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Adipose levels of dioxins and risk of breast cancer

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Abstract

Objective: Our objective was to evaluate the breast cancer risk associated with body burden levels of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs).
Methods: We conducted a hospital-based case-control study among 79 women diagnosed with invasive breast cancer and 52 controls diagnosed with benign breast conditions. We collected breast adipose tissue and analyzed it for all 17 2,3,7,8-substitituted PCDD/PCDFs. We used unconditional logistic regression to calculate age- and race-adjusted exposure-specific odds ratios (ORs) and 95% confidence intervals (CI) for each individual PCDD/PCDF congener as well as for the summary measures (I-TEQ, Adj-TEQ).
Results: Dioxin levels were consistent with reports from other small, contemporary studies of body burdens in the U.S. None of the odds ratios for any of the congeners or summary measures differed significantly from one. Especially for the PCDF congeners, point estimates tended to be below one. One notable exception was octachlorodibenzo-*p*-dioxin (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: 0.64–4.12, respectively), though the test for trend was not significant ($p = 0.36$).
Conclusion: Breast cancer risk was not associated with adipose levels of PCDD/PCDFs. More study is suggested among women of color who may have higher body burden levels of these compounds.

Abbreviations: 1234678-HpCDD – 1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin; 1234678-HpCDF – 1,2,3,4,6,7,8-Heptachlorodibenzofuran; 123478-HxCDF – 1,2,3,4,7,8-Hexachlorodibenzofuran; 123678-HxCDD – 1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin; 123678-HxCDF – 1,2,3,6,7,8-Hexachlorodibenzofuran; 12346789-OCDD –1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin; OCDD – 1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin; 12378-PeCDD – 1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin; 23478-PeCDF – 2,3,4,7,8-Pentachlorodibenzofuran; 2378-TCDD – 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; TCDD – Tetrachlorodibenzo-*p*-dioxin; Adj-TEQ – Adjusted Toxic Equivalents (congeners below detection are omitted); β – beta; °C – degrees celsius; CI – confidence interval; CDTSC – California Department of Toxic Substances Control; DL – detection limit; HML – Hazardous Materials Laboratory; IARC – International Agency for Research on Cancer; I-TEQ – International Toxic Equivalents; m – meter; mm – millimeter; Na_2SO_4 – sodium sulfate; OR – odds ratio; PBDEs – polybrominated diphenyl ethers ; PCBs – polychlorinated biphenyls; PCDDs – polychlorinated dibenzo-*p*-dioxins; PCDFs – polychlorinated dibenzofurans; pg/g – picograms per gram – 10^{-12}; SMR – standardized mortality ratio; SWHS – Seveso Women's Health Study; TEQ – Toxic Equivalents; U.S. – United States; μL – microliter; μm – micrometer

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Introduction

With approximately half of all breast cancer cases not explained by known risk factors [1], considerable interest has recently focused on potential environmental influences [2–26]. Dioxins (polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans or PCDD and PCDFs) are ubiquitous environmental contaminants. Formed as unintentional by-products of industrial or thermal processes [27], dioxins are lipophilic, persist in the environment and bioaccumulate in human tissues [28, 29]. Of the 211 members (congeners) of these classes, 17 are considered toxic due to their stereochemical configuration caused by the position of chlorine atoms in the molecule (2,3,7,8–substitution). Toxicity varies among the 17 congeners with the degree of chlorination, with 2378-tetrachlorodibenzo-*p*-dioxin (TCDD) and 23478-pentachlorodibenzo-*p*-dioxin (PeCDD) having 10,000-fold higher toxicity than the 12346789-octachlorodibenzo-*p*-dioxin (OCDD) and the 12346789-octachlorodibenzofuran (OCDF). The sum of the 17 congeners, weighted by their respective toxicity factors, is used to express the toxic equivalents (TEQ). Many congeners are potent animal carcinogens, shown to disrupt endocrine pathways [27, 30, 31] and to possess anti-estrogenic activity [32–35]. TCDD, the most widely studied compound in this class, was recently classified as a known human carcinogen by the International Agency for Research on Cancer (IARC) [30]. Despite this classification, the health effects associated with dioxin exposures in humans, especially in women, have not been fully characterized.

To date, few epidemiologic studies have examined the relationship between dioxins and breast cancer in human populations. Most of these have examined risks in occupational cohorts [36–40] or in populations exposed to dioxins from large-scale industrial environmental releases [41–45]. Results from these studies have been conflicting and largely limited by small case numbers, a lack of individual-level exposure measures and an inability to account for established breast cancer risk factors.

The results presented here are from a hospital-based, case-control analysis of organochlorine levels measured in the breast adipose of women who underwent surgical breast biopsies in the San Francisco Bay Area of California, United States (U.S.). Included in this analysis are measurements on all 17 2,3,7,8-substituted dioxins and furans.

