

MRI REPORT

Chlorinated Dibenzo-*p*-Dioxin (PCDD) and Chlorinated Dibenzofuran (PCDF) Residue Levels in Food

Executive Summary

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PREFACE

This executive summary presents a synopsis of the residue levels of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in measured foodstuffs collected within two California urban areas. This summary presents the experimental design, the analytical procedures and the results of the chemical and statistical analyses. This research effort was conducted for the State of California's Research Division of the Air Resources Board, Mr. Ralph Propper, Project Officer.

The chemical analysis efforts were completed under the direction of Dr. John Stanley and Mr. Paul Cramer, with assistance from Ms. Kathy Boggess, Ms. Maureen Greene, Mr. Michael McGrath, and Mr. Kelly Thornburg. The statistical analysis efforts were completed under the direction of Ms. Karin Bauer with assistance from Ms. Jean Pelkey.

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INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), particularly isomers with chlorine substitution in the 2,3,7,8-substituted positions are recognized as potentially toxic environmental contaminants. These compounds are the by-products from the production of specific chlorinated aromatic compounds and as a result of incineration processes. The use of the commercial products, disposal of product wastes and uncontrolled incineration activities have resulted in widespread contamination of these compounds in the general environment.

The release of PCDDs and PCDFs as emissions from multiple incineration sources has been heavily studied over the past 10 years. It is recognized that these compounds are contaminants in emissions arising from a variety of sources including municipal and hospital incinerators fired on refused-derived fuels, metal reclamation facilities, hazardous waste incinerators, and automobiles.

As a result, the State of California's Air Resources Board has designated these compounds as toxic air contaminants. The growing requirements for effective waste management minimizing the use of landfill sites and the lack of alternate treatment or recovery processes for hazardous materials has generated a growing demand for this technology. The impacts of the emissions released from the increase in the number of facilities is not known. However, the Air Resources Board has initiated a number of research efforts to determine the impact on the environment and human health. The research conducted to date include (1) assessment of background levels of PCDDs and PCDFs in air from a number of areas within the state affected by differing pollutant sources (agricultural burning to hazardous waste incineration), (2) direct measurement of emissions from major incineration sources, (3) determination of intake of PCDDs and PCDFs through the food chain, and (4) determination of actual body burden levels of PCDDs and PCDFs in the California population.

This executive summary focuses on the results of a study that was conducted to assess the residue levels of the 2,3,7,8-substituted PCDDs and PCDFs in fatty foods that are available to the general California population. The data reported are essential in developing models that relate all possible intakes of PCDDs and PCDFs that will give rise to a specific body burden level. The data presented can be used for comparison with food sources that have been directly impacted by a PCDD or PCDF contaminated source (commercial product, hazardous waste, or emissions from an incineration source).

The data summarized in this report were generated from the analysis of 50 composite food samples collected within the San Francisco and Los Angeles areas. Foodstuffs that were analyzed included fish (freshwater and saltwater), beef, chicken, pork, milk, and eggs. The remainder of this report provides a synopsis of the experimental design, analytical procedures, and the resulting PCDD and PCDF residue levels for each of the foodstuffs.

SUMMARY

The research program described in this report required the random collection of multiple samples of seven (7) specific foodstuffs from the San Francisco and Los Angeles areas. The foodstuffs included saltwater fish, freshwater fish, beef (hamburger), chicken, pork (bacon), bovine milk, and eggs. The individual food samples collected were composited for analysis of the residue levels of PCDDs and PCDFs (specifically the 2,3,7,8-substituted compounds). The composites consisted of up to 31 individually collected items, and five to eight composites were analyzed for each foodstuff. Detectable levels of specific PCDDs and PCDFs were identified in all but the egg samples that were analyzed. Overall the freshwater fish composites were found to have the highest incidence of detectable levels. The order of highest to lowest incidence of detection follows: freshwater fish > saltwater fish > pork and chicken > beef and milk > eggs.

