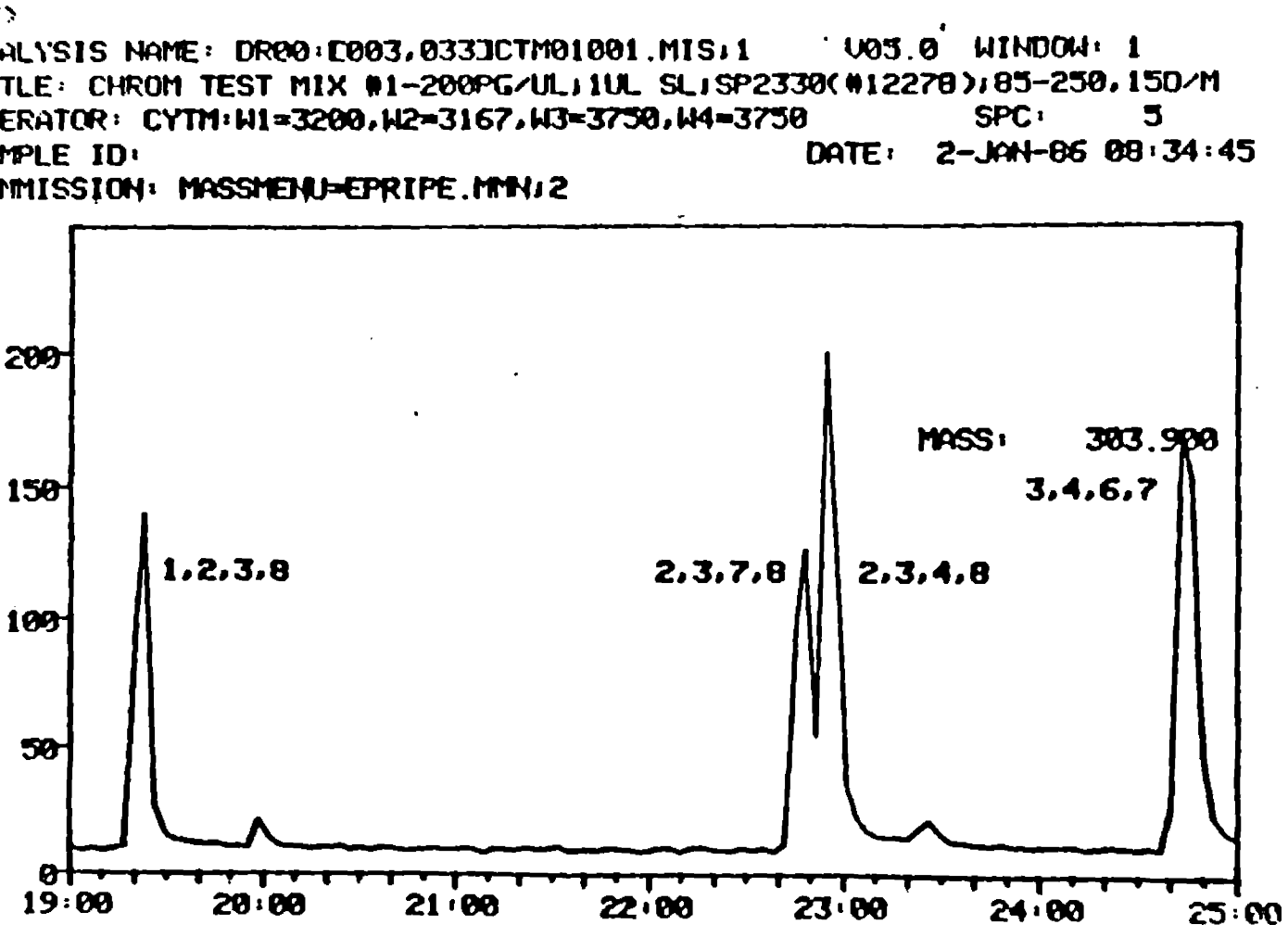

Figure 5. Selected ion current profile for mass 304 from GC/MS analysis of performance check solution containing TCDFs as noted on the plot, using a 60 m SP-2330 fused silica capillary column (Ref. 2).

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TABLE 18. ANALYTICAL SEQUENCE IN GC/MS DETERMINATION OF PCDFs AND PCDDs[a]

Time Window	Start (min)	Stop (min)	GC Column Temp (C)	Temp Prog Rate (C/min)	Cycle Time (ms)	Dwell Time (ms)	Compounds Monitored[b]	Ions Monitored[c] (m/z) Mass 1	Mass 2	Isotope Ratio (Mass 1/Mass 2)
1	14:00	26:00	85	15	3200	197	TCDF	303.9	305.9	0.77
							13C-TCDF	315.9	317.9	0.77
							TCDD	319.9	321.9	0.77
							13C-TCDD	331.9	333.9	0.77
							PnCDF	337.9	339.9	0.61
							13C-PnCDF	349.9	351.9	0.61
							PnCDD	355.9	357.9	1.54
							HxDPE[a]	373.8	--	--
							HpDPE[a]	407.8	--	--
2	26:00	35:30	250	--	3167	195	PnCDF	337.9	339.9	0.61
							PnCDF[b]	274.9	--	--
							PnCDD	355.9	357.9	1.54
							PnCDD[b]	290.9	--	--
							HxCDF	373.8	375.8	1.23
							13C-HxCDF	385.9	387.9	1.23
							HxCDF[b]	310.9	--	--
							13C-HxCDF	389.8	391.8	1.23
							HxCDF[b]	310.9	--	--
							HxCDD	389.9	391.8	1.23
							HxCDD[b]	326.9	--	--
							HpDPE[a]	407.8	--	--
							ODPE[a]	443.8	--	--
3	35:30	56:00	250	--	3750	372	HxCDF	373.8	375.8	1.23
							HxCDD	389.9	391.8	1.23
							HpCDF	407.8	409.8	1.03
							HpCDD	423.8	425.8	1.03
							ODPE[a]	443.8	--	--
							NDPE[a]	477.7	--	--
4	56:00	66:00	250	--	3750	534	OCDF	441.7	443.7	0.88
							OCDD	457.7	459.7	0.88
							13C-OCDD	469.8	471.8	0.88
							DDPE[a]	511.7	--	--

[a]See Ref. 2.

[b]HxPE, HpDPE, ODPE, NDPE, DDPE designate hexa-, hepta-, octa-, nona-, and decachlorobiphenyl ethers resp.

[c]Mass monitored to identify fragment ion (M-COCl).

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The major criteria which were used for confirming the presence of specific PCDFs/PCDDs in the samples analyzed were the following: (1) correct retention times for each PCDF/PCDD of interest relative to the appropriate isotopically-labeled internal standard(s), (2) intensity ratio for $(M)^+/(M+2)^+$ within 10% of theoretically-expected ratio (Table 18), and (3) signal-to-noise response of each PCDF/PCDD of interest greater than 2.5:1 for both ion masses monitored. Each sample extract was instrumentally analyzed in triplicate.

Quantification--Ideally, each of the 210 separate isomers of PCDF and PCDD should be quantified using the instrument response of the corresponding labeled internal standard as a reference. Since only a limited number of these standards were available in practice, the approach generally followed was to use an appropriate set of internal standards which included representative isomers from each chlorinated class of PCDFs and PCDDs, and further assumed that the data obtained for these was representative of all isomers in each group.

Relative response factors were determined from the analysis of standard solutions containing the internal standrds and isomers representative of each chlorination class. Response factors at IITRI were calculated from the following equation:

$$RF = A_X Q_{1s} / A_{1s} Q_X$$

where A_X = sum of the integrated ion abundances of the masses for the unlabeled compound
A_{1s} = sum of the integrated ion abundances of the masses for the appropriate labeled compound
Q_{1s} = amount of the appropriate labeled compound (ng)
Q_X = amount of the labeled compound (ng)

Quantification of PCDFs and PCDDs in the sample extract was achieved by calculating the ratios of the mass spectral responses obtained for the ions characteristic of the labeled PCDFs/PCDDs to those of the appropriate internal standards, corrected for differences in response factor. The equation used for quantification was:

$$\text{Concentration (ng/g)} = (A_X \times Q_{1s})/A_{1s} \times W \times R_F$$

where W = weight of sample (g)
R_F = response factor

Because the labeled internal standard was added prior to sample extraction and analysis, and the internal standard was quantified at the same time as the native components, any losses of PCDD/PCDF incurred during the analysis were accounted for by the above approach.

Detection limits, i.e. the minimum detectable concentrations required to produce a signal 2.5 times the average background signal, were calculated for each

PCDF/PCDD congener class. For each class, the average width of the baseline noise-band for the native compounds and the peak height for a known concentration of the associated ^{13}C-labeled analog were measured manually. Measurements of the noise band were made in a region of the selected ion current profile that was free of interferences and as close as practicable in the plot to the peak for the corresponding ^{13}C-labeled compound. The detection limit DL was calculated using the relationship:

$$DL = (2.5)\ (A_X \times Q_{IS})/A_{IS} \times R_F \times W)$$

where A_X = height of the noise band of the selected mass for the unlabeled compound

A_{IS} = height of the peak corresponding to the labeled compound of the same congener class.

Procedures Used by Radian (1)

GC/MS analysis of the sample extracts was performed on a Hewlett-Packard 5985/87 in the selected ion monitoring mode for quantitation and identification of PCDD/PCDFs. Each sample was extracted in triplicate and each extract was instrumentally analyzed in triplicate yielding nine determinations on each sample. The first series of extracts were run on a Hewlett-Packard 50 m, Ultra-2 column (a methylphenyl silicone coating equivalent to DB-5). On the Ultra-2 column, an initial temperature of 80°C was used. The second and third determinations were performed using a J&W 60 m, 0.32 mm I.D., 1 μm film, DB-5 column. The GC/MS conditions are shown on Table 19. Dried sample extracts were dissolved in 50 μL of hexane and a 1.0 μL volume was injected onto a cool SGE on-column injector.

Each of the three extracts was analyzed a fourth time using a Supelco SP-2331, 60 m, 0.32 mm I.D., 0.2 μm film capillary column to separate 2,3,7,8-TCDF and 2,3,4,8-TCDF. For this separation, samples were evaporated to dryness and then reconstituted with 40 μL of nonane. A two-stage temperature program of 170°-200° at 20°/min followed by 200°-275° at 4°/min was used.

Chromatographic Test Mixture

Part of this project involved separating the more toxic PCDD/PCDF isomers from coeluters. The chromatographic test mixture prepared at Battelle Memorial Institute contained the following analytes at 200 ng/L each:

1,3,6,8-TCDD	1,2,3,4,8-PnCDF
1,2,8,9-TCDD	1,2,4,6,8-PnCDF
1,2,3,4-TCDD	2,3,4,6,7-PnCDF
2,3,7,8-TCDD	1,2,3,7,8-PnCDF
1,3,7,8-TCDD	2,3,4,7,8-PnCDF
3,4,6,7-TCDF	1,2,4,6,7,8-HxCDF
2,3,4,8-TCDF	2,3,4,6,7,8-HxCDF
1,2,3,8-TCDF	1,2,3,4,7,8-HxCDF
2,3,7,8-TCDF	1,2,3,6,7,8-HxCDF

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This test mixture was run daily to evaluate the chromatography. Chromatograms of all the analytes in the test mixture are shown on Figures 6 through 9 for DB-5 and

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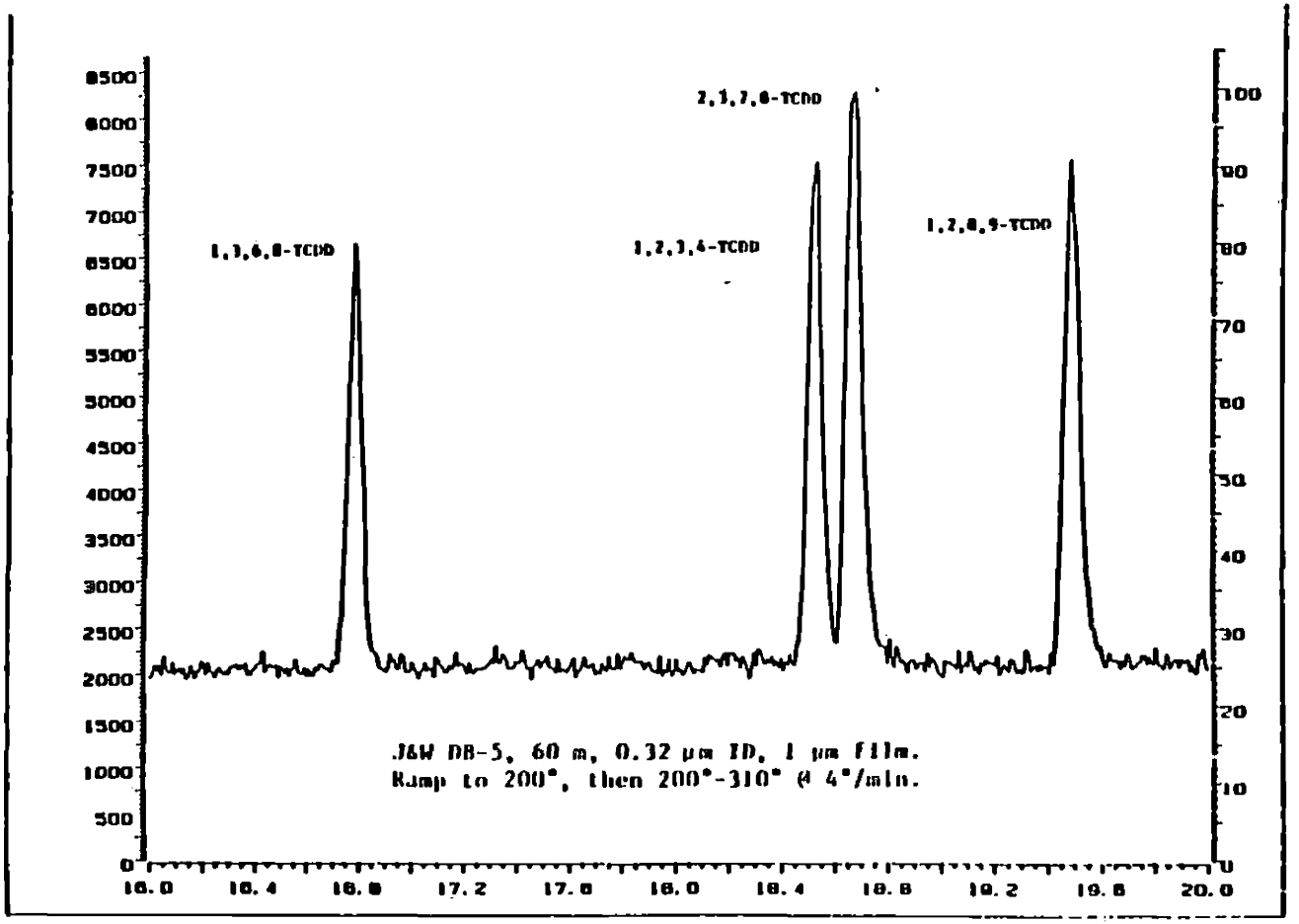

Figure 6. TCDD Test Mixture Chromatogram on DB-5 (Ref. 1)

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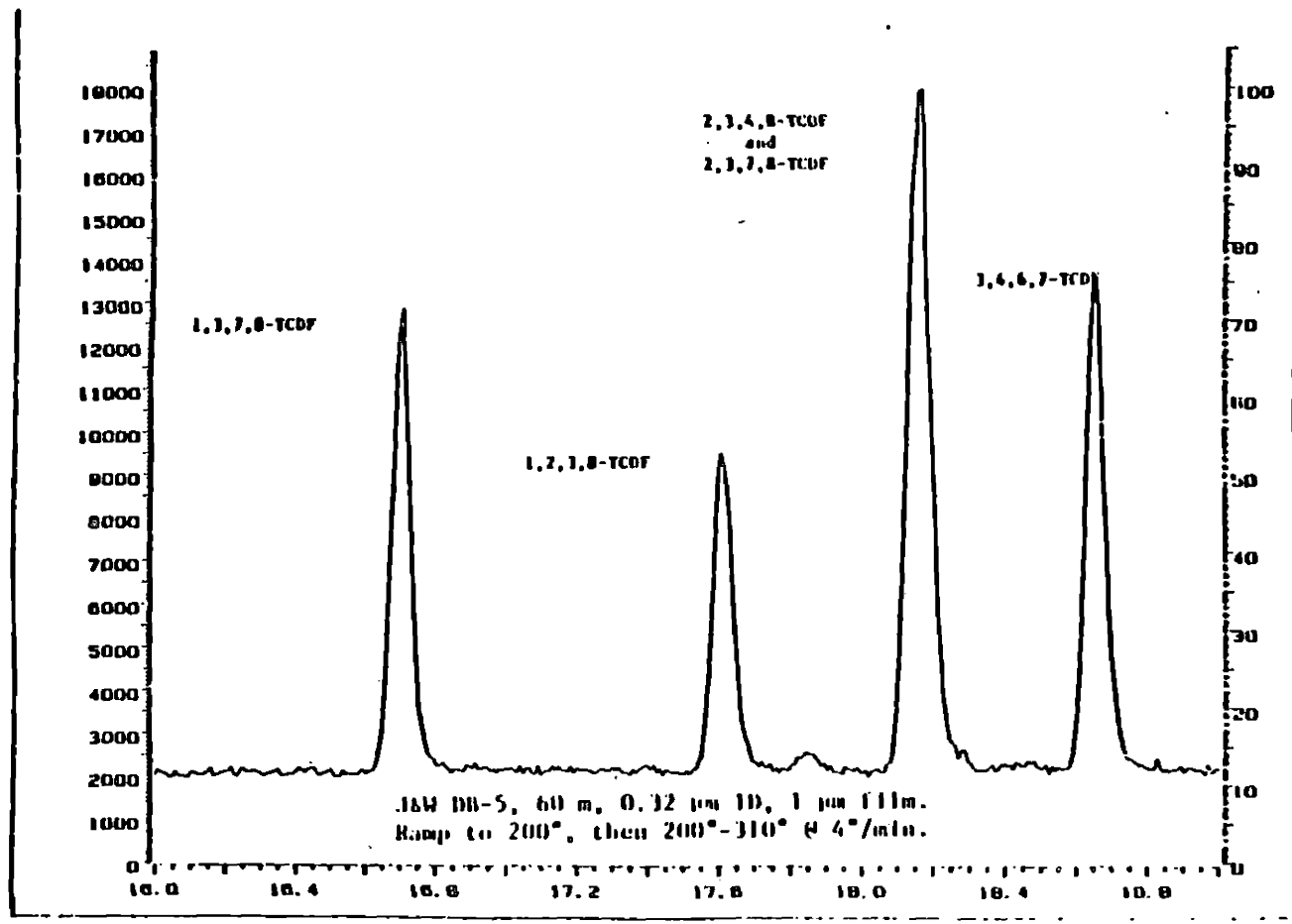

Figure 7. TCDF Test Mixture Chromatogram on DB-5 (Ref. 1)

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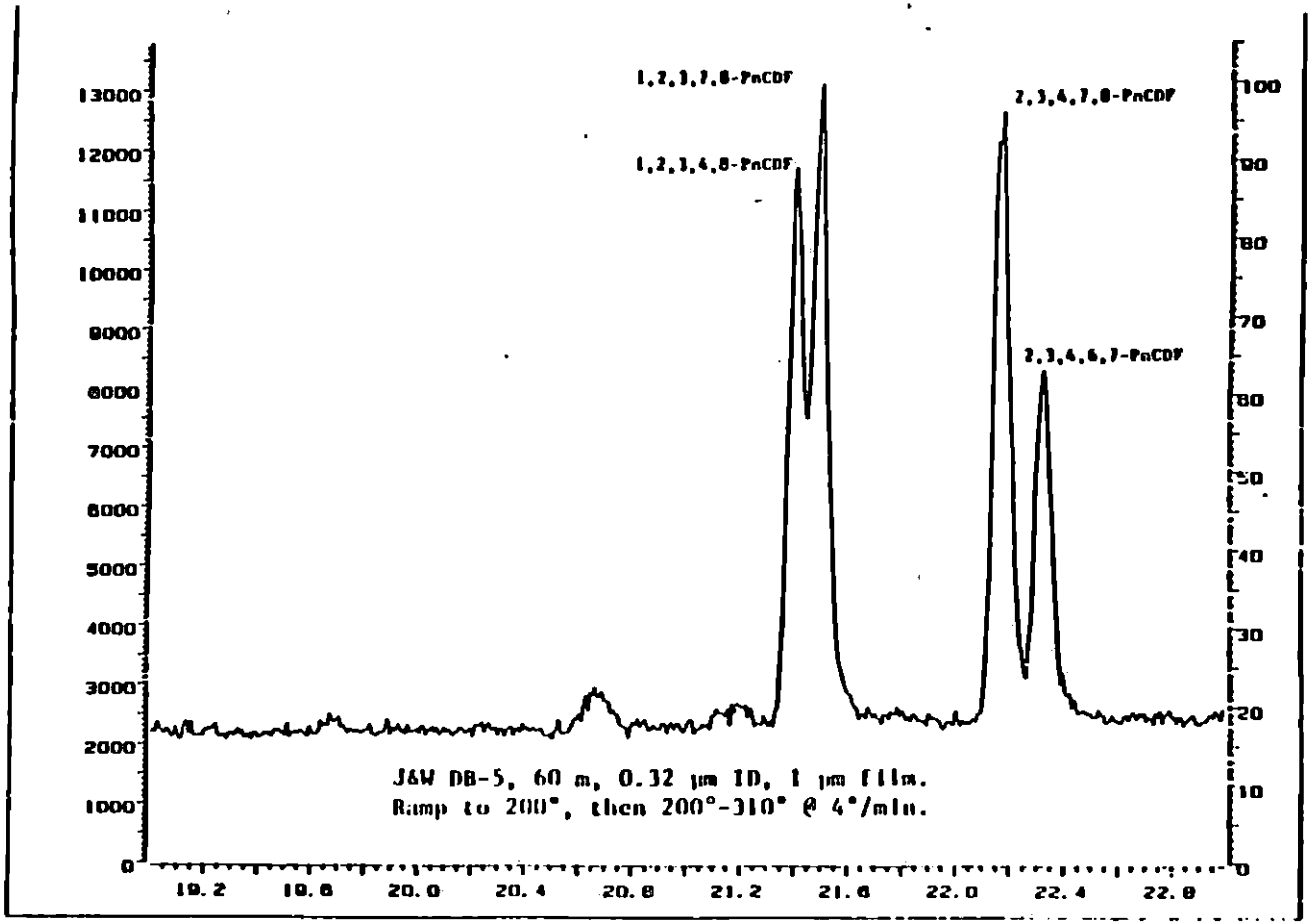

Figure 8. PnCDF Test Mixture Chromatogram on DB-5 (Ref. 1)

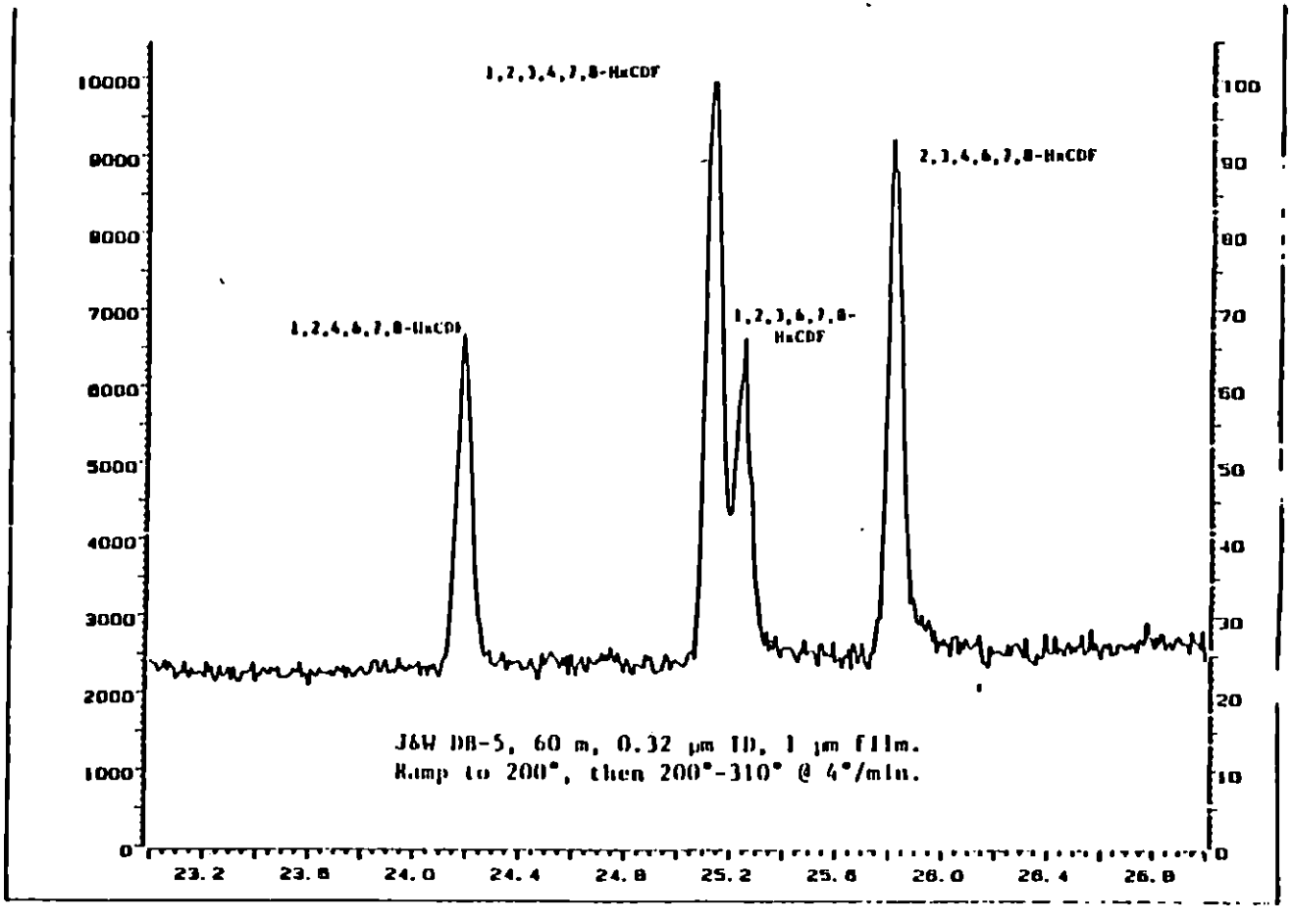

Figure 9. HxCDF Test Mixture Chromatogram on DB-5 (Ref. 1)

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TABLE 19. HRGC-MS ANALYTICAL CONDITIONS[a]

Mass Spectrometer:	Hewlett-Packard 5985 with 5987 Upgrade
Data System:	HP1000 RTE/6
Source Temperature:	250°C
Internal Source Pressure:	3×10^{-5} Torr
Mode:	Electron Impact
Electron Energy:	70 eV
Electron Multiplier Voltage:	2650-2800 volts
Resolution:	Unit
Interface Temperature:	275°C
Scan Mode:	Selected Ion Monitoring
Cycle Time:	0.5 sec
Column:	60 meters x 0.32 mm I.D., J&W DB-5 fused silica capillary column, 1.0 μm film
Carrier Gas:	Hydrogen at 5 psi
Carrier Linear Velocity:	35 cm/sec
Injector:	Cool On-column (65°C)
Interface to MS:	SGE Source Coupling at 275°C
GC Program:	Initial hold of 0.5 min at 65°C then 65°-200° at 30°/min, then 200°-310° at 4°/min
Injection Volume:	1 μL
Tailing Factor:	0.7-2.0

[a]Ref. 1.

Figures 10 through 13 for SP-2331. One analyte, 1,2,4,6,8-PnCDF, could not be found in the chromatographic test mixture. On DB-5, 2,3,7,8-TCDF and 2,3,4,8-TCDF were not resolved, but on SP-2331 this pair could be separated with a 65% valley. On SP-2331, 1,2,3,4,8-PnCDF and 1,2,3,7,8-PnCDF could not be resolved, but on DB-5 this pair could be separated with a 55% valley.

Actual samples were more difficult to resolve than the chromatographic test mixture. The analytes 1,2,3,4,8-PnCDF and 1,2,3,7,8-PnCDF could not be resolved in many cases, particularly when the 1,2,3,4,8-PnCDF concentration was much higher than the 1,2,3,7,8-PnCDF concentration. In one sample (41159-11-14), the

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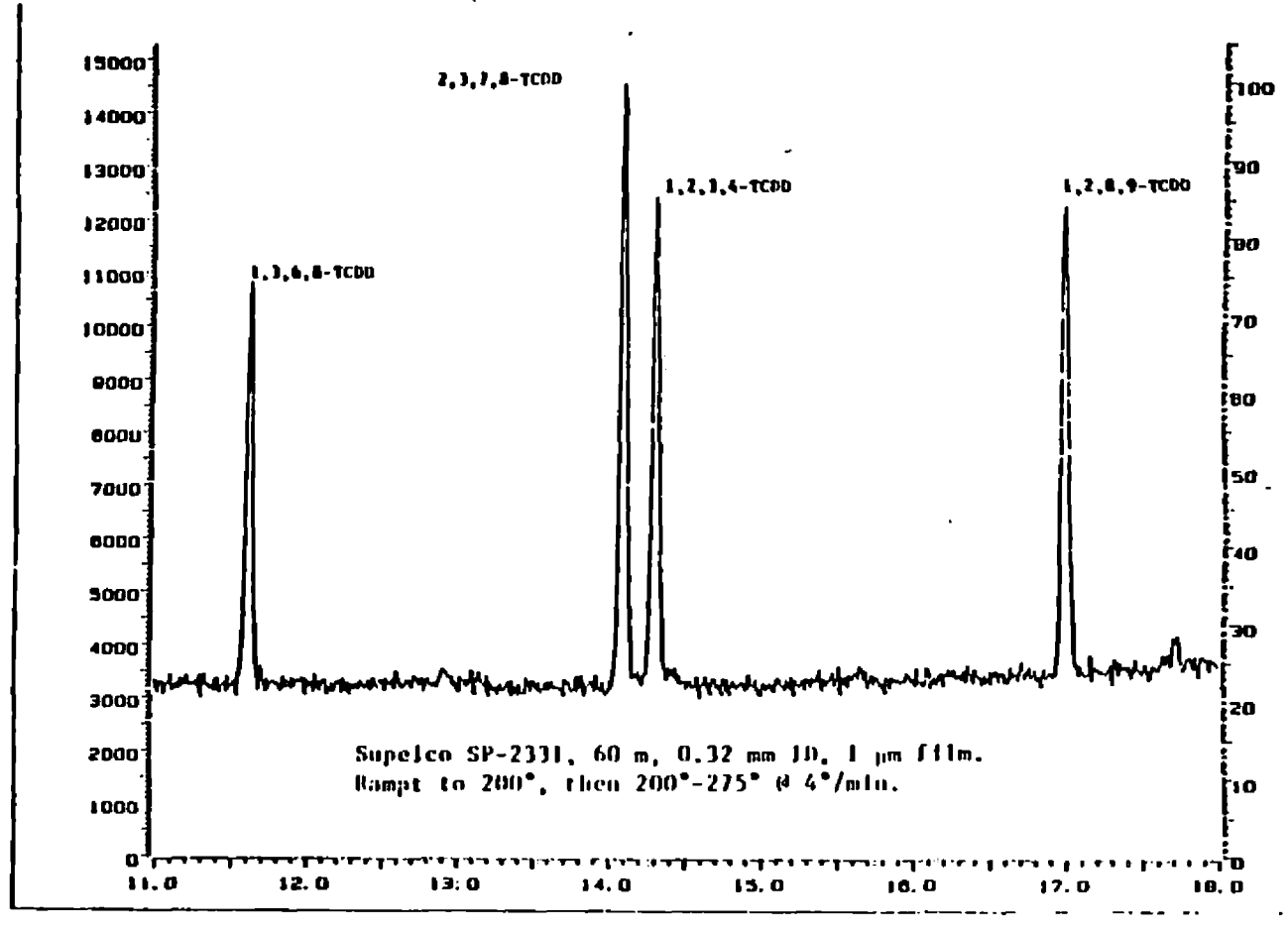

Figure 10. TCDD Test Mixture Chromatogram on SP-2331 (Ref. 1)

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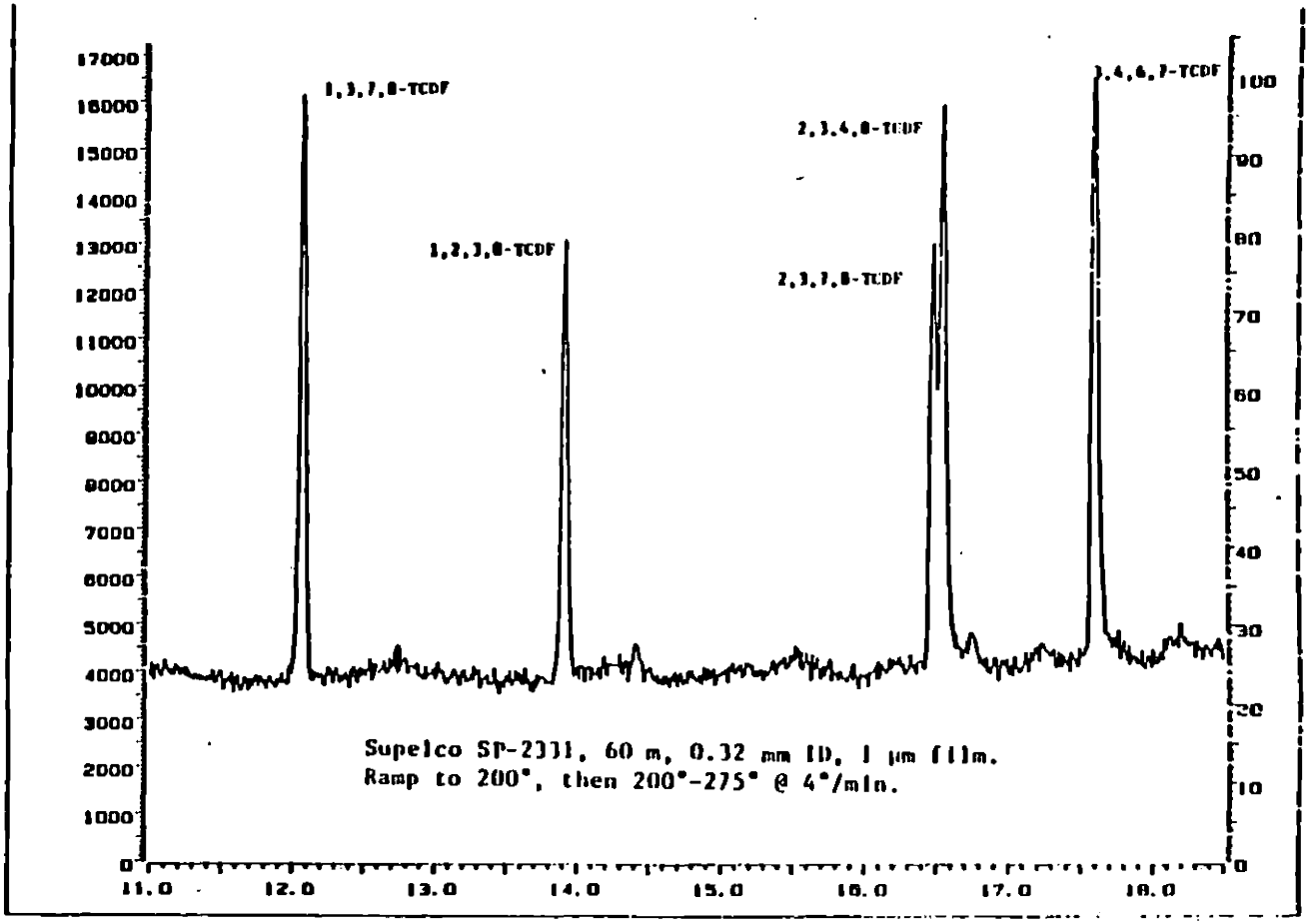