Materials and methods

Study population

We recruited study subjects from among women undergoing surgical breast biopsies, lumpectomies or mastectomies at three hospitals in the San Francisco Bay Area, during the mid-1990s. Cases were women with histologically confirmed invasive breast cancer and controls were women diagnosed with benign histological changes. Because of the strong association of atypical hyperplasia and carcinoma *in situ* with subsequent occurrence of breast cancer, we excluded patients with these conditions from the control group. Additionally, we excluded women with previous (non-skin) cancer diagnoses and women who reported receiving previous radiation or Tamoxifen therapy from both the case and control groups. Participants filled out a self-administered dietary questionnaire, completed an in-person interview and allowed their surgeons to extract a small amount of breast adipose tissue during their diagnostic biopsy or surgical treatment. We obtained tumor estrogen- and progesterone-receptor status from the women's medical record. All study-eligible women signed a form giving their consent to participate in the study and to allow study staff access to their medical records. This project was reviewed and approved by the California Health and Human Services Agency Committee for the Protection of Human Subjects and by the Institutional Review Boards (IRBs) of the three participating hospitals and of the U.S. Army Medical Research and Materiel Command; all IRBs judged the study protocol to be in compliance with their ethical standards as well as with the U.S. Code of Federal Regulations, Title 45, Part 46 on the Protection of Human Subjects.

Specimen collection and laboratory methods

For women undergoing surgical breast biopsy or wide local excision (lumpectomy), participating study surgeons obtained about one gram of breast adipose tissue from beyond the edges of the biopsy or excision cavity. For women undergoing mastectomy, the surgeons obtained similar amounts of breast adipose tissue from a site distant from the tumor in order not to interfere with pathologic analysis. The samples of adipose tissue were immediately placed in chemically clean glass jars with Teflon-lined screw caps and labeled with a number, with no other identifiers. Samples were frozen to below

−20 °C and transported to the California Department of Toxic Substances Control (CDTSC), Hazardous Materials Laboratory (HML) in Berkeley, California, where they were stored at −20 °C until analysis.

We analyzed samples for all 17 2,3,7,8-substituted dioxins and furans (PCDD/PCDFs) as described earlier [46]. Samples were thawed, weighed, mixed with sodium sulfate (Na_2SO_4), homogenized with dichloromethane/hexane (1:1) and spiked with ^{13}C-labeled internal standards. We processed approximately nine-tenths of the extract for PCDD/PCDF analysis, while the rest was analyzed for PCBs, polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (data not shown). We serially processed samples through columns containing Na_2SO_4 and AX21 Carbon. We eluted PCDD/PCDFs from the carbon column with toluene and cleaned up the eluate through alumina and acid silica columns; ^{13}C-labeled recovery standards were added and the final extract concentrated to 10 μl.

We analyzed PCDD/PCDFs by high-resolution gas chromatography/high resolution mass spectrometry (Finnigan MAT 90, San Jose, CA) with a DB-5 ms column (60 m, 0.25 mm inner diameter, 0.25 μm film thickness; J&W Scientific, Folsom, CA). We analyzed samples in batches of six. Most batches consisted of three cases and three controls of similar ages. Lab staff were blinded to case status. In addition to the six samples, each batch contained a reagent blank. We performed duplicate analyses and incorporated laboratory control samples (SRM 1945, whale blubber, National Institute of Standards and Testing, Gaithersburg, MD) in the analysis. The relative percent difference (RPD) of duplicate analyses provided a measure of precision. Measurements of duplicate samples on average differed by 11.4% with RPDs ranging from 5.0% for 123678-HxCDD to 23.8% for 1234578-HpCDD [47]. We determined lipid content gravimetrically in an aliquot of the extract and results were expressed as picograms per gram (pg/g) lipid to adjust for differences in fatty tissue composition of the samples.

Statistical methods

We compared the characteristics of cases and controls by constructing frequency distributions and calculating Pearson chi-square statistics to test for differences by case status. Where appropriate, we calculated *p*-values for trend from the Mantel chi-square [48]. To evaluate the differences in congener distributions across categories of age and race/ethnicity, we calculated *p*-values from the Kruskal–Wallis and Wilcoxon Rank Sum tests [49], respectively.

In addition to the 17 individual 2,3,7,8-substituted congeners, we calculated the International Toxic Equivalents (I-TEQs) [50]. We also devised a new summary measure, the adjusted TEQ (Adj-TEQ), incorporating only those nine congeners reported here that were consistently measured above the detection limit (DL) in this study. Because of the small size of the tissue samples and their often low lipid content (ranging from 97% to less than 10%, with a mean of 67%), some PCDD/PCDF congeners were below the DL. For those congeners, we used half the DL to calculate I-TEQs.