The compounds detected in the fish samples included the 2,3,7,8-substituted tetra- through octachlor- PCDDs but the PCDFs were limited to primarily the 2,3,7,8-TCDF. The tetra compounds were not consistently detected in any of the other foodstuffs except milk. The residue levels detected in the beef, chicken, and pork were generally limited to the hexa- through octachloro-compounds. Estimates of the detection limits on a sample-to-sample basis are provided for each specific compound when they were not detected. These method detection limits were calculated using the observed noise signals and hence should provide an upper estimate of residue levels for consideration in risk assessments.

Although many of the analyses resulted in estimated detection limits for specific compounds, there is evidence that further modifications of the methods, either increased sample size or advances in instrumentation, would result in measurable levels of the PCDDs and PCDFs.

The accuracy of the residue levels reported in this study is supported from the analytical data generated from quality control samples that were prepared from the specific food matrices and were analyzed along with the design samples. In addition the analysis of laboratory method blanks that included all reagents and procedures for preparing the actual samples demonstrated that there was not background contribution from the laboratory. These data support the identification of the PCDDs and PCDFs in the foodstuffs at the low parts-per-trillion level.

APPROACH

Survey Design

The study design focused on the collection and analysis of seven foodstuffs which included saltwater fish, freshwater fish, beef (hamburger), pork (bacon), chicken, bovine milk, and eggs. These foods were selected for analyses as a result of their high fatty contents since it is recognized that PCDDs and PCDFs tend to accumulate in lipophilic matrices.

The original study design required the collection of the seven foodstuffs from two specific geographic urban areas (San Francisco and Los Angeles). One of the considerations was to acquire foods that were produced in California. A survey of agricultural and food agencies conducted prior to completing the study design indicated that almost all of the eggs, milk, and poultry are from California sources. The eggs and milk are labeled as such. Most of the beef solid is from California sources and saltwater fish are generally from the California coastal waters. Fresh fish and pork, however, are from sources outside of the California region.

A total of 50 samples of each foodstuff were targeted for collection from San Francisco and Los Angeles. Since resources for analysis were limited, the samples were prepared for analysis as composites. Compositing the foodstuffs in a statistical manner presents the following advantages:

- A sample with a representative average level of PCDD and PCDF in a specific foodstuff can be obtained.
- The chemical analysis costs were reduced in comparison to the analysis of individual samples.
- The compositing approach leads to an increased probability of detection of the compounds resulting from elevated levels in individual samples.

A total of 50 composite food samples were analyzed for the target compounds. Of the 50 composites, 31 are of foodstuff sampled in Los Angeles and 19 of foodstuff sampled in San Francisco. The relative size between the two sets of composites reflects approximately the relative size in population of the two cities. The distribution of the 50 composites across cities and foodstuff categories is shown in Table 1.

The individual foodstuff samples in each category were composited separately for San Francisco and Los Angeles. Approximately equal numbers of samples were used for composites in a given foodstuff category. The number of individual food samples reflects the number of sites at which these samples were collected. These figures were also used as weights in subsequent statistical analyses.

Table 1. Composites Per Foodstuff and City
 (No. of sites sampled and included in the
 composites is indicated in parentheses)

	Los Angeles	San Francisco	Total
Saltwater fish	3(31)	2(17)	5(48)
Freshwater fish	3(31)	2(17)	5(48)
Pork	5(31)	3(20)	8(51)
Beef	5(31)	3(19)	8(50)
Chicken	5(31)	3(20)	8(51)
Egg	5(31)	3(20)	8(51)
Milk	5(31)	3(18)	8(49)
Total	31	19	50

Analysis Procedures

All samples were prepared such that approximately 10 g of fatty material from a particular foodstuff was available for determination of the PCDDs and PCDFs. Additional details on the sample preparation procedures for specific foods and the high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) determinations are presented.

Laboratory Sample Preparation Procedures for Foodstuffs

Additional details on the preparation of each of the specific foodstuffs are described below.

Milk--

Milk samples consisted of whole milk, half and half, and whipping cream samples. Known amounts from each sample in a composite were combined into a single sample so that each contributed equal amounts of milk fat. The goal was to approximate a total of 10 g of milk fat. For most composites, this corresponded to a volume of 70 mL.