Figure 11. TCDF Test Mixture Chromatogram on SP-2331 (Ref. 1)

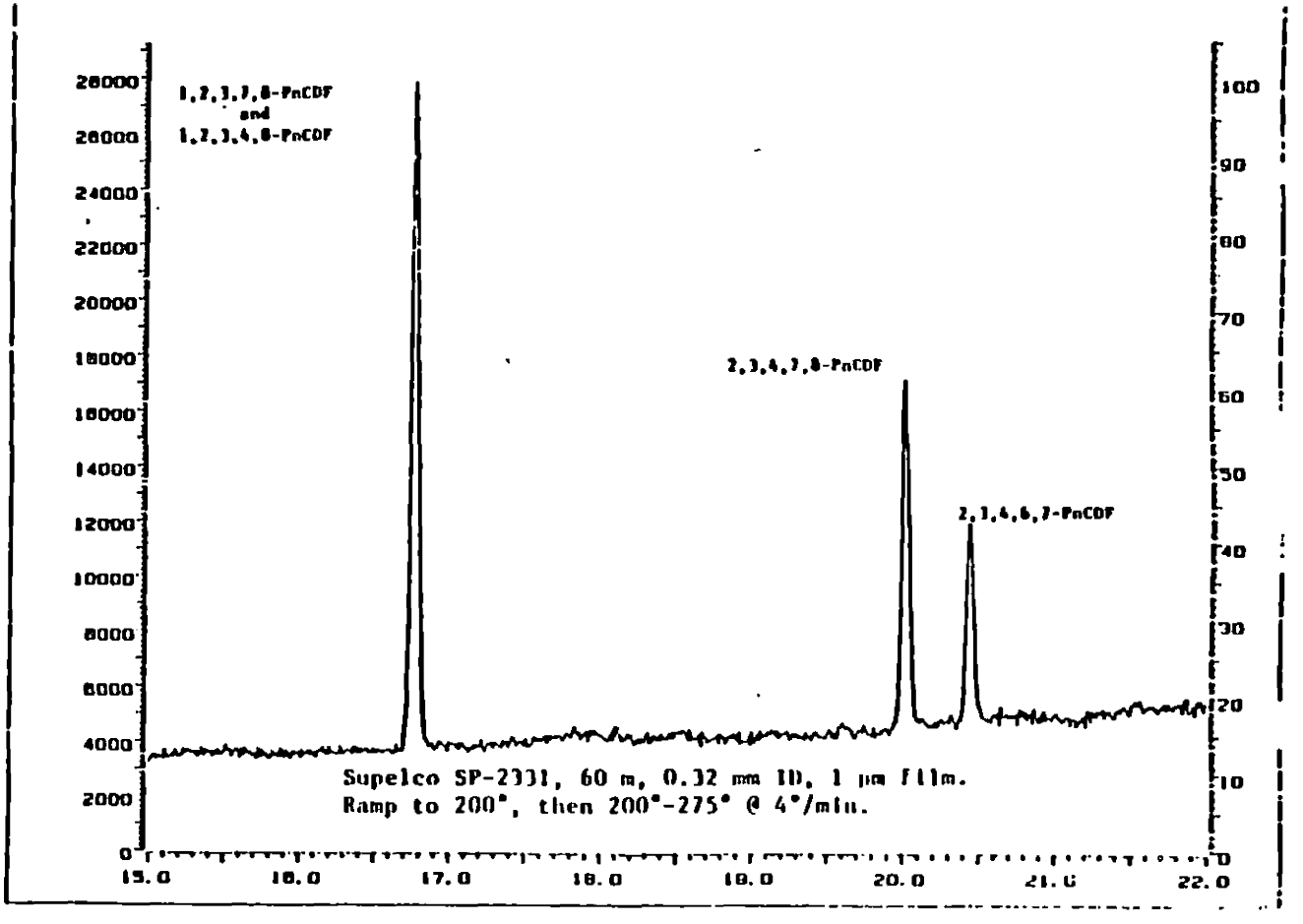

Figure 12. PnCDF Test Mixture Chromatogram on SP-2331 (Ref. 1)

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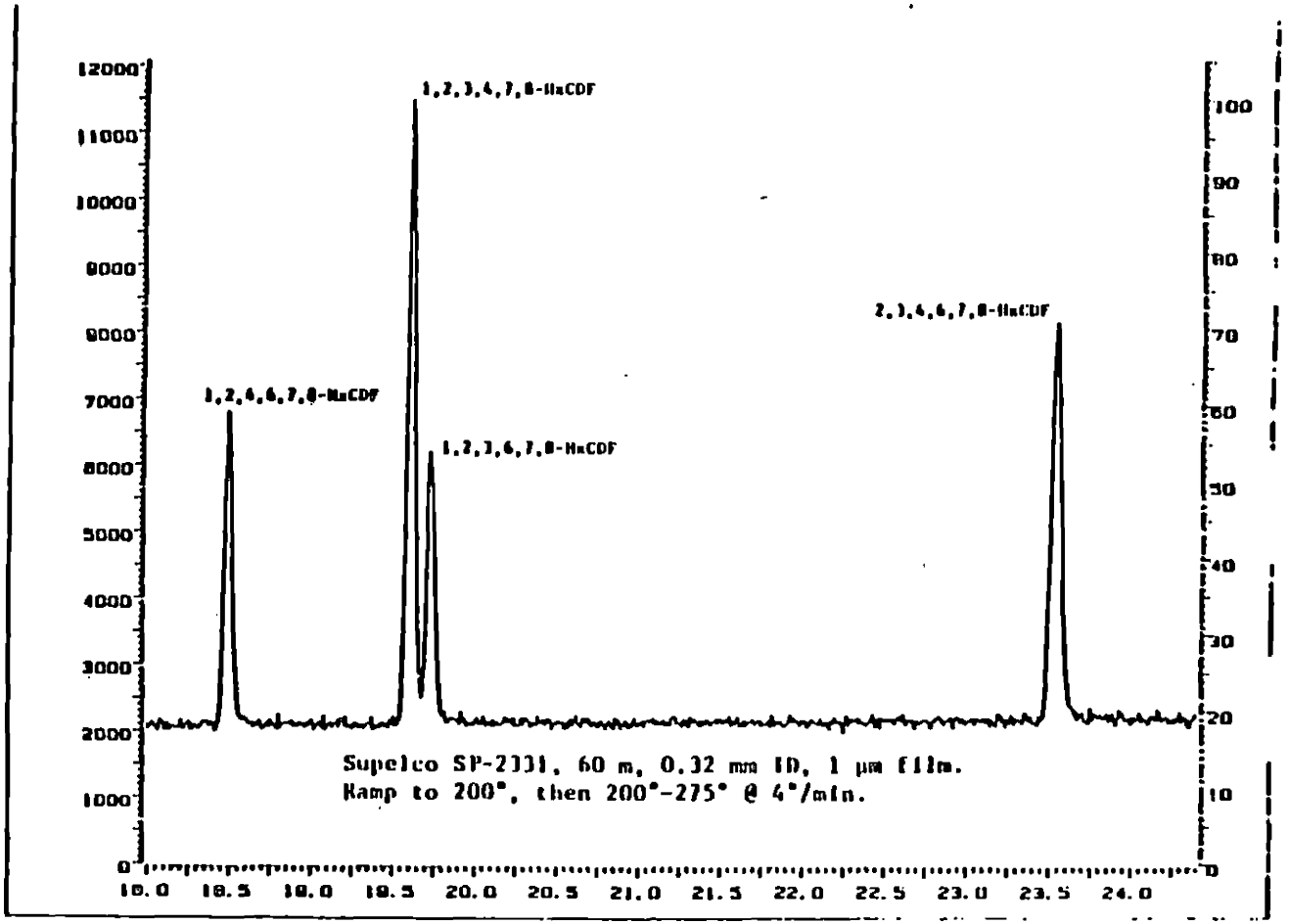

Figure 13. HxCDF Test Mixture Chromatogram on SP-2331 (Ref. 1)

2,3,7,8-TCDF and 2,3,4,8-TCDF pair could not be resolved accurately due to a coeluting interference. Several of the in-service liquid extracts were reported by Radian to be very complex and required the removal of 12 to 18 inches of the front of the column after every injection to give adequate resolution for subsequent samples. Column degradation for DB-5 and SP-2331 could be observed from the daily test mixture chromatograms over the course of this work.

Calibration

Four C^{12}/C^{13} area ratio calibration curves were generated during this study, the first on Ultra-2, the second and third on DB-5 and the fourth on SP-2331. Concentrations of 40, 150, 500, 2500, and 7500 ng/mL were calibrated for the first three calibration curves with each calibration point being the average of three determinations. The fourth calibration curve on SP-2331 also was determined in triplicate for concentrations of 40, 150, 500, and 2500 ng/mL. Calibration curves were generated and statistical analysis performed for the following analyte/C^{13} internal standard sets:

2,3,7,8-TCDD	2,3,7,8-TCDD-^{13}C
2,3,7,8-TCDF	2,3,7,8-TCDF-^{13}C
1,2,3,7,8-PnCDF	1,2,3,7,8-PnCDF-^{13}C
2,3,4,7,8-PnCDF	
1,2,3,4,7,8-HxCDF	
2,3,4,6,7,8-HxCDF	1,2,3,4,7,8-HxCDF-^{13}C
1,2,3,4,6,7,8-HpCDF	
OCDF	OCDD-^{13}C

Identification of PCDD/PCDFs

Identification of native PCDFs and PCDDs was performed by measuring chlorine isotope ratios and monitoring the presence of the fragment ions from loss of COCl. For PCDFs, two chlorine isotope peaks were monitored in addition to the quantitating ion. Only the most abundant chlorine isotope peak was monitored for the first series of extracts. The acceptance criteria for PCDD/PCDF positive identification required agreement better than ±10% between the theoretical and experimentally determined chlorine isotope ratios. The presence of either a second chlorine isotope peak or a M-COCl peak also was necessary for positive identification.

In the few cases where there was substantial interference in the ion profile of the largest chlorine isotope peak, the second largest chlorine isotope peak was used for evaluation of the isotope ratio acceptance criteria. In a few cases, a ±15% chlorine isotope ratio was accepted. If a peak was not within ±10% of the criteria on one determination but was within ±10% on previous determinations, the determination was accepted.

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Quantitation

Quantitation was performed by isotope dilution using the ^{13}C-labeled internal standards added before extraction. Concentrations were determined from C^{12}/C^{13} ratios of sample analyte to internal standard and a linear fit to the five-point calibration curves. A separate calibration curve was generated for 2,3,4,7,8-PnCDF. All other PnCDFs were quantitated from the 1,2,3,7,8-PnCDF calibration curve. A separate calibration curve was produced for 2,3,4,6,7,8-HxCDF. All other HxCDFs and HpCDFs were quantitated from the 1,2,3,4,7,8-HxCDF calibration curve. OCDD and OCDF were quantitated from the OCDD-^{13}C calibration curve. Congener class total concentrations were calculated by summing concentrations of each peak which passed the identification criteria for the congener class. Surrogate recoveries were determined using the mean 500 ng/mL response factor from the calibration checks made at the start and end of the analyses.

One sample (41159-11-13), had a severe interference which coeluted with the TCDF-^{13}C peaks. TCDFs in this sample were quantitated verus the area of the PnCDF-^{13}C quantitating ion with a normalization factor of 1.1 (the average ratio of TCDF-^{13}C to PnCDF-^{13}C response).

Detection Limits

Instrumental detection limits were defined as the analyte concentration which gave a 3:1 signal-to-noise ratio. Method detection limits for all analytes were approximately 3 ng/g except OCDF and OCDD which were 10 ng/g due to peak broadening at the later retention times. The lowest point on the calibration curves, 40 ng/mL, corresponds to a sample concentration of 8 ng/g.

Procedures Used by Battelle (4)

Analysis--

The sample extracts were instrumentally analyzed in triplicate and quantified for PCDD/PCDF using combined capillary column gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The HRGC/HRMS consisted of a Carlo Erba Model 4160 Gas Chromatograph interfaced directly into the ion source of a VG Model 7070 high resolution mass spectrometer. The chromatographic column was a 60 M DB-5 fused silica column using helium carrier gas at a flow velocity of 30 cm/sec. The mass spectrometer was operated in the electron impact (EI) ionization mode at a mass resolution of 9000-12000 (M/ΔM, 10 percent valley definition). All HRGC/HRMS data were acquired by multiple-ion-detection (MID) using a VG Model 2035 Data System.

Recovery of Internal Standards--

The recoveries of the internal standards were calculated by comparison to an external standard, 1,2,3,4-TCDD-$^{13}C_{12}$ which was added following the column cleanup steps. Relative response factors were determined from triplicate analyses of a

standard mixture containing the labelled internal standards and the 1,2,3,4-TCDD-$^{13}C_{12}$. The equation used to calculate the recoveries was:

$$\text{Recovery (\%)} = \frac{A_{is} \times Q_{rs} \times 100}{A_{rs} \times Q_{is} \times R_f}$$

Where:

A_{is} = Sum of integrated areas for internal standard;
Q_{rs} = Quantity of recovery standard in ng;
Q_{is} = Quantity of internal standard in ng;
A_{rs} = Sum of integrated areas for recovery standard; and
R_f = Response factor.

Quantification

The PCDD/PCDF isomers and congener class concentrations were determined by comparing the sum of the two ion masses monitored for each class to the sum of the two ion masses monitored for the corresponding internal standard. It was assumed that the response factors for each of the individual isomers in a class were the same as the response factors calculated for the specific isomers present in the standard solutions. The response factors were calculated from six replicate analyses of standard solutions at four concentrations. The sum of the two most intense ion masses in the molecular ion region of each isomer were used to generate the response factors.

The formula used for quantifying the PCDD/PCDF isomers was:

$$\text{Quantity/Sample (ng/g)} = \frac{A_c \times Q_{is}}{A_{is} \times R_f \times W}$$

Where:

Quantity = Total quantity in ng of target isomer or congener class
A_c = Sum of integrated areas for the target isomer or congener class
Q_{is} = Quantity of internal standard in ng
A_{is} = Total integrated areas for the internal standard
R_f = Response factor.

Each pair of resolved peaks in the selected-ion-current chromatograms was evaluated manually to determine if it met the criteria for a PCDD or PCDF isomer. By examining each pair of peaks separately, quantitative accuracy was improved over what is obtained when all of the peaks in a selected chromatographic window are averaged. When averaged data are used, it is possible for pairs of peaks with high and low chlorine isotope ratios to produce averaged data that meet the chlorine isotope ratio criterion. For example, two pairs of peaks having chlorine isotope ratios of 0.56 and 0.96, both outside of the acceptable range, would have an average ratio of 0.76. By checking the isotope ratio of these peaks separately, they would be eliminated from the class total.

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The criterion that were used to identify PCDD and PCDF isomers were:

1. Simultaneous responses at both ion-masses.
2. Chlorine isotope ratio within +/-15% of the theoretical value.
3. Chromatographic retention times within windows determined from analyses of standard mixtures.
4. Signal-to-noise ratio equal to or greater than 2.5 to 1.0.

The individual isomers for which a isotopically labelled analog was available included the additional criterion that they eluted within 2 seconds of the internal standard.

The limit of detection (LOD) was calculated for samples in which isomers of a particular chlorine congener class were not detected. The formula used for calculating the LOD was:

$$\text{LOD/g (ng)} = \frac{H_c \times Q_{1s} \times 2.5}{H_{1s} \times R_f \times W}$$

Where:

LOD = Single isomer limits of detection for a congener class in ng;
H_c = Height of congener class isomer;
Q_{1s} = Quantity of internal standard in ng;
H_{1s} = Peak height of internal standard;
R_f = Response factor; and
W = Weight in grams of sample.

Calibration Curve

Calibration curves were generated for each of the native/isotopically labelled isomer pairs. The calibration standards were analyzed at least five times at each of four concentration levels between 0.04 and 2.5 parts-per-million (ppm). The repsonse factors were calculated by dividing the sum of the areas for the two most abundant ion masses in the molecular ion cluster of each native PCDD/PCDF isomer by the sum of the areas for the corresponding ion masses from the isotopically labelled standard. The isomer pairs for which response factors were calculated included:

2,3,7,8-TCDD vs 2,3,7,8-TCDD-$^{13}C_{12}$
2,3,7,8-TCDF vs 2,3,7,8-TCDF-$^{13}C_{12}$
OCDD vs OCDD-$^{13}C_{12}$
OCDF vs OCDD-$^{13}C_{12}$
1,2,3,7,8-Penta-CDF vs 1,2,3,7,8-Penta-CDF-$^{13}C_{12}$
2,3,4,7,8-Penta-CDF vs 1,2,3,7,8-Penta-CDF-$^{13}C_{12}$
1,2,3,4,7,8-Hexa-CDF vs 1,2,3,4,7,8-Hexa-CDF-$^{13}C_{12}$
2,3,4,6,7,8-Hexa-CDF vs 1,2,3,4,7,8-Hexa-CDF-$^{13}C_{12}$
1,2,3,4,6,7,8-Hepta-CDF vs OCDD-$^{13}C_{12}$

It was assumed that each of the isomers of a particular chlorine congener class had the same response factor.

Detection Limits

Detection limits were calculated for samples that did not contain PCDD/PCDF isomers in a particular class. These detection limits were calculated using the quantification equation with the addition of a factor of 2.5 to account for the signal-to-noise criterion.

The noise level was measured as the average of the peak-to-peak noise that occurred in the baseline of the particular MID channel. If at all possible, the noise was measured at the retention time of the isotopically labelled internal standard. The height of the internal standard peak was measured to the average of the noise that occurred on the peak top. The reported limits of detection are for single PCDD/PCDF isomers and have been corrected for recovery losses during sample workup.

Baseline Analyses--

The three baseline samples, Aroclor 1260, Aroclor 1254, and Aroclor 1016 were analyzed using the methodology described above. The Aroclor 1016 presented the greatest analytical problem due to the relatively high amount of the sample that passed through the column cleanup steps. Even after the four column cleanup steps, a noticeable polychlorinated biphenyl (PCB) background was evident in the single ion current chromatograms.

In-Service Analyses--

The seven in-service samples were also analyzed using the methodology described above.

QUALITY CONTROL

Each participating laboratory followed their own extensive quality control program which has been previously reported (1-4).

SECTION 3
RESULTS AND DISCUSSION OF STATISTICAL ANALYSIS

EVALUATION OF CALIBRATION MODEL

The calibration data from four of the five participating laboratories in the Round Robin Study was evaluated in terms of the regressions employed, i.e. their appropriateness and linearity over the range of interest for PCDD and PCDF congener analysis (data from Lab C was not available for regression analysis).

For each of the labs, the calibration model is:

$$LOG_{10}\ (y) = a + b\ LOG_{10}\ (x)$$

Where x = concentration

$$y = \begin{cases} \text{response ratio for LAB-A} \\ \text{peak area ratio for LAB-B} \\ \text{area ratios for LAB-D} \\ \text{normalized counts for LAB-E} \end{cases}$$

Exponentiating, the model becomes

$$y = 10^a \cdot x^b$$

Since b is approximately equal to one for each lab and compound, the model can be simplified to

$$y = CX$$

where $C = 10^a$

Tables 20 to 24 give the estimates of C for each lab and compound.

Where

$$C = \frac{y}{x}$$

The intercepts in Tables 21 through 24, except for 2,3,7,8-TCDF (Table 24) were all significantly different from zero at the 0.01 level. Thus, if laboratories employed regression models using a zero intercept, considerable error in the calibration would result. For example, the regression model used by Lab-E was inappropriate (See Appendix A). Discussions with the Lab-E Project Director at this laboratory indicated that the isotopic response ratios of $^{13}C/^{12}C$-PCDF and PCDD congeners were used for quantification, thus, the results reported did not employ the reported regressions. All other laboratories used regression models in which the regression was not forced through the zero intercept.

TABLE 20. CONVERSION FACTORS

	LAB-A	LAB-B	LAB-D	LAB-E
2,3,7,8-TCDD	0.002227	0.003117	0.001994	0.6901
2,3,7,8-TCDF	0.001916	0.003795	0.002293	1.0950
1,2,3,7,8-PNCDF	0.001661	0.003915	0.002513	0.8904
2,3,4,7,8-PNCDF	0.001713	0.003916	0.002601	0.7414
1,2,3,4,7,8-HXCDF	0.001947	0.003657	0.002211	0.6701
2,3,4,6,7,8-HXCDF	0.002217	0.006736	0.002275	0.4082
1,2,3,4,6,7,8-HPCDF	0.002677	0.004121	0.001679	0.3281
OCDF	0.0009404	0.000611	0.001395	0.0459

TABLE 21. CALIBRATION CURVES: LAB-A

$$Y = LOG_{10}\left(\frac{\text{Average Response Factor} \times \text{Concentration}}{500}\right)$$

$$X = LOG_{10}\ (\text{Concentration})$$

	NUMBER OF OBSERVATIONS	INTERCEPT	SLOPE	R^2
2,3,7,8-TCDD	6	-2.704**	1.020**	0.9994
2,3,7,8-TCDF	6	-2.731**	1.005**	0.9997
1,2,3,7,8-PNCDF	6	-2.819**	1.016**	0.9999
2,3,4,7,8-PNCDF	6	-2.702**	0.973**	0.9996
1,2,3,4,7,8-HXCDF	6	-2.769**	1.023**	0.9999
2,3,4,6,7,8-HXCDF	6	-2.632**	0.991**	0.9998
1,2,3,4,6,7,8-HPCDF	6	-2.600**	1.011**	0.9995
OCDF	6	-2.906**	0.949**	0.9980

**Significantly different from zero at .01 level.

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TABLE 22. CALIBRATION CURVES: LAB-B

$$Y = LOG_{10}\left(\frac{\text{Average Response Factor x Concentration}}{300}\right)$$

$$X = LOG_{10}\ (\text{Concentration})$$

	NUMBER OF OBSERVATIONS	INTERCEPT	SLOPE	R^2
2,3,7,8-TCDD	25	-2.475**	0.985**	0.9991
2,3,7,8-TCDF	25	-2.432**	1.005**	0.9997
1,2,3,7,8-PNCDF	25	-2.418**	1.005**	0.9994
2,3,4,7,8-PNCDF	25	-2.414**	1.003**	0.9996
1,2,3,4,7,8-HXCDF	25	-2.468**	1.014**	0.9996
2,3,4,6,7,8-HXCDF	25	-2.184**	1.006**	0.9991
1,2,3,4,6,7,8-HPCDF	25	-2.410**	1.011**	0.9994
OCDF	25	-3.470**	1.091**	0.9918

* Significantly different from zero at .05 level.

** Significantly different from zero at .01 level.

TABLE 23. CALIBRATION CURVES: LAB-D

$Y = LOG_{10}$ (Average Area Ratio)
$X = LOG_{10}$ (Concentration)

	NUMBER OF OBSERVATIONS	INTERCEPT	SLOPE	R^2
2,3,7,8-TCDD	4	-2.691**	0.996**	0.9999
2,3,7,8-TCDF	4	-2.609**	0.988**	0.9999
1,2,3,7,8-PNCDF	4	-2.600**	1.000**	0.9999*
2,3,4,7,8-PNCDF	4	-2.636**	1.020**	0.9995
1,2,3,4,7,8-HXCDF	4	-2.634**	0.991**	0.9999*
2,3,4,6,7,8-HXCDF	4	-2.617**	0.989**	0.9999*
1,2,3,4,6,7,8-HPCDF	4	-2.778**	1.001**	0.9999*
OCDF	3	-2.762**	0.966**	0.9999*

* Significantly different from zero at .05 level.

** Significantly different from zero at .01 level.

TABLE 24. CALIBRATION CURVES: LAB-E

$Y = LOG_{10}$ (Raw Counts)
$X = LOG_{10}$ (Concentration)

	NUMBER OF OBSERVATIONS	INTERCEPT	SLOPE	R^2
2,3,7,8-TCDD	18	-0.286**	1.047**	0.9945
2,3,7,8-TCDF	18	0.004	1.014**	0.9994
1,2,3,7,8-PNCDF	18	-0.144**	1.037**	0.9977
2,3,4,7,8-PNCDF	18	-0.230**	1.038**	0.9960
1,2,3,4,7,8-HXCDF	18	-0.259**	1.032**	0.9958
2,3,4,6,7,8-HXCDF	18	-0.547**	1.058**	0.9893
1,2,3,4,6,7,8-HPCDF	18	-0.668**	1.067**	0.9875
OCDF	18	-1.762**	1.142	0.9779

* Significantly different from zero at .05 level.

** Significantly different from zero at .01 level.

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The slopes were very nearly unity and the R^2 of the regression indicated excellent linearity over the three orders of magnitude concentration range used for calibrating the instrumentation (Tables 21-24).

RESULTS OF ROUND ROBIN

Statistical analysis of the PCDD and PCDF concentration data first required the establishment of a standard reporting format for all of the laboratories. To satisfy this requirement, the following data conversions were performed:

1) For LAB-C 1,2,3,6,7,8-HXCDF was excluded because it was not reported separately but in combination with 1,2,3,4,7,8-HXCDF.
2) If measured amounts were not reported, then detection limits were substituted, if available.
3) For those labs which separately reported 2,3,7,8- and 2,3,4,8- TCDF, or 1,2,3,7,8- and 1,2,3,4,8-PCDF, or 1,2,3,4,7,8- and 1,2,3,4,7,9-HXCDF, the reported concentrations of the pairs were added together (in order to conform with the reporting format of the other laboratories).

Table B-1 in Appendix B gives a listing of the data. Measured concentrations (ng/g) for each of the individual instrumental determinations of a single extract are shown, along with the means and standard deviations of the determinations, by laboratory, sample, and compound. Since LAB-E reported only one instrumental determination of a single extract for each sample and compound, only that measured concentration is given. LAB-D reported results for three extracts for each sample with three instrumental determinations for each extract. These extract results are listed separately. LAB-A reported six determinations for OCDD in sample 11 and 1,2,3,7,8,9-HXCDF in sample 12. Large ranges in concentrations are evident -- for example, by comparing the means among compounds within a sample 1 for LAB-A 1,2,3,4,6,7,8-HPCDF has an average concentration of 354 while OCDD has an average concentration of 2.08. Also, the standard deviations (precision) show great variation in magnitude. Because of this variation in standard deviations, equal variances could not be assumed. Since the standard deviations tended to increase as the concentration levels increased, basic data transformations of taking logarithms and square roots were tried. These, unfortunately, did not correct the problem. This limited the types of statistical tests that could be performed since many depend on the equality of variances.

The coefficients of variations (CVs) which represents precision of the analytical methods for the labs, excluding LAB-E, are given in Table 25. Only those concentrations reported as measured amounts rather than detection limits were included in the calculations. There appears to be little relation between the measured concentration and the CV, i.e., the CV does not tend to increase or decrease as the measured concentration increases. These CVs are summarized in

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TABLE 25. COEFFICIENTS OF VARIATION BY COMPOUND, LAB, AND SAMPLE

Sample	LAB-A	LAB-B	LAB-C	LAB-D-1	LAB-D-2	LAB-D-3
COMPOUND = TOTAL TCDF						
1	16.1	9.4	15.8	5.6	6.5	1.9
2	3.8	0.9	8.7	2.4	4.2	1.0
3	1.3	3.1	27.0	6.6	4.4	1.3
11	47.0	8.6	61.0	13.7	12.0	6.2
12	58.9	4.9	27.7	6.8	3.1	18.3
13	18.5	12.1	26.0	11.3	11.5	12.6
14	23.9	30.0	30.1	10.5	3.6	2.1
15	27.6	.[a]	.	.	.	.
16	21.7	.	.	.	.	.
17	4.3	22.3	22.2	11.9	9.4	0.6
COMPOUND = TOTAL PNCDF						
1	10.8	3.1	41.5	7.6	3.6	20.9
2	10.2	0.7	13.9	0.8	2.7	1.1
3	2.0	5.8	8.3	11.8	10.6	5.0
11	6.9	9.7	50.4	15.4	2.0	0.2
12	19.2	4.7	56.9	13.6	2.6	2.3
13	3.2	10.8	10.2	17.5	6.5	8.0
14	2.9	23.7	31.5	28.5	3.1	1.2
15	10.9	.	.	.	.	.
16	8.8*	61.6	.	.	.	.
17	3.1	22.5	10.8	12.1	11.1	8.8
COMPOUND = TOTAL HXCDF						
1	13.1	1.0	137.1	1.3	1.0	1.7
2	5.8	2.3	83.3	1.7	0.9	4.8
3	3.5	2.1	64.6	10.9	30.3	1.9
11	2.6	14.1	99.3	11.9	1.8	1.8
12	4.6	3.7	20.3	8.5	5.7	5.1
13	14.6	27.5	10.7	22.5	18.4	26.9
14	1.9	4.0	55.3	12.4	6.2	1.0
15	36.2	.	.	44.1	5.6	24.1
16	16.8	54.0	.	47.0	.	.
17	1.7	14.0	42.0	9.2	1.3	0.3
COMPOUND = TOTAL HPCDF						
1	4.4	10.9	96.2	9.7	5.1	2.4
2	11.8	20.4	76.1	3.2	4.3	6.3
3	3.6	16.8	65.6	2.7	6.6	7.1
11	8.6	19.2	52.2	18.3	0.8	5.0
12	14.3	20.2	28.6	13.9	5.3	4.2
13	21.4	39.4	62.4	.	3.5	.
14	2.6	14.1	21.9	19.4	18.2	1.9
15	56.2	62.9*	.	38.7	12.8	10.0
16	.	58.9	.	70.1	.	.
17	6.5	22.6	18.3	15.1	5.4	1.2

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TABLE 25 (cont'd.)

Sample	LAB-A	LAB-B	LAB-C	LAB-D-1	LAB-D-2	LAB-D-3
COMPOUND = TOTAL OCDF						
1	7.9	.	.	26.5*	.	.
2	10.8	45.8	.	1.6	4.1	5.5
3	3.3	30.6	32.6	2.9	6.0	5.4
11	5.7	23.8	14.4	13.6	8.5	5.0
12	8.8	12.7	10.8	12.2	2.4	4.1
13	10.7	.	60.7	39.0	4.4	4.2
14	2.5	40.3	20.0	4.5	11.8	16.8
15	34.1	.	.	4.8	2.6	4.7
16	.	.	.	47.8	27.4	.
17	8.9	8.8	8.5	12.3	3.6	2.1
COMPOUND = TOTAL TCDD						
1	11.8	17.0	.	2.3	4.0	4.3
2	16.0	2.2	2.5	3.0	2.3	3.1
3	3.3	9.2	15.8	1.7	5.3	1.6
14	56.6*	.	.	.	.	.
16	43.9*	.	.	.	.	.
COMPOUND = OCDD						
11	25.1**	14.5	91.7	.	.	.
12	.	18.1	.	.	.	.
13	19.0	6.9	.	.	.	.
14	3.0	46.6	.	.	.	.
15	11.5	24.4	.	.	.	.
16	12.2	9.9	11.8	.	8.3	.
17	20.5	28.5	30.1	.	.	.
COMPOUND = 2,3,7,8-TCDD						
1	11.8	17.0	11.4	2.3	4.0	4.3
2	16.0	2.2	2.5	3.0	2.3	3.1
3	3.3	9.2	15.8	1.7	5.3	1.6
COMPOUND = 2,3,7,8 + 2,3,4,8-TCDF						
1	15.5	9.4	15.8	5.6	6.5	1.9
2	4.5	0.9	8.7	2.4	4.2	1.0
3	0.8	1.0	19.5	1.1	3.8	2.2
11	24.3	45.7	16.4	9.6	0.0	2.9
12	21.1	4.3	24.7	11.5	5.4	15.9
13	16.5	4.4	15.6	9.9	5.6	2.2
14	16.3	9.1	6.7	9.7	2.1	1.2
15	9.3	.	.	.	.	.
16	16.4		.	.	.	.
17	3.7	22.0	15.6	14.5	6.4	1.1

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TABLE 25 (cont'd.)

Sample	LAB-A	LAB-B	LAB-C	LAB-D-1	LAB-D-2	LAB-D-3
COMPOUND = 1,2,3,7,8 + 1,2,3,4,8-PNCDF						
1	12.0	2.1	11.5	1.4	3.0	0.7
2	11.5	1.8	3.1	1.3	2.1	1.0
3	0.9	4.1	13.6	4.0	6.8	3.7
11	12.9	6.3	31.6	10.4	2.1	2.2
12	26.1	4.8	23.1	16.6	4.5	2.9
13	8.4	7.1	12.8	6.3	7.5	0.0
14	3.8	4.6	7.3	15.6	17.3	5.7
15	11.7	.	.	.	.	.
16	.	61.1	.	.	.	.
17	4.0	14.9	2.2	11.5	18.4	4.5
COMPOUND = 2,3,4,7,8-PNCDF						
1	11.1	4.2	60.1	13.8	2.1	2.1
2	15.3	1.8	23.4	0.4	3.3	1.4
3	2.9	4.4	37.8	0.7	5.2	2.8
11	2.9	5.8	9.4	32.4	3.7	2.7
12	22.2	10.1	5.1	22.0	3.2	5.3
13	8.7	6.3	17.2	10.9	1.8	2.5
14	1.4	5.8	14.8	20.1	1.3	1.9
15	11.0	.	.	.	.	.
16	14.1*	60.1	.	.	.	.
17	7.2	13.7	7.3	22.0	4.9	4.9
COMPOUND = 1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF						
1	13.2	0.6	36.7	1.3	2.4	3.7
2	40.2	34.6	.	.	.	.
3	3.2	3.5	8.6	1.9	2.0	3.0
11	2.1	13.9	100.9	8.0	1.7	1.7
12	1.9	6.6	16.0	10.1	0.6	4.7
13	17.0	23.1	38.6	7.5	2.3	8.4
14	0.6	1.2	30.8	9.9	16.3	3.4
15	35.8	.	.	57.3	4.3	27.2
16	5.8	59.2	.	38.6	.	19.2
17	5.4	14.1	43.0	10.3	0.8	0.9
COMPOUND = 1,2,3,7,8,9-HXCDF						
3	5.4	.	80.9	4.7	5.1	1.5
11	.	.	82.3	11.0	4.4	2.5
12	27.1**	.	20.3	6.9	5.4	1.5
13	33.2	.	36.5	.	24.1	15.7*
14	4.8	15.7	88.5	14.0	26.9	1.3
15	42.4	.	.	.	.	.
16	.	61.9	.	.	.	.
17	.	.	53.3	4.8	2.3	6.6

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TABLE 25 (cont'd.)