To compare the chemical distributions between cases and controls we used the Wilcoxon Rank Sum test. We used unconditional logistic regression models [48] to estimate exposure-specific odds ratios (OR) and 95% confidence intervals (CI), adjusted for four categories of age (<40, 40–49, 50–59 and ≥ 60 years) and two categories of race/ethnicity (non-Hispanic white and 'other'). Subsequent models also adjusted for lactation history. We attempted to adjust the models for a variety of other potential confounding factors but, due to small numbers, many of the models failed to converge. Due to colinearity, we considered each PCDD/PCDF congener and summary measure (I-TEQ, Adj-TEQ) in a separate model. We examined exposure categories in tertiles, based on the distribution among the controls, with the lowest tertile serving as the referent category for calculating odds ratios. To test for trend, we entered each PCDD/PCDF congener into a logistic model as a continuous variable and the *p*-value for the β coefficient was evaluated. Previous studies of organochlorine exposures and breast cancer have suggested that the breast cancer risk related to some of these chemicals may be limited to certain subpopulations [2, 5, 11, 13, 51]. To evaluate potential effect modifications in our data, we repeated these analyses, stratifying by race/ethnicity, parity, lactation history, menopausal status and tumor estrogen receptor status.

All analyses were performed in SAS 8.2 [52].

Results

The study initially identified 201 women as potentially eligible participants. Of these, 131 (79 cases, 52 controls) provided a breast adipose specimen, completed the interview and dietary questionnaire, and met all the inclusion criteria of the study. Participants were excluded for the following reasons: 12 had a previous cancer; eight were diagnosed with atypical hyperplasia; 19 were diagnosed with ductal carcinoma *in situ*; two

were not proficient in English; 12 refused the interview or were lost to follow-up; and 17 had specimens that were too small for analysis. The demographic characteristics of those who initially agreed to participate (n = 201) and those who were eventually included in the present analysis (n = 131) did not differ (data not shown).

Table 1 presents the distribution of selected characteristics by case status. Both cases and controls were predominantly non-Hispanic white and, in general, had high family incomes. Cases were more likely than controls to be older (mean difference in age = 7 years, $p < 0.001$) and post-menopausal (55% of cases post-menopausal, 35% of controls post-menopausal,

Table 1. Distribution of selected characteristics of breast cancer cases (n = 79) and benign breast controls (n = 52) with valid PCDD/PCDF data with *p*-values for the Pearson chi-square (χ^2)

	Cases (n = 79)[a] n (%)	Controls (n = 52)[a] n (%)	*p*-value (χ^2)
Age group			
< 40 years	5 (6)	10 (19)	
40–49 years	29 (37)	27 (52)	
50–59 years	27 (34)	11 (21)	
≥60 years	18 (23)	4 (8)	< 0.001[b]
Race/ethnicity			
Non-Hispanic white	56 (71)	41 (79)	
Hispanic	5 (6)	4 (8)	
Black	4 (5)	4 (8)	
Asian/Pacific Islander	9 (12)	0 (0)	
Other	5 (6)	3 (5)	0.16
Family income (annual)			
< $50,000	20 (29)	10 (19)	
$50,000–$99,999	21 (30)	22 (42)	
≥$100,000	29 (41)	20 (39)	0.66[b]
Age at Menarche			
≤12 years	41 (52)	30 (58)	
> 12 years	38 (48)	22 (42)	0.51
Parity			
Parous	59 (75)	34 (67)	
Nulliparous	20 (25)	17 (33)	0.32
Age at first live birth[c]			
< 30 years	47 (81)	24 (71)	
≥30years	11 (19)	10 (29)	0.25
Lifetime duration of lactation[c]			
0 months	15 (26)	4 (12)	
1–5 months	16 (26)	7 (21)	
6–11 months	13 (22)	10 (29)	
≥12 months	15 (26)	13 (38)	0.05[b]
Menopausal status			
Post-menopausal	42 (55)	17 (35)	
Pre-menopausal	35 (45)	32 (65)	0.03
Oral contraceptive use			
Ever for ≥6 months	55 (70)	42 (81)	
Never for ≥6 months	23 (30)	10 (19)	0.19
Hormone replacement therapy			
Yes	36 (47)	19 (36)	
No	41 (53)	33 (64)	0.25

[a] Numbers do not add up to totals due to missing data for some women.
[b] *p*-value for the Mantel's test for trend.
[c] Among parous women.

