MRI REPORT

Determination of Body Burdens of Polychlorinated Dibenzo-*p*-Dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) in California Residents

Executive Summary

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The statements and conclusions in this report are those of the contractor and not necessarily those of the State of California Air Resources Board. The material reported herein is not to be construed as actual or implied endorsement of such products.

PREFACE

This executive summary presents a synopsis of the body burden of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) for humans from 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, Ralph Propper, Project Officer.

The sampling and analysis efforts were completed under the direction of Dr. John Stanley, Ms. Kay Turman, Ms. Kathy Boggess, and Mr. Paul Cramer, with assistance from Mr. Michael McGrath, Mr. Jay Wilner, Ms. Donna Miller, and Mr. Kelly Thornburg. The statistical survey design and analysis efforts were completed under the direction of Ms. Karin Bauer with assistance from Ms. Jean Pelkey. Dr. Eli Mishuck of IWG Corporation, San Diego, California, provided valuable assistance in the recruitment of medical facilities for tissue collection in the Los Angeles area.

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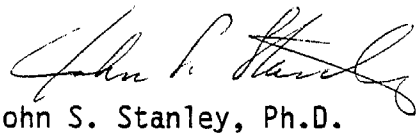

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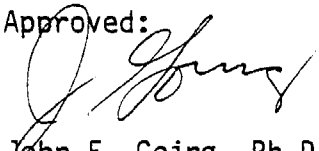

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TABLE OF CONTENTS

LIST OF TABLES

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 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 extensively 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 an increased demand for incineration as a disposal technology. The impact of the emissions released from an increasing 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 includes (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 body burdens of the 2,3,7,8-substituted PCDDs and PCDFs in California residents. 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 other studies that have focused on human exposure to these compounds. The data will also be valuable for comparison in future human monitoring programs conducted within the state of California.

The data summarized in this report were generated from the analysis of 57 human adipose tissue samples collected within the San Francisco and Los Angeles areas. The remainder of this report provides a synopsis of the survey design, analytical procedures, and the resulting statistics on PCDD and PCDF body burden levels from the study group.

SUMMARY

The research program described in this report required the collection of human adipose tissue specimens via a stratified survey design. Stratification factors included two geographical locations within California (Los Angeles and San Francisco), three age groups (12 to 34, 35 to 49, and 50+ years), and sex. This resulted in a survey design covering 12 specific strata. The initial survey design specified a total of 60 samples to be distributed among the 12 strata. A total of 57 adipose tissue specimens were collected for analysis of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The PCDDs and PCDFs of interest focused on the 2,3,7,8-substituted compounds.

Detectable levels of the specific PCDDs and PCDFs were measured in the majority of samples analyzed. The PCDD and PCDF isomer patterns detected are consistent with the incidence of these compounds detected in other studies conducted within the continental United States, North America, and Europe. Although the samples collected did not meet the full requirements of the survey design, sufficient data were available to demonstrate the prevalence of these compounds in the general California population.

None of the factors considered in the survey design--geographic location, age, and sex--were statistically significant at the 5% significance level. There is no statistically significant difference in mean concentrations between cities, between sexes, or among age groups at the 95% confidence level, regardless of whether the data are analyzed in the individual design cells or in combinations of these cells.

The data presented herein provide a preliminary estimate of the body burden levels of PCDDs and PCDFs in the California population. In order to fully assess (or measure) the impacts that specific contaminant sources might have on body burden levels of these compounds, it will be necessary to drastically increase the number of individual data points. This will be necessary in order to detect initial differences arising from exposure to these contaminants.

If additional monitoring efforts are anticipated, it is recommended that a rigorous and consistent sample collection and analysis program be initiated. Such a program must anticipate the importance of the participation of a long-term collection facility, the development of the necessary quality control samples to demonstrate long-term accuracy and precision, and a detailed study design.

As a recommendation focused on the study reported in this document, it should be noted that the concentration data have been analyzed for each compound individually. However, correlations between compounds exist and have not been considered here. Relationships of geographic location, age groups, or sex, and the levels of all detected compounds should be investigated by means of a multivariate analysis approach. The results from a series of principal component analyses and cluster analyses could possibly indicate some clustering of samples when considering all compounds simultaneously.

APPROACH

Survey Design

A total of 57 adipose tissue specimens were obtained from donors in California. These were stratified by city (Los Angeles and San Francisco), by sex, and by age group (12 to 34 years, 35 to 49 years, and 50 and above). The survey design developed at the beginning of the project specified quotas in the 12 cells defined by the three stratification variables with a target sample size of 60 samples. The allocation and quotas are summarized in Table 1 below.