Sample	LAB-A	LAB-B	LAB-C	LAB-D-1	LAB-D-2	LAB-D-3
COMPOUND = 1,2,3,6,7,8-HXCDF						
1	14.9	0.0	.	4.1	5.9	8.1
2	3.4	2.5	.	5.3	2.7	7.6
3	4.0	3.2	.	5.2	9.3	2.0
11	1.0	19.6	.	7.0	20.8	1.3
12	2.7	5.3	.	8.1	7.9	27.6
13	12.3	27.2	.	.	10.2	.
14	1.7	2.6	.	7.6	14.7	16.0
15	34.9	.	.	.	.	.
16	.	64.9	.	.	.	.
17	3.0	2.0	.	7.9	22.5	8.7
COMPOUND = 2,3,4,5,7,8-HXCDF						
1	6.2	1.5	159.1	2.2	1.6	1.3
2	7.3	4.2	86.9	0.4	0.4	3.7
3	4.8	4.7	80.9	1.2	11.4	2.1
11	12.7	13.4	121.3	15.3	4.0	0.5
12	25.0	19.5	30.6	21.7	3.2	5.4
13	8.8	31.5	41.2	.	10.2	23.6
14	5.2	13.7	125.1	11.2	26.2	2.0
16	.	51.3	.	.	.	.
17	12.3	19.2	52.7	5.8	1.6	5.5
COMPOUND = 1,2,3,4,6,7,8-HPCDF						
1	4.0	10.9	96.2	9.7	5.1	2.4
2	11.7	20.4	76.1	3.2	4.3	6.3
3	4.1	12.1	144.9	1.4	5.4	5.7
11	10.3	17.8	58.9	17.6	0.7	4.0
12	9.9	18.7	36.9	14.9	5.5	4.9
13	21.1	31.6	60.9	.	7.5	.
14	1.6	11.5	39.9	20.4	17.6	1.2
15	.	60.6*	.	21.7	6.7	10.3
16	.	53.4	.	57.4	.	.
17	3.4	23.9	29.2	14.9	4.8	1.6

* Based on two rather than three determinations.

** Based on six rather than three determinations.

[a]. = No measurable value reported, i.e., not detected, therefore no CV was calculated.

Table 26 showing the minimum, median, and maximum CVs over all compounds by laboratory and sample. Overall, Lab-C shows the largest median and maximum values for CVs, but there is also variation among the other laboratories.

TABLE 26. MINIMUM, MEDIAN, AND MAXIMUM CVs BY LAB AND SAMPLE OVER ALL COMPOUNDS

	Sample							
	1	2	3	11	12	13	14	17
MINIMUM								
LAB-A	4.01	3.45	0.83	0.99	1.90	3.25	0.57	1.71
LAB-B	0.00	0.67	1.02	5.83	3.71	4.41	1.24	1.96
LAB-C	11.36	2.55	8.33	9.36	5.13	10.20	6.68	2.16
LAB-D-1	1.25	0.40	0.74	6.97	6.84	6.25	4.48	4.84
LAB-D-2	0.98	0.41	2.04	0.00	0.58	1.75	1.28	0.83
LAB-D-3	0.71	0.96	1.31	0.17	1.55	0.00	0.98	0.34
MEDIAN								
LAB-A	11.78	11.15	3.26	8.63	19.21	15.54	2.86	4.34
LAB-B	4.25	2.25	4.56	14.10	6.62	17.63	12.62	19.22
LAB-C	41.53	13.87	29.82	58.92	23.94	31.23	30.49	22.18
LAB-D-1	4.85	2.44	2.68	13.60	12.22	11.08	12.39	11.94
LAB-D-2	4.00	2.70	5.39	2.08	4.54	7.53	14.65	4.90
LAB-D-3	2.40	3.08	2.21	2.53	4.92	8.24	1.93	2.07
MAXIMUM								
LAB-A	16.06	40.29	5.42	46.96	58.89	33.18	56.57	20.47
LAB-B	16.99	45.81	30.59	45.73	20.24	39.41	46.63	28.49
LAB-C	159.06	86.90	144.88	121.26	56.85	62.39	125.10	53.35
LAB-D-1	26.52	5.30	11.82	32.44	22.05	39.04	28.47	22.04
LAB-D-2	6.53	4.29	30.30	20.80	7.86	24.12	26.89	22.51
LAB-D-3	20.86	7.64	7.11	6.21	27.56	26.91	16.84	8.84

The spiked versus measured amounts in samples 1 and 2 are compared in Tables 27 and 28. The percent accuracy by compound, lab, and sample for the arithmetic mean, the geometric mean, the minimum, and the maximum are given in Table 27. The percent accuracy was determined by calculating the summary statistic, dividing by the spiked amount, then multiplying by 100. For example,

$$\% \text{ accuracy mean} = \frac{\text{mean}}{\text{spike}} \times 100$$

With the exception of Total PNCDF; Total HXCDF; 2,3,4,7,8-PNCDF; 2,3,4,6,7,8-HXCDF, and 1,2,3,4,6,7,8-HPCDF for LAB-C, these numbers generally fall in the 80 to 120 range.

To summarize the percent found, Table 28 gives the minimum, median, and maximum percent found by laboratory. Percent found expresses each determination as a percentage of the spiked amount similar to the formula above. LAB-C had by far

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TABLE 27. PERCENT ACCURACY FOR SPIKED SAMPLES BY COMPOUND, SAMPLE, AND LAB

Lab	Sample 1				Sample 2			
	Arithmetic Mean	Geometric Mean	Minimum	Maximum	Arithmetic Mean	Geometric Mean	Minimum	Maximum
COMPOUND = TOTAL TCDF								
Lab-A	89.0	88.2	72.5	97.5	118.9	118.8	114.7	123.6
Lab-B	78.5	78.3	73.5	87.0	115.7	115.7	115.1	117.0
Lab-C	95.0	94.2	80.0	110.0	119.5	119.2	107.5	126.4
Lab-D Ext 1	88.8	88.7	84.0	94.0	118.2	118.2	115.1	120.8
Lab-D Ext 2	92.7	92.5	88.0	99.5	112.6	112.5	107.5	117.0
Lab-D Ext 3	92.7	92.7	91.0	94.5	113.8	113.8	113.2	115.1
Lab-E	95.0	95.0	95.0	95.0	117.0	117.0	117.0	117.0
COMPOUND = TOTAL PNCDF								
Lab-A	94.0	93.6	83.7	104.0	98.7	98.4	87.3	106.3
Lab-B	99.8	99.7	97.7	103.3	98.6	98.6	98.1	99.4
Lab-C	124.4	118.0	86.7	183.3	164.6	163.5	145.6	189.9
Lab-D Ext 1	96.8	96.6	88.3	101.0	95.1	95.1	94.3	95.9
Lab-D Ext 2	102.3	102.3	98.3	105.7	98.2	98.2	96.5	101.3
Lab-D Ext 3	87.8	86.4	66.7	99.3	96.2	96.2	94.9	96.8
Lab-E	106.7	106.7	106.7	106.7	117.1	117.1	117.1	117.1
COMPOUND = TOTAL HXCDF								
Lab-A	84.3	83.8	73.7	95.8	114.4	114.3	108.6	121.6
Lab-B	98.2	98.2	97.3	99.2	122.2	122.1	120.0	125.4
Lab-C	538.2	253.7	82.1	1389.5	1527.9	1152.3	421.6	2918.9
Lab-D Ext 1	102.3	102.3	101.5	103.8	102.9	102.9	101.6	104.9
Lab-D Ext 2	101.2	101.2	100.4	102.3	105.9	105.9	104.9	106.5
Lab-D Ext 3	99.8	99.8	97.9	101.1	105.2	105.1	99.5	108.6
Lab-E	103.2	103.2	103.2	103.2	81.1	81.1	81.1	81.1
COMPOUND = TOTAL HPCDF								
Lab-A	88.9	88.9	85.3	93.0	119.2	118.7	100.9	135.4
Lab-B	91.6	91.2	80.5	100.0	100.5	99.0	76.8	112.7
Lab-C	65.3	39.2	8.3	132.5	35.2	28.3	12.4	64.6
Lab-D Ext 1	102.9	102.6	97.0	114.5	104.4	104.3	102.5	108.2
Lab-D Ext 2	108.4	108.3	102.8	113.8	114.1	114.1	109.7	119.4
Lab-D Ext 3	93.8	93.8	91.5	96.0	99.9	99.7	94.3	106.7
Lab-E	112.5	112.5	112.5	112.5	119.8	119.8	119.8	119.8
COMPOUND = OCDF								
Lab-A	--[a]	--	--	--	91.2	90.8	81.1	100.8
Lab-B	--	--	--	--	102.5	94.7	55.3	149.2
Lab-C	--	--	--	--	--	--	--	--
Lab-D Ext 1	--	--	--	--	96.0	96.0	94.7	97.7
Lab-D Ext 2	--	--	--	--	92.9	92.9	89.4	97.0
Lab-D Ext 3	--	--	--	--	97.0	96.9	93.2	101.0
Lab-E	--	--	--	--	42.4	42.4	42.4	42.4

TABLE 27. (cont'd.)

Lab	Sample 1 Arithmetic Mean	Sample 1 Geometric Mean	Sample 1 Minimum	Sample 1 Maximum	Sample 2 Arithmetic Mean	Sample 2 Geometric Mean	Sample 2 Minimum	Sample 2 Maximum
COMPOUND = TOTAL TCDD								
Lab-A	75.3	75.0	68.0	85.2	90.9	90.1	74.7	102.8
Lab-B	82.7	81.9	68.0	96.0	101.3	101.2	100.0	103.0
Lab-C	10.4	10.4	10.4	10.4	85.5	85.5	83.0	86.0
Lab-D Ext 1	98.7	98.6	96.0	100.0	95.6	95.6	92.5	98.1
Lab-D Ext 2	100.0	99.9	96.0	104.0	95.6	95.6	94.3	98.1
Lab-D Ext 3	92.0	91.9	96.0	96 0	93.7	93.7	90.6	96.2
Lab-E	56.0	56.0	56.0	56.0	71.7	71.7	71.7	71.7
COMPOUND = OCDD								
Lab-A	--	--	--	--	--	--	--	--
Lab-B	--	--	--	--	--	--	--	--
Lab-C	--	--	--	--	--	--	--	--
Lab-D Ext 1	--	--	--	--	--	--	--	--
Lab-D Ext 2	--	--	--	--	--	--	--	--
Lab-D Ext 3	--	--	--	--	--	--	--	--
Lab-E	--	--	--	--	--	--	--	--
COMPOUND = 2,3,7,8-TCDD								
Lab-A	75.3	75.0	68.0	85.2	90.9	90.1	74.7	102.8
Lab-B	82.7	81.9	68.0	96.0	101.3	101.2	100.0	103.0
Lab-C	81.3	81.0	76.0	92.0	85.5	85.5	83.0	86.0
Lab-D Ext 1	98.7	98.6	96.0	100.0	95.6	95.6	92.5	98.1
Lab-D Ext 2	100.0	99.9	96.0	104.0	95.6	95.6	94.3	98.1
Lab-D Ext 3	92.0	91.9	88.0	96.0	93.7	93.7	90.6	96.2
Lab-E	56.0	56.0	56.0	56.0	71.7	71.7	71.7	71.7
COMPOUND = 2,3,7,8 + 2,3,4,8-TCDF								
Lab-A	88.8	88.1	73.0	97.5	117.5	117.4	114.2	123.6
Lab-B	78.5	78.3	73.5	87.0	115.7	115.7	115.1	117.0
Lab-C	95.0	94.2	80.0	110.0	119.5	119.2	107.5	126.4
Lab-D Ext 1	88.8	88.7	84.0	94.0	118.2	118.2	115.1	120.8
Lab-D Ext 2	92.7	92.5	88.0	99.5	112.6	112.5	107.5	117.0
Lab-D Ext 3	92.7	92.7	91.0	94.5	113.8	113.8	113.2	115.1
Lab-E	94.5	94.5	94.5	94.5	118.5	118.5	118.5	118.5
COMPOUND = 1,2,3,7,8 + 1,2,3,4,8-PNCDF								
Lab-A	90.3	89.8	80.7	102.0	100.9	100.4	87.5	108.3
Lab-B	101.6	101.5	100.0	104.0	104.0	104.0	101.9	105.1
Lab-C	100.0	99.6	93.3	113.3	116.0	116.0	113.9	120.3
Lab-D Ext 1	97.6	97.5	96.0	98.7	99.4	99.4	98.1	100.6
Lab-D Ext 2	91.6	91.6	89.3	94.7	97.0	97.0	95.6	99.4
Lab-D Ext 3	93.3	93.3	92.7	94.0	98.5	98.5	97.5	99.4
Lab-E	106.7	106.7	106.7	106.7	120.3	120.3	120.3	120.3

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TABLE 27 (cont'd.)

Lab	Sample 1 Arithmetic Mean	Sample 1 Geometric Mean	Sample 1 Minimum	Sample 1 Maximum	Sample 2 Arithmetic Mean	Sample 2 Geometric Mean	Sample 2 Minimum	Sample 2 Maximum
COMPOUND = 2,3,4,7,8-PNCDF								
Lab-A	94.0	93.6	84.7	105.3	93.7	92.9	77.2	103.2
Lab-B	98.0	97.9	94.7	102.7	93.0	93.0	91.1	94.3
Lab-C	146.7	130.8	80.0	246.7	206.8	203.1	164.6	259.5
Lab-D Ext 1	96.0	95.4	80.7	104.0	90.9	90.9	90.5	91.1
Lab-D Ext 2	108.7	108.7	107.3	111.3	99.4	99.3	97.5	103.2
Lab-D Ext 3	104.4	104.4	102.0	106.0	93.9	93.9	92.4	94.9
Lab-E	106.7	106.7	106.7	106.7	113.9	113.9	113.9	113.9
COMPOUND = 1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF								
Lab-A	91.0	90.5	79.6	103.6	--	--	--	--
Lab-B	95.9	95.9	95.2	96.4	--	--	--	--
Lab-C	104.0	98.4	60.0	128.0	--	--	--	--
Lab-D Ext 1	106.4	106.4	104.8	107.2	--	--	--	--
Lab-D Ext 2	103.1	103.0	100.4	105.2	--	--	--	--
Lab-D Ext 3	104.4	104.4	100.0	107.2	--	--	--	--
Lab-E	116.0	116.0	116.0	116.0	--	--	--	--
COMPOUND = 1,2,3,7,8,9-HXCDF								
Lab-A	--	--	--	--	--	--	--	--
Lab-B	--	--	--	--	--	--	--	--
Lab-C	--	--	--	--	--	--	--	--
Lab-D Ext 1	--	--	--	--	--	--	--	--
Lab-D Ext 2	--	--	--	--	--	--	--	--
Lab-D Ext 3	--	--	--	--	--	--	--	--
Lab-E	--	--	--	--	--	--	--	--
COMPOUND = 1,2,3,6,7,8-HXCDF								
Lab-A	83.1	82.4	70.2	94.8	106.7	106.6	103.0	110.4
Lab-B	124.0	124.0	124.0	124.0	117.6	117.6	115.1	120.8
Lab-D Ext 1	102.7	102.6	98.0	106.0	108.8	108.7	103.8	115.1
Lab-D Ext 2	97.3	97.2	94.0	104.0	106.9	106.9	103.8	109.4
Lab-D Ext 3	86.0	85.8	82.0	94.0	113.2	113.0	103.8	120.8
Lab-E	62.0	62.0	62.0	62.0	75.5	75.5	75.5	75.5
COMPOUND = 2,3,4,6,7,8-HXCDF								
Lab-A	91.6	91.5	85.7	97.1	114.1	113.9	106.1	122.7
Lab-B	94.5	94.5	93.1	96.0	123.0	122.9	118.9	128.8
Lab-C	1188.6	313.5	80.0	3371.4	2015.2	1478.0	515.2	3939.4
Lab-D Ext 1	98.1	98.1	96.6	100.6	100.5	100.5	100.0	100.8
Lab-D Ext 2	101.3	101.3	99.4	102.3	105.6	105.6	105.3	106.1
Lab-D Ext 3	98.7	98.7	97.1	99.4	102.0	102.0	97.7	104.5
Lab-E	97.1	97.1	97.1	97.1	83.3	83.3	83.3	83.3

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TABLE 27 (cont'd.)

Lab	Sample 1				Sample 2			
	Arithmetic Mean	Geometric Mean	Minimum	Maximum	Arithmetic Mean	Geometric Mean	Minimum	Maximum
COMPOUND = 1,2,3,4,6,7,8-HPCDF								
Lab-A	88.4	88.4	85.3	92.3	118.9	118.4	109.3	134.8
Lab-B	91.6	91.2	80.5	100.0	100.5	99.0	76.8	112.7
Lab-C	65.3	39.2	8.3	132.5	35.2	28.3	12.4	64.6
Lab-D Ext 1	102.9	102.6	97.0	114.5	104.4	104.3	102.5	108.2
Lab-D Ext 2	108.4	108.3	102.8	113.8	114.1	114.1	109.7	119.4
Lab-D Ext 3	93.8	93.8	91.5	96.0	99.9	99.7	94.3	106.7
Lab-E	112.5	112.5	112.5	112.5	119.0	119.8	119.8	119.8

[a]Below limit of detection.

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TABLE 28. MINIMUM, MEDIAN, AND MAXIMUM PERCENT FOUND (ACCURACY) FOR ALL PCDF AND PCDD CONGENERS IN SPIKED SAMPLES 1 AND 2

	Sample 1			Sample 2		
LAB	Minimum	Median	Maximum	Minimum	Median	Maximum
LAB-A	68.0	87.8	105.3	74.7	106.8	135.4
LAB-B	68.0	96.0	124.0	55.3	112.0	149.2
LAB-C	8.2	94.2	3371.4	12.4	120.3	3939.4
LAB-D	80.7	98.7	114.5	90.5	100.8	120.8
LAB-D	88.0	100.8	113.8	89.4	104.9	119.4
LAB-D	66.7	94.5	107.2	90.6	98.7	120.8
LAB-E	56.0	103.2	116.0	42.4	113.9	120.2

the largest ranges. Among the other laboratories, LAB-B had the highest maximum values, while LAB-E had the lowest minimums.

To provide a better comparison of the analytical method performance among the laboratories, the following steps were taken:

1) Samples 15 and 16 were dropped from further analysis because few compounds were found above the detection limit.
2) To eliminate the missing data for LABS-B and C caused by nonreporting of detection limits, the following values, based upon detection limits reported by LAB-D, were imputed:

Compound	Limit of Detection (ng/g)
Total TCDD	3
OCDD	10
2,3,7,8-TCDD	3
2,3,4,7,8-PCDF	3

Analyses of variance were then run on the altered data to determine the sources of variation. Table 29 gives the total variance by compounds for each laboratory and then breaks it down into sample (different types analyzed) and error (instrumental measurement) variances. Since LAB-E reported only one observation per compound and sample, its variance could not be broken down, but the total variance can be compared with the other labs. Generally, the TCDF, PNCDF, and OCDD compounds showed smaller variances, while OCDF showed the largest variances. There was some variability among laboratories in amount of total variance with LAB-E having a tendency to be higher, but no clear cut patterns emerge. Lab-C had the largest measurement error for almost every congener. The greatest percent of variation, however, was due to sample differences as reflected in comparing percent sample variance with percent measurement error variance.

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TABLE 29. VARIANCE COMPONENTS BY COMPOUND AND LAB

	Variance Components			As Percent of Total	
	Total	Sample	Measurement Error	Sample	Measurement Error
TOTAL TCDF					
LAB-A	1.23	1.14	0.09	93.0	7.0
LAB-B	1.24	1.22	0.02	98.3	1.7
LAB-C	1.33	1.22	0.12	91.1	8.9
LAB-D Ext.-1	1.52	1.51	0.01	99.4	0.6
LAB-D Ext.-2	1.31	1.30	0.01	99.5	0.5
LAB-D Ext.-3	1.37	1.36	0.01	99.5	0.5
LAB-E	1.66	-[a]	-	-	-
TOTAL PNCDF					
LAB-A	1.55	1.54	0.01	99.4	0.6
LAB-B	1.37	1.36	0.02	98.9	1.1
LAB-C	1.57	1.42	0.15	90.3	9.7
LAB-D Ext.-1	1.71	1.68	0.02	98.6	1.4
LAB-D Ext.-2	1.55	1.55	0.00	99.7	0.3
LAB-D Ext.-3	1.60	1.59	0.01	99.5	0.5
LAB-E	1.90	-	-	-	-
TOTAL HXCDF					
LAB-A	3.34	3.33	0.01	99.8	0.2
LAB-B	2.96	2.94	0.01	99.5	0.5
LAB-C	3.50	2.86	0.65	81.6	18.4
LAB-D Ext.-1	4.42	4.41	0.01	99.7	0.3
LAB-D Ext.-2	3.18	3.16	0.02	99.4	0.6
LAB-D Ext.-3	3.38	3.36	0.01	99.7	0.3
LAB-E	4.20	-	-	-	-
TOTAL HPCDF					
LAB-A	4.20	4.19	0.01	99.7	0.3
LAB-B	3.19	3.15	0.05	98.5	1.5
LAB-C	4.65	4.06	0.59	87.3	12.7
LAB-D Ext.-1	6.06	6.04	0.02	99.7	0.3
LAB-D Ext.-2	3.76	3.75	0.01	99.8	0.2
LAB-D Ext.-3	5.55	5.49	0.06	98.9	1.1
LAB-E	5.35	-	-	-	-
OCDF					
LAB-A	10.94	10.93	0.01	99.9	0.1
LAB-B	6.50	6.43	0.07	98.9	1.1
LAB-C	6.46	6.30	0.16	97.5	2.5
LAB-D Ext.-1	8.52	8.48	0.04	99.5	0.5
LAB-D Ext.-2	6.41	6.40	0.00	99.9	0.1
LAB-D Ext.-3	8.19	8.19	0.00	99.9	0.1
LAB-E	9.86	-	-	-	-

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TABLE 29 (cont'd.)

	Variance Components			As Percent of Total	
	Total	Sample	Measurement Error	Sample	Measurement Error
TOTAL TCDD					
LAB-A	5.61	4.34	1.27	77.4	22.6
LAB-B	2.22	2.22	0.00	99.8	0.2
LAB-C	2.24	2.24	0.00	99.8	0.2
LAB-D Ext.-1	2.25	2.25	0.00	99.9+	0.0
LAB-D Ext.-2	2.30	2.30	0.00	99.9+	0.0
LAB-D Ext.-3	2.19	2.19	0.00	99.9+	0.0
LAB-E	7.52	-	-	-	-
OCDD					
LAB-A	2.73	2.15	0.58	78.7	21.3
LAB-B	0.24	0.15	0.09	63.1	36.9
LAB-C	0.59	0.53	0.06	89.2	10.8
LAB-D Ext.-1	-	-	-	-	-
LAB-D Ext.-2	-	-	-	-	-
LAB-D Ext.-3	-	-	-	-	-
LAB-E	0.84	-	-	-	-
2,3,7,8-TCDD					
LAB-A	8.05	6.87	1.18	85.4	14.6
LAB-B	5.46	5.45	0.00	99.9	0.1
LAB-C	2.12	2.12	0.00	99.8	0.2
LAB-D Ext.-1	2.25	2.25	0.00	99.9+	0.0
LAB-D Ext.-2	2.30	2.30	0.00	99.9+	0.0
LAB-D Ext.-3	2.19	2.19	0.00	99.9+	0.0
LAB-E	7.52	-	-	-	-
2,3,7,8 + 2,3,4,8-TDCF					
LAB-A	1.13	1.11	0.02	98.0	2.0
LAB-B	1.32	1.29	0.03	97.6	2.4
LAB-C	1.70	1.67	0.03	98.4	1.6
LAB-D Ext.-1	1.57	1.56	0.01	99.5	0.5
LAB-D Ext.-2	1.43	1.42	0.00	99.8	0.2
LAB-D Ext.-3	1.43	1.43	0.00	99.8	0.2
LAB-E	1.65	-	-	-	-
1,2,3,7,8 + 1,2,3,4,8-PNCDF					
LAB-A	1.70	1.69	0.02	99.0	1.0
LAB-B	1.54	1.54	0.00	99.7	0.3
LAB-C	2.10	2.08	0.03	98.8	1.2
LAB-D Ext.-1	1.94	1.93	0.01	99.5	0.5
LAB-D Ext.-2	1.92	1.91	0.01	99.5	0.5
LAB-D Ext.-3	1.65	1.65	0.00	99.9	0.1
LAB-E	2.02	-	-	-	-

TABLE 29 (cont'd.)

	Variance Components			As Percent of Total	
	Total	Sample	Measurement Error	Sample	Measurement Error
2,3,4,7,8-PNCDF					
LAB-A	1.27	1.26	0.01	98.9	1.1
LAB-B	1.20	1.20	0.01	99.5	0.5
LAB-C	1.65	1.58	0.08	95.4	4.6
LAB-D Ext.-1	1.30	1.26	0.04	97.0	3.0
LAB-D Ext.-2	1.32	1.32	0.00	99.9	0.1
LAB-D Ext.-3	1.29	1.29	0.00	99.9	0.1
LAB-E	1.76	-	-	-	-
1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF					
LAB-A	6.47	6.44	0.03	99.6	0.4
LAB-B	7.25	7.22	0.03	99.6	0.4
LAB-C	4.48	4.24	0.24	94.6	5.4
LAB-D Ext.-1	5.93	5.93	0.01	99.9	0.1
LAB-D Ext.-2	5.69	5.69	0.00	99.9	0.1
LAB-D Ext.-3	5.78	5.78	0.00	99.9+	0.0
LAB-E	8.71	-	-	-	-
1,2,3,7,8,9-HXCDF					
LAB-A	7.86	7.48	0.38	95.2	4.8
LAB-B	3.50	3.50	0.00	99.9	0.1
LAB-C	4.68	4.04	0.65	86.2	13.8
LAB-D Ext.-1	4.88	4.86	0.02	99.7	0.3
LAB-D Ext.-2	4.46	4.44	0.02	99.6	0.4
LAB-D Ext.-3	4.86	4.85	0.01	99.8	0.2
LAB-E	6.06	-	-	-	-
1,2,3,6,7,8-HXCDF					
LAB-A	2.99	2.99	0.01	99.8	0.2
LAB-B	2.48	2.46	0.01	99.4	0.6
LAB-C	-	-	-	-	-
LAB-D Ext.-1	3.01	3.00	0.00	99.9	0.1
LAB-D Ext.-2	2.32	2.30	0.02	99.2	0.8
LAB-D Ext.-3	2.85	2.83	0.02	99.3	0.7
LAB-E	3.79	-	-	-	-
2,3,4,6,7,8-HXCDF					
LAB-A	3.27	3.25	0.01	99.5	0.5
LAB-B	2.62	2.59	0.03	99.0	1.0
LAB-C	4.53	3.18	1.38	69.6	30.4
LAB-D Ext.-1	3.11	3.10	0.01	99.6	0.4
LAB-D Ext.-2	1.77	1.76	0.01	99.2	0.8
LAB-D Ext.-3	1.85	1.84	0.01	99.5	0.5
LAB-E	4.27	-	-	-	-

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TABLE 29 (cont'd.)

	Variance Components			As Percent of Total	
	Total	Sample	Measurement Error	Sample	Measurement Error
1,2,3,4,6,7,8-HPCDF					
LAB-A	4.05	4.04	0.01	99.8	0.2
LAB-B	3.01	2.97	0.04	98.7	1.3
LAB-C	3.97	3.10	0.87	78.1	21.9
LAB-D Ext.-1	4.43	4.41	0.02	99.6	0.4
LAB-D Ext.-2	3.32	3.32	0.01	99.8	0.2
LAB-D Ext.-3	4.00	3.94	0.06	98.5	1.5
LAB-E	5.28	-	-	-	-

[a]Below limit of detection.

For LAB-D, the variance component for repetitive extraction of the same sample could be included because data for three extracts was reported (see Table 30). Here as before, at least 95% of the variance was in the samples with extract and instrumental measurement error contributing little.

To gain further understanding of the differences in variance, Table 31 gives the standard deviations (the square root of the variances) by compound, sample, and laboratory (recalling that each lab used different analytical procedures as described in Section 2.0). The laboratories were then ranked by compound and sample according to the size of the standard deviation, i.e. for TCDF in Sample 1, LAB-D-3 had the smallest standard deviation while LAB-C had the largest. Generally, LAB-C had larger standard deviations (and therefore poorer accuracy and precision from their analytical method). While the other laboratories, excluding LAB-C, show no large differences, LAB-D tended to be lower (and therefore the best accuracy and precision from their analytical method). Also shown are the results of Bartlett's tests for homogeneity of variance among laboratories, by compound and sample (6). Here again, there is variability in the results. For some of the compound-sample combinations, the labs were significantly different at the .01 level while for other combinations the labs were not significantly different at the .05 level.

Table 32 gives the results of Levene's test for homogeneity of variance among laboratories (over samples), by compound (6). For every compound, the laboratories were significantly different from one another at the .05 level, and for all but one compound (1,2,3,6,7,8-HXCDF), they were significantly different from one another at the .01 level.

Table 33 provides approximate 95-percent prediction intervals of levels reported from future analyses of the samples. The seven laboratories (LAB-D included three times) were treated as a random sample from a large population of laboratories, and a variance component, denoted as VL, of the log concentrations due to laboratories (and therefore analytical method used) was thus estimated. A measurement error variance component, VM, was also estimated. This component is the within-extract variance of the log concentrations. Data from the LAB-E were not used in determining this component of variance, since there were no replicate observations. The two components were used to calculate the prediction intervals via the following formula:

$$\exp \{x \pm 2V\},$$

where

x = mean of the log concentrations (across laboratories and extracts), and

V = square root of (VL + VM).

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TABLE 30. VARIANCE COMPONENTS BY COMPOUND FOR LAB-D

Compounds	Variance Components				As Percent of Total		
	Total	Sample	Extract	Error	Sample	Extract	Error
Total TCDF	1.40	1.38	0.01	0.01	98.9	0.6	0.5
Total PNCDF	1.62	1.61	0.00	0.01	99.2	0.0	0.8
Total HXCDF	3.66	3.59	0.05	0.01	98.3	1.3	0.4
Total HPCDF	5.13	5.00	0.09	0.03	97.6	1.8	0.6
OCDF	7.73	7.41	0.30	0.02	95.9	3.9	0.2
Total TCDD	2.25	2.25	0.00	0.00	99.9+	0.0	0.0
OCDD	0.00	0.00	0.00	0.00	99.9+	0.0	0.0
2,3,7,8-TCDD	2.25	2.25	0.00	0.00	99.9+	0.0	0.0
2,3,7,8 + 2,3,4,8-TCDF	1.48	1.47	0.00	0.00	99.5	0.2	0.3
1,2,3,7,8 + 1,2,3,4,8-PNCDF	1.84	1.82	0.01	0.01	99.2	0.4	0.4
2,3,4,7,8-PNCDF	1.31	1.30	0.00	0.01	98.9	0.0	1.1
1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	5.80	5.80	0.00	0.00	99.9	0.0	0.1
1,2,3,7,8,9-HXCDF	4.73	4.71	0.01	0.01	99.5	0.2	0.3
1,2,3,6,7,8-HXCDF	2.73	2.69	0.02	0.01	98.6	0.8	0.5
2,3,4,6,7,8-HXCDF	2.25	2.16	0.08	0.01	96.1	3.4	0.5
1,2,3,4,6,7,8-HPCDF	3.92	3.87	0.02	0.03	98.7	0.5	0.7

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TABLE 31. STANDARD DEVIATIONS, THEIR RANKS, AND BARTLETTS TEST BY COMPOUND AND SAMPLE

	Standard Deviations							Ranks					
Sample	LAB-A	LAB-B	LAB-C	LAB-D-1	LAB-D-2	LAB-D-3	BT	LAB-A	LAB-B	LAB-C	LAB-D-1	LAB-D-2	LAB-D-3
COMPOUND = TOTAL TCDF													
1	28.58	14.80	30.00	10.02	12.10	3.51		5	4	6	2	3	1.0
2	2.36	0.58	5.51	1.53	2.52	0.58		4	1	6	3	5	2
3	20.82	49.32	366.92	98.15	65.51	20.55	**	2	3	6	5	4	1
11	108.01	8.33	65.06	19.67	20.31	10.44	*	6	1	5	3	4	2
12	182.17	6.35	20.81	4.58	3.06	19.60	**	6	3	5	2	1	4
13	143.93	60.92	130.00	97.12	92.67	109.61		6	1	5	3	2	4
14	283.08	216.61	217.03	92.48	30.75	18.45	*	6	4	5	3	2	1
17	23.39	78.17	65.06	59.87	39.55	3.21	*	2	6	5	4	3	1
COMPOUND = TOTAL PNCDF													
1	30.51	9.29	155.03	21.94	11.14	54.93	*	4	1	6	3	2	5
2	31.75	2.08	72.11	2.52	8.39	3.46	**	5	1	6	2	4	3
3	26.46	66.37	100.00	166.96	134.52	67.88		1	2	4	6	5	3
11	123.42	166.86	860.31	312.68	41.07	3.52	**	3	4	6	5	2	1
12	74.81	15.28	177.30	42.45	7.09	7.23	**	5	3	6	4	1	2
13	11.53	27.10	25.17	46.48	20.84	26.51		1	5	3	6	2	4
14	300.00	1722.88	2542.31	2886.65	275.13	113.15	**	3	4	5	6	2	1
17	35.12	218.48	64.29	161.04	131.23	109.55		1	6	2	5	4	3
COMPOUND = TOTAL HXCDF													
1	52.63	4.51	3504.6	6.08	4.73	7.94	**	5	1	6	3	2	4
2	12.22	5.29	2354.6	3.21	1.73	9.29	**	5	3	6	2	1	4
3	86.22	48.17	7689.6	271.01	622.22	42.15	**	3	2	6	4	5	1
11	172.14	913.30	24055.4	828.67	126.26	131.05	**	3	5	6	4	1	2
12	43.02	36.37	257.0	85.04	49.90	44.66		2	4	6	5	4	3
13	6.46	17.35	15.3	3.61	9.45	11.93		2	6	5	1	3	4
14	200.00	413.63	12165.5	1217.54	590.93	96.11	**	2	3	6	5	4	1
17	30.55	283.28	6600.8	206.65	30.29	8.06	**	3	5	6	4	2	1
COMPOUND = TOTAL HPCDF													
1	15.57	40.00	251.02	40.13	22.03	9.02	**	2	4	6	5	3	1
2	73.90	107.98	140.80	17.32	25.77	32.96	**	4	5	6	1	2	3
3	85.44	175.84	1172.40	64.84	147.14	148.19	**	2	5	6	1	3	4
11	793.87	1592.80	4838.73	1855.90	92.60	548.43	**	3	4	6	5	1	2
12	295.01	345.56	600.00	332.58	125.01	95.04		3	5	6	4	2	1
13	2.16	8.14	12.40	0.00	0.58	4.04	*	3	5	6	1	2	4
14	52.92	290.13	737.11	387.58	370.24	40.78		2	3	6	5	4	1
17	107.86	422.94	305.51	322.10	139.41	28.04	*	2	6	4	5	3	1
COMPOUND = OCDF													
1	0.27	0.00	.	4.58	0.58	0.00	**	3	1.5	.	5	4.0	1.5
2	13.01	62.00	.	2.08	5.03	7.00	**	4	5.0	.	1	2.0	3.0
3	166.53	829.67	1357.69	114.04	221.20	228.29	**	2	5.0	6	1	3.0	4.0
11	2260.53	8644.25	3464.10	3667.22	879.17	1420.07	.	3	6.0	4	5	1.0	2.0
12	640.86	1049.04	493.29	817.82	127.80	232.46		4	6.0	3	5	1.0	2.0
13	1.61	0.00	20.23	6.51	1.15	1.15	**	4	1.0	6	5	2.5	2.5
14	4.58	173.66	36.06	8.62	22.74	36.67	*	1	6.0	4	2	3.0	5.0
17	387.66	546.51	404.15	598.87	14.29	89.69		3	5.0	4	6	1.0	2.0

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TABLE 3I (cont'd.)