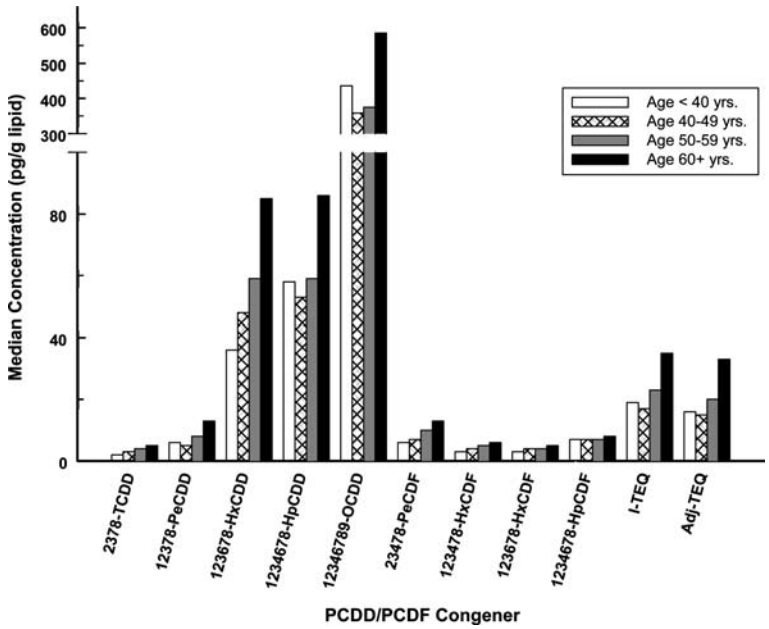

Fig. 1. PCDD/PCDF median concentrations by age group. Distributions significantly vary by age group (Kruskal–Wallis p-value < 0.05) for all congeners except 1234678-HpCDD ($p = 0.06$), 12346789-OCDD ($p = 0.23$), and 1234678-HpCDF ($p = 0.45$).

$p = 0.03$). Compared to controls, cases had shorter lactation histories ($p = 0.05$). The samples from post-menopausal women had significantly higher fat content than those from pre-menopausal women (data not shown).

Figure 1 displays the median PCDD/PCDFs levels across categories of age. With the exception of OCDD, 1234678-HpCDF, and 1234678-HpCDD, the concentrations of each congener significantly varied with age ($p < 0.05$) with levels generally increasing with greater age. The distribution of PCDD/PCDFs among non-Hispanic whites *versus* women of color (*i.e.*, the 'other' race/ethnicity category) are shown in Table 2. The 'other' category is comprised of Hispanics, blacks, Asian/Pacific Islanders and women who classified themselves as American Indian/Aleutian or Eskimo, mixed race/ethnicity or some other race/ethnicity. Generally, PCDD/PCDF concentrations were more consistently above the detection limits and higher among women in the 'other' race/ethnicity category than among the non-Hispanic white women, although differences were only statistically significant for 12378-PeCDD and 123478-HxCDF.

Table 3 summarizes the distribution of chemicals by case status. Wilcoxon Rank Sum tests indicated no significant differences between the two groups. Age- and race- adjusted exposure-specific odds ratios and 95% confidence intervals are presented in Table 4. No odds ratios for any of the PCDD/PCDFs were significantly different from one and, in general, point estimates tended to be below one, especially for the PCDF congeners. 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: 0.64–4.12, respectively). The confidence intervals, however, were wide and the test for trend was not significant ($p = 0.36$). Further adjustment for lactation history did not substantially change the patterns of risk estimates (data not shown).

To evaluate potential effect modifications in the data, we conducted several subgroup analyses, adjusting for age and stratifying by menopausal status, parity, race/ethnicity, lactation history and tumor estrogen receptor status of cases. Due to small numbers, these analyses generated highly unstable risk estimates with extremely wide confidence intervals and models in some strata failed to converge. With the possible exception of race/ethnicity, these analyses did not reveal substantially different patterns of risk between any of the subgroups examined (data not shown). There was a provocative suggestion that the elevated risk for OCDD was being driven by an elevated risk among women in the 'other' race/ethnicity category since the risk estimates for this compound among non-Hispanic whites were below one (data not shown). These analyses, however, were based on very small numbers.

Table 2. Distribution of selected PCDD/PCDF congeners (concentrations in pg/g lipid) by race/ethnicity

Congener	Non-Hispanic whites					Others[a]					*p*-Value[c]
	n[b]	% >DL	Med	Min	Max	n[b]	% >DL	Med	Min	Max	
2378-TCDD	92	65	3	0.2	20	34	82	4	0.4	19	0.24
12378-PeCDD	92	65	6	0.3	37	34	85	10	0.4	123	0.03
123678-HxCDD	97	99	55	6.5	205	34	100	54	20.6	189	0.84
1234678-HpCDD	97	99	58	1.3	334	34	100	64	30.8	220	0.07
12346789-OCDD	91	100	388	29.5	3234	28	100	428	161.0	3293	0.31
23478-PeCDF	97	95	9	2.0	28	34	100	7	3.8	100	0.20
123478-HxCDF	91	78	4	1.1	21	33	97	5	2.1	103	0.05
123678-HxCDF	92	80	4	0.5	18	33	97	4	1.8	103	0.07
1234678-HpCDF	96	86	7	0.6	32	34	100	8	1.4	166	0.07
I-TEQ[d]	97	100	19	7.3	84	34	100	22	8.8	221	0.36
Adj-TEQ[e]	97	100	17	6.4	77	34	100	21	7.3	170	0.28