Table 1. Planned Survey Design for the PCDD/PCDF Body Burden Study

	Los Angeles		San Francisco	
	Male	Female	Male	Female
12-34 years	6	6	2	2
35-49 years	7	7	4	4
50 and above	7	7	4	4
Total	20	20	10	10

Table 2 summarizes the distribution of the 57 residents from which samples were obtained across the three stratification factors through the collection process.

Table 2. Survey Results for the PCDD/PCDF Body Burden Study

	Los Angeles		San Francisco	
	Male	Female	Male	Female
12-34 years	5	1	0	1
35-49 years	3	4	3	10
50 and above	12	6	6	6
Total	20	11	9	17
% within city	65	35	35	65

Analysis Procedures

All samples were prepared such that approximately 5 to 10 g of adipose tissue 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 Adipose Tissue--

After the adipose tissue samples were weighed and a known amount of a series of nine ^{13}C-labeled internal quantitation standards was added, the tissues were ground in the presence of methylene chloride. The adipose matrix was extracted with three successive aliquots of methylene chloride. The extract was dried by eluting through anhydrous sodium sulfate, and the extract was adjusted to a final volume. A known aliquot was removed to determine extractable lipid, and the remaining extract was solvent-exchanged to hexane.

The hexane fat extract was subsequently subjected to an acidic silica gel slurry cleanup procedure. Specifically, 100 g of 40% sulfuric acid-impregnated silica gel was mixed with the hexane/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 was 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 Carbopak C on Celite. (Sample batch 1 was chromatographed in a column consisting of Amoco AX-21 in 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 µL, two internal recovery standards in tridecane were then added, and the extract further evaporated to final volume (10 µL).

HRGC/HRMS Analysis Procedures

The sample extracts were analyzed using either a Kratos MS-50TC or a VG 70 250S 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 µL; 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 µA; 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.

RESULTS

This section summarizes the occurrence that compounds were detected and the estimated average PCDD and PCDF body burden levels in California residents.

Overall Results on Occurrences of Compounds in Adipose Samples

A total of 57 specimens were collected, of which 49 have been included in the statistical evaluation reported herein. The 49 specimens were analyzed in batches 1 to 4, with the following allocation of specimens to batches.

Batch 1: 18 Los Angeles specimens
Batch 2: 9 Los Angeles and 7 San Francisco specimens
Batch 3: 6 San Francisco specimens
Batch 4: 2 Los Angeles and 7 San Francisco specimens
Batch 5: 2 Los Angeles and 6 San Francisco specimens (results not included in statistical data evaluation)

It is anticipated that the results from the eight specimens from batch 5 will not affect overall concentration levels. However, they will increase overall sample sizes and thus slightly reduce the confidence intervals of the mean concentrations.

Ideally, the specimens would have all been collected first, then randomly assigned to the batches to circumvent a potential confounding effect of the batch and city factors. Because of collection difficulties, however, the samples were analyzed as they arrived, resulting in the above assignment. This potential problem has been investigated throughout the statistical analyses performed on the concentration data.

Table 3 presents the overall results on the occurrences of compounds in the individual samples. As demonstrated in Table 3, the distribution of nondetects and detects varied across cities. The higher occurrence of nondetects in the Los Angeles samples versus the San Francisco samples is mostly due to a high incidence of nondetects in the first sample batch. This variation is attributed to slight differences in the analytical procedure and instrumentation and does not reflect a difference based on geographic location. The compounds, ordered by most frequent occurrence, correspond to the compounds related to human body burdens of PCDDs and PCDFs, as often cited in other studies.

Statistical Analysis of Concentration Data

The objective of the statistical analysis of the levels of the compounds was twofold. First, determine whether the levels between Los Angeles and

Table 3. Overall Results on Occurrences of Compounds in 49 Specimens, by City and Across Both Cities (ND = not detected, PQ = positive quantifiable)