After the sample composite was prepared, a known amount of a series of nine ^{13}C -labeled internal quantitation standards was added, and the mixture was denatured with 3% sodium oxalate, ethanol, and diethyl ether. The sample was then extracted with three portions of hexane and the hexane combined for further cleanup.

The hexane/milk fat extract was subsequently fortified with ^{13}C -labeled dioxins and furans and subjected to an acidic silica gel slurry cleanup procedure. Specifically, 100 g of 40% sulfuric acid-impregnated silica gel was mixed with the hexane/milk fat mixture for 2 hr. Afterwards, the hexane was decanted through a funnel of sodium sulfate into a 4-g acid silica gel/1-g neutral silica gel column. The fraction collected in a Kuderna-Danish (K-D) evaporating flask. The acidic silica gel was slurried an additional two times with 50 mL of hexane for 15 min each time and the rinses placed on the column. After all the solvent from the slurry had passed through the column, an additional 50 mL of hexane was placed on the column and combined with the other eluent in the K-D flask.

The extract was reduced in volume to approximately 2 mL and applied to the top of a chromatography column comprised of 4 g sodium sulfate, 4 g neutral alumina, and 4 g sodium sulfate. The column was eluted with 10 mL of 8% dichloromethane in hexane. This portion was archived. The PCDDs and PCDFs were eluted in 15 mL of 60% dichloromethane in hexane. This fraction was collected and reduced in volume to approximately 2 mL and applied to the final column.

The final cleanup column consisted of 1 g of 5% Amoco AX-21 carbon on neutral silica gel. The column was prerinsed with 4 mL toluene, 2 mL dichloromethane/methanol/benzene (75:20:5), and 4 mL cyclohexane/dichloromethane (50:50). The fraction from the alumina column was transferred to the AX-21/silica gel column with two 1-mL

rinses of hexane. The column was eluted with 10 mL of the cyclohexane/dichloromethane solution and 5 mL of the dichloromethane/methanol/benzene solution. These fractions were combined and archived. The columns were then turned over and eluted with 20 mL of toluene. The toluene was reduced in volume to approximately 100 μ L, two internal recovery standards in tridecane were then added, and the extract further evaporated to final volume (10 μ L).

Eggs--

Two eggs from each dozen samples collected were combined to form a composite. The eggs were mixed with sodium sulfate and allowed to dry overnight. After drying, the powder was extracted with hexane, and the hexane/egg fat mix was fortified with the nine ^{13}C mass-labeled internal quantitation standards and slurried with 150 g of acidic silica gel for 2 hr. The remaining cleanup procedure was as described for the milk samples.

Meats (Beef, Pork, Poultry) and Fish--

All meats and fish were initially combined in equal amounts according to the composite design. The composites were then ground two to three times with dichloromethane and sodium sulfate. The dichloromethane was decanted into a round-bottom flask and the dichloromethane removed by roto-evaporation until only lipid remained. Ten (10) grams of the lipid were then dissolved in 200 mL of hexane and fortified with nine ^{13}C mass-labeled internal quantitation standards. The mixture was then processed through the cleanup procedures described previously (acid silica gel slurry, acid/neutral silica gel column, neutral alumina column, and AX-21/silica gel column).

HRGC/HRMS Analysis Procedures

The sample extracts were analyzed using a Kratos MS-50TC high resolution mass spectrometer operated at a minimum mass resolution of 10,000. The components of the sample extract were separated on a nonpolar DB-5 column (60 m x 0.25 mm). Instrumental conditions included: splitless injection; injector temperature 270°C, interface temperature 300°C; injection size 1-2 μ L; temperature program 200°C (2 min), then 5°C/min to 270°C (10 min), then 5°C/min to 330°C (5 min). HRMS parameters: accelerating voltage 8,000 V; tray current 500 μ A; electron energy, -1,800 V; source temperature 280°C; mass resolution \geq 10,000. The HRGC/HRMS determination required the monitoring of five distinct sets of ions. Each set of ions was characteristic for a specific degree of chlorination of the PCDDs and PCDFs. Each set of ions included two ions characteristic of each unlabeled and each labeled target PCDD and PCDF, an ion characteristic of a reference compound, PFK, and an ion to determine the presence of potentially overlapping chlorinated diphenyl ether interferences.