Med = median; Min = minimum; Max = maximum; DL = detection limit.
[a] Other race/ethnicity includes Hispanics (n = 9), blacks (n = 8), Asians/Pacific Islanders (n = 9) and other non-white races (n = 8).
[b] The n varies due to missing chemical data for some women.
[c] *p*-value calculated from the Wilcoxon Rank Sum test for the difference between non-Hispanic whites and 'others'.
[d] International Toxic Equivalent.
[e] The TEQ that includes only the nine congeners that were consistently measured above the detection limit.

Discussion

This study represents the largest investigation conducted to date of breast cancer risk associated with body burdens of dioxins in women. Overall, the results do not provide evidence for an association between body burdens of PCDD/PCDFs and breast cancer risk. A review of the literature indicates that this is only the second case-control study designed to examine the association of dioxin body burdens in women and breast cancer risk. The first study, conducted in Sweden among 22 invasive breast cancer cases and 19 controls with benign breast conditions, also found no association between breast cancer risk and adipose levels of 17 different PCDD/PCDF congeners [53]. Similar to the present study's results, however, the Swedish study noted a suggestive association for OCDD levels, although with considerably larger point estimates (adjusted OR = 3.8, 95% CI: 0.4–39.0 for concentrations of 401–1000 pg/g lipid and OR = 5.2, 95% CI: 0.4–72 for concentrations greater than 1000 pg/g lipid compared to OCDD levels less than 400 pg/g lipid). OCDD adipose concentrations were higher in the Swedish subjects than in those in the present study. Repeating the present study's analyses using the same cut points as those used in the Swedish study did not result in similarly large odds ratios (adjusted OR = 1.37, 95% CI: 0.60–3.11 for concentrations of 401–1000 pg/g lipid and OR = 1.81, 95% CI: 0.4–8.17 for concentrations greater than 1000 pg/g lipid compared to OCDD levels less than 400 pg/g lipid).

Neither the Swedish [53] nor the present study found an association between TCDD and breast cancer risk. TCDD is considered the most toxic of the dioxin congeners but also exhibits anti-estrogenic activity [32]. As expected for populations with no known exposures to PCDD/PCDFs, adipose concentrations of TCDD in both studies were much lower than those observed for OCDD [54]. Findings from both studies highlight the importance of examining individual congeners when evaluating dioxin-associated health risks. OCDD contributes very little weight to the I-TEQ and, if a true association exists, it could be obscured by reliance solely on the I-TEQ.

The concentrations found in our study are consistent with dioxin levels reported from other small, contemporary studies on dioxin body burdens in the U.S. [55]. The patterns of dioxin congeners (relative concentrations) are also consistent with patterns in populations that have no known dioxin exposures [55]. These dioxin levels are lower than levels measured during the late 1980s in adipose specimens from a small group of San Francisco Bay Area women [56]. This is consistent with the downward trend reported in most industrialized countries.

Previous occupational and environmental studies of dioxin body burdens in women have been conducted almost exclusively among Caucasian populations. The present study suggests that women of color may bear higher body burden levels of a number of congeners. Unfortunately, we did not have sufficient statistical power to formally examine breast cancer risks by race/

Table 3. Distribution of selected PCDD/PCDF congeners among cases and controls (concentrations in pg/g lipid)

Congener	Cases					Controls					*p*-Value[b]
	N[a]	% > DL	Med	Min	Max	N[a]	% > DL	Med	Min	Max	
2378-TCDD	76	75	4	0.2	19	50	62	3	0.3	20	0.14
12378-PeCDD	79	78	7	0.3	123	47	61	6	0.4	25	0.18
123678-HxCDD	79	99	54	6.5	205	52	100	55	20.6	179	0.58
1234678-HpCDD	79	99	60	1.3	334	52	100	57	22.7	198	0.34
12346789-OCDD	67	100	409	29.5	3293	52	100	360	113.0	3234	0.28
23478-PeCDF	79	99	8	3.1	100	52	92	8	2.0	26	0.74
123478-HxCDF	73	84	5	1.1	103	51	87	4	1.8	18	0.73
123678-HxCDF	74	85	4	0.5	103	51	81	3	1.0	13	0.35
1234678-HpCDF	79	91	7	0.6	117	51	88	8	3.4	166	0.08
I-TEQ[c]	79	100	20	7.3	221	52	100	20	9.4	60	0.56
Adj-TEQ[d]	79	100	18	6.4	170	52	100	17	8.2	51	0.47

pg/g = picograms per gram; Med = median; Min = minimum; Max = maximum; DL = detection limit.
[a] The N varies due to missing chemical data for some women.
[b] *p*-value calculated from Wilcoxon Rank Sum test for the difference between cases and controls.
[c] International Toxic Equivalent.
[d] The TEQ that includes only the nine congeners that were consistently measured above the detection limit.

ethnicity. Our results, while predicated on very small numbers, suggest that further research should focus on populations of color.