	Standard Deviations							Ranks					
Sample	LAB-A	LAB-B	LAB-C	LAB-D-1	LAB-D-2	LAB-D-3	BT	LAB-A	LAB-B	LAB-C	LAB-D-1	LAB-D-2	LAB-D-3
COMPOUND = TOTAL TCDD													
1	2.22	3.51	.	0.58	1.00	1.00		4	5	.	1	2.5	2.5
2	7.70	1.15	1.15	1.53	1.15	1.53	*	6	3	1.5	5	1.5	4.0
3	2.09	9.02	15.28	1.73	5.51	1.53	*	3	5	6.0	2	4.0	1.0
11	34.97	0.00	0.00	0.00	0.00	0.00		6	3	3.0	3	3.0	3.0
12	3.87	0.00	0.00	0.00	0.00	0.00		6	3	3.0	3	3.0	3.0
13	0.34	0.00	0.00	0.00	0.00	0.00		6	3	3.0	3	3.0	3.0
14	0.47	0.00	0.00	0.00	0.00	0.00		6	3	3.0	3	3.0	3.0
17	0.24	0.00	0.00	0.00	0.00	0.00		6	3	3.0	3	3.0	3.0
COMPOUND = OCDD													
1	2.61	0.00	.	0	0	0		5	2.5	0	2.5	2.5	2.5
2	2.14	0.00	.	0	0	0		5	2.5	.	2.5	2.5	2.5
3	4.68	0.00	.	0	0	0		5	2.5	.	2.5	2.5	2.5
11	3.03	2.52	4.16	0	0	0		5	4.0	6.0	2.0	2.0	2.0
12	0.40	7.64	0.00	0	0	0	**	5	6.0	2.5	2.5	2.5	2.5
13	0.44	0.58	0.00	0	0	0	*	5	6.0	2.5	2.5	2.5	2.5
14	0.03	4.04	4.62	0	0	0	*	4	5.0	6.0	2.0	2.0	2.0
17	4.86	5.51	4.62	0	0	0		5	6.0	4.0	2.0	2.0	2.0
COMPOUND = 2,3,7,8-TCDD													
1	2.22	3.51	2.31	0.58	1.00	1.00		4	6	5.0	1	2.5	2.5
2	7.70	1.15	1.15	1.53	1.15	1.53	*	6	3	1.5	5	1.5	4.0
3	2.09	9.02	15.28	1.73	5.51	1.53	*	3	5	6.0	2	4.0	1.0
11	34.74	0.00	0.00	0.00	0.00	0.00		6	3	3.0	3	3.0	3.0
12	1.11	0.00	0.00	0.00	0.00	0.00		6	3	3.0	3	3.0	3.0
13	0.34	0.00	0.00	0.00	0.00	0.00		6	3	3.0	3	3.0	3.0
14	0.04	0.00	0.00	0.00	0.00	0.00		6	3	3.0	3	3.0	3.0
17	0.24	0.00	0.00	0.00	0.00	0.00		6	3	3.0	3	3.0	3.0
COMPOUND = 2,3,7,8 + 2,3,4,8-TCDF													
1	27.47	14.80	30.00	10.02	12.10	3.51		5	4.0	6	2	3.0	1
2	2.80	0.58	5.51	1.53	2.52	0.58		5	1.0	6	3	4.0	2
3	10.00	11.59	219.39	12.22	42.03	25.42	**	1	2.0	6	3	5.0	4
11	12.94	16.46	5.51	3.46	0.00	1.15		5	6.0	4	3	1.0	2
12	15.50	1.73	5.77	3.06	1.73	5.51		6	1.5	5	3	1.5	4
13	38.35	4.58	41.63	25.01	12.06	5.77		5	1.0	6	4	3.0	2
14	66.46	25.11	25.17	32.15	7.09	4.00		6	3.0	4	5	2.0	1
17	7.00	28.73	26.46	27.51	11.02	2.00	*	2	6.0	4	5	3.0	1
COMPOUND = 1,2,3,7,8 + 1,2,3,4,8-PNCDF													
1	16.27	3.21	17.32	2.08	4.16	1.00	**	5	3	6	2	4	1
2	18.29	2.89	5.77	2.00	3.21	1.53	**	6	3	5	2	4	1
3	3.06	13.58	32.15	12.74	20.23	12.17		1	4	6	3	5	2
11	105.71	58.50	11.59	104.41	21.13	22.81	*	6	4	1	5	2	3
12	25.10	5.51	6.24	19.86	4.58	3.00	*	6	3	4	5	2	1
13	1.51	1.53	1.12	1.00	1.15	0.00		5	6	3	2	4	1
14	42.12	49.15	62.45	221.59	210.79	56.04		1	2	4	6	2	3
17	11.04	42.23	1.53	37.40	49.00	14.47		2	5	1	4	6	3

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TABLE 31 (cont'd.)

	Standard Deviations							Ranks					
Sample	LAB-A	LAB-B	LAB-C	LAB-D-1	LAB-D-2	LAB-D-3	BT	LAB-A	LAB-B	LAB-C	LAB-D-1	LAB-D-2	LAB-D-3
COMPOUND = 2,3,4,7,8-PNCDF													
1	15.72	6.24	132.29	19.92	3.46	3.21	**	4	3	6	5	2	1
2	22.61	2.65	76.36	0.58	5.20	2.08	**	5	3	6	1	4	2
3	8.50	11.59	237.14	2.08	14.01	6.08	**	3	4	6	1	5	2
11	3.06	6.51	11.55	34.82	4.00	3.08	**	1	4	5	6	3	2
12	11.79	4.62	2.00	7.94	1.15	2.08		6	4	2	5	1	3
13	5.60	4.00	11.36	6.51	1.00	1.53		4	3	6	5	1	2
14	26.46	98.00	321.46	312.67	21.13	33.05	**	2	4	6	5	1	3
17	11.93	22.61	20.42	44.66	9.64	9.29		3	5	4	6	2	1
COMPOUND = 1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF													
1	30.12	1.53	95.4	3.46	6.11	9.64	**	5	1	6	2	3	4
2	1.71	0.58	.	0.00	0.00	0.00		5	4	.	2	2	2
3	39.45	33.72	152.8	22.74	23.86	35.23		5	3	6	1	2	4
11	99.90	517.84	13519.3	394.71	80.89	83.93	**	3	5	6	4	1	2
12	11.59	35.80	120.5	67.98	3.06	25.87	*	2	4	6	5	1	3
13	3.12	4.93	10.8	1.53	0.58	2.08	*	4	5	6	2	1	3
14	25.02	46.74	1644.2	419.97	660.20	148.33	**	1	2	6	4	5	3
17	56.70	253.24	2294.2	145.23	11.53	13.32	**	3	5	6	4	1	2
COMPOUND = 1,2,3,7,8,9-HXCDF													
1	0.27	.	.	0.00	0.00	0.00		4	.	.	2	2	2
2	0.24	.	.	0.00	0.00	0.00		4	.	.	2	2	2
3	2.05	.	558.84	1.53	1.73	0.58	**	4	.	5	2	3	1
11	1.36	0	847.68	28.62	11.68	6.81	**	2	1	6	5	4	3
12	8.11	0	9.45	2.52	2.08	0.58	**	5	1	6	4	3	2
13	0.09	0	6.93	1.15	1.53	1.00		2	1	6	4	5	3
14	8.14	31	3865.66	173.14	289.83	15.72	**	1	3	6	4	5	2
17	0.16	0	1024.30	2.89	1.53	4.51	**	2	1	6	4	3	5
COMPOUND = 1,2,3,6,7,8-HXCDF													
1	6.17	0.00	.	2.08	2.89	3.46		5	1	.	2	3	4
2	1.95	1.53	.	3.06	1.53	4.58		3	2	.	4	1	5
3	13.32	12.06	.	15.72	27.30	6.00		3	2	.	4	5	1
11	3.61	100.72	.	22.28	64.49	4.04	**	1	5	.	3	4	2
12	1.18	4.36	.	3.00	3.51	8.08	*	1	4	.	2	3	5
13	0.48	2.08	.	0.00	0.58	0.58		2	5	.	1	3	4
14	17.32	29.69	.	63.66	110.39	110.53		1	2	.	3	4	5
17	1.94	2.31	.	4.51	18.61	6.03	*	1	2	.	3	5	4
COMPOUND = 2,3,4,6,7,8-HXCDF													
1	10.02	2.52	3308.35	3.79	2.89	2.31	**	5	2	6	4	3	1
2	11.02	6361	2311.45	0.58	0.58	4.93		5	4	6	2	1	3
3	14.36	14.29	4659.68	3.79	39.58	6.43	**	4	3	6	1	5	2
11	5.67	10.58	909.45	30.05	8.08	1.00	**	2	4	6	5	3	1
12	3.05	3.06	11.72	5.00	1.15	2.08		3	4	6	5	1	2
13	0.16	1.15	11.14	0.00	1.15	2.52		2	4	6	1	3	5
14	20.43	62.55	3481.99	105.72	227.28	19.73	**	2	3	6	4	5	1
17	7.27	13.45	1457.17	6.03	1.73	6.00	**	4	5	6	3	1	2

TABLE 31 (cont'd.)

	Standard Deviations							Ranks					
Sample	LAB-A	LAB-B	LAB-C	LAB-D-1	LAB-D-2	LAB-D-3	BT	LAB-A	LAB-B	LAB-C	LAB-D-1	LAB-D-2	LAB-D-3
COMPOUND = 1,2,3,4,6,7,8-HPCDF													
1	14.19	40.08	251.02	40.13	22.03	9.02	**	2	4	6	5	3	1
2	72.95	107.98	140.80	17.32	25.77	32.98	**	4	5	6	1	2	3
3	34.64	61.58	460.74	10.69	42.67	42.03	**	2	5	6	1	4	3
11	384.32	698.55	1708.80	672.92	29.02	166.79	**	3	5	6	4	1	2
12	60.62	118.97	200.33	101.36	37.00	31.97		3	5	6	4	2	1
13	0.81	3.06	3.53	0.00	0.58	4.04		3	4	5	1	2	6
14	11.02	96.70	370.05	133.06	119.93	8.72	**	2	6	4	5	6	1
17	27.10	250.47	70.95	153.68	59.37	17.93	*	2	6	4	5	3	1

* Bartlett's test shows significant difference at the .05 level.
** Bartlett's test shows significant difference at the .01 level.

TABLE 32. LEVENE'S TEST OF DIFFERENCES AMONG LABS BY COMPOUND-OVER SAMPLES

Compound	F-Value	Degrees of Freedom
Total TCDF	8.89**	5
Total PNCDF	11.15**	5
Total HXCDF	23.12**	5
Total HPCDF	17.39**	5
OCDF	6.97**	5
Total TCDD	17.82**	5
OCDD	12.81**	5
2,3,7,8-TCDD	14.96**	5
2,3,7,8 and 2,3,4,8-TCDF	6.60**	5
1,2,3,7,8 and 1,2,3,4,8-PNCDF	4.39**	5
2,3,4,7,8-PNCDF	9.76**	5
1,2,3,4,7,8 and 1,2,3,4,7,9-HXCDF	17.80**	5
1,2,3,7,8,9-HXCDF	16.10**	5
1,2,3,6,7,8-HXCDF	2.96*	4
2,3,4,6,7,8-HXCDF	39.93**	5
1,2,3,4,6,7,8-HPCDF	22.80**	5

* Significantly different at the .05 level.
** Significantly different at the .01 level.

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TABLE 33. APPROXIMATE 95 PERCENT PREDICITON INTERVALS BY COMPOUND AND SAMPLE[a]

Compound	Sample 1	2	3	11	12	13	14	17
Total TCDF	144 222	57 67	1046 2321	57 362	37 321	389 1247	532 1389	249 729
Total PNCDF	206 431	220 517	1004 1682	1125 3079	143 590	179 442	5846 13417	562 1999
Total HXCDF	155 1862	40 2118	798 10654	3041 21535	726 1320	11 198	5897 21657	610 13977
Total HPCDF	103 1105	137 1532	819 4341	1581 41116	1467 3090	2 52	1391 3486	242 11063
OCDF	2 89	67 208	2280 6436	9641 64743	3822 10453	7 75	96 457	442 24465
Total TCDD	6 60	40 60	63 132	<1 21	<1 14	<1 15	<1 20	<1 10
OCDD	1 59	1 40	2 32	7 19	5 30	<1 70	1 43	5 32
2,3,7,8-TCDD	15 30	40 60	63 132	<1 25	<1 20	<1 13	<1 32	<1 11
2,3,7,8 + 1,2,3,4,8-PNCDF	144 221	57 67	977 1355	24 61	15 81	90 459	251 459	119 241
1,2,3,7,8 + 1,2,3,4,8-PNCDF	123 168	138 193	226 425	41 7913	27 264	8 30	742 1577	74 746
2,3,4,7,8-PNCDF	99 248	90 321	164 618	84 145	28 59	46 80	1324 2389	106 403
1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	182 347	1 15	825 1838	2138 12579	447 809	13 36	3144 5860	486 5023
1,2,3,7,8,9-HXCDF	<1 34	<1 25	2 965	1 8869	4 167	<1 61	64 7800	<1 6136
1,2,3,6,7,8-HXCDF	31 72	45 72	254 412	218 603	20 97	2 10	561 1385	43 134
2,3,4,6,7,8-HXCDF	40 1041	24 1912	55 3975	26 879	9 68	1 51	196 2882	10 2237
1,2,3,4,6,7,8-HPCDF	103 1104	137 1530	96 3258	580 16405	418 976	1 17	482 1130	79 5652

[a]Values are in ppb.

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With about 95 percent confidence of being correct (i.e. 1 our of 20 measurements having a chance of being outside of this range), each of the reported intervals is expected to cover a future laboratory's analytical results for the given compound and sample. This assumes that the concentrations are log normally distributed. Note that the data are not adequate for assessing this assumption; also the data for generating these intervals are extremely limited and can be sensitive to spurious observations. Many of the intervals are so wide that they are likely to be of little use. If the analytical method used by Lab-C was omitted, these intervals would improve, but a quantitaive estimate of this improvement would be difficult to determine since the amount of data for statistical analysis would be insufficient.

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SECTION 4
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APPENDIX A

EVALUATION OF CALIBRATION REGRESSION USED BY U. UMEÅ

RESEARCH TRIANGLE INSTITUTE

Center for Medical, Environmental and Energy Statistics

September 2, 1986

MEMORANDUM

TO: Edo Pellizzari

FROM: Andy Clayton

SUBJECT: Problems with Calibration of Dioxin/Furan Data

REFERENCE: Analys results EPRI-prodjekt 2028 (6/23/86)

Two problems with the calibration procedure used by the Umeå Universitet laboratory are noted. These are the following:

1. The regression of x on y is performed, where x is the (injected) amount of dioxin/furan, and y is the (observed) normalized count. Although there has been much controversy over the past 20-25 years concerning whether it is more appropriate to regress x on y or y on x, I believe that most statisticians agree that regressing y on x is the preferred approach.

2. Ordinary least squares (OLS) is used to fit the calibration curve (assumed to be a straight line). This is a much more serious problem. OLS is appropriate when the magnitude of the measurement errors is approximately the same across the entire range of concentrations of interest. This is clearly not the situation here. As an example, consider the data for 2378-TCDF. When x=7500, the y values range from 8593.7 to 9037.3, a difference of 443.6. On the other hand, when x=10, the range of observed counts is only 2.9. Clearly, the measurement error increases with increasing concentration, so that OLS is inappropriate. The severity of the problem is demonstrated by the estimates obtained for 2378-TCDF. Using the values of a and b from the referenced document, it is seen that a normalized count of 10 units will yield a predicted concentration of (.853)(10) + 29.89 = 38.42 pg. Examination of the calibration data indicates that the true concentration should be roughly 10 pg. For lower concentrations (say, less than 200 pg), OLS has thus produced a curve that (in this case) results in a significant overestimation of the true concentration. It is precisely in this range where the transformer data occur.

One solution to the above problems is to perform the calibration ourselves and to apply the new calibration to normalized counts obtained from the reported concentrations and the reported curve parameter estimates. Some imprecision will occur due to the fact that the concentrations are reported to only two significant digits. Alternatively, we can request the raw (normalized count) data, perform an appropriate calibration, and re-estimate the concentrations for the transformers.

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APPENDIX B

DATA USED IN COMPARISONS BETWEEN LABS

TABLE B-1. DATA USED IN COMPARISONS BETWEEN LABS

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
1	NYSDOH	TOTAL TCDF	145.00	195.00	194.00	178.00	28.60
		TOTAL PNCDF	251.00	312.00	283.00	282.00	30.50
		TOTAL HXCDF	396.00	455.00	350.00	400.00	52.60
		TOTAL HPCDF	354.00	341.00	372.00	356.00	15.60
		OCDF	3.48	3.55	3.06	3.37	0.27
		TOTAL TCDD	17.00	21.30	18.20	18.80	2.22
		OCDD	5.09	0.49	0.67	2.08	2.61
		2,3,7,8-TCDD	17.00	21.30	18.20	18.80	2.22
		2,3,7,8 + 2,3,4,8-TCDF	146.00	195.00	192.00	178.00	27.50
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	121.00	153.00	132.00	135.00	16.30
		2,3,4,7,8-PNCDF	127.00	158.00	138.00	141.00	15.70
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	225.00	259.00	199.00	228.00	30.10
		1,2,3,7,8,9-HXCDF	0.53	0.07	0.05	0.22	0.27
		1,2,3,6,7,8-HXCDF	42.10	47.40	35.10	41.50	6.17
		2,3,4,6,7,8-HXCDF	150.00	161.00	170.00	160.00	10.00
		1,2,3,4,6,7,8-HPCDF	351.00	341.00	389.00	354.00	14.20
2	NYSDOH	TOTAL TCDF	60.00	82.70	85.50	63.00	2.36
		TOTAL PNCDF	276.00	336.00	324.00	312.00	31.70
		TOTAL HXCDF	201.00	209.00	225.00	212.00	12.20
		TOTAL HPCDF	578.00	712.00	591.00	627.00	73.90
		OCDF	133.00	107.00	121.00	120.00	13.00
		TOTAL TCDD	39.60	50.40	54.50	48.20	7 70
		OCDD	1.44	0.41	4.52	2.12	2.14
		2,3,7,8-TCDD	39.60	50.40	54.50	48.20	7 70
		2,3,7,8 + 2,3,4,8-TCDF	60.60	60.50	65.50	62.30	2.80
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	136.00	171.00	169.00	159.00	18.30
		2,3,4,7,8-PNCDF	122.00	159.00	163.00	148.00	22.60
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	2.77	3.85	6.12	4.24	1.71
		1,2,3,7,8,9-HXCDF	0.21	0.05	0.52	0.26	0.24
		1,2,3,6,7,8-HXCDF	58.50	54.60	56.50	56.50	1.95
		2,3,4,6,7,8-HXCDF	140.00	150.00	162.00	151.00	11.00
		1,2,3,4,6,7,8-HPCDF	575.00	709.00	592.00	625 00	73.00
3	NYSDOH	TOTAL TCDF	1630.00	1620.00	1590.00	1613.00	20.80
		TOTAL PNCDF	1330.00	1290.00	1340.00	1320.00	26.50
		TOTAL HXCDF	2490.00	2380.00	2550.00	2473.00	86.20
		TOTAL HPCDF	2470.00	2300.00	2400.00	2390.00	85.40
		OCDF	4850.00	5090.00	5170.00	5037.00	167.00
		TOTAL TCDD	65.10	65.50	61.70	64.10	2.09
		OCDD	3.73	0.72	9.89	4 78	4.68
		2,3,7,8-TCDD	65.10	65.50	61.70	64.10	2.09
		2,3,7,8 + 2,3,4,8-TCDF	1210.00	1220.00	1200.00	1210.00	10 00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	346.00	342.00	348.00	345.00	3.06
		2,3,4,7,8-PNCDF	304.00	286.00	291.00	294.00	8.50
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	1228.00	1209.00	1285.00	1241.00	39 40
		1,2,3,7,8,9-HXCDF	37.80	35.80	39.90	37 80	2.05
		1,2,3,6,7,8-HXCDF	339.00	321.00	347.00	336.00	13 30
		2,3,4,6,7,8-HXCDF	313.00	286.00	291.00	297.00	14 40
		1,2,3,4,6,7,8-HPCDF	865.00	805.00	865.00	845.00	34.60

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784042

TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
11	NYSDOH	TOTAL TCDF	232.00	121.00	337.00	230.00	108.00
		TOTAL PNCDF	1930.00	1690.00	1760.00	1793.00	123.00
		TOTAL HXCDF	6840.00	6570.00	6320.00	6643.00	172.00
		TOTAL HPCDF	10100.00	8610.00	8880.00	9197.00	794.00
		OCDF	39000.00	37700.00	42100.00	39600.00	2261.00
		TOTAL TCDD	1.32	62.80	2.76	22.20	35.00
		OCDD	11.80	9.00	14.30	12.10	3.03
			10.00	10.40	17.00		
		2,3,7,8-TCDD	1.32	62.20	2.76	22.10	34.70
		2,3,7,8 + 2,3,4,8-TCDF	46.90	42.80	67.70	53.20	12.90
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	638.00	734.00	788.00	820.00	106.00
		2,3,4,7,8-PNCDF	101.00	107.00	105.00	104.00	3.06
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	4782.00	4614.00	4625.00	4677.00	99.80
		1,2,3,7,8,9-HXCDF	2.36	4.14	5.00	3.83	1.36
		1,2,3,6,7,8-HXCDF	360.00	367.00	362.00	363.00	3.61
		2,3,4,6,7,8-HXCDF	51.20	42.40	40.60	44.70	5.67
		1,2,3,4,6,7,8-HPCDF	3890.00	3280.00	3990.00	3720.00	384.00
12	NYSDOH	TOTAL TCDF	228.00	182.00	518.00	309.00	182.00
		TOTAL PNCDF	430.00	303.00	435.00	389.00	74.80
		TOTAL HXCDF	983.00	897.00	938.00	939.00	43.00
		TOTAL HPCDF	2400.00	1940.00	1650.00	2063.00	295.00
		OCDF	6800.00	8000.00	7010.00	7270.00	641.00
		TOTAL TCDD	0.35	0.43	7.10	2.63	3.87
		OCDD	27.40	28.60	27.10	27.00	0.40
		2,3,7,8-TCDD	0.05	0.12	2.00	0.72	1.11
		2,3,7,8 + 2,3,4,8-TCDF	66.60	61.10	90.90	73.50	15.50
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	116.00	66.80	101.00	96.10	25.10
		2,3,4,7,8-PNCDF	60.60	39.60	59.40	53.20	11.80
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	621.00	598.00	612.00	610.00	11.60
		1,2,3,7,8,9-HXCDF	37.90	17.70	36.10	29.90	8.11
			33.90	22.20	31.50		
		1,2,3,6,7,8-HXCDF	43.00	44.60	42.30	43.30	1.16
		2,3,4,6,7,8-HXCDF	15.20	9.10	12.30	12.20	3.05
		1,2,3,4,6,7,8-HPCDF	662.00	545.00	631.00	613.00	60.60
13	NYSDOH	TOTAL TCDF	686.00	700.00	942.00	776.00	144.00
		TOTAL PNCDF	368.00	351.00	346.00	355.00	11.50
		TOTAL HXCDF	39.70	51.90	42.00	44.50	6.48
		TOTAL HPCDF	8.30	12.50	9.51	10.10	2.16
		OCDF	13.40	15.30	16.60	15.10	1.61
		TOTAL TCDD	0.16	0.39	0.83	0.46	0.34
		OCDD	1.10	0.38	0.29	0.59	0.44
		2,3,7,8-TCDD	0.16	0.39	0.83	0.46	0.34
		2,3,7,8 + 2,3,4,8-TCDF	197.00	226.00	273.00	232.00	38.40
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	19.80	17.30	17.10	18.10	1.51
		2,3,4,7,8-PNCDF	61.30	61.50	71.10	64.60	5.80
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	18.80	21.90	18.20	18.30	3.12
		1,2,3,7,8,9-HXCDF	0.19	0.36	0.24	0.26	0.09
		1,2,3,6,7,8-HXCDF	3.40	4.36	3.94	3.90	0.48
		2,3,4,6,7,8-HXCDF	1.70	2.00	1.75	1.82	0.16
		1,2,3,4,6,7,8-HPCDF	3.40	4.80	3.38	3.86	0.81

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784043

TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
14	NYSDOH	TOTAL TCDF	1500.00	953.00	1100.00	1184.00	283.00
		TOTAL PNCDF	10500.00	10200.00	10600.00	10500.00	300.00
		TOTAL HXCDF	10500.00	10900.00	10700.00	10700.00	200.00
		TOTAL HPCDF	2000.00	2020.00	2100.00	2040.00	52.90
		OCDF	190.00	187.00	181.00	186.00	4.58
		TOTAL TCDD	0.85	0.42	0.98	0.46	0.47
		OCDD	1.04	0.98	1.01	1.01	0.03
		2,3,7,8-TCDD	0.05	0.02	0.09	0.05	0.04
		2,3,7,8 + 2,3,4,8-TCDF	480.00	349.00	395.00	408.00	66.50
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	1106.00	1052.00	1135.00	1098.00	42.10
		2,3,4,7,8-PNCDF	1930.00	1920.00	1970.00	1940.00	26.50
		1,2,3,4,7,8 + 1,2,3,4,7,8-HXCDF	4413.00	4432.00	4382.00	4409.00	25.00
		1,2,3,7,8,9-HXCDF	162.00	177.00	175.00	171.00	8.14
		1,2,3,6,7,8-HXCDF	1030.00	1030.00	1000.00	1020.00	17.30
		2,3,4,6,7,8-HXCDF	386.00	404.00	398.00	389.00	20.40
		1,2,3,4,6,7,8-HPCDF	690.00	678.00	700.00	689.00	11.00
15	NYSDOH	TOTAL TCDF	0.53	0.38	0.31	0.41	0.11
		TOTAL PNCDF	1.72	1.84	2.14	1.93	0.21
		TOTAL HXCDF	1.78	1.14	2.43	1.78	0.65
		TOTAL HPCDF	0.51	0.23	0.19	0.31	0.17
		OCDF	0.46	0.30	0.24	0.33	0.11
		TOTAL TCDD	0.09	0.08	20.00	6 72	11.50
		OCDD	3.02	3.36	3.80	3.40	0.39
		2,3,7,8-TCDD	0.09	0.06	20.00	6.72	11.50
		2,3,7,8 + 2,3,4,8-TCDF	0.20	0.24	0.23	0.22	0.02
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	0.37	0.39	0.31	0.36	0.04
		2,3,4,7,8-PNCDF	0.45	0.51	0.41	0.46	0.05
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	1.28	0.60	1.16	1.01	0.36
		1,2,3,7,8,9-HXCDF	0.22	0.11	0.28	0.20	0.09
		1,2,3,6,7,8-HXCDF	0.25	0.13	0.27	0.22	0 08
		2,3,4,6,7,8-HXCDF	0.21	0.14	0.07	0.14	0.07
		1,2,3,4,6,7,8-HPCDF	0.28	0.08	0.09	0 15	0.11
16	NYSDOH	TOTAL TCDF	0.15	0.18	0.23	0.19	0.04
		TOTAL PNCDF	0.34	0.60	0.53	0.49	0.13
		TOTAL HXCDF	0.56	0.52	0.71	0.60	0.10
		TOTAL HPCDF	0.65	0.23	0.21	0.36	0.25
		OCDF	0.63	2.40	3.80	2.28	1.59
		TOTAL TCDD	0.81	0.19	0.10	0.30	0.27
		OCDD	20.80	16.80	17.10	18.20	2.23
		2,3,7,8-TCDD	0.60	0.12	0.09	0.27	0.29
		2,3,7,8 + 2,3,4,8-TCDF	0.15	0.18	0.13	0.15	0 03
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	0.68	0.18	0.14	0.33	0.30
		2,3,4,7,8-PNCDF	0.34	0.09	0.11	0.18	0.14
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	0.77	0.71	0.69	0.72	0.04
		1,2,3,7,8,9-HXCDF	0.21	0.19	0.16	0.19	0 03
		1,2,3,6,7,8-HXCDF	0.21	0.19	0.16	0.19	0.03
		2,3,4,6,7,8-HXCDF	0.32	0.20	0.18	0.23	0.08
		1,2,3,4,6,7,8-HPCDF	0.65	0.23	0.21	0.36	0.25

784044

GENP 011233

TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
17	NYSDOH	TOTAL TCDF	553.00	552.00	512.00	539.00	23.40
		TOTAL PNCDF	1170.00	1130.00	1100.00	1133.00	35.10
		TOTAL HXCDF	1780.00	1780.00	1620.00	1767.00	30.60
		TOTAL HPCDF	1780.00	1580.00	1610.00	1657.00	108.00
		OCDF	3962.00	4410.00	4734.00	4369.00	388.00
		TOTAL TCDD	1.16	0.62	0.71	0.90	0.24
		OCDD	18.70	24.20	26.40	23.80	4.86
		2,3,7,8-TCDD	1.16	0.62	0.71	0.90	0.24
		2,3,7,8 + 2,3,4,8-TCDF	195.00	184.00	182.00	187.00	7.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	289.00	277.00	287.00	278.00	11.00
		2,3,4,7,8-PNCDF	170.00	151.00	173.00	165.00	11 90
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	1001.00	1031.00	1111.00	1047.00	56.70
		1,2,3,7,8,9-HXCDF	0.89	0.88	0.59	0.78	0.16
		1,2,3,6,7,8-HXCDF	64.90	65.20	61.70	63.90	1.94
		2,3,4,6,7,8-HXCDF	70.00	58.00	56.00	61.30	7.57
		1,2,3,4,6,7,8-HPCDF	815.00	781.00	784.00	787.00	27.10
1	IITRI	TOTAL TCDF	174.00	130.00	147.00	157.00	14.80
		TOTAL PNCDF	293.00	295.00	310.00	299.00	9.29
		TOTAL HXCDF	471.00	462.00	461.00	467.00	4.51
		TOTAL HPCDF	322.00	400.00	377.00	366.00	40.10
		OCDF	54.00	54.00	54.00	54.00	0.00
		TOTAL TCDD	24.00	17.00	21.00	20.70	3 51
		OCDD	10.00	10.00	10.00	10.00	0 00
		2,3,7,8-TCDD	24.00	17.00	21.00	20.70	3.51
		2,3,7,8 + 2,3,4,8-TCDF	174.00	150.00	147.00	157.00	14.80
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	151.00	150.00	156.00	152.00	3.21
		2,3,4,7,8-PNCDF	142.00	145.00	154.00	147.00	6 24
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	241.00	238.00	240.00	240.00	1 53
		1,2,3,7,8,9-HXCDF	.	.	.	.	
		1,2,3,6,7,8-HXCDF	62.00	62.00	62.00	62.00	0.00
		2,3,4,6,7,8-HXCDF	168.00	163.00	165.00	165.00	2.52
		1,2,3,4,6,7,8-HPCDF	322.00	400.00	377.00	366.00	40.10
2	IITRI	TOTAL TCDF	61.00	62.00	61.00	61.30	0.58
		TOTAL PNCDF	311.00	310.00	314.00	312.00	2.08
		TOTAL HXCDF	224.00	232.00	222.00	226.00	5.29
		TOTAL HPCDF	593.00	589.00	404.00	529.00	108.00
		OCDF	197.00	136.00	73.00	135.00	62.00
		TOTAL TCDD	53.00	53.00	55.00	53.70	1.15
		OCDD	5.20	5.20	5.20	5.20	0.00
		2,3,7,8-TCDD	53.00	53.00	55.00	53.70	1 15
		2,3,7,8 + 2,3,4,8-TCDF	61.00	62.00	61.00	61.30	0 58
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	161.00	166.00	166.00	164.00	2.89
		2,3,4,7,8-PNCDF	149.00	144.00	148.00	147.00	2.65
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	2.00	1.00	2.00	1.67	0.58
		1,2,3,7,8,9-HXCDF	.	.	.	.	
		1,2,3,6,7,8-HXCDF	62.00	61.00	64.00	62.30	1 53
		2,3,4,6,7,8-HXCDF	160.00	170.00	157.00	162.00	6.81
		1,2,3,4,6,7,8-HPCDF	593.00	589.00	404.00	529.00	108.00