		LOS ANGELES (29 samples)				SAN FRANCISCO (20 samples)				BOTH CITIES (49 samples)			
Compound	No.	ND	PQ	%ND	%PQ	ND	PQ	%ND	%PQ	ND	PQ	%ND	%PQ
2378-TCDF *1	1	12	17	41%	59%	1	18	5%	95%	13	35	27%	73%
2378-TCDD *2	2	4	25	14%	86%	0	19	0%	100%	4	44	8%	92%
12378-PeCDF	3	25	4	86%	14%	15	5	75%	25%	40	9	82%	18%
23478-PeCDF	4	4	25	14%	86%	3	17	15%	85%	7	42	14%	86%
12378-PeCDD	5	4	25	14%	86%	0	20	0%	100%	4	45	8%	92%
123478-HxCDF	6	8	21	28%	72%	0	20	0%	100%	8	41	16%	84%
123678-HxCDF	7	12	17	41%	59%	0	20	0%	100%	12	37	24%	76%
234678-HxCDF	8	15	14	52%	48%	2	18	10%	90%	17	32	35%	65%
123789-HxCDF	9	27	2	93%	7%	17	3	85%	15%	44	5	90%	10%
123478/123678-HxCDD	10	0	29	0%	100%	0	20	0%	100%	0	49	0%	100%
123789-HxCDD	11	6	23	21%	79%	0	20	0%	100%	6	43	12%	88%
1234678-HpCDF *3	12	10	17	37%	63%	0	20	0%	100%	10	37	21%	79%
1234789-HpCDF	13	27	2	93%	7%	14	6	70%	30%	41	8	84%	16%
1234678-HpCDD	14	0	29	0%	100%	0	20	0%	100%	0	49	0%	100%
OCDF	15	18	11	62%	38%	3	17	15%	85%	21	28	43%	57%
OCDD	16	0	29	0%	100%	0	20	0%	100%	0	49	0%	100%

*1: 2378-TCDF concentration was not computed for one San Francisco sample

*2: 2378-TCDD concentration was not computed for one San Francisco sample

*3: Interference in analysis could not be removed for two Los Angeles samples

San Francisco, between male and female, and among age groups are significantly different. Then, calculate the average concentration levels and their confidence intervals for the 12 design cells determined by the survey design. If some or all factors (city, sex, or age) are found to be nonsignificant, then the cells can be collapsed and statistics computed across larger cells.

The concentration data were log-transformed and analyzed by analysis of variance. The statistics presented in subsequent tables are in the original scale, i.e., in pg/g. After removal of the batch effect, none of the factors considered in the survey design was significant at the 5% significance level. Thus there is no statistically significant difference in mean concentration levels between cities, between sexes, or between age groups at the 95% confidence level, regardless of the level of detail included in the analysis.

Table 4 presents the most condensed format for presentation of the data, which is simple concentration statistics generated from all 49 samples (batches 1 through 4) by specific compound. The statistics presented in Table 4 include the number of detects per number of samples analyzed, minimum and maximum detected levels, the geometric means, and the upper and lower 95% confidence limits of the geometric means. All statistics are based on detected concentration levels only.

Table 4. Concentration Statistics by Compound

Compound (No.)	Number of detects/ number of specimens	Minimum detected level (pg/g)	Lower 95% limit of geometric mean (pg/g)	Geometric mean of detected levels (pg/g)	Upper 95% limit of geometric mean (pg/g)	Maximum detected level (pg/g)
2378-TCDF (1)	35 of 48	0.634	1.49	1.79	2.14	6.49
2378-TCDD (2)	44 of 48	1.80	3.69	4.22	4.83	12.5
12378-PeCDF (3)	9 of 49	0.257	0.303	0.435	0.625	0.924
23478-PeCDF (4)	42 of 49	0.726	4.29	5.43	6.89	25.3
12378-PeCDD (5)	45 of 49	1.98	8.20	9.64	11.3	39.1
123478-HxCDF (6)	41 of 49	1.69	5.60	6.53	7.61	17.3
123678-HxCDF (7)	37 of 49	1.83	3.54	4.10	4.75	12.7
234678-HxCDF (8)	32 of 49	0.528	1.13	1.47	1.90	10.5
123789-HxCDF (9)	5 of 49	0.275	0.329	0.586	1.04	0.942
123478/123678 HxCDD (10)	49 of 49	15.6	55.9	64.6	74.6	164
123789-HxCDD (11)	43 of 49	4.47	7.87	9.14	10.6	31.7
1234678-HpCDF (12)	37 of 47	4.08	7.30	8.38	9.63	25.8
1234789-HpCDF (13)	8 of 49	0.236	0.314	0.445	0.630	0.743
1234678-HpCDD (14)	49 of 49	3.17	52.7	65.9	82.4	334
OCDF (15)	28 of 49	0.275	0.639	0.735	0.846	1.37
OCDD (16)	49 of 49	137	424	494	576	1,320

Note: Significant figures for each compound are limited to 3