The decision, by IARC, to classify TCDD as a known human carcinogen was largely predicated on reported higher incidence of all cancer sites in men occupationally exposed to TCDD [30]. Animal studies have shown that TCDD causes a broad spectrum of antiestrogenic responses [27, 29, 32–35]. Consequently, carcinogenic effects may differ in men and women, especially for cancers that are endocrine-modulated, such as breast cancer. The few occupational studies conducted in women have yielded inconsistent results, especially with respect to breast cancer risk [36–39, 57]. These studies have been based on very small numbers, with no study having more than 20 cases of breast cancer [36–39, 57] and have been hampered by indirect exposure measures and potential confounding from other occupational exposures and/or unmeasured lifestyle factors. Given the small number of women employed in industries with potential TCDD exposures, occupational studies of women are unlikely to elucidate the role, if any, that dioxin exposures play in breast cancer etiology.

Many epidemiologic investigations of dioxins, including numerous cancer incidence [41, 42] and mortality studies [44, 58, 59], have focused on health effects in the population living in Seveso, Italy, in 1976, at the time of a large industrial accident which exposed nearby residents to the highest known residential TCDD exposures [60]. The most recent of these cancer mortality studies, which included 20 years of follow-up, reported no increase in breast cancer deaths among women living in the most highly contaminated region [44], although only two breast cancer deaths in this area were observed during the study. In 1996, the Seveso Women's Health Study (SWHS), a retrospective cohort study of the female population residing around Seveso at the time of the accident, was initiated to offer more comprehensive evaluations of exposure and health outcomes of interest in women [60]. A recent study of breast cancer incidence in this cohort, which defined exposures based on TCDD levels in archived serum collected shortly after the accident, reported a statistically significant increased incidence of breast cancer associated with TCDD sera levels [41]. These results, which persisted after adjustment for breast cancer covariates, though based on only 15 cases, offer some of the most convincing evidence for an association between breast cancer incidence and TCDD exposure. Similarly, a recent study conducted in Russia reported a higher overall risk of breast cancer (SMR = 2.1, 95% CI: 1.6–2.7) among women living in an area with intense dioxin contamination from a chemical plant in Chapaevsk [45]. While the results from our study may appear to stand in contrast to these recent findings, it is important to remember that the present study was conducted among women who were not known to have had significant dioxin exposures.

There are a number of limitations to the present study worth noting. One concern, raised by the rather similar distribution of breast cancer risk factors among cases and controls, is that the use of women undergoing surgery for benign breast conditions as the control group may result in over-matching. Other than lactation history, cases and controls did not significantly differ

Table 4. Age- and race-adjusted odd ratios (OR) and 95% confidence intervals (CI) associated with selected PCDD/PCDFs estimated from unconditional logistic regression models (n = 79 cases and n = 52 controls)[a]

Congener	Cases (n)	Controls (n)	OR	95% CI	*p*-Value (trend)
2378-TCDD (pg/g)					
≤2.1	19	18	1.00	(Ref.)	
2.2–3.8	22	16	1.05	(0.40–2.76)	
≥3.9	35	16	1.17	(0.44–3.09)	0.70
12378-PeCDD (pg/g)					
≤2.7	15	16	1.00	(Ref.)	
2.8–9.7	35	16	2.29	(0.85–6.19)	
≥9.8	29	15	0.99	(0.33–3.00)	0.78
123678-HxCDD (pg/g)					
≤40.1	25	18	1.00	(Ref.)	
40.2–59.2	19	17	0.73	(0.28–1.87)	
≥59.3	35	17	0.86	(0.34–2.20)	0.89
1234678-HpCDD (pg/g)					
≤44.0	25	18	1.00	(Ref.)	
44.1–66.2	21	17	0.94	(0.37–2.44)	
≥66.3	33	17	1.09	(0.44–2.70)	0.60
12346789-OCDD (pg/g)					
≤307.0	20	18	1.00	(Ref.)	
307.1–475.3	19	17	1.22	(0.47–3.16)	
≥475.4	28	17	1.62	(0.64–4.12)	0.36
23478-PeCDF (pg/g)					
≤6.7	29	18	1.00	(Ref.)	
6.8–9.9	21	17	0.60	(0.23–1.55)	
≥10.0	29	17	0.62	(0.23–1.62)	0.83
123478-HxCDF (pg/g)					
≤3.7	28	19	1.00	(Ref.)	
3.8–5.5	21	16	0.85	(0.33–2.17)	
≥5.6	24	16	0.55	(0.21–1.48)	0.95
123678-HxCDF (pg/g)					
≤2.8	22	17	1.00	(Ref.)	
2.9–4.2	21	18	0.67	(0.25–1.76)	
≥4.3	31	16	0.94	(0.35–2.47)	0.82
1234678-HpCDF (pg/g)					
≤6.4	35	17	1.00	(Ref.)	
6.5–10.3	24	18	0.58	(0.23–1.43)	
≥10.4	20	16	0.43	(0.16–1.13)	0.25
I-TEQ (pg/g)[b]					
≤16.0	25	18	1.00	(Ref.)	
16.1–23.0	22	17	0.78	(0.30–1.98)	
≥23.1	32	17	0.76	(0.29–1.98)	0.87
Adj-TEQ (pg/g)[c]					
≤14.0	24	18	1.00	(Ref.)	
14.1–20.9	22	17	0.72	(0.28–1.88)	
≥21.0	33	17	0.73	(0.27–1.95)	0.99

