Organohalogen body burdens in a breast cancer case-control study

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Introduction

Due to their lipophilic properties, dioxins (PCDD/PCDFs) and other organohalogen compounds bioaccumulate in the food chain, with diet accounting for over 90% of non-occupational exposures. To date, few epidemiologic studies have examined the relationship between dioxins and breast cancer in human populations. Most have examined risks in occupational cohorts[1-5] or in populations exposed to dioxins from the Seveso accident[6-8]. Results from these studies have been conflicting and have largely been limited by a lack of individual-level measures of exposure, small numbers of cases, and inability to account for established breast cancer risk factors. Very little is known about the potential health effects of low-level environmental dioxin contamination. We present data on PCDD/PCDFs, organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) in adipose tissues of women participating in a breast cancer case-control study centered in the San Francisco Bay Area. In addition, we examine distributions of these chemicals in breast and abdominal adipose of women undergoing mastectomies with concurrent breast reconstruction. If concentrations were equivalent, use of abdominal adipose would greatly enhance the pool for controls for future epidemiological studies.

Methods and Materials

Study populations. Women undergoing surgical breast biopsies, lumpectomies or mastectomies at two hospitals in the San Francisco Bay Area of California were recruited in the study. Small amounts of breast adipose tissue were collected during surgery and women were interviewed regarding demographics, exposures, medical and reproductive histories. The case control analysis was based on 79 women diagnosed with invasive breast cancer and 52 controls diagnosed with benign breast conditions. To study the distribution of chemicals in breast and abdominal adipose, we focused on a subset of women (n=21) undergoing mastectomies with simultaneous breast

reconstruction using abdominal tissue. Most were cases, although one with benign disease and three with ductal carcinoma in situ were also included.

Sample Analysis. Samples were stored at -20 ^{o}C until analysis. Samples were thawed, weighed, mixed with Na_2SO_4, homogenized with 1:1 dichloromethane:hexane, and spiked with ^{13}C-labeled internal standards (all seventeen 2,3,7,8-PCDD / PCDFs; PCBs #77, 126, 169, 28, 52, 47, 101, 105, 118, 153, 180, 194, 209; HCB, β-HCH, DDE, DDT, Dieldrin, Mirex and PBDE 77). Approximately 1/10 of the extract was analysed for OCPs, PCBs and PBDEs, and 9/10 analysed for PCDD/Fs and coplanar PCBs. Lipid content was determined gravimetrically in an aliquot of the extract. Samples were serially processed through columns containing Na_2SO_4 and AX21 Carbon. The first fraction off the carbon column was further cleaned up by GPC and Florisil, recovery standards were added and the sample concentrated to 10 μL for PCB, OCP and PBDE analysis. PCDD/Fs and coplanar PCBs were eluted from the carbon column with toluene and the eluate cleaned up with alumina and acid silica columns; recovery standards were added and the sample concentrated to 10 μL.

PCDD/Fs and PCBs were analyzed by HRGC/HRMS (Finnigan MAT 90) with a 60m, 0.25 mm ID, 0.25 μm film thickness, DB-5ms column. PFK was used for the lock masses and the MS was operated in an EI mode with multiple ion monitoring. OCPs and PBDEs were analyzed by LRMS in ECNI mode (Finnigan 4510) with a 60m, 0.25 mm ID, 0.25 μm film thickness, DB5ms column, with methane as the reagent gas. The ion source pressure was 0.6 Torr and ion source temperature was 100 ^{o}C. The electron energy was typically 70eV and the electron current was kept at 0.3 mA.

Results and Discussion

All results were expressed on a lipid basis. Because of the small size of the adipose samples, and their often low lipid content, many PCDD/PCDF congeners were below the detection limit (DL). For those congeners, half the DL was used to calculate I-TEQs[9]. In addition, a new summary measure (Adjusted TEQ) was devised incorporating only those congeners that were consistently measured above the DL. The eight congeners that comprised the Adj-TEQ are shown in Table 1. The conventional I-TEQs correlated well with the Adj-TEQs (R^2=0.98, $p<0.0001$) and, therefore, Adj-TEQs were used in statistical analyses to minimize uncertainties.

Major OCP and PCDD/PCDF concentrations are shown in Table 1 for cases and controls. Patterns and levels were, in general, similar to those reported from other industrialized regions.

TEQs correlated significantly with most PCBs and with HCB and β-HCH, but not with other OCPs. Most OCPs correlated with each other, while DDE and trans-nonachlor also correlated with many PCB congeners. Age, country of birth, parity and breastfeeding history were examined as predictors of body burdens. The data suggest surrogate markers of exposure that may optimize future studies.

Unconditional logistic regression was used to calculate age- and race- adjusted exposure-specific odds ratios (ORs) and 95% confidence intervals (95% CI) for each individual PCDD/PCDF congener as well as for the summary measures (I-TEQ, Adj-TEQ). None of the odds ratios for any of the congeners or summary measures were significantly different from one. One notable

exception was OCDD for which the odds ratio for the second and third tertiles appeared modestly elevated (OR=1.22 95% CI:0.47 – 3.16 and OR=1.62, 95% CI:0.64 – 6.12, respectively). The confidence intervals, however, included one and the test for trend was not significant. Additional models, adjusting for breastfeeding history did not substantially change the risk estimates. When the data were stratified by race/ethnicity, there was a statistically significant increase in breast cancer risk associated with increasing levels of OCDD only among non-whites (OR=4.73, 95% CI: 0.35 – 65.70, OR=51.28, 95% CI: 2.60 – 999.99, for the second and third tertiles of OCDD exposure compared to the lowest tertile, p(trend)=0.02). These results, however, were based on very small numbers (n=28). In contrast, the OCDD odds ratios among whites (n=91), were below one and the confidence intervals included the null.