STATISTICAL DATA ANALYSIS

This section summarizes the occurrence that compounds were detected and the products used for estimating average PCDD and PCDF residue levels in each foodstuff.

Overall Results on Occurrences of Compounds in Compositing Samples--

The frequencies of detects and nondetects in the 50 composite samples, regardless of collection site, are summarized in Table 2. The table shows the number of composite samples with residue levels below ("Non Detects") or above ("Detects") the limit of detection for each of the compounds in each of the seven food categories. The last two columns show the frequencies across all foodstuff groups for both cities.

Of the target compounds, only six were not detected in any of the 50 composites. The compounds that were not detected included the PeCDF and HxCDF isomers. Also, detectable levels of 1,2,3,4,6,8,9-HpCDF were found in only one composite sample (133 pg/g in a freshwater fish composite from San Francisco). In order, the compounds with detectable levels in a number of composites are as follows:

OCDD	in 39 (78%) composites
1,2,3,4,6,7,8-HpCDD	in 36 (72%) composites
2,3,7,8-TCDF	in 23 (46%) composites
1,2,3,4,6,7,8-HpCDF	in 20 (40%) composites
1,2,3,4,7,8/1,2,3,6,7,8-HxCDD	in 14 (28%) composites
2,3,7,8-TCDD	in 12 (24%) composites
OCDF	in 7 (14%) composites
1,2,3,7,8-PeCDD	in 6 (12%) composites
1,2,3,7,8,9-HxCDD	in 6 (12%) composites
1,2,3,4,7,8,9-HpCDF	in 1 (2%) composite

Overall, freshwater fish samples were found to have the highest incidence of detectable levels of one or more compounds, and egg samples the least. In order from highest to lowest incidence of any compound at a detectable level, the foodstuffs are (1) freshwater fish, (2) saltwater fish, (3) and (4) pork and chicken, (5) and (6) beef and milk, and (7) egg. This pattern was also reflected for the foods collected in each city separately.

Overall Results on Concentration Levels--

Two approaches were taken to summarize the results in terms of actual levels (pg/g on a lipid basis). First, only those samples with levels

Table 2. Percent Detects and Nondetects Per City and Foodstuff, Across Compounds (percentage figures are based on the total number of analyses within each cell determined by a foodstuff-city combination)

	Los Angeles		San Francisco		Both Cities	
	Nondetects (%)	Detects (%)	Nondetects (%)	Detects (%)	ND ^a (%)	PQ ^b (%)
Saltwater fish	79	21	66	34	74	26
Freshwater fish	56	44	66	34	60	40
Pork	76	24	77	23	77	23
Beef	83	18	79	21	81	19
Chicken	78	23	77	23	77	23
Egg	99	1	94	6	97	3
Milk	80	20	83	17	81	19
All Foods	80	20	79	21	80	20

^a ND = Not detected.

^b PQ = Positive quantifiable.

above detection limits were included in the computations of weighted means, standard deviations, and coefficients of variation. Second, all results, above and below detection limits, were considered.

Treatment of Levels Below Detection Limit--

Limit of detection values were available whenever a sample concentration was below detection limit. These values were used when calculating statistics based on all data.

Estimated Average Concentrations of PCDDs and PCDFs in Foods

Table 3 provides the basic (weighted) statistics of concentration levels for all compounds based only on those composites with detectable levels of a particular compound. Table 3 summarizes the data across cities. Only those compounds found present in composites from both cities are reported. Thus, if a compound is not shown in a table, it was not detected in any composite samples from either city.

Tables 4 presents these same statistics based on all data. In each case, limit of detection values were used whenever a particular compound was below the detection limit.

Because of small sample sizes (five composites for Los Angeles and three for San Francisco), the average concentrations were not compared between the two cities by means of a t-test. For the same reason, upper confidence limits were not computed for concentration levels. Rather, the maximum concentration level of a given compound found in a given foodstuff category was given for each city.