pg/g = picograms per gram.
[a] The n varies due to missing chemical data for some women.
[b] International Toxic Equivalent.
[c] The TEQ that includes only the nine congeners that were consistently measured above the detection limit.

on a number of breast cancer-related risk factors. In fact, some characteristics which are known to increase a woman's risk of breast cancer (e.g., early age at menarche, nulliparity, late age at first live birth) were slightly more common among controls than cases. While, as part of our protocol, we excluded from the

control group women with atypical hyperplasia, which is known to be associated with increased breast cancer risk [61–65], it is possible that other benign conditions of the breast included in the control group may be related to subsequent breast cancer risk as well [64, 66]. If such benign conditions are on the causal pathway between the exposures of interest and the development of invasive breast cancer, it could hinder our ability to detect a risk. An earlier analysis of a subset of the women in this study, which compared organochlorine concentrations in the adipose of breast and abdominal tissue, suggested that concentrations in one tissue could be derived from measurements in the other tissue [47]. Together these findings suggest that future studies should consider selecting controls among other surgical patients, such as those undergoing abdominal procedures, rather than relying only on patients undergoing breast surgeries.

Another limitation of the present study, though not unique to this analysis, is the measurement of dioxin adipose concentrations at (or near) the time of diagnosis. Despite the long half lives of PCDD/PCDFs, these adipose concentrations may not be fully representative of lifetime exposures or exposures during potential critical windows of vulnerability. OCDD, the only congener for which we found a suggestive association, has a longer half-life than the less chlorinated dioxins [67] and thus, may better reflect lifetime and early-life body burdens. There is increasing evidence that early-life exposures, in particular those experienced *in utero* or during adolescence, may be especially important in breast cancer etiology [68, 69].

Despite these limitations, our study has a number of strengths as well. While quite small, it represents the largest investigation of dioxin body burdens and breast cancer risk in the general population, for those not exposed occupationally or at high levels through large-scale industrial environmental releases. By separately examining nine different congeners, this study provided the opportunity to evaluate potentially differing effects for compounds with different toxicities and endocrine modulating activities. Future work using a more representative control series and focused on congener-specific associations in women of color may be warranted.

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References

1. Madigan MP, Ziegler RG, Benichou J, Byrne C, Hoover RN (1995) Proportion of breast cancer cases in the United States explained by well-established risk factors. *J Natl Cancer Inst* **87**: 1681–1685.
2. Aronson KJ, Miller AB, Woolcott CG, *et al.* (2000) Breast adipose tissue concentrations of polychlorinated biphenyls and other organochlorines and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* **9**: 55–63.
3. Bagga D, Anders KH, Wang HJ, Roberts E, Glaspy JA (2000) Organochlorine pesticide content of breast adipose tissue from women with breast cancer and control subjects. *J Natl Cancer Inst* **92**: 750–753.
4. Dello Iacovo R, Celentano E, Strollo AM, Iazzetta G, Capasso I, Randazzo G (1999) Organochlorines and breast cancer. A study on Neapolitan women. *Adv Exp Med Biol* **472**: 57–66.
5. Dewailly E, Dodin S, Verreault R, *et al.* (1994) High organochlorine body burden in women with estrogen receptor-positive breast cancer. *J Natl Cancer Inst* **86**: 232–234.
6. Dorgan JF, Brock JW, Rothman N, *et al.* (1999) Serum organochlorine pesticides and PCBs and breast cancer risk: results from a prospective analysis (USA). *Cancer Causes Control* **10**: 1–11.
7. Guttes S, Failing K, Neumann K, Kleinstein J, Georgii S, Brunn H (1998) Chlororganic pesticides and polychlorinated biphenyls in breast tissue of women with benign and malignant breast disease. *Arch Environ Contam Toxicol* **35**: 140–147.
8. Helzlsouer KJ, Alberg AJ, Huang HY, *et al.* (1999) Serum concentrations of organochlorine compounds and the subsequent development of breast cancer. *Cancer Epidemiol Biomarkers Prev* **8**: 525–532.
9. Hoyer AP, Grandjean P, Jorgensen T, Brock JW, Hartvig HB (1998) Organochlorine exposure and risk of breast cancer. *Lancet* **352**: 1816–1820.
10. Hunter DJ, Hankinson SE, Laden F, *et al.* (1997) Plasma organochlorine levels and the risk of breast cancer. *N Engl J Med* **337**: 1253–1258.