784045

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TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
3	IITRI	TOTAL TCDF	1550.00	1647.00	1583.00	1593.00	49.30
		TOTAL PNCDF	1114.00	1217.00	1093.00	1141.00	66.40
		TOTAL HXCDF	2338.00	2283.00	2242.00	2288.00	48.20
		TOTAL HPCDF	1209.00	1072.00	860.00	1047.00	176.00
		OCDF	3618.00	2530.00	1989.00	2712.00	830.00
		TOTAL TCDD	107.00	89.00	97.00	97.70	9.02
		OCDD	9.90	9.90	9.90	9.90	0.00
		2,3,7,8-TCDD	107.00	89.00	97.00	97.70	9.02
		2,3,7,8 + 2,3,4,8-TCDF	1121.00	1144.00	1135.00	1133.00	11.60
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	323.00	346.00	322.00	330.00	13.60
		2,3,4,7,8-PNCDF	266.00	275.00	252.00	264.00	11.60
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	987.00	921.00	942.00	950.00	33.70
		1,2,3,7,8,9-HXCDF	.	.	.	.	.
		1,2,3,6,7,8-HXCDF	381.00	395.00	371.00	382.00	12.10
		2,3,4,6,7,8-HXCDF	318.00	290.00	299.00	302.00	14.30
		1,2,3,4,6,7,8-HPCDF	587.00	511.00	444.00	507.00	61.60
11	IITRI	TOTAL TCDF	103.00	99.00	87.00	96.30	8.33
		TOTAL PNCDF	1601.00	1911.00	1649.00	1720.00	167.00
		TOTAL HXCDF	5449.00	6789.00	7194.00	6477.00	913.00
		TOTAL HPCDF	7035.00	7798.00	10095.00	8309.00	1593.00
		OCDF	29722.00	33265.00	46148.00	36378.00	8644.00
		TOTAL TCDD	.	.	.	.	.
		OCDD	17.00	20.00	15.00	17.30	2.52
		2,3,7,8-TCDD	0.50	0.50	0.50	0.50	0 00
		2,3,7,8 + 2,3,4,8-TCDF	35.00	26.00	27.00	36.00	16.50
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	867.00	984.00	926.00	926.00	58.50
		2,3,4,7,8-PNCDF	118.00	105.00	112.00	112.00	6.51
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	3130.00	3953.00	4086.00	3723.00	518.00
		1,2,3,7,8,9-HXCDF	.	.	.	.	.
		1,2,3,6,7,8-HXCDF	401.00	545.00	595.00	514.00	101.00
		2,3,4,6,7,8-HXCDF	87.00	67.00	83.00	79 00	10.60
		1,2,3,4,6,7,8-HPCDF	3212.00	3925.00	4609.00	3915.00	699.00
12	IITRI	TOTAL TCDF	136.00	125.00	125.00	129.00	6.35
		TOTAL PNCDF	329.00	339.00	309.00	326.00	15.30
		TOTAL HXCDF	939.00	1002.00	1002.00	981.00	36.40
		TOTAL HPCDF	1511.00	1504.00	2106.00	1707.00	346.00
		OCDF	7617.00	7702.00	9475.00	8265.00	1049.00
		TOTAL TCDD	.	.	.	.	.
		OCDD	7.00	22.00	17.00	15.30	7.64
		2,3,7,8-TCDD	1.00	1.00	1.00	1.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	41.00	38.00	41.00	40.00	1.73
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	110.00	115.00	121.00	115.00	5.51
		2,3,4,7,8-PNCDF	43.00	43.00	51.00	45.70	4.62
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	501.00	549.00	571.00	540.00	35.80
		1,2,3,7,8,9-HXCDF	.	.	.	.	.
		1,2,3,6,7,8-HXCDF	80.00	87.00	79.00	82.00	4.36
		2,3,4,6,7,8-HXCDF	13.00	15.00	19.00	15.70	3.06
		1,2,3,4,6,7,8-HPCDF	569.00	583.00	772.00	635.00	119.00

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784046

TABLE 8-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
13	IITRI	TOTAL TCDF	572.00	473.00	461.00	502.00	60.90
		TOTAL PNCDF	276.00	253.00	222.00	250.00	27.10
		TOTAL HXCDF	54.00	52.00	63.00	63.00	17.30
		TOTAL HPCDF	17.00	15.00	30.00	20.70	8.14
		OCDF	42.00	42.00	42.00	42.00	0.00
		TOTAL TCDD	.	.	.	.	.
		OCDD	9.00	8.00	8.00	8.33	0.58
		2,3,7,8-TCDD	0.50	0.50	0.50	0.50	0.00
		2,3,7,8 + 2,3,4,8-TCDF	105.00	99.00	108.00	104.00	4.58
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	23.00	22.00	20.00	21.70	1.53
		2,3,4,7,8-PNCDF	63.00	59.00	67.00	63.00	4.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	19.00	18.00	27.00	21.30	4.93
		1,2,3,7,8,9-HXCDF	.	.	.	.	.
		1,2,3,6,7,8-HXCDF	8.00	7.00	10.00	7.67	2.08
		2,3,4,6,7,8-HXCDF	3.00	3.00	5.00	3.67	1.15
		1,2,3,4,6,7,8-HPCDF	9.00	7.00	13.00	9.67	3.06
14	IITRI	TOTAL TCDF	970.00	617.00	576.00	721.00	217.00
		TOTAL PNCDF	9238.00	8589.00	8002.00	7276.00	1723.00
		TOTAL HXCDF	10598.00	9918.00	10666.00	10394.00	414.00
		TOTAL HPCDF	1725.00	2196.00	2254.00	2058.00	290.00
		OCDF	293.00	626.00	374.00	431.00	174.00
		TOTAL TCDD	.	.	.	.	.
		OCDD	8.00	13.00	5.00	8.67	4.04
		2,3,7,8-TCDD	0.50	0.50	0.50	0.50	0.00
		2,3,7,8 + 2,3,4,8-TCDF	297.00	248.00	282.00	276.00	25.10
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	1115.00	1039.00	1023.00	1059.00	49.20
		2,3,4,7,8-PNCDF	1772.00	1584.00	1728.00	1694.00	98.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	3802.00	3711.00	3775.00	3763.00	46 70
		1,2,3,7,8,9-HXCDF	174.00	187.00	233.00	198 00	31 00
		1,2,3,6,7,8-HXCDF	1195.00	1155.00	1137.00	1162.00	29 70
		2,3,4,6,7,8-HXCDF	387.00	472.00	509.00	456.00	62.60
		1,2,3,4,6,7,8-HPCDF	728.00	905.00	884.00	839.00	96 70
15	IITRI	TOTAL TCDF	.	.	.	.	
		TOTAL PNCDF	.	16.00		16.00	
		TOTAL HXCDF	.	.	.	.	.
		TOTAL HPCDF	13.00	5.00	.	9.00	5.66
		OCDF	31.00	31.00	31.00	31.00	0.00
		TOTAL TCDD	.	.	.	.	.
		OCDD	13.00	10.00	8.00	10.30	2.52
		2,3,7,8-TCDD	1.00	1.00	1.00	1.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	0.50	0.50	0.50	0.50	0.00
		2,3,4,7,8-PNCDF	.	7.00	.	7.00	.
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	1.00	1.00	1.00	1 00	0 00
		1,2,3,7,8,9-HXCDF	.	.		.	
		1,2,3,6,7,8-HXCDF	.	.			
		2,3,4,6,7,8-HXCDF	.	.	.	.	.
		1,2,3,4,6,7,8-HPCDF	5.00	2.00	0.50	2.50	2.29

784047

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TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
16	IITRI	TOTAL TCDF	.	.	.	.	.
		TOTAL PNCDF	358.00	87.00	215.00	220.00	136.00
		TOTAL HXCDF	819.00	191.00	381.00	397.00	314.00
		TOTAL HPCDF	114.00	33.00	62.00	69.70	41.00
		OCDF	37.00	37.00	37.00	37.00	0.00
		TOTAL TCDD	.	.	.	.	.
		OCDD	25.00	23.00	28.00	25.30	2.52
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	5.00	5.00	5.00	5.00	0.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	72.00	19.00	40.00	43.70	26.70
		2,3,4,7,8-PNCDF	111.00	29.00	65.00	68.30	41.10
		1,2,3,4,7,8 + 1,2,3,4,7,8-HXCDF	243.00	65.00	144.00	151.00	89.20
		1,2,3,7,8,9-HXCDF	15.00	4.00	8.00	9.00	5.57
		1,2,3,6,7,8-HXCDF	87.00	21.00	46.00	51.30	33.30
		2,3,4,6,7,8-HXCDF	32.00	11.00	19.00	20.70	10.60
		1,2,3,4,6,7,8-HPCDF	43.00	19.00	23.00	27.00	14.40
17	IITRI	TOTAL TCDF	431.00	344.00	275.00	350.00	78.20
		TOTAL PNCDF	1221.00	887.00	810.00	973.00	218.00
		TOTAL HXCDF	2311.00	2006.00	1745.00	2021.00	283.00
		TOTAL HPCDF	1912.00	2273.00	1430.00	1872.00	423.00
		OCDF	6046.00	6846.00	5801.00	6231.00	547.00
		TOTAL TCDD	.	.	.	.	.
		OCDD	22.00	23.00	13.00	19.30	5.51
		2,3,7,8-TCDD	0.50	0.50	0.50	0.50	0.00
		2,3,7,8 + 2,3,4,8-TCDF	152.00	142.00	98.00	131.00	28.70
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	320.00	292.00	237.00	283.00	42.20
		2,3,4,7,8-PNCDF	180.00	176.00	139.00	165.00	22.60
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	1218.00	1131.00	920.00	1090.00	153.00
		1,2,3,7,8,9-HXCDF	.	.	.	.	.
		1,2,3,6,7,8-HXCDF	119.00	115.00	119.00	118.00	2.31
		2,3,4,6,7,8-HXCDF	74.00	81.00	55.00	70.00	13.50
		1,2,3,4,6,7,8-HPCDF	1095.00	1270.00	776.00	1047.00	250.00
1	BATTELLE	TOTAL TCDF	220.00	160.00	190.00	190.00	30.00
		TOTAL PNCDF	310.00	550.00	260.00	373.00	155.00
		TOTAL HXCDF	680.00	6800.00	390.00	2557.00	3505.00
		TOTAL HPCDF	530.00	33.00	220.00	261.00	251.00
		OCDF	.	.	.	.	.
		TOTAL TCDD	.	.	2.60	2.60	.
		OCDD	.	.	0.78	0.78	.
		2,3,7,8-TCDD	19.00	19.00	23.00	20.30	2.31
		2,3,7,8 + 2,3,4,8-TCDF	220.00	160.00	190.00	190.00	30.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	140.00	170.00	140.00	150.00	17.30
		2,3,4,7,8-PNCDF	170.00	370.00	120.00	220.00	132.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	310.00	320.00	150.00	260.00	95.40
		1,2,3,7,8,9-HXCDF	.	.	.	.	.
		2,3,4,6,7,8-HXCDF	200.00	5900.00	140.00	2080.00	3308.00
		1,2,3,4,6,7,8-HPCDF	530.00	33.00	220.00	261.00	251.00

GENP 011237

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TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
2	BATTELLE	TOTAL TCDF	57.00	67.00	66.00	63.30	5.51
		TOTAL PNCDF	800.00	500.00	460.00	520.00	72.10
		TOTAL HXCDF	5400.00	2300.00	780.00	2827.00	2355.00
		TOTAL HPCDF	65.00	150.00	340.00	185.00	141.00
		OCDF	.	.	.	.	.
		TOTAL TCDD	46.00	46.00	44.00	45.30	1.15
		OCDD	.	.	.	.	.
		2,3,7,8-TCDD	46.00	46.00	44.00	45.30	1.15
		2,3,7,8 + 2,3,4,8-TCDF	57.00	67.00	66.00	63.30	5.51
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	180.00	180.00	190.00	183.00	5.77
		2,3,4,7,8-PNCDF	410.00	310.00	260.00	327.00	76.40
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	.	.	.	.	.
		1,2,3,7,8,9-HXCDF	.	.	.	.	.
		2,3,4,6,7,8-HXCDF	5200.00	2100.00	680.00	2660.00	2311.00
		1,2,3,4,6,7,8-HPCDF	65.00	150.00	340.00	185.00	141.00
3	BATTELLE	TOTAL TCDF	1400.00	1700.00	970.00	1357.00	367.00
		TOTAL PNCDF	1100.00	1300.00	1200.00	1200.00	100.00
		TOTAL HXCDF	20000.00	4700.00	11000.00	11900.00	7690.00
		TOTAL HPCDF	1700.00	3000.00	660.00	1787.00	1172.00
		OCDF	5000.00	4900.00	2600.00	4167.00	1358.00
		TOTAL TCDD	110.00	100.00	80.00	96.70	15.30
		OCDD	.	.	.	.	.
		2,3,7,8-TCDD	110.00	100.00	80.00	96.70	15.30
		2,3,7,8 + 2,3,4,8-TCDF	1200.00	1300.00	860.00	1127.00	219.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	260.00	200.00	250.00	237.00	32.10
		2,3,4,7,8-PNCDF	590.00	410.00	880.00	627.00	237.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	1900.00	1800.00	1800.00	1767.00	153.00
		1,2,3,7,8,9-HXCDF	1200.00	93.00	780.00	691.00	559.00
		2,3,4,6,7,8-HXCDF	10000.00	770.00	6500.00	5757.00	4660.00
		1,2,3,4,6,7,8-HPCDF	55.00	850.00	49.00	318.00	461.00
11	BATTELLE	TOTAL TCDF	170.00	110.00	40.00	107.00	65.10
		TOTAL PNCDF	2300.00	2100.00	720.00	1707.00	860.00
		TOTAL HXCDF	52000.00	11000.00	9700.00	24233.00	24055.00
		TOTAL HPCDF	3800.00	13000.00	11000.00	9267.00	4839.00
		OCDF	22000.00	28000.00	22000.00	24000.00	3464.00
		TOTAL TCDD	.	.	.	.	.
		OCDD	18.00	12.00	0.00	10.00	9.17
		2,3,7,8-TCDD	.	.	.	.	.
		2,3,7,8 + 2,3,4,8-TCDF	31.00	30.00	40.00	33.70	5.51
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	35.00	49.00	26.00	36.70	11.60
		2,3,4,7,8-PNCDF	130.00	130.00	110.00	123.00	11.50
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	29000.00	8100.00	5100.00	13400.00	13519.00
		1,2,3,7,8,9-HXCDF	2000.00	430.00	660.00	1030.00	848.00
		2,3,4,6,7,8-HXCDF	1800.00	240.00	210.00	750.00	909.00
		1,2,3,4,6,7,8-HPCDF	1100.00	4500.00	3100.00	2900.00	1709.00

784049

FLD 004645

TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
12	BATTELLE	TOTAL TCDF	62.00	64.00	99.00	75.00	20.80
		TOTAL PNCDF	79.00	230.00	310.00	206.00	117.00
		TOTAL HXCDF	1500.00	1300.00	990.00	1263.00	257.00
		TOTAL HPCDF	1500.00	2100.00	2700.00	2100.00	600.00
		OCDF	4000.00	4800.00	4900.00	4567.00	493.00
		TOTAL TCDD	.	.	.	.	.
		OCDD	.	.	.	.	.
		2,3,7,8-TCDD	.	.	.	.	.
		2,3,7,8 + 2,3,4,8-TCDF	20.00	20.00	30.00	23.30	5.77
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	22.00	25.00	34.00	27.00	6.24
		2,3,4,7,8-PNCDF	39.00	41.00	37.00	39.00	2.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	680.00	740.00	840.00	753.00	121.00
		1,2,3,7,8,9-HXCDF	54.00	50.00	36.00	46.70	9.45
		2,3,4,6,7,8-HXCDF	47.00	25.00	43.00	38.30	11.70
		1,2,3,4,6,7,8-HPCDF	350.00	530.00	750.00	543.00	200.00
13	BATTELLE	TOTAL TCDF	580.00	570.00	350.00	500.00	130.00
		TOTAL PNCDF	250.00	270.00	220.00	247.00	25.20
		TOTAL HXCDF	140.00	130.00	160.00	143.00	15.30
		TOTAL HPCDF	26.00	26.00	5.60	19.90	12.40
		OCDF	46.00	44.00	10.00	33.30	20.20
		TOTAL TCDD	.	.	.	.	.
		OCDD	.	.	.	.	.
		2,3,7,8-TCDD	.	.	.	.	.
		2,3,7,8 + 2,3,4,8-TCDF	300.00	280.00	220.00	267.00	41.60
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	10.00	8.30	7.90	8.73	1.12
		2,3,4,7,8-PNCDF	71.00	74.00	53.00	66.00	11.40
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	37.00	16.00	31.00	28.00	10.80
		1,2,3,7,8,9-HXCDF	15.00	15.00	27.00	19.00	6.93
		2,3,4,6,7,8-HXCDF	25.00	17.00	39.00	27.00	11.10
		1,2,3,4,6,7,8-HPCDF	7.10	8.50	1.80	5.80	3.53
14	BATTELLE	TOTAL TCDF	970.00	580.00	610.00	720.00	217.00
		TOTAL PNCDF	11000.00	6700.00	6500.00	8067.00	2542.00
		TOTAL HXCDF	14000.00	36000.00	16000.00	22000.00	12166.00
		TOTAL HPCDF	3100.00	2800.00	4200.00	3367.00	737.00
		OCDF	150.00	170.00	220.00	180.00	36.10
		TOTAL TCDD	.	.	.	.	.
		OCDD	.	18.00	.	18.00	.
		2,3,7,8-TCDD	.	.	.	.	.
		2,3,7,8 + 2,3,4,8-TCDF	400.00	380.00	350.00	377.00	25.20
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	870.00	900.00	780.00	850.00	62.40
		2,3,4,7,8-PNCDF	2300.00	2400.00	1800.00	2167.00	321 00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	4700.00	7200.00	4100.00	5333.00	1644.00
		1,2,3,7,8,9-HXCDF	1700.00	8800.00	2600.00	4367.00	3866.00
		2,3,4,6,7,8-HXCDF	620.00	6800.00	930.00	2783 00	3482.00
		1,2,3,4,6,7,8-HPCDF	920.00	560.00	1300.00	927.00	370 00

784050

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TABLE 8-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
15	BATTELLE	TOTAL TCDF	.	.	.	.	.
		TOTAL PNCDF	.	.	.	.	.
		TOTAL HXCDF	.	.	.	.	.
		TOTAL HPCDF	.	.	.	.	.
		OCDF	.	12.00	.	12.00	.
		TOTAL TCDD	.	.	.	.	.
		OCDD	.	.	.	.	.
		2,3,7,8-TCDD	.	.	.	.	.
		2,3,7,8 + 2,3,4,8-TCDF	.	.	.	.	.
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	.	.	.	.	.
		2,3,4,7,8-PNCDF	.	.	.	.	.
		1,2,3,4,7,8 + 1,2,3,4,7,8-HXCDF	.	.	.	.	.
		1,2,3,7,8,9-HXCDF	.	.	.	.	.
		2,3,4,6,7,8-HXCDF	.	.	.	.	.
		1,2,3,4,6,7,8-HPCDF	.	.	.	.	.
16	BATTELLE	TOTAL TCDF	.	.	.	.	.
		TOTAL PNCDF	.	.	.	.	.
		TOTAL HXCDF	.	.	.	.	.
		TOTAL HPCDF	.	.	.	.	.
		OCDF	.	.	.	.	.
		TOTAL TCDD	.	.	.	.	.
		OCDD	.	13.00	11.00	12.00	1.41
		2,3,7,8-TCDD	.	.	.	.	.
		2,3,7,8 + 2,3,4,8-TCDF	.	.	.	.	.
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	.	.	.	.	.
		2,3,4,7,8-PNCDF	.	.	.	.	.
		1,2,3,4,7,8 + 1,2,3,4,7,8-HXCDF	.	.	.	.	.
		1,2,3,7,8,9-HXCDF	.	.	.	.	.
		2,3,4,6,7,8-HXCDF	.	.	.	.	.
		1,2,3,4,6,7,8-HPCDF	.	.	.	.	.
17	BATTELLE	TOTAL TCDF	360.00	230.00	290.00	293.00	65.10
		TOTAL PNCDF	670.00	550.00	570.00	597.00	64.30
		TOTAL HXCDF	8100.00	19000.00	20000.00	15700.00	6601.00
		TOTAL HPCDF	1600.00	1400.00	2000.00	1667.00	306.00
		OCDF	4300.00	4800.00	5100.00	4733.00	404.00
		TOTAL TCDD	.	.	.	.	.
		OCDD	18.00	16.00	10.00	15.30	4.62
		2,3,7,8-TCDD	.	.	.	.	.
		2,3,7,8 + 2,3,4,8-TCDF	190.00	140.00	180.00	170.00	26.50
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	69.00	72.00	71.00	70.70	1.53
		2,3,4,7,8-PNCDF	300.00	260.00	290.00	283.00	20.80
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	2700.00	6400.00	6900.00	5333.00	2294.00
		1,2,3,7,8,9-HXCDF	760.00	2300.00	2700.00	1920.00	1024.00
		2,3,4,6,7,8-HXCDF	1100.00	3400.00	3800.00	2767 00	1457.00
		1,2,3,4,6,7,8-HPCDF	180.00	230.00	320.00	243.00	70.90

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TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
1	RADIAN EXT1	TOTAL TCDF	166.00	186.00	177.00	176.00	10.00
		TOTAL PNCDF	265.00	303.00	303.00	290.00	21.90
		TOTAL HXCDF	493.00	483.00	482.00	486.00	6.08
		TOTAL HPCDF	456.00	388.00	389.00	412.00	40.10
		OCDF	19.00	10.00	13.00	14.00	4.58
		TOTAL TCDD	24.00	25.00	25.00	24.70	0.58
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	24.00	25.00	25.00	24.70	0.58
		2,3,7,8 + 2,3,4,8-TCDF	166.00	186.00	177.00	176.00	10.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	144.00	147.00	148.00	146.00	2.08
		2,3,4,7,8-PNCDF	121.00	156.00	155.00	144.00	19.90
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	268.00	268.00	262.00	266.00	3.46
		1,2,3,7,8,9-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,6,7,8-HXCDF	52.00	49.00	53.00	51.[illegible]	2.08
		2,3,4,6,7,8-HXCDF	176.00	169.00	170.00	172.00	3.79
		1,2,3,4,6,7,8-HPCDF	456.00	388.00	389.00	412.00	40.10
2	RADIAN EXT1	TOTAL TCDF	61.00	64.00	63.00	62.70	1.53
		TOTAL PNCDF	298.00	303.00	301.00	301.00	2.52
		TOTAL HXCDF	194.00	189.00	188.00	190.00	3.21
		TOTAL HPCDF	569.00	539.00	539.00	549.00	17.30
		OCDF	125.00	126.00	129.00	127.00	2.08
		TOTAL TCDD	52.00	49.00	51.00	50.70	1.53
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	52.00	49.00	51.00	50.70	1.53
		2,3,7,8 + 2,3,4,8-TCDF	61.00	64.00	63.00	62.70	1.53
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	155.00	159.00	157.00	157.00	2.00
		2,3,4,7,8-PNCDF	143.00	144.00	144.00	144.00	0.58
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	6.00	6.00	6.00	6.00	0.00
		1,2,3,7,8,9-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,6,7,8-HXCDF	61.00	57.00	55.00	57.70	3.06
		2,3,4,6,7,8-HXCDF	133.00	132.00	133.00	133.00	0.58
		1,2,3,4,6,7,8-HPCDF	569.00	539.00	539.00	549.00	17.30
3	RADIAN EXT1	TOTAL TCDF	1393.00	1416.00	1431.00	1480.00	98.10
		TOTAL PNCDF	1605.00	1310.00	1322.00	1412.00	167.00
		TOTAL HXCDF	2348.00	2795.00	2306.00	2483.00	271.00
		TOTAL HPCDF	2492.00	2416.00	2363.00	2424.00	64.80
		OCDF	4029.00	3910.00	3801.00	3913.00	114.00
		TOTAL TCDD	100.00	100.00	97.00	99.00	1.73
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	100.00	100.00	97.00	99.00	1.73
		2,3,7,8 + 2,3,4,8-TCDF	1141.00	1157.00	1165.00	1154.00	12.20
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	300.00	323.00	321.00	315.00	12.70
		2,3,4,7,8-PNCDF	281.00	282.00	278.00	280.00	2.08
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	1142.00	1186.00	1174.00	1167.00	22.70
		1,2,3,7,8,9-HXCDF	31.00	34.00	33.00	32.70	1.53
		1,2,3,6,7,8-HXCDF	323.00	294.00	298.00	305.00	15.70
		2,3,4,6,7,8-HXCDF	317.00	324.00	323.00	321.00	3.79
		1,2,3,4,6,7,8-HPCDF	781.00	780.00	762.00	774.00	10.70

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TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
11	RADIAN EXT1	TOTAL TCDF	147.00	162.00	123.00	144.00	19.70
		TOTAL PNCDF	2206.00	2217.00	1670.00	2031.00	313.00
		TOTAL HXCDF	7143.00	7709.00	6077.00	6976.00	829.00
		TOTAL HPCDF	11221.00	11206.00	8000.00	10143.00	1836.00
		OCDF	29720.00	28359.00	22798.00	26959.00	3667.00
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	38.00	36.00	32.00	36.00	3.46
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	1018.00	894.00	1106.00	1007.00	104.00
		2,3,4,7,8-PNCDF	137.00	116.00	69.00	107.00	34.80
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	4874.00	5346.00	4562.00	4927.00	395.00
		1,2,3,7,8,9-HXCDF	275.00	273.00	227.00	260.00	28.60
		1,2,3,6,7,8-HXCDF	334.00	331.00	294.00	320.00	22.30
		2,3,4,6,7,8-HXCDF	207.00	219.00	162.00	196.00	30.00
		1,2,3,4,6,7,8-HPCDF	4221.00	4210.00	3050.00	3827.00	673.00
12	RADIAN EXT1	TOTAL TCDF	72.00	66.00	83.00	87.00	4.58
		TOTAL PNCDF	322.00	348.00	265.00	312.00	42.50
		TOTAL HXCDF	987.00	1088.00	919.00	998.00	85.00
		TOTAL HPCDF	2588.00	2580.00	2008.00	2392.00	333.00
		OCDF	8118.00	7627.00	6326.00	6690.00	818.00
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	26.00	30.00	24.00	26.70	3.06
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	102.00	141.00	115.00	119.00	19.90
		2,3,4,7,8-PNCDF	39.00	42.00	27.00	36.00	7.94
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	651.00	748.00	617.00	672.00	68 00
		1,2,3,7,8,9-HXCDF	37.00	34.00	39.00	36.70	2.52
		1,2,3,6,7,8-HXCDF	37.00	40.00	34.00	37.00	3.00
		2,3,4,6,7,8-HXCDF	23.00	28.00	18.00	23.00	5.00
		1,2,3,4,6,7,8-HPCDF	738.00	743.00	565.00	682.00	101.00
13	RADIAN EXT1	TOTAL TCDF	812.00	975.00	802.00	863.00	97.10
		TOTAL PNCDF	239.00	320.00	240.00	266.00	46.50
		TOTAL HXCDF	20.00	15.00	13.00	16.00	3.61
		TOTAL HPCDF	3.00	3.00	3.00	3.00	0.00
		OCDF	23.00	17.00	10.00	16.70	6.51
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	227.00	277.00	251.00	252.00	25.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	15.00	16.00	17.00	16.00	1.00
		2,3,4,7,8-PNCDF	60.00	66.00	53.00	59.70	6.51
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	22.00	20.00	19.00	20.30	1.53
		1,2,3,7,8,9-HXCDF	5.00	3.00	3.00	3.67	1.15
		1,2,3,6,7,8-HXCDF	3.00	3.00	3.00	3.00	0.00
		2,3,4,6,7,8-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,4,6,7,8-HPCDF	3.00	3.00	3.00	3.00	0.00

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TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
14	RADIAN EXT1	TOTAL TCDF	818.00	986.00	835.00	880.00	92.50
		TOTAL PNCDF	13100.00	9983.00	7333.00	10139.00	2887.00
		TOTAL HXCDF	9436.00	11187.00	8846.00	9823.00	1218.00
		TOTAL HPCDF	2010.00	2384.00	1609.00	2001.00	388.00
		OCDF	200.00	194.00	183.00	192.00	8.62
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	308.00	368.00	316.00	331.00	32.10
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	1439.00	1628.00	1156.00	1416.00	222.00
		2,3,4,7,8-PNCDF	1803.00	1659.00	1204.00	1555.00	313.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	4127.00	4580.00	3856.00	4221.00	420.00
		1,2,3,7,8,9-HXCDF	1168.00	1432.00	1107.00	1235.00	173.00
		1,2,3,6,7,8-HXCDF	822.00	912.00	789.00	841.00	63.70
		2,3,4,6,7,8-HXCDF	903.00	1068.00	871.00	947.00	106.00
		1,2,3,4,6,7,8-HPCDF	671.00	774.00	510.00	652.00	133.00
15	RADIAN EXT1	TOTAL TCDF	50.00	3.00	3.00	18.70	27.10
		TOTAL PNCDF	50.00	3.00	3.00	18.70	27.10
		TOTAL HXCDF	34.00	14.00	21.00	23.00	10.10
		TOTAL HPCDF	47.00	33.00	21.00	33.70	13.00
		OCDF	106.00	103.00	113.00	107.00	5.13
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	20.00	3.00	3.00	8.67	9.81
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	20.00	3.00	3.00	8.67	9.81
		2,3,4,7,8-PNCDF	20.00	3.00	3.00	8.67	9.81
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	50.00	17.00	24.00	30.30	17.40
		1,2,3,7,8,9-HXCDF	20.00	3.00	3.00	8.67	9.81
		1,2,3,6,7,8-HXCDF	20.00	3.00	3.00	8.67	9.81
		2,3,4,6,7,8-HXCDF	20.00	3.00	3.00	8.67	9.81
		1,2,3,4,6,7,8-HPCDF	30.00	21.00	21.00	24.00	5.20
16	RADIAN EXT1	TOTAL TCDF	3.00	3.00	3.00	3.00	0.00
		TOTAL PNCDF	3.00	3.00	3.00	3.00	0.00
		TOTAL HXCDF	21.00	9.00	11.00	13.70	6.43
		TOTAL HPCDF	50.00	11.00	24.00	28.30	19.90
		OCDF	131.00	44.00	107.00	94.00	44.90
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	3.00	3.00	3.00	3.00	0.00
		2,3,4,7,8-PNCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	24.00	12.00	14.00	16.70	6.43
		1,2,3,7,8,9-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,6,7,8-HXCDF	3.00	3.00	3.00	3.00	0.00
		2,3,4,6,7,8-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,4,6,7,8-HPCDF	18.00	5.00	11.00	11.30	6.51

TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
17	RADIAN EXT1	TOTAL TCDF	438.00	509.00	557.00	501.00	59.90
		TOTAL PNCDF	1280.00	1508.00	1197.00	1328.00	161.00
		TOTAL HXCDF	2242.00	2462.00	2049.00	2251.00	207.00
		TOTAL HPCDF	2277.00	2346.00	1758.00	2128.00	322.00
		OCDF	4857.00	5492.00	4295.00	4881.00	599.00
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	163.00	189.00	218.00	190.00	27.50
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	300.00	388.00	307.00	325.00	37.40
		2,3,4,7,8-PNCDF	207.00	245.00	158.00	203.00	44.70
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	1416.00	1547.00	1257.00	1407.00	145.00
		1,2,3,7,8,9-HXCDF	58.00	63.00	58.00	59.70	2.89
		1,2,3,6,7,8-HXCDF	62.00	57.00	53.00	57.30	4.51
		2,3,4,6,7,8-HXCDF	99.00	111.00	104.00	105.00	6.03
		1,2,3,4,6,7,8-HPCDF	1094.00	1145.00	857.00	1032.00	154.00
1	RADIAN EXT2	TOTAL TCDF	199.00	181.00	176.00	185.00	12.10
		TOTAL PNCDF	309.00	317.00	295.00	307.00	11.10
		TOTAL HXCDF	477.00	486.00	479.00	481.00	4.73
		TOTAL HPCDF	411.00	435.00	455.00	434.00	22.00
		OCDF	11.00	10.00	10.00	10.30	0.58
		TOTAL TCDD	25.00	26.00	24.00	25.00	1.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	25.00	26.00	24.00	25.00	1.00
		2,3,7,8 + 2,3,4,8-TCDF	199.00	181.00	176.00	185.00	12.10
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	142.00	136.00	134.00	137.00	4.16
		2,3,4,7,8-PNCDF	167.00	161.00	161.00	163.00	3.46
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	259.00	263.00	251.00	258.00	6.11
		1,2,3,7,8,9-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,6,7,8-HXCDF	47.00	47.00	52.00	48.70	2.89
		2,3,4,6,7,8-HXCDF	174.00	179.00	179.00	177.00	2.89
		1,2,3,4,6,7,8-HPCDF	411.00	435.00	455.00	434.00	22.00
2	RADIAN EXT2	TOTAL TCDF	62.00	60.00	57.00	59.70	2.52
		TOTAL PNCDF	320.00	305.00	306.00	310.00	8.39
		TOTAL HXCDF	197.00	197.00	194.00	196.00	1.73
		TOTAL HPCDF	577.00	598.00	628.00	600.00	25.80
		OCDF	126.00	122.00	118.00	123.00	5.03
		TOTAL TCDD	50.00	52.00	50.00	50.70	1.15
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	50.00	52.00	50.00	50.70	1.15
		2,3,7,8 + 2,3,4,8-TCDF	62.00	60.00	57.00	59.70	2.52
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	157.00	151.00	152.00	153.00	3.21
		2,3,4,7,8-PNCDF	163.00	154.00	154.00	157.00	5.20
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	6.00	6.00	6.00	6.00	0.00
		1,2,3,7,8,9-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,6,7,8-HXCDF	57.00	58.00	55.00	56.70	1.53
		2,3,4,6,7,8-HXCDF	140.00	139.00	139.00	139.00	0.58
		1,2,3,4,6,7,8-HPCDF	577.00	598.00	628.00	600.00	25.80