As an overall summary, three tables were generated:

Table 3 lists the weighted mean concentrations for all compound and foodstuff combinations where levels were above detection limits.

Table 4 lists the same statistics as Table 3 with the exception that levels below detection limits have been replaced by the actual detection limit values. Thus all compounds are listed. However, no data were available for 1,2,3,4,7,8-HxCDF in saltwater fish because of interferences arising from an octachlorodiphenyl ether.

Table 5 which lists the maximum concentration levels of those compounds detected in at least one composite food sample, regardless of sampling site. Only 10 compounds are listed. If a compound is not shown in the first column of this table, then it was not been detected in any of the 50 composites.

Table 3. Summary of Weighted Mean Concentrations for Compounds Above the Detection in Specific Food Composites

COMPOUND (No.)	WEIGHTED MEAN CONCENTRATIONS BASED ON DETECTS ONLY											
	SALTWATER FISH	FRESHWATER FISH	PORK	REEF	CHICKEN	EGG	MILK					
	CONC. (PG/G)	CONC. (PG/G)	CONC. (PG/G)	CONC. (PG/G)	CONC. (PG/G)	CONC. (PG/G)	CONC. (PG/G)	MEAN	MEAN	MEAN	MEAN	MEAN
2378-TCDF (1)	21.96	3.19		0.99	0.67	0.10	2.74					
2378-TCDD (2)	1.13	5.59			0.78		1.46					
12378-PeCDD (5)	2.40	10.28										
123478/123678-HxCDD (10)	2.35	31.29	3.14	2.07	2.29		0.59					
123789-HxCDD (11)		16.14			3.14							
1234678-HpCDF (12)	2.21		4.26	0.84	6.51	0.59	0.70					
1234789-HpCDF (13)		133.00										
1234678-HpCDD (14)	2.31	79.29	13.05	5.68	8.97	1.76	3.11					
10CDF (15)			3.05		15.75							
10CDD (16)	13.82	510.07	76.18	9.48	34.69	11.71	4.24					

Table 4. Summary of Weighted Mean Concentrations for All PCDD and PCDF Compounds Based on All Measured Levels and Estimated Detection Limits

COMPOUND (No.)	WEIGHTED MEAN CONCENTRATIONS BASED ON ALL DATA--LOD VALUES USED FOR NONDETECTS													
	SALTWATER FISH		FRESHWATER FISH		PORK		BEEF		CHICKEN		EGG		MILK	
	CONC. (PG/B)	MEAN	CONC. (PG/B)	MEAN	CONC. (PG/B)	MEAN	CONC. (PG/B)	MEAN	CONC. (PG/B)	MEAN	CONC. (PG/B)	MEAN	CONC. (PG/B)	MEAN
2378-TCDF (1)	21.96		3.19		0.35		0.55		0.42		0.20		2.74	
2378-TCDD (2)	1.21		5.02		0.33		0.26		0.50		0.27		0.69	
12378-PCDF (3)	1.62		1.04		0.68		0.60		0.28		0.54		0.39	
23478-PCDF (4)	1.28		1.16		0.62		0.57		0.26		0.39		0.38	
12378-PCDD (5)	1.81		10.28		2.29		2.78		1.64		1.93		0.98	
123478-HxCDF (6)			4.66		1.58		0.67		0.51		1.95		0.70	
123678-HxCDF (7)	1.51		1.14		0.65		0.63		0.45		1.86		0.68	
234678-HxCDF (8)	2.91		1.35		0.77		0.75		0.54		2.43		0.82	
123789-HxCDF (9)	1.96		1.47		0.84		0.82		0.59		2.70		0.88	
123478/123678-HxCDD (10)	2.64		25.68		1.66		2.07		1.51		7.17		1.00	
123789-HxCDD (11)	2.36		13.37		1.72		2.08		1.78		3.29		1.01	
1234678-HpCDF (12)	2.05		1.33		4.04		1.37		4.86		2.85		0.87	
1234789-HpCDF (13)	2.69		26.47		2.99		1.72		1.34		4.00		1.25	
1234678-HpCDD (14)	2.08		64.70		13.05		5.38		8.17		2.71		2.86	
TCDF (15)	5.71		2.48		2.51		1.73		5.10		4.03		2.67	
TCDD (16)	13.82		510.07		76.18		9.63		31.03		12.19		3.78	