To our knowledge, this is only the second case-control study designed to examine breast cancer risk and body burdens in women with no known occupational or accidental exposures. A Swedish study among 22 cases of invasive breast cancer and 19 controls with benign breast conditions, found no association with adipose levels of 17 PCDD/PCDF congeners and breast cancer risk[10]. Similar to our results, however, the authors noted a suggestive association between breast cancer risk and OCDD levels[10].

We used intra-class correlation and the Wilcoxon test for the paired analyses (Table 2) of breast and abdominal tissues from 21 women. Results showed, with some notable exceptions, no bias and no statistically significant differences in concentrations[11]. This finding allows future studies to use either abdominal or breast adipose tissue for measurements of lipophilic chemicals, greatly enhancing selection of subjects[11].

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Table 1. Distribution of selected OCPs (ng/g lipid) and PCDD/PCDF congeners (pg/g lipid) among cases and controls.

	Cases						Controls					
	n	**Mean**	**SD**	**Med**	**Min**	**Max**	**n**	**Mean**	**SD**	**Med**	**Min**	**Max**
AGE (yrs)	71	52.7	10.7	51	34	85	51	46	9	45	28	69
LIPID (%)	69	80.5	15.1	84.3	20.7	96	52	67	21	76	10	97
OCPs (ng/g lipid)												
DDE	69	1,090	930	850	120	4,910	51	844	527	720	169	3,290
t-NONACHLOR	69	109	112	88	23	690	51	122	114	85	10	560
OXYCHLORDANE	68	56	45	48	8	340	51	58	45	44	6	260
DDT	60	44	39	33	2	220	41	45	43	34	8	260
β-HCH	65	71	89	50	0.5	623	46	42	43	31	1	216
HCB	69	48	28	40	14	190	51	43	28	34	9	170
DIELDRIN	69	30	16	25	8	90	50	34	33	27	5	230
PCDD/F (pg/g lipid)												
2378TCDD	64	5	4	3	0.2	19	51	4	3	3	0.2	20
12378PeCDD	67	11	16	7	0.3	123	48	7	7	5	0.4	25
123678HxCDD	67	65	48	51	6.5	232	53	58	29	55	1.9	179
1234678HpCDD	66	82	67	61	1.3	334	53	66	40	58	1.4	198
OCDD	67	580	542	409	30	3,290	53	482	462	358	1.7	3,230
23478PeCDF	67	12	13	9	3.1	100	53	10	5	8	1	26
123478HxCDF	62	7	13	5	1.6	103	52	6	6	4	0.4	48
123678HxCDF	63	6	13	4	0.5	103	52	4	2	3	0.4	13
I-TEQ	67	28	29	19	7.3	221	53	22	12	19	3.5	60

Table 2. Tests to assess agreement between Abdominal and Breast measurements of major analytes (Interclass Correlation coefficient and Wilcoxon signed rank test) and linear regression to predict Breast concentrations from Abdominal concentrations.

	Agreement between measurements		Breast = a + b × Abdominal		
	Interclass Correlation Coefficient	Wilcoxon Signed Rank Test for (Ho: Abdominal = Breast)	intercept (a)	slope (b)	R^2
I-TEQ	0.843	**Reject**	2.053	0.844	0.731
2378TCDD	0.471	Accept	1.913*	0.448	0.238
12378PeCDD	0.426	**Reject**	1.478	0.674	0.275
123678HxCDD	0.930	Accept	3.614	0.921	0.867
1234678HpCDD	0.976	Accept	0.584	1.049	0.957
OCDD	0.968	Accept	13.20	0.968	0.936
23478TCDF	0.942	Accept	0.372	0.926	0.894
123478PeCDF	0.837	Accept	0.902	0.821	0.708
123678HxCDF	0.824	Accept	0.801	0.764	0.685
1234678HpCDF	0.763	Accept	0.527	0.968	0.614
DDE	0.934	Accept	167.0	0.927	0.885
t-Nonachlor	0.671	Accept	49.26	0.485	0.538
Oxychlordane	0.804	Accept	18.50	0.522	0.743
DDT	0.915	Accept	-4.360	1.222	0.904
β-HCH	0.978	Accept	9.813	0.883	0.971
HCB	0.987	Accept	2.911	1.005	0.981
Dieldrin	0.981	Accept	0.353	0.977	0.964
PBDE-47	0.987	Accept	-0.923	1.024	0.976
PBDE-99	0.970	Accept	0.308	0.940	0.942
PBDE-100	0.996	Accept	-0.543	1.015	0.992
PBDE-153	0.901	Accept	1.870	0.649	0.997
PBDE-154	0.632	Accept	3.880	0.565	0.410
PCB-153	0.878	Accept	-0.263	0.920	0.790
PCB-138	0.884	Accept	11.11	0.896	0.781
PCB-180	0.800	Accept	32.81	0.649	0.697
PCB-118	0.906	Accept	4.685	0.820	0.834

* All intercepts are not significantly different from 0 ($p > 0.05$), except fo