784055

GENP 011244

TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
3	RADIAN EXT2	TOTAL TCDF	1407.00	1538.00	1474.00	1473.00	65.50
		TOTAL PNCDF	1267.00	1398.00	1129.00	1265.00	135.00
		TOTAL HXCDF	1335.00	2400.00	2425.00	2053.00	622.00
		TOTAL HPCDF	2095.00	2253.00	2389.00	2246.00	147.00
		OCDF	3772.00	3806.00	3407.00	3662.00	221.00
		TOTAL TCDD	101.00	102.00	111.00	105.00	5.51
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	101.00	102.00	111.00	105.00	5.51
		2,3,7,8 + 2,3,4,8-TCDF	1150.00	1093.00	1068.00	1104.00	42.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	309.00	307.00	273.00	296.00	20.20
		2,3,4,7,8-PNCDF	279.00	272.00	252.00	268.00	14.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	1157.00	1195.00	1151.00	1168.00	23.90
		1,2,3,7,8,9-HXCDF	36.00	33.00	33.00	34.00	1.73
		1,2,3,6,7,8-HXCDF	274.00	280.00	324.00	293.00	27.30
		2,3,4,6,7,8-HXCDF	320.00	393.00	330.00	348.00	39.60
		1,2,3,4,6,7,8-HPCDF	751.00	787.00	836.00	791.00	42.70
11	RADIAN EXT2	TOTAL TCDF	183.00	179.00	146.00	169.00	20.30
		TOTAL PNCDF	2058.00	1999.00	2078.00	2045.00	41.10
		TOTAL HXCDF	7115.00	7158.00	6921.00	7065.00	126.00
		TOTAL HPCDF	11075.00	10990.00	11175.00	11080.00	92.60
		OCDF	10192.00	11292.00	9554.00	10346.00	879.00
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	38.00	38.00	38.00	38.00	0.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	1003.00	1000.00	1038.00	1014.00	21.10
		2,3,4,7,8-PNCDF	111.00	103.00	107.00	107.00	4.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	4672.00	4720.00	4748.00	4780.00	80.90
		1,2,3,7,8,9-HXCDF	278.00	255.00	263.00	265.00	11.70
		1,2,3,6,7,8-HXCDF	253.00	380.00	297.00	310.00	64.50
		2,3,4,6,7,8-HXCDF	209.00	193.00	199.00	200.00	8.08
		1,2,3,4,6,7,8-HPCDF	4165.00	4171.00	4218.00	4185.00	29.00
12	RADIAN EXT2	TOTAL TCDF	103.00	97.00	99.00	99.70	3.06
		TOTAL PNCDF	271.00	266.00	280.00	272.00	7.09
		TOTAL HXCDF	855.00	845.00	936.00	879.00	49.90
		TOTAL HPCDF	2339.00	2234.00	2483.00	2352.00	125.00
		OCDF	5429.00	5226.00	5193.00	5283.00	128.00
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	33.00	33.00	30.00	32.00	1.73
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	96.00	102.00	105.00	101.00	4.58
		2,3,4,7,8-PNCDF	35.00	37.00	35.00	35.70	1.15
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	528.00	530.00	534.00	531.00	3.06
		1,2,3,7,8,9-HXCDF	38.00	37.00	41.00	38.70	2.08
		1,2,3,6,7,8-HXCDF	46.00	45.00	41.00	44.70	3.51
		2,3,4,6,7,8-HXCDF	35.00	37.00	35.00	35.70	1.15
		1,2,3,4,6,7,8-HPCDF	672.00	634.00	708.00	671.00	37.00

784056

GENP 011245

TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
13	RADIAN EXT2	TOTAL TCDF	702.00	627.00	683.00	604.00	92.70
		TOTAL PNCDF	337.00	296.00	323.00	319.00	20.60
		TOTAL HXCDF	62.00	48.00	44.00	51.30	9.45
		TOTAL HPCDF	17.00	16.00	17.00	16.70	0.58
		OCDF	27.00	25.00	27.00	26.30	1.15
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	204.00	214.00	228.00	215.00	12.10
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	16.00	14.00	16.00	15.30	1.15
		2,3,4,7,8-PNCDF	57.00	56.00	58.00	57.00	1.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	25.00	24.00	25.00	24.70	0.58
		1,2,3,7,8,9-HXCDF	6.00	6.00	5.00	6.33	1.53
		1,2,3,6,7,8-HXCDF	6.00	6.00	5.00	5.67	0.58
		2,3,4,6,7,8-HXCDF	12.00	10.00	12.00	11.30	1.15
		1,2,3,4,6,7,8-HPCDF	7.00	8.00	8.00	7.67	0.58
14	RADIAN EXT2	TOTAL TCDF	845.00	851.00	901.00	866.00	30.70
		TOTAL PNCDF	9050.00	8561.00	9024.00	8678.00	275.00
		TOTAL HXCDF	9844.00	9961.00	8884.00	9563.00	591.00
		TOTAL HPCDF	2218.00	2273.00	1606.00	2032.00	370.00
		OCDF	174.00	186.00	218.00	193.00	22.70
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	350.00	336.00	341.00	342.00	7.09
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	1347.00	1337.00	977.00	1220.00	211.00
		2,3,4,7,8-PNCDF	1674.00	1632.00	1657.00	1654.00	21.10
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	4422.00	4427.00	3281.00	4043.00	660.00
		1,2,3,7,8,9-HXCDF	1244.00	1246.00	743.00	1078.00	290.00
		1,2,3,6,7,8-HXCDF	668.00	714.00	878.00	753.00	110.00
		2,3,4,6,7,8-HXCDF	979.00	992.00	592.00	854.00	227.00
		1,2,3,4,6,7,8-HPCDF	739.00	759.00	542.00	680.00	120.00
15	RADIAN EXT2	TOTAL TCDF	3.00	3.00	3.00	3.00	0.00
		TOTAL PNCDF	3.00	3.00	3.00	3.00	0.00
		TOTAL HXCDF	10.00	11.00	10.00	10.30	0.58
		TOTAL HPCDF	34.00	34.00	27.00	31.70	4.04
		OCDF	56.00	59.00	56.00	57.70	1.53
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	3.00	3.00	3.00	3.00	0.00
		2,3,4,7,8-PNCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	13.00	14.00	13.00	13.30	0.58
		1,2,3,7,8,9-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,6,7,8-HXCDF	3.00	3.00	3.00	3.00	0.00
		2,3,4,6,7,8-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,4,6,7,8-HPCDF	14.00	15.00	16.00	15.00	1.00

784057

GENP 011246

TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
16	RADIAN EXT2	TOTAL TCDF	3.00	3.00	3.00	3.00	0.00
		TOTAL PNCDF	3.00	3.00	3.00	3.00	0.00
		TOTAL HXCDF	3.00	3.00	3.00	3.00	0.00
		TOTAL HPCDF	3.00	3.00	3.00	3.00	0.00
		OCDF	22.00	26.00	37.00	28.30	7.77
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	11.00	13.00	12.00	12.00	1.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	3.00	3.00	3.00	3.00	0.00
		2,3,4,7,8-PNCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	6.00	6.00	6.00	6.00	0.00
		1,2,3,7,8,9-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,6,7,8-HXCDF	3.00	3.00	3.00	3.00	0.00
		2,3,4,6,7,8-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,4,6,7,8-HPCDF	3.00	3.00	3.00	3.00	0.00
17	RADIAN EXT2	TOTAL TCDF	462.00	383.00	419.00	421.00	39.60
		TOTAL PNCDF	1328.00	1116.00	1088.00	1177.00	131.00
		TOTAL HXCDF	2358.00	2302.00	2310.00	2323.00	30.30
		TOTAL HPCDF	2411.00	2609.00	2680.00	2567.00	139.00
		OCDF	414.00	385.00	386.00	398.00	14.30
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	164.00	164.00	166.00	171.00	11.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	316.00	218.00	266.00	267.00	49.00
		2,3,4,7,8-PNCDF	204.00	186.00	201.00	197.00	9.64
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	1384.00	1407.00	1397.00	1396.00	11.50
		1,2,3,7,8,9-HXCDF	66.00	69.00	67.00	67.30	1.53
		1,2,3,6,7,8-HXCDF	100.00	63.00	85.00	82.70	18.60
		2,3,4,6,7,8-HXCDF	110.00	107.00	110.00	109.00	1.73
		1,2,3,4,6,7,8-HPCDF	1186.00	1242.00	1283.00	1230.00	59.40
1	RADIAN EXT3	TOTAL TCDF	185.00	189.00	182.00	185.00	3.51
		TOTAL PNCDF	298.00	292.00	200.00	263.00	54.90
		TOTAL HXCDF	465.00	480.00	477.00	474.00	7.94
		TOTAL HPCDF	366.00	384.00	376.00	375.00	9.02
		OCDF	10.00	10.00	10.00	10.00	0.00
		TOTAL TCDD	24.00	22.00	23.00	23.00	1.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	24.00	22.00	23.00	23.00	1.00
		2,3,7,8 + 2,3,4,8-TCDF	185.00	189.00	182.00	185.00	3.51
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	140.00	139.00	141.00	140.00	1.00
		2,3,4,7,8-PNCDF	156.00	153.00	159.00	157.00	3.21
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	250.00	268.00	265.00	261.00	9.64
		1,2,3,7,8,9-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,6,7,8-HXCDF	47.00	41.00	41.00	43.00	3.46
		2,3,4,6,7,8-HXCDF	170.00	174.00	174.00	173.00	2.31
		1,2,3,4,6,7,8-HPCDF	366.00	384.00	376.00	375.00	9.02

GENP 011247

784058

TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
2	RADIAN EXT3	TOTAL TCDF	60.00	60.00	61.00	60.30	0.58
		TOTAL PNCDF	308.00	300.00	306.00	304.00	3.46
		TOTAL HXCDF	201.00	184.00	199.00	195.00	9.29
		TOTAL HPCDF	561.00	496.00	519.00	525.00	33.00
		OCDF	125.00	123.00	136.00	128.00	7.00
		TOTAL TCDD	50.00	51.00	48.00	49.70	1.53
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	50.00	51.00	48.00	49.70	1.53
		2,3,7,8 + 2,3,4,8-TCDF	60.00	60.00	61.00	60.30	0.58
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	156.00	154.00	157.00	156.00	1.53
		2,3,4,7,8-PNCDF	150.00	146.00	149.00	148.00	2.08
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	6.00	6.00	6.00	6.00	0.00
		1,2,3,7,8,9-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,6,7,8-HXCDF	64.00	55.00	61.00	60.00	4.58
		2,3,4,6,7,8-HXCDF	137.00	129.00	138.00	135.00	4.93
		1,2,3,4,6,7,8-HPCDF	561.00	496.00	519.00	525.00	33.00
3	RADIAN EXT3	TOTAL TCDF	1593.00	1575.00	1552.00	1573.00	20.80
		TOTAL PNCDF	1431.00	1311.00	1316.00	1353.00	67.90
		TOTAL HXCDF	2244.00	2171.00	2244.00	2220.00	42.10
		TOTAL HPCDF	2254.00	1976.00	2026.00	2085.00	148.00
		OCDF	4130.00	3996.00	4441.00	4189.00	226.00
		TOTAL TCDD	96.00	93.00	94.00	94.30	1.53
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	96.00	93.00	94.00	94.30	1.53
		2,3,7,8 + 2,3,4,8-TCDF	1154.00	1121.00	1171.00	1149.00	25 40
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	333.00	331.00	311.00	325.00	12.20
		2,3,4,7,8-PNCDF	296.00	282.00	282.00	287.00	8.08
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	1214.00	1144.00	1186.00	1181.00	35.20
		1,2,3,7,8,9-HXCDF	39.00	38.00	38.00	38.30	0.58
		1,2,3,6,7,8-HXCDF	292.00	304.00	298.00	298.00	6.00
		2,3,4,6,7,8-HXCDF	317.00	305.00	315.00	312.00	6.43
		1,2,3,4,6,7,8-HPCDF	788.00	704.00	729.00	740.00	42.00
11	RADIAN EXT3	TOTAL TCDF	156.00	173.00	175.00	168.00	10.40
		TOTAL PNCDF	2080.00	2087.00	2084.00	2084.00	3.51
		TOTAL HXCDF	7276.00	7041.00	7058.00	7125.00	131.00
		TOTAL HPCDF	11581.00	10545.00	10751.00	10959.00	548.00
		OCDF	28338.00	29446.00	26629.00	28138.00	1420.00
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	38.00	40.00	40.00	39.30	1.15
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	998.00	1014.00	1043.00	1018.00	22.80
		2,3,4,7,8-PNCDF	110.00	112.00	116.00	113.00	3.06
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	5013.00	4662.00	4674.00	4916.00	83.90
		1,2,3,7,8,9-HXCDF	274.00	261.00	271.00	269.00	6.81
		1,2,3,6,7,8-HXCDF	310.00	303.00	303.00	305.00	4.04
		2,3,4,6,7,8-HXCDF	199.00	200.00	201.00	200.00	1.00
		1,2,3,4,6,7,8-HPCDF	4347.00	4015.00	4153.00	4172.00	167.00

784059

GENP 011248

TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
12	RADIAN EXT3	TOTAL TCDF	105.00	89.00	128.00	107.00	19.60
		TOTAL PNCDF	322.00	310.00	309.00	314.00	7.23
		TOTAL HXCDF	660.00	921.00	834.00	872.00	44.70
		TOTAL HPCDF	2244.00	2344.00	2154.00	2247.00	95.00
		OCDF	5784.00	5959.00	5496.00	5740.00	232.00
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	32.00	31.00	41.00	34.70	5.51
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	103.00	106.00	100.00	103.00	3.00
		2,3,4,7,8-PNCDF	37.00	40.00	41.00	39.30	2.08
		1,2,3,4,7,8 + 1,2,3,4,7,8-HXCDF	530.00	581.00	548.00	553.00	25.90
		1,2,3,7,8,9-HXCDF	37.00	38.00	37.00	37.30	0.58
		1,2,3,6,7,8-HXCDF	34.00	34.00	20.00	29.30	8.08
		2,3,4,6,7,8-HXCDF	37.00	41.00	38.00	38.70	2.08
		1,2,3,4,6,7,8-HPCDF	656.00	678.00	615.00	650.00	32.00
13	RADIAN EXT3	TOTAL TCDF	790.00	997.00	831.00	873.00	110.00
		TOTAL PNCDF	307.00	324.00	359.00	330.00	26.50
		TOTAL HXCDF	46.00	31.00	54.00	44.30	11.90
		TOTAL HPCDF	3.00	3.00	10.00	5.33	4.04
		OCDF	26.00	28.00	28.00	27.30	1.15
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	271.00	271.00	261.00	268.00	5.77
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	20.00	20.00	20.00	20.00	0.00
		2,3,4,7,8-PNCDF	59.00	61.00	62.00	60.70	1.53
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	23.00	24.00	27.00	24.70	2.08
		1,2,3,7,8,9-HXCDF	4.00	5.00	3.00	4.00	1.00
		1,2,3,6,7,8-HXCDF	4.00	3.00	3.00	3.33	0.58
		2,3,4,6,7,8-HXCDF	11.00	8.00	13.00	10.70	2.52
		1,2,3,4,6,7,8-HPCDF	3.00	3.00	10.00	5.33	4.04
14	RADIAN EXT3	TOTAL TCDF	915.00	879.00	904.00	899.00	18.40
		TOTAL PNCDF	9159.00	9232.00	9010.00	9134.00	113.00
		TOTAL HXCDF	9817.00	9905.00	9713.00	9812.00	96.10
		TOTAL HPCDF	2131.00	2136.00	2063.00	2110.00	40.80
		OCDF	197.00	196.00	260.00	218.00	36.70
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	345.00	337.00	341.00	341.00	4.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	952.00	956.00	1051.00	986.00	56.00
		2,3,4,7,8-PNCDF	1712.00	1766.00	1706.00	1728.00	33.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	4385.00	4496.00	4200.00	4354.00	148.00
		1,2,3,7,8,9-HXCDF	1222.00	1218.00	1193.00	1211.00	15.70
		1,2,3,6,7,8-HXCDF	662.00	599.00	814.00	692.00	111.00
		2,3,4,6,7,8-HXCDF	976.00	972.00	940.00	963.00	19.70
		1,2,3,4,6,7,8-HPCDF	707.00	705.00	691.00	701.00	8.72

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TABLE B-1 (cont'd.)

Sample	Lab	Compound	Det. 1	Det. 2	Det. 3	Mean	Std. Dev.
15	RADIAN EXT3	TOTAL TCDF	3.00	3.00	3.00	3.00	0.00
		TOTAL PNCDF	3.00	3.00	3.00	3.00	0.00
		TOTAL HXCDF	28.00	20.00	18.00	22.00	5.29
		TOTAL HPCDF	45.00	51.00	55.00	50.30	5.03
		OCDF	92.00	100.00	100.00	97.30	4.62
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	3.00	3.00	3.00	3.00	0.00
		2,3,4,7,8-PNCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	31.00	19.00	21.00	23.70	6.43
		1,2,3,7,8,9-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,6,7,8-HXCDF	3.00	3.00	3.00	3.00	0.00
		2,3,4,6,7,8-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,4,6,7,8-HPCDF	22.00	24.00	27.00	24.30	2.52
16	RADIAN EXT3	TOTAL TCDF	3.00	7.00	3.00	4.33	2.31
		TOTAL PNCDF	3.00	3.00	3.00	3.00	0.00
		TOTAL HXCDF	8.00	3.00	3.00	4.67	2.89
		TOTAL HPCDF	3.00	3.00	3.00	3.00	0.00
		OCDF	10.00	10.00	10.00	10.00	0.00
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	3.00	7.00	3.00	4.33	2.31
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	3.00	3.00	3.00	3.00	0.00
		2,3,4,7,8-PNCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	11.00	8.00	8.00	9.00	1.73
		1,2,3,7,8,9-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,6,7,8-HXCDF	3.00	3.00	3.00	3.00	0 00
		2,3,4,6,7,8-HXCDF	3.00	3.00	3.00	3.00	0.00
		1,2,3,4,6,7,8-HPCDF	3.00	3.00	3.00	3.00	0.00
17	RADIAN EXT3	TOTAL TCDF	513.00	512.00	518.00	514.00	3.21
		TOTAL PNCDF	1263.00	1119.00	1334.00	1239.00	110.00
		TOTAL HXCDF	2360.00	2374.00	2360.00	2365.00	8.08
		TOTAL HPCDF	2412.00	2362.00	2365.00	2380.00	28.00
		OCDF	4412.00	4354.00	4236.00	4334.00	89.70
		TOTAL TCDD	3.00	3.00	3.00	3.00	0.00
		OCDD	10.00	10.00	10.00	10.00	0.00
		2,3,7,8-TCDD	3.00	3.00	3.00	3.00	0.00
		2,3,7,8 + 2,3,4,8-TCDF	182.00	184.00	186.00	184.00	2.00
		1,2,3,7,8 + 1,2,3,4,8-PNCDF	315.00	313.00	339.00	322.00	14.50
		2,3,4,7,8-PNCDF	187.00	182.00	200.00	190.00	9.29
		1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	1406.00	1432.00	1424.00	1421.00	13.30
		1,2,3,7,8,9-HXCDF	64.00	68.00	73.00	68.30	4.51
		1,2,3,6,7,8-HXCDF	70.00	75.00	63.00	69.30	6.03
		2,3,4,6,7,8-HXCDF	109.00	103.00	115.00	109.00	6.00
		1,2,3,4,6,7,8-HPCDF	1173.00	1143.00	1141.00	1152.00	17.90

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APPENDIX C

PROJECT SUMMARY

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PROJECT SUMMARY

INTRODUCTION

During the past four years Electric Power Research Institute (EPRI) has sponsored a research program to evaluate methodology for the analysis of selected individual congeners of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in dielectric fluids. This program included the synthesis of native and isotopically labeled PCDD and PCDF analytical standards (1). Analytical standards and spiked matrix samples were prepared by a contract laboratory and then distributed to four other participating laboratories as part of a round robin study. These samples were analyzed blind by five independent contract laboratories.

The study was conducted in two phases. Phase I involved the analysis of PCDF's and PCDD's in five spiked baseline samples; for example, Aroclor-1016, Aroclor-1242, Aroclor-1260, tri- and tetrachlorobenzene mixture, aged mineral oil, and four in-service dielectric fluids (1-4). Since validated analytical methods did not exist before this program was initiated, this phase of the program was necessary to assess any deficiencies and optimize the analytical methodology. Each participating laboratory used their best available in-house extraction and analysis techniques (1-4). After evaluating these results and implementing several improvements to the methodology, then Phase II was initiated.

Phase II was designed to provide quantitative data on PCDF and PCDD congeners in selected in-service dielectric fluids and to make a statistical interlaboratory comparison of the analytical methods. The specific aims were (1) to determine the accuracy of analysis by each participating laboratory for a selected list of PCDF's and PCDD's in spiked samples, (2) to determine the precision of analysis by each laboratory for PCDF's and PCDD's in spiked and in-service utility samples, (3) to perform an interlaboratory comparison and thus a comparison of methods employed for accuracy and precision, and finally (4) to measure the levels of PCDF's and PCDD's in a few selected in-service utility samples.

METHODS

Five independent laboratories participated in the interlaboratory study. Each laboratory was provided with aliquots of the three baseline and seven in-service utility samples (4). Also, native and isotopically labeled PCDD and PCDF analytical standards were prepared by one laboratory (4) and distributed to each participating laboratory. Subsequently, each laboratory employed their best

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available in-house extraction and analysis techniques (Table 1). Each laboratory added five carbon-13 labeled PCDD or PCDF congeners as internal standards to the sample. Some laboratories employed a liquid-liquid extraction step, either hexane/acetone or hexane/sulfuric acid/water, whereas others applied aliquots of the sample directly to a chromatographic column. All laboratories performed a purification step using open column chromatography (Table 1). For example, Lab-A used acid alumina, PX-21 carbon and neutral alumina chromatography, respectively, for purification; Lab-B used LH20, basic alumina and acidic alumina; and Lab-C used a combination of sulfuric acid silica/silica sodium hydroxide followed by basic alumina and florisil. Lab-D used a sandwich of acid silica, basic silica and silica followed by PX-21 carbon, acid silica/silica and then acidic alumina; Lab-E used sulfuric acid impregnated silica, PX-21 carbon and florisil. Various solvent evaporation techniques were also used. Specifically, Lab-A used heat and vacuum; Lab-B used rotary evaporation, while Labs-C and -D used nitrogen blowdown.

During extract analysis, Lab-A used both high and low resolution MS, in the electron impact mode. Lab-B, D and E used only low resolution mass spectrometry while Lab-C used exclusively high resolution mass spectrometry. All laboratories employed high resolution gas chromatography (HRGC), although different stationary phases and GC operating parameters were used.

Calibration curves were generated over the linear response range of each gas chromatograph/mass spectrometry system at five concentrations. All labs used the same initial stock calibration solution prepared by one laboratory containing eight native and five isotopically labeled PCDD's and PCDF's (1).

The samples analyzed in the round robin study are shown in Table 2. Two samples were spiked with a selected number of congeners at known concentrations into mineral oil and Aroclor 1016, respectively. A third sample, Aroclor 1260, was spiked at known levels with these same congeners but it also contained endogenous PCDF's and PCDD's. Samples 11-17 (Table 2) were in-service utility samples that were selected to represent the various types of utility uses (5). In some cases the dielectric was Arochlor 1260, or mineral oil containing PCBs. Dielectric fluids from transformers, capacitors, or precipitators were selected. Some samples represented devices that had been in use for many years (Table 2).

A large database was produced in this study. An example of the measurements that were performed for dioxins and furans by Lab-B on sample No. 14 is shown in Table 3. All of the five participating laboratories reported either measurable values or limits of detection for each of these compounds in each of the ten samples. With the exception of one laboratory, each laboratory performed triplicate instrumental analysis on a single extract from each sample. The mean and standard deviation were calculated (Table 3). Lab-E reported only one

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TABLE 1. ANALYTICAL PROCEDURES EMPLOYED BY ROUND ROBIN LABS

Step	Lab-A	Lab-B	Lab-C
1. Add standards	+	+	+
2. Extraction	• Hex/acetone	-	• Hex/H_2SO_4; H_2O
3. Chromatography	• Acid alumina • PX-21 carbon • Neutral alumina	• LH-20 • Basic alumina • Acidic alumina	• Silica/H_2SO_4-silica/ NaOH-silica • Basic alumina (2Xs) • Florisil
4. Solvent evaporation	• Δ and vacuum	• Roto-evaporation	• N_2 blow-down
5. Analysis	• HRGC/LRMS (EI) • HRGC/HRMS (EI)	• HRGC/LRMS (EI)	• HRGC/HRMS (EI)

Step	Lab-D	Lab-E
1. Add standards	+	+
2. Extraction	-	-
3. Chromatography	• Acid silica/basic silica/silica • PX-21 carbon • Acid silica/silica • Acid alumina	• H_2SO_4-silica • PX-21 carbon • Florisil
4. Solvent evaporation	• N_2 blow-down	
5. Analysis	• HRGC/LRMS (EI)	• HRGC/LRMS (EI)

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TABLE 2. SAMPLES ANALYZED IN ROUND ROBIN STUDY

Sample No.	Dielectric	Utility Appliance	Service Life (yr)
1	Spiked mineral oil	-	-
2	Spiked Aroclor 1016	-	-
3	Spiked Aroclor 1260	-	-
11	Aroclor 1260	Transformer (LCN)[a]	31
12	Aroclor 1260	Transformer (LCN)	28
13	?	Capacitor	?
14	?	Precipitator	27
15	Mineral oil (150 ppm PCB)	Transformer (Arc furnace)	8
16	Mineral oil (100 ppm PCB)	Transformer (LCN)	?
17	Aroclor 1260	Transformer (LCN)	20

[a]LCN = load circuit network.

TABLE 3. EXAMPLE OF MEASUREMENTS PERFORMED FOR DIOXINS/FURANS BY A LAB ON ONE SAMPLE (LAB B, SAMPLE 14)- PPB

Compound	Measurement 1	2	3	Mean	S.D.
2,3,7,8-TCDD	(0.5)[a]	(0.5)	(0.5)	(0.5)	(0.0)
Total TCDD	(0.5)	(0.5)	(0.5)	(0.5)	(0.0)
OCDD	8	13	5	8.7	4.0
2,3,7,8 + 2,3,4,8-TCDF	297	248	282	276	25
Total TCDF	970	617	576	721	217
1,2,3,7,8 + 1,2,3,4,8-PNCDF	1115	1039	1023	1059	492
2,3,4,7,8-PNCDF	1171	1584	1726	1694	98
Total PNCDF	9236	6589	6002	7276	1723
1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	3802	3711	3775	3763	47
1,2,3,7,8,9-HXCDF	174	187	233	198	31
1,2,3,6,7,8-HXCDF	1195	1155	1137	1162	30
2,3,4,6,7,8-HXCDF	387	472	509	456	63
Total HXCDF	10,598	9918	10,656	10,394	414.0
1,2,3,4,6,7,8-HPCDF	728	905	884	839	97
Total HPCDF	1725	2196	2254	2058	290
OCDF	293	626	374	431	174

[a]() = Limit of detection.

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measurement on each sample extract. Lab-D, on the otherhand, extracted each sample three times and then performed three instrumental determinations on each of the extracts, thus producing nine measurements per sample.

RESULTS AND DISCUSSION

Accuracy of Measurement

The accuracy of measurement for specific selected congeners, for each laboratory and by sample matrix was examined. To gain insight into measurement accuracy, samples were spiked with known amounts of each congener and the percent accuracy of the mean of triplicate measurements was calculated and expressed as the mean divided by the spiked amount times one hundred.

Table 4 shows an example of these data for just one chemical, 2,3,7,8-TCDD. These statistical data were obtained for each compound measured. The percent accuracy for spiked samples for 2,3,7,8-TCDD by sample and laboratory is shown. Lab-D performed triplicate extraction/purification on each of the samples which also provided an estimate of precision for the entire analytical procedure. The mean value of the accuracy for 2,3,7,8-TCDD is given in Table 4 and the range for all determinations is shown in parenthesis. For these two samples the percent accuracy is in general very good. Lab-E only performed a single determination and experienced a lower percent accuracy than the other four laboratories.

Accuracy of analysis for 2,3,4,6,7,8-hexachlorodibenzofuran congener is shown in Table 5. The percent accuracy of measurement for this PCDF, by laboratory and sample is given in Table 5. The accuracy of determination was quite good for four of the five laboratories. Lab-C reported more variable recoveries with a range of 80-3371 in sample No. 1. Data such as these were obtained for each congener to determine the percent accuracy in two samples. With the exception noted above, acceptable accuracies were obtained.

A third sample also was spiked at known concentrations with these congeners, but there were endogenous levels present in the Aroclor 1260. Thus, the percent accuracy could not be calculated.

The median and range of percent accuracy across all the chemicals that were spiked into the two samples is summarized in Table 6. As indicated, the accuracy is reasonable in most cases with a few exceptions where the range was quite large. The determination of the hexa CDF congeners by Lab-C yielded this large overall variability.

Precision of Measurement

Similarly to accuracy, the precision of measurement by congener, laboratory and sample matrix was also investigated. The coefficients of variation for the determination of 1,2,3,7,8- plus 1,2,3,4,8-pentachlorodibenzofuran (not chromatographically resolved) by compound and laboratory are given in Table 7. In

TABLE 4. PERCENT ACCURACY FOR SPIKED SAMPLES FOR 2,3,7,8-TCDD BY SAMPLE AND LABORATORY

Laboratory	Sample 1	Sample 2
A	75 (68-85)	91 (75-103)
B	83 (68-96)	101 (100-104)
C	81 (76-92)	85 (83-87)
D-1	99 (96-100)	96 (92-98)
D-2	100 (96-104)	96 (94-98)
D-3	92 (88-96)	94 (91-96)
E	56[a]	72[a]

[a]Single determination.

TABLE 5. PERCENT ACCURACY FOR SPIKED SAMPLES FOR 2,3,4,6,7,8-HXCDF BY SAMPLE AND LABORATORY

Laboratory	Sample 1	Sample 2
A	92 (86-97)	114 (106-123)
B	94 (93-96)	123 (119-129)
C	1189 (80-3371)	2015 (515-3939)
D-1	98 (97-101)	100 (100-101)
D-2	101 (99-102)	106 (105-106)
D-3	99 (97-99)	102 (98-104)
E	97[a]	83

[a]Single determination.

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TABLE 6. MEDIAN AND RANGE OF PERCENT ACCURACY ACROSS ALL COMPOUNDS FOR SPIKED SAMPLES

Laboratory	Sample 1	Sample 2
A	88 (68-105)	107 (75-135)
B	96 (68-124)	112 (55-149)
C	94 (8-3371)	120 (12-3939)
D-1	99 (81-114)	101 (90-121)
D-2	101 (88-114)	105 (89-119)
D-3	94 (67-107)	99 (91-121)
E	103 (56-116)	114 (42-120)

TABLE 7. COEFFICIENTS OF VARIATION FOR 1,2,3,7,8 + 1,2,3,4,8-PNCDF BY COMPOUND AND LABORATORY

	Laboratory No.					
Sample	A	B	C	D-1	D-2	D-3
1	12.0	2.1	11.5	1.4	3.0	0.7
2	11.5	1.8	3.1	1.3	2.1	1.0
3	0.9	4.1	13.6	4.0	6.8	3.7
11	12.9	6.3	31.6	10.4	2.1	2.2
12	26.1	4.8	23.1	16.6	4.5	2.9
13	8.4	7.1	12.8	6.3	7.5	0.0
14	3.8	4.6	7.3	15.6	17.3	5.7
15	11.7	-	-	-	-	-
16	-	61.1	-	-	-	-
17	4.0	14.9	2.2	11.5	18.4	4.5

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general across all the samples, the coefficients of variation (i.e., precision) were quite low. Lab-D performed triplicate extractions on each baseline and utility sample and then performed triplicate instrumental determinations on each extract. Good precision for both extraction and measurement were observed. Another example is shown in Table 8 for 1,2,3,4,6,7,8-heptachlorodibenzofuran by compound and laboratory. For this particular congener, a greater variability was observed amongst Labs-B and C.

The median and range of coefficients of variation across all compounds by laboratory and samples are given in Table 9. A small range in the coefficients of variation was observed except for Lab-C which had relatively poor precision.

Components Contributing to Variability

A more detailed statistical analysis was performed to determine the contribution of instrumental measurement, method extraction/purification, and sample type to the total variability of a reported value for a PCDD or PCDF congener. The variance components for a set of measurements made on 2,3,7,8-TCDD across all except two samples, samples 15 and 16 (most data were non-measurable values), are listed in Table 10. The total variance and the percentage of the total variance apportioned to either the sample or the instrumental measurement step are shown. The greatest variance is attributed to the sample type as compared to the instrumental measurement step. Lab-A, as compared to the other laboratories, exhibited a higher percentage of variability for the measurement step (14.6%). The remaining three laboratories exhibited the major variability (99+%) from the sample type.

Table 11 gives the variance components for 2,3,4,5,6,7,8-hexachlorodibenzofuran across all sample types analyzed. Again, the total variance and the percent of the total variance for each of the laboratories are given for the sample and the measurement step. Except for Lab-C, the predominant variance is associated with the sample type. For Lab-C, 30% of the variability in these results was associated with the instrumental measurement step.

The variance components by congener for Lab-D are depicted in Table 12. Lab-D performed triplicate extraction on the utility sample and triplicate instrumental determinations on each sample extract. Thus, it was possible to apportion the variability due to sample type, extraction, and measurement. The largest contribution to variation in the reported values was associated with the types of sample or sample matrix analyzed. Variability resulting from the extraction steps was smaller while an insignificant variation was attributed to the instrumental determination step.