Table 5. Summary of the Maximum Concentration Levels for Compounds That Were Detected in at Least One Food Composite

COMPOUND (No.)	MAXIMUM CONCENTRATIONS OF DETECTS ONLY													
	SALTWATER FISH		FRESHWATER FISH		PORK		BEEF		CHICKEN		EGG		MILK	
	CONC. (PB/G)	MAXIMUM	CONC. (PB/G)	MAXIMUM	CONC. (PB/G)	MAXIMUM	CONC. (PB/G)	MAXIMUM	CONC. (PB/G)	MAXIMUM	CONC. (PB/G)	MAXIMUM	CONC. (PB/G)	MAXIMUM
2378-TCDF (1)	28.20	7.96					1.56		0.67		0.10			6.11
2378-TCDD (2)	1.89	9.78							1.67					1.46
12378-PeCDD (5)	2.40	23.60												
123478/123678-HxCDD (10)	3.82	84.10		3.50		3.96		2.29						0.59
123789-HxCDD (11)		38.90						4.30						
1234678-HpCDF (12)	2.21			10.60		1.15		24.60			0.59			0.70
1234789-HpCDF (13)		133.00												
1234678-HpCDD (14)	3.15	201.00		45.50		8.95		35.20			1.76			4.25
POCDF (15)				9.36				26.00						
POCDD (16)	22.70	1490.00		254.00		11.90		96.20			11.71			6.12

PCDD and PCDF Intake Via Food Consumption

Dietary intake of PCDDs and PCDFs can be estimated through consideration of the consumption of specific food products for the average person. In order to determine the dietary intake for the average Californian or U.S. citizen, several different agricultural and food organizations were contacted. These sources of reference information included the California Egg Commission, the California Beef Council, the California Milk Advisory Board, the California Pork Producers, the California Department of Food and Agriculture, the National Pork Producers Association, the National Livestock and Meat Board, and the U.S. Department of Agriculture. In most instances, the data on food consumption were traced back to the USDA information sources on national averages. Additional detail beyond the USDA estimates for regional or state usages would require conduct of specific surveys. Data generated from the USDA sources are typically based on documented production and imports divided by the total population. Table 6 provides a summary of the consumption information gathered. Some comparisons of estimates from specific California agencies are provided with the USDA statistics.

In addition to identifying the total consumption of specific food products, it is also necessary to estimate the average intake of lipophilic materials which the PCDDs and PCDFs are expected to be associated. Table 7 provides a summary of the expected lipid consumption based on specific food products. The data presented were taken from a publication of the National Livestock and Meat Board or estimated from the percentage of lipid extractable materials as determined from the laboratory procedures of this study.

Table 6. Average Annual Consumption of Food Products on a National and/or California Basis

Food product	Retail	Edible	Units/Year	Information Source
Beef	72.7	48.7	1b/person	USDA (1988)
Pork	63.1	42.3	1b/person	USDA (1988)
Chicken	64.1	44.2	1b/person	USDA (1988)
Fish	15.4	15.4	1b/person	USDA (1988)
	20	20	1b/person	California Seafood
Milk	228	228	1b/person	USDA (1987)
	228	228	1b/person	California Milk Board
Egg	243	243	eggs/person	USDA (1988)
	240	240	eggs/person	California Egg Commission

Table 7. Average Lipid Consumption Based on Specific Food Product Usage

Food Product	Lipid Consumption	Information Source
Beef	1,230 g/person/year	Breidenstein and Williams ^a
Pork	807 g/person/year	Breidenstein and Williams
Milk	~ 5,000 g/person/year	CARB Project A6-197-32.
Eggs	~ 600 g/person/year	CARB Project A6-197-32

^a "Contribution of Red Meat to the U.S. Diet," National Livestock and Meat Board.