The measured values between each lab were tested to determine whether they were significantly different from one to another. The Levene's test of

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TABLE 8. COEFFICIENTS OF VARIATION FOR 1,2,3,4,6,7,8-HPCDF BY COMPOUND AND LABORATORY

Sample	Laboratory No.					
	A	B	C	D-1	D-2	D-3
1	4.0	10.9	96.2	9.7	5.1	2.4
2	11.7	20.4	76.1	3.2	4.3	6.3
3	4.1	12.1	144.9	1.4	5.4	5.7
11	10.3	17.8	58.9	17.6	0.7	4.0
12	9.9	18.7	36.9	14.9	5.5	4.9
13	21.1	31.6	60.9	-	7.5	-
14	1.6	11.5	39.9	20.4	17.6	1.2
15	-	60.6	-	21.7	6.7	10.3
16	-	53.4	-	57.4	-	-
17	3.4	23.9	29.2	14.9	4.8	1.6

TABLE 9. MEDIAN AND RANGE OF COEFFICIENTS OF VARIATION ACROSS ALL COMPOUNDS BY LABORATORY AND SAMPLE

Laboratory	Sample							
	1	2	3	11	12	13	14	17
A	11.8 (4-16)	11.2 (3-40)	3.3 (1-5)	8.6 (1-47)	19.2 (2-59)	15.5 (3-33)	2.9 (1-57)	4.3 (2-20)
B	4.2 (0-17)	2.2 (1-46)	4.6 (1-30)	14.1 (6-46)	6.6 (4-20)	17.6 (4-39)	12.6 (1-47)	19.2 (2-28)
C	41.5 (11-159)	13.9 (2-87)	29.8 (8-145)	48.9 (9-121)	23.9 (5-57)	31.2 (10-62)	30.5 (7-125)	22.2 (2-53)
D-1	4.8 (1-27)	2.4 (0-5)	2.7 (1-12)	13.6 (7-32)	12.2 (7-22)	11.1 (6-39)	12.4 (4-28)	11.9 (5-22)
D-2	4.0 (1-7)	2.7 (0.4)	5.4 (2-30)	2.1 (0-21)	4.5 (1-8)	7.5 (2-24)	14.6 (1-27)	4.9 (1-23)
D-3	2.4 (1-21)	3.1 (1-8)	2.2 (1-7)	2.5 (0-6)	4.9 (2-28)	8.2 (0-27)	1.9 (1-17)	2.1 (0.9)

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TABLE 12. VARIANCE COMPONENTS BY COMPOUND FOR LAB-D

Compound	Variance Components				% of Total		
	Total	Sample	Extraction	Meas.	Sample	Extraction	Meas.
2,3,7,8-TCDD	2.25	2.25	0.00	0.00	99.9+	0.0	0.0
Total TCDD	2.25	2.25	0.00	0.00	99.9+	0.0	0.0
OCDD	0.00	0.00	0.00	0.00	99.9	0.0	0.0
2,3,7,8 + 2,3,4,6-TCDF	1.48	1.47	0.00	0.00	99.5	0.2	0.3
Total TCDF	1.40	1.38	0.01	0.01	98.9	0.6	0.5
1,2,3,7,8 + 1,2,3,4,8-PNCDF	1.84	1.82	0.01	0.01	99.2	0.4	0.4
2,3,4,7,8-PNCDF	1.31	1.30	0.00	0.01	98.9	0.0	1.1
Total PNCDF	1.62	1.61	0.00	0.01	99.2	0.0	0.8
1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	5.80	5.80	0.00	0.00	99.9	0.0	0.1
1,2,3,7,8,9-HXCDF	4.73	4.71	0.01	0.01	99.5	0.2	0.3
1,2,3,6,7,8-HXCDF	2.25	2.16	0.08	0.01	96.1	3.4	0.5
2,3,4,6,7,8-HXCDF	2.25	2.16	0.08	0.01	96.1	3.4	0.5
Total HXCDF	3.66	3.59	0.05	0.01	98.3	1.3	0.4
1,2,3,4,6,7,8-HPCDF	3.92	3.87	0.02	0.03	98.7	0.5	0.7
Total HPCDF	5.13	5.00	0.09	0.03	97.6	1.8	0.6
OCDF	7.73	7.41	0.30	0.03	95.9	3.9	0.2

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TABLE 10. VARIANCE COMPONENTS FOR 2,3,7,8-TCDD ACROSS ALL SAMPLES (1,2,3,11,12,13,14,17)

	Variance Components			% of Total	
Laboratory	Total	Sample	Measurement	Sample	Measurement
A	8.05	6.87	1.18	85.4	14.6
B	5.46	5.45	0.00	99.9	0.1
C	2.12	2.12	0.00	99.8	0.2
D-1	2.25	2.25	0.00	99.9+	0.0
D-2	2.30	2.30	0.00	99.9+	0.0
D-3	2.19	2.19	0.00	99.9+	0.0
E	7.52	-	-	-	-

TABLE 11. VARIANCE COMPONENTS FOR 2,3,4,6,7,8-HXCDF ACROSS ALL SAMPLES (1,2,3,11,12,13,14,17)

	Variance Components			% of Total	
Laboratory	Total	Sample	Measurement	Sample	Measurement
A	3.27	3.25	0.01	99.5	0.5
B	2.62	2.59	0.03	99.0	1.0
C	4.53	3.15	1.38	69.6	30.4
D-1	3.11	3.10	0.01	99.6	0.4
D-2	1.77	1.76	0.01	99.2	0.8
D-3	1.85	1.84	0.01	99.5	0.5
E	4.27	-	-	-	-

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differences amongst the laboratories by compound across the samples was applied to the data (6). For available data, the F value for these measurements and the degrees of freedom were calculated and are shown in Table 13. In every case, the values reported by each of the laboratories were significantly different from one to another at the 95% confidence level for each of the congeners except 1,2,3,6,7,8-hexachlorodibenzofuran, which was significantly different at the 99% confidence level. This test indicates that each laboratory reported statistically different quantitative values for each of these congeners in these samples.

Prediction of Variability in Future Measurements

In practical terms, what would be the predicted range of values if the analyses were repeated by a laboratory using one of the methods previously employed. Shown in Table 14 are the approximate 95% prediction intervals for the laboratories if they were to repeat the analysis for each of these chemicals in the same utility samples. In other words, if one of the five laboratories were to repeat the analysis for 2,3,7,8-TCDD in sample No. 1, the reported value would fall within a range of 15-30 ppb. If 20 determinations were made, one value would be outside of this range. For 2,3,7,8-TCDD a factor of two variation would be anticipated. In some cases, the variability for other congeners may be as much as 20-fold or more. This variability does not appear to correlate to the congener concentrations actually present in the samples. For example, even though, in samples 11-17 the amount of 2,3,7,8-TCDD is quite low and the variability may be as much as a factor of 20-30 as indicated here, the reported values for 1,2,3,7,8,9-hexachlorodibenzofuran could vary over the range of 1-8869. In samples 11-17, a large variability in levels for the individual isomers are predicted as well as for the total, for example, the total hexachlorodibenzofuran.

TABLE 13. LEVENE'S TEST OF DIFFERENCES AMONG LABS BY COMPOUND ACROSS SAMPLES

Compound	F-Value	Degrees of Freedom
2,3,7,8-TCDD	14.96**	5
Total TCDD	17.82**	5
OCDD	12.81**	5
2,3,7,8 + 2,3,4,8-TCDF	6.60**	5
Total TCDF	8.89**	5
1,2,3,7,8 + 1,2,3,4,8-PNCDF	4.39**	5
2,3,4,7,8-PNCDF	9.76**	5
Total PNCDF	11.15**	5
1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	17.80**	5
1,2,3,7,8,9-HXCDF	16.10**	5
1,2,3,6,78-HXCDF	2.96*	4
2,3,4,6,7,8-HXCDF	36.93**	5
Total HXCDF	23.12**	5
1,2,3,4,6,7,8-HPCDF	22.80**	5
Total HPCDF	17.39**	5
OCDF	6.97**	5

*Significant at 0.01 level (99%).
**Significant at 0.05 level (95%).

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TABLE 14. APPROXIMATE 95 PERCENT PREDICTION INTERVALS
(PPB)

Compound	1	2	3	11	12	13	14	17
2,3,7,8-TCDD	15- 30	40- 60	63- 132	1- 25	1- 20	1- 13	1- 32	1- 11
OCDD	1- 59	1- 40	2- 32	7- 19	5- 30	1- 70	1- 43	5- 32
2,3,7,8 + 2,3,4,8-TCDF	144- 221	57- 67	977- 1355	24- 61	15- 81	90- 459	251- 459	119- 241
1,2,3,7,8 + 1,2,3,4,8-PNCDF	123- 168	138- 193	226- 425	41- 7913	27- 264	8- 30	742- 1577	74- 746
2,3,4,7,8-PNCDF	99- 248	90- 321	164- 618	84- 145	28- 59	46- 80	1324- 2389	106- 403
1,2,3,4,7,8 + 1,2,3,4,7,9-HXCDF	182- 347	1- 15	825- 1838	2138- 12.579	447- 809	13- 36	3144- 5860	486- 5023
1,2,3,7,8,9-HXCDF	1- 34	1- 25	2- 965	1- 8869	4- 167	1- 61	64- 7800	1- 6136
1,2,3,6,7-HXCDF	31- 72	45- 72	254- 412	218- 603	20- 97	20- 10	561- 1385	43- 131
2,3,4,6,7,8-HXCDF	40- 1041	24- 1912	55- 3975	26- 879	9- 68	1- 51	196- 2882	10- 2237
1,2,3,4,6,7,8-HPCDF	103- 1104	137- 1530	96- 3,258	580- 16,405	418- 976	1- 17	482- 1130	79- 5652
OCDF	2- 89	67- 208	2280- 6436	9641- 64,743	3822- 10,453	7- 75	96- 457	442- 24,465
Total TCDF	144- 222	57- 67	1046 2321	57- 362	37- 321	389- 1247	532 1389	249 729
Total PNCDF	206- 431	220- 517	1004- 1682	1125- 3079	143- 590	179- 442	5846- 13,471	562- 1,999
Total HXCDF	155- 1862	40- 2118	798- 10,654	3041- 21,535	726- 1320	11- 198	5897- 21,657	610- 13,977
Total HPCDF	103- 1105	137- 1532	819- 4341	1581- 41,116	1467- 3090	2- 52	1391- 3483	242- 11,063
Total TCDD	6- 60	40- 60	63- 132	1- 21	1- 14	1- 15	1- 20	1- 10

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W. Ligon
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SOURCES OF DIOXINS IN THE ENVIRONMENT: A STUDY OF PCDDs AND PCDFs IN ANCIENT, FROZEN ESKIMO TISSUE

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ABSTRACT

According to the trace chemistries of fire theory, small amounts of polychlorodibenzodioxins (PCDDs), including the most toxic 2,3,7,8-tetrachlorodibenzodioxin, and polychlorodibenzofurans (PCDFs) are generated from combustion of natural products. The implication is that PCDDs and PCDFs have existed in nature prior to the large scale manufacture of chemicals that has occurred during the past few decades. However, on the basis of Hites' et al. studies of lake sediment, it has been concluded that nearly all PCDDs and PCDFs are either synthetic in origin, or arise from the incomplete combustion of synthetic chemicals. In the present work, few if any PCDDs and PCDFs were found in human tissue samples and other samples that have been preserved for greater than 400 years. The samples were from a Barrow, Alaska, Inupiat Eskimo household that was destroyed by an ice overflow, trapping two women. The analytical chemistry evidence is consistent with the Czuczwa and Hites' conclusion that the majority of PCDD/Fs in the environment today are of anthropogenic origin.

INTRODUCTION

Polychlorodibenzodioxins (PCDDs) and polychlorodibenzofurans are highly toxic chemicals which are persistent in the environment. Relatively recently, it was proposed that the PCDDs and PCDFs including the highly toxic 2,3,7,8-TCDD are produced in small or trace amounts by combustion of natural,

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A Competitive Binding Assay for 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin and Related Ligands of the *Ah* Receptor

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SUMMARY

A sensitive competitive binding assay for the detection of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other ligands of the *Ah* receptor was developed using a stable preparation of the *Ah* receptor, the 40–55% ammonium sulfate fraction of liver cytosol from C57BL/6J mice, and the radioligand [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin (specific radioactivity, 2176 Ci/mmol, and binding affinity, K_D = 6.5 pM). Conditions are described which maximize assay precision and sensitivity, while minimizing sample counting time, ensuring ligand solubility, and permitting attainment of binding equilibrium for competing ligands. Assay conditions were developed to allow calculation of the binding affinity for competing ligands and to ensure that an unknown competitor could be quantified in terms of "TCDD binding equivalents." Standard assay conditions consisted of incubation of 8 pM radioligand and 18–20 pM *Ah* receptor with 5–1000 pM TCDD, in a 1-ml volume, for 16 hr at 4°. Statistical analysis of the standard curve of bound radioligand *versus* the log of the concentration of competing TCDD indicated the minimal detectable concentration of TCDD to be 10 pM (3.2 pg in a 1-ml assay $\alpha \leq 0.01$). The simplicity, sensitivity, and reproducibility of this competitive binding assay should prove useful as a screen to detect planar halogenated aromatic hydrocarbons and other ligands of the *Ah* receptor. The availability of this ^{125}I-labeled dioxin congener also permitted the characterization of *Ah* receptor-ligand binding over a range of ligand and receptor concentrations not possible with currently available ^{3}H-ligands.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin is one of the most potent small molecule toxins known (1–3) and serves as the prototype for a large number of planar halogenated aromatic hydrocarbons which elicit their biological effects by a common mechanism (e.g., certain isomers of halogenated dibenzo-*p*-dioxins, dibenzofurans, azo[*xy*]benzenes, and biphenyls). The risk presented by the widespread dispersion of these compounds into the environment is a function of 1) their toxic potency, 2) their resistance to chemical and biological degradation (4), and 3) their lipophilicity and hence potential for accumulation in the food chain (5).

Analysis of trace concentrations of these compounds in environmental and biological samples has been made possible through recent advances in chromatographic and mass spectral technologies. Current methodologies involve varying degrees of sample preparation, separation of isomers by liquid or gas chromatography, and identification and quantification of congeners by mass spectrometry. Extremely low concentrations of TCDD in environmental samples, picogram per gram levels (i.e., parts per trillion), are now routinely quantified using this technology (6). Despite the sensitivity of mass spectrometer-based methods, their use is limited by cost and availability of instrumentation.

Bioassays have played a prominent early role in the identification and monitoring of chlorinated dibenzo-*p*-dioxins: e.g., the formation of pericardial edema in the newborn chick (7), and the production of chloracne in the rabbit pinna (8). More recently, cell culture bioassays [e.g., the induction of aryl hydrocarbon hydroxylase activity in rat hepatoma cells (9), and keratinization in XB/3T3 cell cultures (10)] have achieved remarkable sensitivity, 10 pg of TCDD, but have not gained widespread use. Radioimmunoassays have been developed to detect TCDD, 2,3,7,8-tetrachlorodibenzofuran, and chlorinated biphenyls with detection limits approaching 25 pg (11–13). The limited use of these radioimmunoassays may be attributable to 1) the need to characterize each antisera for its reactivity toward a large number of isomers and cross-reacting com-

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ABBREVIATIONS: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; MOPS, 3-(*N*-morpholino)propanesulfonic acid; EDTA, (ethylenedinitrilo)-tetraacetic acid); K_D, equilibrium dissociation constant; *L*, ligand (labeled or unlabeled); *R*, receptor; *RL*, receptor-radioligand complex; *RL'*, transformed receptor-radioligand complex; $[L]_T$, total radioligand concentration; $[R]_T$, total receptor concentration; B_{max}, concentration of receptor determined by Scatchard analysis; *B*, bound radioligand at equilibrium; B_0, receptor-bound radioligand in the presence of no competing ligand; B_x, *receptor-bound radioligand in the presence of competing ligand*; *C*, competing ligand; $[C]_F$, concentration of "free" competing ligand at equilibrium; k_{-1}, dissociation rate constant; NSB, nonspecific binding (radioligand binding in the presence of a 200–1000-fold excess of 2,3,7,8-tetrachlorodibenzofuran); EC_{20}, EC_{50}, EC_{80}, competing ligand concentration which produces 20%, 50%, or 80% reduction in specific binding, respectively; $t_{\frac{1}{2}}$, half-life of dissocation.

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pounds, 2) difficulties in the solubility of radioligand (or antigen), and 3) the limited availability of the radioligands.

TCDD and related halogenated aromatic hydrocarbons are all approximate isostereomers and produce a characteristic pattern of morphological and biochemical changes. The biological effects elicited by these compounds appear to result from their stereospecific binding to a soluble protein, the *Ah* receptor, and the resultant gene expression which is initiated by this ligand-receptor complex (14–17). In support of this model: for chlorinated dibenzo-*p*-dioxins, the apparent binding affinities (K_D) of congeners for the *Ah* receptor correspond to their rank-ordered potencies to produce biological responses (e.g., induction of monooxygenase activity, lethality, chloracne, and tumor promotion). Secondly, in mice there is a genetic polymorphism in the *Ah* locus that determines the receptor. Strains which express a high or low affinity receptor are more or less sensitive to the effects of agonists, respectively, and a variety of toxic responses (e.g., thymic involution, porphyria, epidermal hyperkeratosis, and teratogenicity) have been shown to segregate with the *Ah* allele which determines the high affinity receptor (for a review-see Ref. 16).

Since these halogenated aromatic hydrocarbons produce their biological effects by virtue of their binding to the *Ah* receptor, a competitive binding assay of sample and radiolabeled ligand for *Ah* receptor occupancy would appear to offer a direct and simple assay system. As an analytical method, a competitive radioligand-*Ah* receptor binding assay has a number of advantages: 1) theoretical foundation based on the law of mass action, 2) elimination of the biological variability inherent in the use of whole animals or cells, and 3) rapid screening not requiring expensive instrumentation.

The sensitivity, and hence utility, of *Ah* receptor-based competitive binding assays has been limited to date by the low specific activity of available radioligands (e.g., [^{3}H]TCDD, 58 Ci/mmol). We have previously reported the synthesis and binding kinetics of a new radioligand of the *Ah* receptor, [^{125}I] 2-iodo-7,8-dibromodibenzo-*p*-dioxin (18). This radioligand possesses a high affinity for the *Ah* receptor (K_D = 6.5 pM) and a high specific activity (2176 Ci/mmol). We now report the use of this radioligand in the development of a highly sensitive competitive binding assay for ligands of the *Ah* receptor.

Rationale

Our approach to the development of this competitive binding assay consisted of: 1) synthesis of a radioligand of high specific activity and high receptor affinity; 2) characterization of a stable and reproducible preparation of the *Ah* receptor; 3) use of competitive binding theory to approximate optimal conditions; and 4) refinement of the conditions by experiment.

Radioligand

The sensitivity of a competitive binding assay is proportional to the [specific activity of the radioligand × $1/K_D$]$^{1/2}$ (19). Thus, use of [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin (2176 Ci/mmol, counting efficiency ~70%, K_D = 1.7 K_D for TCDD) should increase assay sensitivity approximately 6-fold compared to use of [1,6-^{3}H]TCDD (58 Ci/mmol, counting efficiency ~40%).

Receptor Preparation

The 40–55% ammonium sulfate precipitate fraction of hepatic cytosol from C57BL/6J mice was chosen because 1) we have recently characterized the kinetics of radioligand binding to this preparation in detail (18), and 2) the receptor concentration in this preparation is slightly enriched compared to cytosol, the lipid contamination is reduced, and binding characteristics are unchanged for over 1 year when stored at −80°.

Mathematical and Statistical Theory Used to Optimize Assay Sensitivity

Equilibrium binding. The binding of a radioligand, L, to its receptor, R, under conditions of equilibrium, can be described by the law of mass action, where the equilibrium dissociation constant, K_D, is equal to the ratio of the constituents: $[R]$ = concentration of free receptor; $[L]$ = concentration of free radioligand; and $[RL]$ = concentration of radioligand-receptor complex. Classically, this interaction is represented as:

$$R + L \rightleftharpoons RL \tag{1}$$

$$K_D = \frac{[R][L]}{[RL]} \tag{2}$$

We have recently examined the binding of the *Ah* receptor from C57BL/6J mouse liver and [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin (18) and found it to be best characterized by the following model:

$$R + L \rightleftharpoons RL \rightleftharpoons RL' \rightleftharpoons R' + L \tag{3}$$

where RL' is a distinct form of the ligand-receptor complex, which has a much slower ligand dissociation rate than does RL.

Under the binding conditions employed (*Ah* receptor from C57BL/6J mice, incubation at 4°, for 16 hr), the transformed receptor-ligand complex, RL', is less than 25% of the total bound ligand; and equations 1 and 2 provide a rough approximation of equilibrium binding.[1]

Optimization of a competitive binding assay. The quantity of radioligand bound to receptor, $[RL]$ or B, at a fixed concentration of radioligand and receptor, is progressively decreased by the addition of increasing concentrations of unlabeled competitor, C, which competes for receptor occupation. The concentration of radioligand bound, as a function of unlabeled ligand added, describes a competitive binding curve and can be derived from the following relationship:

$$\left(\frac{[RL]}{[L]}\right)^2 + \left(\frac{[RL]}{[L]}\right)\left(1 + \frac{[L]_T}{K_D} - \frac{[R]_T}{K_D}\right) - \frac{[R]_T}{K_D} = 0 \tag{4}$$

where $[L]_T$ = concentration of total ligand, and $[R]_T$ = concentration of total receptor.

A major goal in developing a competitive binding assay is to optimize the precision and sensitivity of the assay. This is accomplished by adjusting the concentrations of radioligand $[L]_T$ and receptor $[R]_T$. Berson and Yalow (20) and Ekins and co-workers (19) independently developed theoretical solutions to calculate optimum reagent concentrations. Although these solutions differ in their definitions of sensitivity and considerations of error, they predict similar optimal conditions. Berson and Yalow define conditions which yield maximal sensitivity to those which give an initial ratio of bound to total radioligand ($B_0/[L]_T$) = 0.33 to 0.5, $[R]_T = 0.5\ K_D$, and $[L]_T$ that approaches zero. Ekins and co-workers define optimal conditions as those which yield $B_0/[L]_T = 0.5$; $[R]_T \leq 1.25\ K_D$ and $[L]_T \leq 2.25\ K_D$, when considering only counting error, or which approach $[R]_T = 0.5\ K_D$ and $[L]_T = 0$, with increasing experimental error. These derivations assume simple mass action and equilibrium conditions which only approximate ligand binding to the *Ah* receptor (i.e., equations 1, 2, and 4). Additionally, they are dependent upon experimental as well as counting errors. Therefore, we used these theoretical values to determine initial ranges of receptor and radioligand concentrations and further optimized assay sensitivity empirically.

[1] This assumption was necessary since the optimization theory used was derived from Eqs. 1, 2, and 4. The assumption was supported by the fact that optimal sensitivity was achieved in accordance with the theoretically determined optimal reagent concentration ranges. (see "Results" and "Discussion").

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Quantification of competing ligands with differing receptor affinity. A host of halogenated aromatic hydrocarbon isomers are ligands for the *Ah* receptor, but differ widely in their binding affinities. We foresee this competitive binding assay as being useful in the quantification of the sum of these compounds present in environmental or biological samples, in terms of "TCDD binding equivalents." Ekins *et al.* (19) and Rodbard and Lewald (21) have examined the complex relationship between the relative binding potency of an unlabeled competitor, C, with an equilibrium dissociation constant K_C, and radioligand, L (with affinity K_D). When the affinity of the radioligand is equal to or greater than that of the competing ligand (and the ratio of bound radioligand/free radioligand is less than 0.5), the estimate of binding potency is nearly linear (i.e., $EC_{\omega C}/EC_{\omega L} = K_C/K_D$). Since we have chosen a radioligand with a receptor affinity approximately one-half that of TCDD, it is very likely the estimate of binding equivalency of unknown ligands will always be nearly linear. We have chosen to optimize the assay sensitivity for detection of TCDD; one may enhance the sensitivity of detecting a known ligand of lower affinity by adjusting the concentration of receptor $[R]_T$ and radioligand $[L]_T$ (19).

Time for equilibrium. To accurately estimate the binding equivalency of a competing unlabeled ligand, it is important that binding equilibrium be achieved for both the unlabeled compound and the radioligand. The time required to achieve equilibrium binding is a function of the dissociation rate constant of the slower dissociating ligand. Since the radioligand has a very high affinity for the *Ah* receptor, comparable to or greater than that of any known competing ligand, we assumed that 5 times the half-life of its dissociation [$t_{1/2} = 1.9$ hr (18)] or $3.5/k_{-1}$ is a good approximation of equilibrium conditions for all potential ligands (22).

Calculation of dissociation constants. The equilibrium dissociation constant of a competing ligand (K_C) is most commonly calculated by the Cheng-Prusoff equation (23), which is applicable to situations where $[L] \approx [L]_T$. These conditions are not met in competitive binding assays which have been optimized for sensitivity (i.e., $B_0/[L]_T = 0.3$–0.5). We estimated the K_C of competing ligands from EC_{ω} values by the equations of Linden (24) which place no constraints on the fraction of radioligand or competitor bound. First the concentration of "free" competing ligand is calculated:

$$[C]_F = EC_{\omega} - [R]_T + \frac{[R]_T}{2}\left[\left(\frac{[L]_T}{K_D + [L]_T}\right) + \left(\frac{K_D}{K_D + [L]_T + [R]_T/2}\right)\right] \quad (5)$$

then $[C_F]$ is substituted into the following equation to determine K_C:

$$K_C = \frac{[C]_F}{1 + \frac{[L]_T}{K_D} + \frac{[R]_T}{K_D}\left(\frac{K_D + [L]_T/2}{K_D + [L]_T}\right)} \quad (6)$$

Materials and Methods

Reagents. [^{125}I]2-Iodo-7,8-dibromodibenzo-*p*-dioxin (2176Ci/mmol) was synthesized and purified as described previously (18). TCDD was a generous gift of Dow Chemical Co. (Midland, MI). 2,3,7,8-Tetrachlorodibenzofuran and 2,3-dichlorodibenzo-*p*-dioxin were a gift from Drs. David Firestone and Albert Pohland (Food and Drug Administration, Washington, DC). [^{14}C]TCDD (specific activity 114 mCi/mmol), and 2,3-dibromodibenzo-*p*-dioxin were synthesized as described (25, 26). 3,4,3′,4′-Tetrachlorobiphenylether was a gift from Dr. Andrew Linde (University of Rochester). Estradiol was purchased from Calbiochem (San Diego, CA). Pregenolone-16α-carbonitrile was purchased from Upjohn Diagnostics (Kalamazoo, MI). Sodium phenobarbital was purchased from Merck Chemical Division (Rahway, NJ). Active charcoal, grade PX-21, was a gift from Amoco Research Corp. (Chicago, IL). Bacto-Gelatin was purchased from Difco Laboratories (Detroit, MI). Glycerol was purchased from J. T. Baker (Phillipsburg, NJ). EDTA was purchased from EM Scientific (Cherry Hill, NJ). Testosterone, cortisol, Na-L-thyroxine, dithiothreitol, β-mercaptoethanol, sodium azide, and MOPS (free acid and sodium salt) were purchased from Sigma Chemical Co. (St. Louis, MO). ρ-Dioxane (anhydrous 99+% pure) and dimethyl sulfoxide (anhydrous, 99% pure, stored under N_2 gas) were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Buffers. MN represents the stock buffer which contains 25 mM MOPS and 0.02% sodium azide, pH 7.5 (at 4°). MEN is the stock buffer plus 1 mM EDTA. MβENG and MDENG represent MEN with the addition of 10% (w/v) glycerol, plus the addition of either 10 mM β-mercaptoethanol or 1 mM dithiothreitol (respectively).

Receptor preparation. The 40–55% ammonium sulfate precipitate of C57BL/6J mouse hepatic cytosol was prepared as described previously (18).

Standard assay protocol. To a series of 12- × 75-mm borosilicate assay tubes was added 5 μl of dimethyl sulfoxide containing TCDD standards (0, 5, 10, 20, 40, 60, 100, 200, 500, and 1000 fmol) or unknowns. The frozen 40–55% ammonium sulfate precipitate (15 mg of protein/tube) was dissolved in ice-cold MDENG buffer and diluted to a concentration of 150 μg of protein/ml (equivalent to an *Ah* receptor concentration of 18–20 fmol/ml). The radioligand in dimethyl sulfoxide was added to the solution of receptor to a concentration of 8.0 fmol/ml (~40,000 dpm/ml, added as a 1 μl stock solution/ml of buffer) before dispensing 1 ml of the solution to each assay tube (without vortexing). The tubes were then incubated at 4° for 16 hr. The assay was terminated by the addition of a 0.5 ml of suspension of charcoal/gelatin (3%/0.3%, w/v) in MN buffer with vigorous stirring on a vortex mixer for 3 sec, followed by incubation for 10 min at 4°C. The tubes were then centrifuged at 2000 × *g* for 10 min at 4°. A 1-ml aliquot of the supernatant fraction of each tube was transferred to 12- × 75-mm polypropylene tubes (VWR Scientific, San Francisco, CA), and the bound radioligand was quantified in a MINAXI Series-5000 gamma scintillation counter (United Technologies/Packard Instrument Co., Downers Grove, IL). Sample counting times were adjusted to obtain a counting error of 3% (range of 0.1–2.0 min, counting efficiency of 75%). Each sample and standard were analyzed in quadruplicate. Total radioligand, $[L]_T$, was determined by the transfer of 0.67 ml of assay solution directly to the polypropylene tubes for counting (no charcoal/gelatin is added to these tubes).

Data analysis. The γ-scintillation counter was connected by a serial communication port to an IBM-XT personal computer equipped with software for analysis of radioimmunoassay data and quality control (SECURIA PLUS, Packard Instrument Co., Downers Grove, IL).

Total radioligand, $[L]_T$, was defined as the concentration of radioligand in solution after the 16-hr incubation. (This value is generally 10% lower than the concentration of radioligand originally added to the tubes, i.e., 8 pM.) NSB was defined as the amount of radioligand bound in the presence of 8 nM 2,3,7,8-tetrachlorodibenzofuran (1000-fold molar excess). The total radioligand bound at each concentration of competing ligand (i.e., 5–1000 fmol of TCDD/ml) minus the NSB was defined as the specific binding: B_0 = specific binding in absence of competing ligand and B_x = specific binding at "x" concentration of competing ligand.

To generate a standard curve, the response parameter, B_x/B_0, i.e., the ratio of specific binding at each concentration of TCDD relative to the specific binding in the absence of competing ligand, was plotted *versus* the log of the concentration of TCDD. For data analysis we chose to fit these results, via an iterative curve-fitting technique, to the four-parameter logistic model described by DeLean *et al.* (27).[3] The concentration of ligand in unkown samples is quantified as "equivalents" of TCDD (in picomolar concentration or femtomoles per milliliter), by interpolation of the fractional bound response metameter on

[3] $B_x/B_0 = \frac{B_0 - NSB}{1 - ([C]/EC_{\omega})^S} + NSB$
where S is slope of log logit plot.

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the standard curve. Outliers were defined as values varying more than 5 standard deviations from the mean ($n = 4$ at each point).

The variation between assays was monitored by following a variety of quality control parameters for the standard curve, which included: 1) B_0, (specifically bound ligand in the absence of competing ligand; 2) B_0/L_T, (specifically bound ligand as a fraction of total radioligand per milliliter); 3) NSB; 4) estimates of EC_{20}, EC_{50}, and EC_{80} (competing ligand concentration which produces a 20%, 50% and 80% reduction in specific binding, respectively;), 5) the calculated slope of the four-parameter logistic model (i.e., slope of the logit-log plot), and 6) a control standard yielding a TCDD concentration of 45 pM.

Results

A representative saturation binding isotherm of [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin binding to the *Ah* receptor in the 40–55% ammonium sulfate precipitate fraction of liver cytosol is shown in Fig. 1A. The data in Fig. 1A were transformed by the method of Scatchard (28) to generate the plot described in Fig. 1B. From an average of three experiments the concentration of binding sites, $B_{max} = 120 \pm 7$ fmol/mg of protein, the apparent $K_D = 16 \pm 3$ pM, and the correlation coefficient = 0.99 ± 0.00. The Hill coefficient of these plots is 0.97 ± 0.02 with a correlation coefficient = 0.99 ± 0.00 (29).

As outlined under "Rationale," the approximate conditions for optimal precision and sensitivity of a competitive binding assay are: 1) $B_0/[L]_T = 0.33$–0.5; 2) $[R]_T \leq 1.25\ K_D$ and $[L]_T \leq 2.25\ K_D$. In Fig. 2, are displayed a series of competitive binding curves generated at three radioligand concentrations ($[L]_T$ = 2.4, 7.7, and 23 pM) and three receptor concentration ($[R]_T$ = 7, 28, and 112 pM), with TCDD as the competing unlabeled ligand. The initial $B_0/[L]_T$ ratios (in the absence of unlabeled ligand) varied from 0.2 to 0.7. For a given radioligand concentration, decreasing the concentration of the receptor decreased the initial $B_0/[L]_T$ and increased the initial slope of the competitive binding assay.

As a test of the relative assay sensitivity obtained at differing

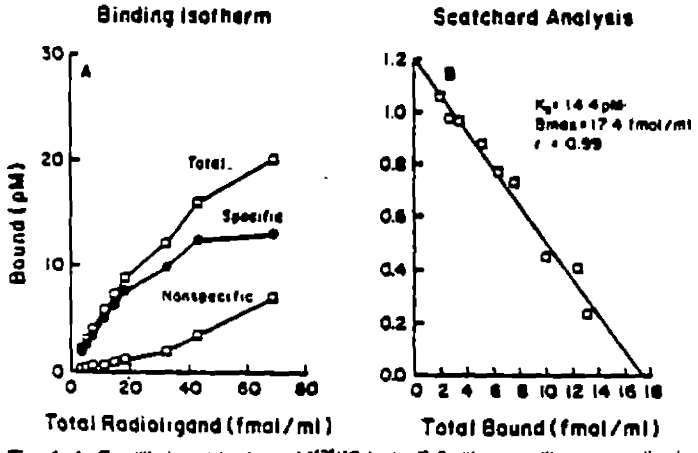

Fig. 1. A. Equilibrium binding of [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin to the 40–55% ammonium sulfate precipitate fraction of hepatic cytosol. The receptor preparation (145 μg of protein/ml) was incubated at 4° for 16 hr, with increasing concentrations of radioligand (3–70 pM). Nonspecific binding was estimated in the presence of a 200-fold excess of 2,3,7,8-tetrachlorodibenzofuran. Each point represents the average of two determinations. B. Scatchard analysis of equilibrium binding data from A. The binding parameters were calculated by a linear least squares estimate of specifically bound/free radioligand versus specifically bound radioligand. The K_D and B_{max} results (inset) are derived from the experiment shown, the mean and standard deviations from three experiments are K_D = 16 ± 3 pM and B_{max} = 18 ± 3 fmol/ml (120 ± 17 fmol/mg of protein).

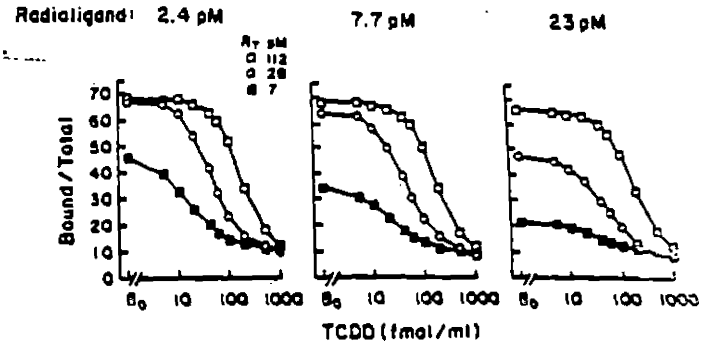

Fig. 2. Effect of radioligand and receptor concentration on binding curves generated with TCDD as competing ligand. Assays were incubated at 4° for a period of 16 hr (see text). All data points are the result of quadruplicate determinations. Ordinate: total radioligand bound divided by total radioligand in solution. Abscissa: concentration of unlabeled competitor (TCDD, picomolar concentration). Receptor concentration: 112 pM (□); 28 pM (○); and 7 pM (■). Left, total radioligand concentration, $[L]_T$, is 2.4 pM (11,600 dpm/ml); center, total radioligand concentration is 7.7 pM (37,200 dpm/ml); right, total radioligand concentration is 23 pM (111,000 dpm/ml).

TABLE 1

Statistical power of the competitive binding assay

Statistical power (see footnote 3) of various assay conditions to detect the decrease in radioligand bound in the presence of 5 pM TCDD, as compared to radioligand bound in the absence of TCDD (i.e., B_0). Ekins *et al.* (19) have shown that the sensitivity of a competitive binding assay is equivalent to the error in B_0 divided by the initial slope of the assay curve, Δ bound/Δ competing ligand added. The statistical power ($1 - \delta$) was calculated using the Student's *t* value with $\alpha \leq 0.01$ (30, 31).

Radioligand Concentration	Statistical Power at Receptor Concentration		
	7 pM	28 pM	112 pM
pM			
2.4	0.99	0.10	0.00
7.7	0.95	0.30	0.00
23	0.60	0.10	0.10

reagent concentrations, we compared the statistical power[3] of these assay conditions to detect a 5 pM concentration of TCDD. As shown in Table 1, the power of the assay increased with a reduction of receptor concentration, at a receptor concentration of 7 pM, the power increased as radioligand concentration decreased.

TCDD and related halogenated aromatic hydrocarbon have very limited solubilities in aqueous solution. We examined the concentration of radioligand that remained in solution, after a 16-hr incubation at 4°, as a function of the concentration of the receptor preparation used. As seen in Fig. 3, only at protein concentrations of 150 μg/ml ($[R]_T$ = 18 pM) or greater, did at least 95% of the added radioligand remain in solution. While maximal sensitivity is achieved at receptor concentrations of 7 pM, we chose to use the higher concentration (18 pM, 150 μg/ml) to increase the solubility of competing ligands.

Based on the observations presented above, the standard conditions for the competitive binding assays were chosen to be: radioligand concentration, $[L]_T$, of 7.2–7.7 pM (≈4 × 10^4 dpm/ml added initially), a receptor concentration of 18–20 pM

[3] The statistical power of an assay (30) is the probability that the decrease in bound radioligand, due to the presence of 5 pM TCDD, will be detected when compared to B_0 (Student's *t* value, $\alpha \leq 0.01$). Thus, assay conditions which yield the greatest statistical power are the conditions which yield the greatest assay sensitivity. Statistical power is equal to $1 - \delta$ (where δ is the probability of making a type II statistical error) (31).

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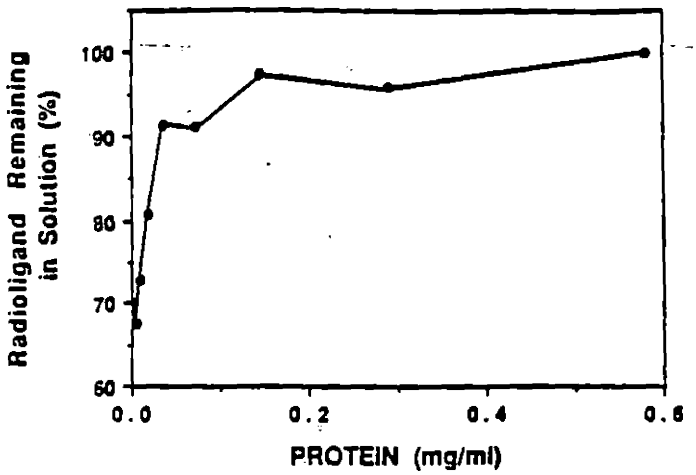

Fig. 3. Effect of protein concentration on radioligand solubility. The radioligand (8.3 pM) was incubated at 4° with varying concentrations of the receptor preparation (0, 5, 10, 19, 38, 75, 150, 300, 600, and 1200 μg of protein/ml. After incubation for 16 hr, total radioligand in solution, $[L]_T$, was determined as described in the text. Each point represents the average of two determinations.

(approximately 150 μg of protein/ml), and an incubation time of 16 hr at 4°. A typical standard curve for the competitive binding of TCDD determined under these conditions is shown in Fig. 4. The initial total bound ligand is 17,176, NSB is 1996 dpm, initial specifically bound ligand, B_o, is 15,180 dpm and $B_o/[L]_T = 0.40$. The concentration of TCDD which produces a reduction in specifically bound radioligand equal to one-half the initial value (EC_{50}) is 41 ± 3 pM (Table 2). Under the standard assay conditions the minimal detectable concentration of TCDD which reproducibly produced a statistically significant reduction in bound radioligand is 10 pM (10 fmol/ml = 3.2 pg/ml, $\alpha \leq 0.05$). Thus, to ensure ligand solubility with an increase in $[R_T]$, we have reduced sensitivity about 2-fold. By reducing the assay volume to 0.25 ml, one can decrease the minimal detectable concentration to 0.8 pg of TCDD (data not shown). The advantage of the 1.0-ml incubation volume is greater bound radioactivity, and thus reduced counting time per sample to achieve the same precision.

We have previously found that [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin has a biphasic dissociation from the *Ah* receptor, with the major component (75%) having a dissociation rate constant, $k_{-1} = 0.36$/hr at 4° ($T_{\frac{1}{2}} = 1.9$ hr^{-1}) (18). Thus, binding equilibrium for competing ligands with dissociation rate constants equal to or greater than that of the radioligand should be achieved by $5 \times t_{\frac{1}{2}}$, or 10 hr (22) (see Rationale). Competing ligands with slower dissociation rates than the radioligand [presumably TCDD, since its K_D is approximately one-half that of the radioligand (Table 3)] may require more than 10 hr to reach binding equilibrium. To confirm that binding equilibrium was obtained for TCDD by 16 hr, we examined the competitive binding curves at 4° as a function of incubation time (Fig. 5). The initial bound radioligand B_o and slope of the competitive binding curved reached a maximum by 16 hr, remained virtually unchanged between 16 and 48 hr, and then progressively declined. The decrease in B_o at longer incubation times is probably attributable to degradation of receptor with time.

The accuracy of the assay is dependent on the standard, i.e., the accuracy in weighing and dilution of small amounts of TCDD. We checked this by determining the competitive bind-

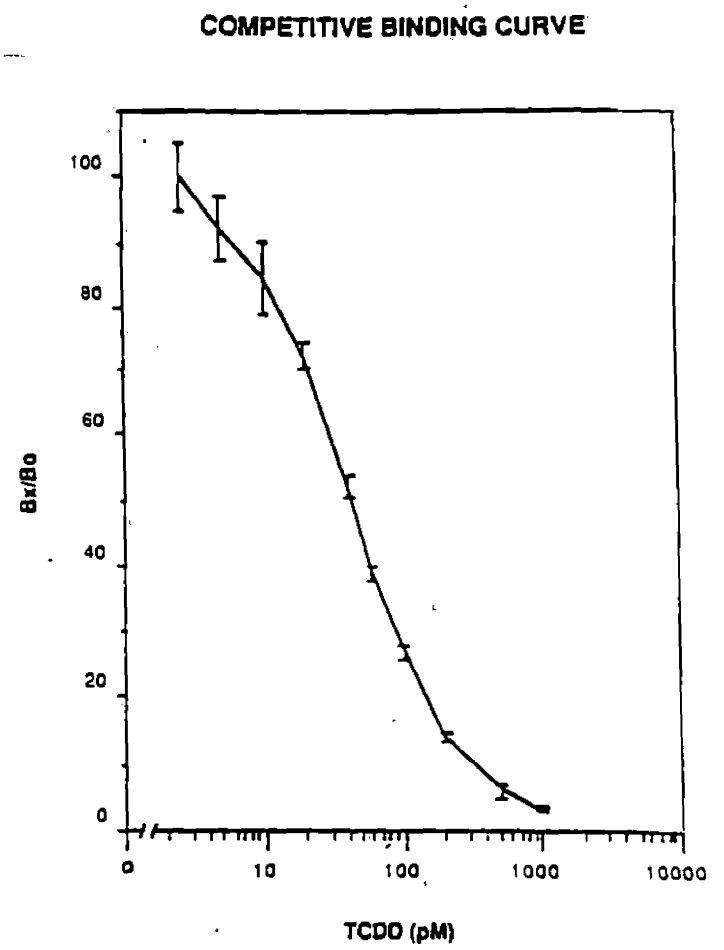

Fig. 4. Competitive binding assay. Standard curve with TCDD as competing ligand. Assay was run, as described in text, with increasing concentrations of TCDD, a total radioligand concentration of 7.2 pM (added 8.2 pM), and a receptor concentration of 18 pM. Incubation was for 16 hr at 4°. Ordinate: B_x/B_o, specifically bound radioligand in the presence of a given concentration of TCDD divided by specifically bound radioligand in the absence of TCDD. Abscissa: concentration of TCDD (picomolar concentration). The first data point is the interpolated concentration of B_o, as calculated by the four parameter logistic model (see text). For this standard curve: $EC_{20} = 13$ pM, $EC_{50} = 40$ pM, $EC_{80} = 138$ pM, B_o divided by total radioligand = 0.36, NSB divided by total radioligand = 0.06.

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TABLE 2

Quality control parameters of the competitive binding assay standard curve

The standard curve for TCDD competition with radioligand for the *Ah* receptor was determined as described under "Materials and Methods." Quality control parameters were compared from assays using freshly prepared radioligand ($n = 9$), and using radioligand after 75% radiodecay (120 days, $n = 1$). Values are means ± standard deviation. All parameters are described in the text. ND, not determined.

Parameter	"New" Radioligand No Radiodecay	Radioligand after 75% Radiodecay
B_o/L_T	0.38 ± 0.06	0.34
NSB/L_T	0.06 ± 0.01	0.06
EC_{20} (pM)	14 ± 3	14
EC_{50} (pM)	41 ± 3	45
EC_{80} (pM)	140 ± 20	160
Logit slope	1.3 ± 0.1	1.2
45 pM TCDD (control pool)	42 ± 6	48
40 pM [^{14}C]TCDD	40 ± 2	ND

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TABLE 3

Relative binding potencies for ligands of the Ah receptor

The competitive binding assay was performed using nonradiolabeled congeners, under standard conditions as described under "Materials and Methods." The $[R]_T$ was 20 pM, and $[L]_T$ = 7–7.6 pM. The true K_D of the radioligand was assumed to be 6.5 pM (18). The EC_{50} is the total ligand concentration which produced a 50% reduction in the specific binding of the radioligand; the relative binding potency is $EC_{50\,standard}/EC_{50\,TCDD}$. The K_C for each compound was determined by the method of Linden (Eqs. 5 and 6, Ref. 24) and the relative K_C = K_C competitor/K_C TCDD.

Competitor	EC_{50}	Relative EC_{50}	K_C	Relative K_C
	pM		pM	
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	41	1	6.8	1
2-Iodo-7,8-Dibromodibenzo-*p*-dioxin	58	1.4	11	1.7
2,3,7,8-Tetrachlorodibenzofuran	85	2.1	18	2.6
2,3-Dibromodibenzo-*p*-dioxin	580	14	140	20
2,3-Dichlorodibenzo-*p*-dioxin	6400	160	1600	230

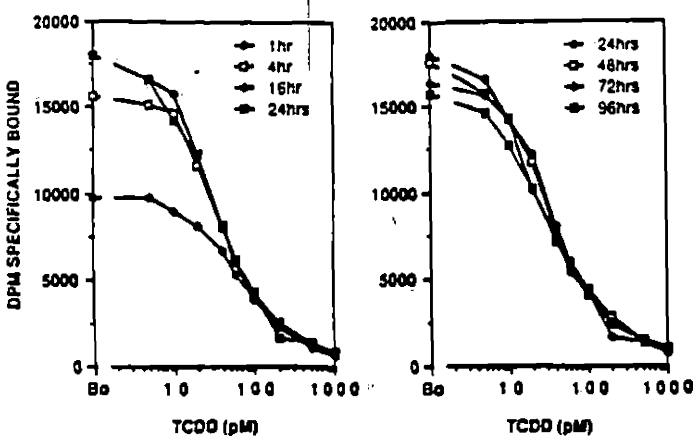

Fig. 5. Effect of incubation time on the competition binding curve. The assay was run as described in the text, with increasing concentrations of TCDD, a total radioligand concentration of 7.5 pM and a receptor concentration of 18 pM. The competition binding curves were analyzed after varying incubation times (key is inset) at 4°. Ordinate: radioligand specifically bound in disintegrations per milliliter. Abscissa: concentration of unlabeled TCDD (picomolar concentration). Each value is the result of triplicate determinations.

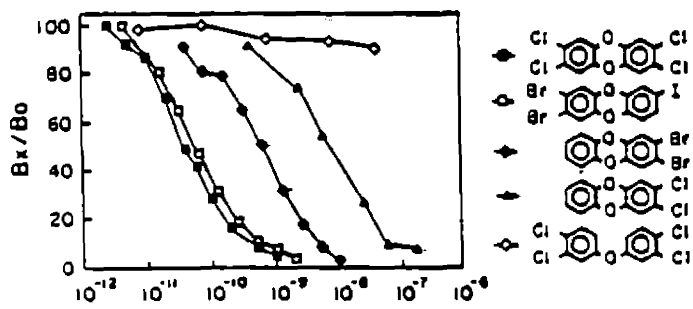

Fig. 6. Competition by various compounds for the specific binding of [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin to the *Ah* receptor. The assay conditions are identical to those described in Fig. 5. All values are the result of quadruplicate determinations. Compounds were dissolved in dimethyl sulfoxide and added to the incubation in 5 μl total volume.

ing of [^{14}C]TCDD. Three independent analyses of a [^{14}C]TCDD standard, quantified by β-scintillation spectrometry to a concentration of 40 pM, gave a value of 40 ± 2 pM from the standard curve (Table 2).

^{125}I decays to tellurium with a half-life of 60 days. Radiodecay of the iodinated ligand may produce unlabeled 2,3-dibromodibenzo-*p*-dioxin in the radioligand stock solution. The K_D of the 2,3-dibromodibenzo-*p*-dioxin was estimated by competitive binding to be approximately 140 pM (see below). We tested whether radioligand decay products might compete for receptor binding and alter the competitive binding curve. Assays using the stock solution of radioligand 120 days after its synthesis (75% of ^{125}I radiodecay), showed no significant deviation in the quality control parameters compared to freshly prepared radioligand (Table 2). Thus, repurification of the radioligand by high performance liquid chromatography does not appear necessary for at least 120 days after synthesis.

Using the standard assay conditions outlined above, competitive binding curves were generated for a variety of halogenated-dioxin congeners (examples are given in Fig. 6). The relative binding affinities of these congeners, as indicated by estimates of EC_{50} and K_D (Table 3), are in agreement with relative binding affinities, and biological potencies which have been published previously [i.e., TCDD > TCDBF > 2,3-dichlorodibenzo-*p*-dioxin > 3,4,3′,4′-tetrachlorobiphenylether (10, 25)]. Additionally, testosterone, phenobarbital, thyroxine, estradiol, cortisol, and pregnenalone-16α-carbonitrile at levels as high as 0.8 μM (i.e., 10^5 times the concentration of radioligand) did not compete with the radioligand for *Ah* receptor occupancy (data not shown).

Discussion

In this report, we describe a sensitive competitive binding assay for the detection and characterization of ligands of the *Ah* receptor. Since the sensitivity of such an assay is a function of the binding affinity and specific activity of the radioligand, considerable attention has been devoted to the selection, labeling, and binding kinetics of this compound, [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin (18).

The higher specific acitivity of this radioligand (2176 Ci/mmol) and the greater counting efficiency of γ-emission over β-emission increases the ratio of counts per minute per femtomole of radioligand about 2 orders of magnitude as compared to [^{3}H]TCDD (58 Ci/mmol). The ^{125}I-labeled ligand offers other minor advantages: 1) it has high radiochemical purity resulting from the easy separation of the iodinated product from the starting material, reaction intermediates, and radiodecay products, 2) specific activity is determined as that of the Na[^{125}I] used for synthesis, and 3) after 75% radiodecay, a stock solution of the ^{125}I-ligand produces the same competitive binding curve, indicating that radiodecay products do not significantly affect receptor-ligand binding (Table 2).

In a previous report (18), we described the effects of protein concentration on the quantification of "free" radioligand and on the calculations of K_D and $[R]_T$. We suggested that at protein concentrations above 70 μg/ml, "nonspecifically bound" radioligand is misclassified as "free" radioligand, leading to significant overestimation of K_D by Scatchard analysis. By minimizing misclassification errors we calculated the K_D of [^{125}I]2-iodo-7,8-dibromodibenzo-*p*-dioxin to be approximately 6.5 pM. For the competitive binding assay, a concentration of 150 μg/ml was employed to ensure ligand solubility. At this protein concentration Scatchard analysis of the saturation binding isotherm yielded an apparent K_D of 16 pM (Fig. 1), suggesting that some misclassification of nonspecifically bound radioligand as free radioligand is occurring. The effect of this misclassification appears to be minimal, as the calculation of K_C for 2-iodo-7,8-

dibromodibenzo-*p*-dioxin (i.e., 11 pM) is only 1.7-fold greater than the previously determined estimate of K_D for the ^{125}I analogue (i.e., 6.5 pM). This 1.7-fold overestimation of K_C suggests that the true K_C for TCDD is approximately 4 pM, 100-fold lower than previous estimates for this compound (25).

It has been previously proposed (32) and demonstrated (33) that a competitive binding assay, using the *Ah* receptor and radioligand, could be used to screen for the presence of competing ligands present in the environment. The extraordinary sensitivity of the present assay, attributable to the high specific activity of the radioligand, revised estimate of receptor affinity, and optimization of assay sensitivity, make it feasible to screen environmental samples for TCDD and related halogenated aromatic hydrocarbons: e.g., chlorinated dibenzo-*p*-dioxin, -dibenzofuran, and -biphenyl isomers. Environmental samples may also contain polycyclic aromatic hydrocarbons, many of which are also ligands of the *Ah* receptor. These compounds can be eliminated from analysis by the appropriate sample cleanup. The method of sample cleanup depends on the sample matrix (e.g., biological tissue, soil, water, etc.) and the presence of interfering substances. The competitive binding assay does not provide chemical identification of competing ligands, but an estimate of their concentrations as "TCDD-binding equivalents" (see Rationale and Table 3). Samples judged to have sufficient concentrations of TCDD-binding equivalents, could be subjected to mass spectrometry for chemical identification.

Finally, one of the most exciting uses of this competitive binding assay, is to screen biological tissue extracts for the postulated endogenous ligand of the *Ah* receptor.

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ASKAREL: REQUIEM FOR AN OLD RELIABLE

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Abstract

For several decades, askarel containing polychlorinated biphenyls (PCB's) was considered an excellent insulating fluid. In the span of a few short years, it has come under attack as a pernicious and ubiquitous environmental contaminant. Regulations concerning this material are evolving rapidly, and a total ban is ultimately expected. Spill prevention, control and disposal are required to preclude high economic loss as well as adverse publicity. Alternates to the use of askarel include new fluids that must also be reviewed for environmental acceptability.

Introduction

Until rather recently, our society used many chemicals with seemingly few environmental problems. In a short span of time, several factors contributed to a substantial change in the environment. A rapidly expanding population, enjoying the fruits of the most advanced technology and greatest affluence in the history of mankind, created a demand for more goods, often based on a host of new chemicals and materials. Consequently, genuine and serious environmental problems were becoming common.

In the latter part of the 1960's, there was a new mood of social awareness and activism. Concurrently, the rapid advances in technology also included significant improvements in the means of detecting and measuring contaminants at remarkably low levels. The stage was set for the sudden change in the status of askarel, an old reliable.

PCB's - The Problem

Polychlorinated biphenyls are produced by substituting one to ten chlorine atoms for hydrogen atoms on the biphenyl structure shown in Figure 1. There are 209 possible combinations or isomers; for example, there are twenty-four different possible arrangements for the molecule containing three chlorine atoms. One of these trichlorobiphenyls is illustrated by Figure 2.

The manufacturing process results in a loosely controlled mixture of various amounts of many of the isomers; hence the characteristics of a particular batch is the sum of the several individual components. Some general conclusions about these components are:

1. They cause abnormalities in cells, organs and glands of many animals. This occurs at rather low levels of the chemical.

2. The material is quite stable and barely reacts, degrades, or decomposes under normal circumstances; it is quite insoluble in water.

3. It is heavier than water and is ingested by bottom-feeding animals. Animals tend to bioaccumulate the chemical, i.e., it is absorbed readily and excreted slowly, with a resultant high level becoming stored in tissues.

4. Traces of two very similar chemicals (see Figure 3), polychlorinated dibenzofuran and polychlorinated dibenzodioxin, have been found in the tissues of animals which have absorbed PCB's. The materials may be present in the original PCB or may be metabolized from PCB by the animal. These compounds are known to be highly toxic.

5. PCB's are ubiquitous and are apparently mobile in the atmosphere. They have even been found in snow at the Antarctic.

6. They are being found at significant levels in human tissues and in mother's milk.

About ten years ago, PCB's were first isolated and identified as a possible contaminant contributing to problems in animals. At that time, this chemical was in wide use. About half was utilized as the primary constituent of askarel in electrical equipment (askarel also contains trichlorobenzene as a solvent). The remainder of the PCB was used in plastics, paints, carbonless paper, and many other products and processes. Since few problems had ever been noted with the material, any scrap or waste was indiscriminately severed or placed in dumps, as most chemicals had been handled for quite some time.

One of the characteristics that made PCB's or askarel attractive was its stability; but this very permanency also meant that the material remained virtually intact for decades. It is generally conceded today that this residual of PCB's will be present as a contaminant for decades, regardless of a complete elimination from the market place.

Initial Control Efforts

PCB's because of their excellent stability and thermal properties, have been used as a heat-transfer fluid in several industries. In Japan during 1968, a food product was contaminated with PCB's from a heat-exchange operation and more than 1200 people were afflicted, some rather severely, with what was called "Yusho" disease. This outbreak, plus other incidents, and a rapidly expanding research effort identifying PCB's as a potential detriment, led the sole U.S. producer (Monsanto Industrial Chemicals Company) to voluntarily restrict sales to closed electrical systems.

This step removed about half the PCB's from the market, and began the elimination of the most common uses whereby PCB's were being introduced to the environment, such as through carbonless office papers. Of course, outdated capacitors and the like continued to be thrown on the junk pile.

In late 1975, the U.S. Environmental Protection Agency sponsored a PCB conference which was both technical and political. The technical program was enlightening; there was little doubt that hard scientific evidence identifying PCB's as a major concern was developing quickly. The political aspect of the conference came from two sources. First, the

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public sector was allowed to make presentations, the majority of which predictably were emotional and often based on misinformation. The second political aspect arose as representatives from various governmental agencies - FDA, Interior, NIOSH, Commerce, etc. - made statements to explain past indifference and to scourge the plague of the 70's, PCB's. There was little doubt that the bureaucracy felt safe in taking a stand, and that regulation or restriction was imminent. There is strong public support for control since the mass of the populace is technically naive and thoroughly frightened by the constant disclosure that everything from licorice to marachino cherries may be mutagenic, teratogenic, or carcinogenic.

In this context, and knowing that there were some substitute materials forthcoming, the equipment manufacturers began phasing out of askarel and into new fluids. Naturally, the fluid supplier (Monsanto) recognized that the market was disappearing and made the decision to terminate all production of the chemical later this year. Thus today, there are three questions to be answered:

1. What should be expected concerning the askarel-filled equipment now in service?

2. Exactly what is available in the future?

3. What assurance is there that the course taken now is correct; can this happen again with a substitute material?

Anticipated Pressure

In these times of OSHA, IRS, FDA, EPA, Consumer Protection Agencies and the like, it is apparent that users of equipment containing askarel can expect the following:

1. Specific regulations will be published for askarel handling, protection, disposal, etc. For example, one state is in the process of promulgating a regulation that includes mandatory labeling of equipment containing more than two pounds of PCB.

2. Users will be asked to inventory askarel on the plant premises and to indicate the location of equipment in reference to waterways.

3. Monitoring of effluents will be required; this is already being specified in NPDES permits in some locales.

4. Protective measures, such as dikes, will be made mandatory.

5. It may be required to include askarel in the spill plans which most plants already have.

6. Any accidental spill will have to be reported, even if it was apparently completely controlled on the plant site. If the spill should involve more than a certain quantity, a regulatory agency may visit the site.

7. If a spill should escape to a waterway, recovery of the lost askarel will be required. There have been some specific (and very expensive) instances where this has been done in the past.

8. There will be a ban on new installations of askarel-filled equipment, which will be rather meaningless since industry is already voluntarily abandoning the market. But there will ultimately also be a ban forbidding the continued use of existing equipment. This too may prove to be meaningless because the unavailability of askarel for topping out or retrofilling transformers may result in early retirement of most askarel-filled equipment. There are substitutes available for topping out, but there is a limit to their use.

Loss Prevention and Control

Until quite recently, askarel spills were virtually ignored. As attention focused on the problem, many incidents came to light. Spills or losses are known to have occurred under these circumstances:

1. As a consequence of moving or handling.

2. Overloaded or faulty capacitor.

3. Overloaded and overheated transformer which loses fluid through the relief valve.

4. Changing out carbonized askarel from a shorted transformer.

5. Leaks from valves on the transformer casing.

6. Loss when sampled for dielectric or moisture test.

7. Improper disposal of sample.

8. Misuse - mistaken for another chemical. This has occurred when waste askarel was placed in a used drum without relabeling.

9. Damage to drums or equipment by other equipment such as forklifts.

10. Freak incidents, such as an icicle piercing a transformer cooling fin, or a capacitor skewered by the arrow of a frustrated bow hunter.

Two actions should be taken immediately to preclude such occurrences. A rather detailed inventory should be taken of every askarel transformer and capacitor as shown in Figure 4. While most plants have some form of inventory for electrical purposes, they do not include information which will allow an evaluation of the environmental risk. It is suggested that oil transformers be included in the inventory since even these present potential environmental costs. Inactive units or stored fluids should also be noted

The second most immediate action is to inform or educate the plant staff. There are two objectives - (1) to prevent or control spills, and (2) to ensure that personnel are not confused or frightened by inadequate knowledge of a potential problem.

It is suggested that an appropriate label - large and bright - be placed on askarel equipment. It is also necessary to ensure that contract personnel are informed to look for these labels, and it may be advisable to specify in writing the responsibility for spills caused by a contractor.

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After making an inventory of askarel equipment, decisions must be made whether to invest in protection or spill control for a particular item. This matter is arbitrary, but it is suggested that the items be rated by vulnerability to damage, quantity of askarel, and proximity to a waterway. Thus the larger transformer located by a road with an adjacent running ditch going toward the river warrants more consideration than the smaller unit inside a building away from any traffic.

The potential for a costly spill can be reduced by preventive measures. For example, place fences or posts around exposed equipment; a light roof may be added to prevent damage from ice or dropped tools. Relocation of the equipment is an alternate. A plug should be threaded into the drain valve of a transformer. Finally, many spills could be prevented by operating equipment at the design rating instead of 110% plus.

Inevitably, equipment will fail or freak accidents will occur; several steps may be taken to contain spilled askarel. Dikes can be installed around equipment preferably with a capacity slightly greater than the largest volume of askarel in one unit. If the location is outdoors in a wet climate, a device known as an imbiber valve, which contains molecular sieves that pass water but retain PCB's, can be installed when the dike is constructed. Plastic shields can be placed around pole-mounted capacitors to restrict a spill to a lesser area.

All drain holes in vaults should be plugged, and ramped sills placed at doors. All joints should be sealed with a silicone putty, and concrete block walls should be sealed with silicone paint or an equivalent coating.

Should askarel be spilled, the precise amount of cleanup or reclamation is arbitrary - there are no standards yet. At the time of the spill in the Duwamish Waterway (Seattle) in 1974, the EPA terminated the operation when the PCB content was reduced to 77 parts per trillion; this should indicate that a thorough collection is expected.

Collection of visible or standing askarel can be initiated with an absorbent compound. Rags with solvent such as trichlorobenzene should be used to wipe down equipment, poles, etc. Soil should be removed to a depth exceeding the obvious level to which the fluid soaked, and radially beyond the last visible signs of fluid. Boards or the like should be replaced. All solid wastes - rags, gloves, soil, shovels, etc. as well as liquids should be set aside for proper disposal.

Should a spill enter an internal sewer, the water flow should be terminated and askarel collected from the low spots. If the sewer discharges into a sewage plant, most of the PCB that is carried in the water will attach to suspended matter in the treatment plant and settle. Of course, these settled materials usually end up in a landfill eventually, but at least most of the PCB apparently remains immobilized.

The worst situation, of course, is if a spill enters a waterway intact. Noone has an answer for the situation where the contaminant enters a fast stream and disperses widely. For lakes or rather static waters, the bottom is dredged. The material first passes through a settling chamber, then through a sand filter, and finally through an activated carbon column. All of the wet solids require disposal. Obviously, every effort should be made to avoid a spill of this nature; the cost is horrendous. The final bill for cleaning the spill of 265 gallons into the Duwamish Waterway was $500,000!

Proper disposal of waste askarel, scrap equipment, or solids wastes is rather well defined. Askarel may be transported as a liquid or gelled with molecular sieves. About twenty facilities are available for burning or landfill. Burning requires quite high temperatures to ensure complete destruction, and the exhaust gases must be scrubbed with caustic to remove the hydrochloric acid that is emitted. Landfill can only be in certain approved sites. Transportation of PCB's to a disposal site requires special preparation and handling. In one incident involving a spill from a 1500 kVA transformer, transportation and incineration cost $2,200. In another incident involving the rupture of a pole-mounted capacitor, transportation and burial cost $150.

Environmental Acceptability of Alternate Compounds

Several alternate fluids are now available in new transformers and capacitors.

Silicone-based fluids are offered for liquid-filled transformers. All evidence thus far indicates that the material is quite innocuous. It should be noted that unlike askarel, the silicone fluid has a specific gravity less than one, and any spill would have to be treated as an ordinary oil spill.

Obtaining detailed environmental information from the vendors on their new capacitor fluids has been difficult, and much of it is not comparable. For example, one vendor will report on the toxic effects on a certain fish species on the basis of a 16 day test, while another uses a 96 hour test. All the new materials offer definite improvements over PCB's however. On the basis of all the data-toxicity, biodegradability, solubility, etc. - which this writer has been able to obtain, the three available fluids should be environmentally ranked:

Most acceptable	- isopropyl biphenyl
Second	- butylated chlorodiphenyl oxide
Third	- di - 2 - ethylhexylphthalate (a phthalate ester)*

One suggested alternative to the replacement of askarel transformers has been to retrofill with the silicone-based fluids. This procedure involves draining out all possible askarel, followed by flushing with trichlorobenzene. A certain amount of askarel will be retained in pockets, insulation, etc.; the residual PCB content is estimated to be 1-3%, and will be very dependent upon the amount of care taken and number of flushings performed. It should be noted that all flushing fluids will require disposal by methods approved for PCB's.

* 95 Citations for toxicity reported by the author in discussion. —

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Conclusions and Recommendations

In this age of environmental awareness and social activism, many chemical substances will be scrutinized intently. Regardless of its relative impact, PCB is currently being cited as the leading hazardous chemical which warrants control. Industry faces a new round of regulations, reports, and costs. As with any such issue, the cost can be best controlled by gathering information, making unemotional judgments, and planning to take the appropriate action before the citations are issued.

Specifically, these steps are recommended:

1. Define the problem in the plant. Locate each item and assess the probability of damage or loss. Estimate the cost of equipment protection and spill control.

2. Get information from the vendors on their new equipment, including very specific data to back any claims of environmental properties.

3. For transformers, determine the efficacy of retrofilling and the probable availability of topping fluid.

4. Proceed with protection, retrofilling, or replacement on a scheduled basis. In many instances, the choice will not be obvious, and it is suggested that it is better to err on the side of greater environmental protection.

5. In some instances where only a few pieces of equipment are involved, consider that total elimination of askarel may cost less than the hidden costs of continually reporting, inspecting, etc.

Noone can be completely sure that the steps taken will preclude a costly spill of this hazardous chemical. Should one occur, however, the plant that has evinced awareness and effort will fare better with the regulatory agencies and the public than a plant which ignored the problem of PCB's.

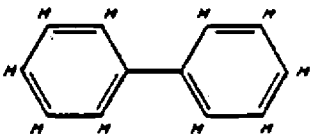


Figure 1 - Biphenyl Molecule

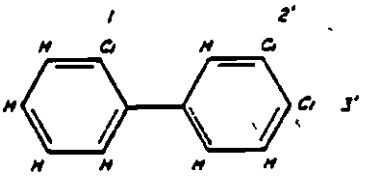
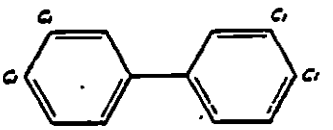
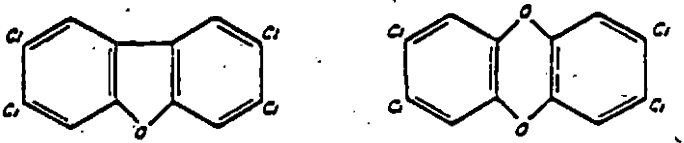


Figure 2

Figure 2 - 1-2',3' Trichlorobiphenyl



Tetrachloro Biphenyl



Tetrachloro Dibenzofuran

Tetrachloro Dibenzodioxin

Figure 3 - Possible PCB Metabolites

Transformers No.	Location	Mfr.	Capacity kVA	Insulating Fluid	Gal.	Risk of Potential Damage or Spill	Degree of Environmental Sensitivity
1	East Side Ply. Plant	West	1500	Inerteen	382	High. Outside bldg., asphalt drive 2 sides, forklift area	Medium. Concrete pad, no curb, 100 ft. from drainage
2 3 4	Sawmill	West	250 ea.	Oil	300 ea.	Low. Outside bldg., fenced, no traffic	Medium. Concrete pad, no curb, 50 ft. from drainage
5	Machine Shop	GE	1000	Pyranol	210	Medium. Work area. Equipment fabrication. Snow plow area	High. 20 ft. from drainage ditch. No Platform
etc.							

Capacitors No.	Location	Mfr.	Rating kVAR	Insulating Fluid-Gal.	Risk of Potential Damage or Spill	Degree of Environmental Sensitivity
1	PCC Shop	McCrew - Edison	15		Low. Inside Control Room.	Low. Room closed, with door curb.
2-25	East Yard	Sprague	4800 (24 x 200)		Medium. Pole-mounted low traffic. Multiple units	High. Coarse gravel underneath. 75' from river.
etc.						

Figure 4. Suggested Inventory Sheets

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