11. Liljegren G, Hardell L, Lindstrom G, Dahl P, Magnuson A (1998) Case-control study on breast cancer and adipose tissue concentrations of congener specific polychlorinated biphenyls, DDE and hexachlorobenzene. *Eur J Cancer Prev* **7**: 135–140.
12. Mendonca GA, Eluf-Neto J, Andrada-Serpa MJ, *et al.* (1999) Organochlorines and breast cancer: a case-control study in Brazil. *Int J Cancer* **83**: 596–600.
13. Millikan R, DeVoto E, Duell EJ, *et al.* (2000) Dichlorodiphenyldichloroethene, polychlorinated biphenyls, and breast cancer among African-American and white women in North Carolina. *Cancer Epidemiol Biomarkers Prev* **9**: 1233–1240.
14. Moysich KB, Ambrosone CB, Vena JE, *et al.* (1998) Environmental organochlorine exposure and postmenopausal breast cancer risk. *Cancer Epidemiol Biomarkers Prev* **7**: 181–188.
15. Mussalo-Rauhamaa H, Hasanen E, Pyysalo H, Antervo K, Kauppila R, Pantzar P (1990) Occurrence of beta-hexachlorocyclohexane in breast cancer patients. *Cancer* **66**: 2124–2128.
16. Olaya-Contreras P, Rodriguez-Villamil J, Posso-Valencia HJ, Cortez JE (1998) Organochlorine exposure and breast cancer risk in Colombian women. *Cad Saude Publica* **14**: 125–132.
17. Romieu I, Hernandez-Avila M, Lazcano-Ponce E, Weber JP, Dewailly E (2000) Breast cancer, lactation history, and serum organochlorines. *Am J Epidemiol* **152**: 363–370.
18. Schecter A, Toniolo P, Dai LC, Thuy LT, Wolff MS (1997) Blood levels of DDT and breast cancer risk among women living in the north of Vietnam. *Arch Environ Contam Toxicol* **33**: 453–456.
19. Stellman SD, Djordjevic MV, Britton JA, *et al.* (2000) Breast cancer risk in relation to adipose concentrations of organochlorine pesticides and polychlorinated biphenyls in Long Island, New York. *Cancer Epidemiol Biomarkers Prev* **9**: 1241–1249.
20. Unger M, Kiaer H, Blichert-Toft M, Olsen J, Clausen J (1984) Organochlorine compounds in human breast fat from deceased with and without breast cancer and in a biopsy material from newly diagnosed patients undergoing breast surgery. *Environ Res* **34**: 24–28.
21. van't Veer P, Lobbezoo IE, Martin-Moreno JM, *et al.* (1997) DDT (dicophane) and postmenopausal breast cancer in Europe: case-control study. *BMJ* **315**: 81–85.
22. Wolff MS, Toniolo PG, Lee EW, Rivera M, Dubin N (1993) Blood levels of organochlorine residues and risk of breast cancer. *J Natl Cancer Inst* **85**: 648–652.
23. Wolff MS, Zeleniuch-Jacquotte A, Dubin N, Toniolo P (2000) Risk of breast cancer and organochlorine exposure. *Cancer Epidemiol Biomarkers Prev* **9**: 271–277.
24. Zheng T, Holford TR, Mayne ST, *et al.* (1999) Beta-benzene hexachloride in breast adipose tissue and risk of breast carcinoma. *Cancer* **85**: 2212–2218.
25. Zheng T, Holford TR, Mayne ST, *et al.* (1999) DDE and DDT in breast adipose tissue and risk of female breast cancer. *Am J Epidemiol* **150**: 453–458.
26. Zheng T, Holford TR, Mayne ST *et al.* (1999) Environmental exposure to hexachlorobenzene (HCB) and risk of female breast cancer in Connecticut. *Cancer Epidemiol Biomarkers Prev* **8**: 407–411.
27. Adami HO, Lipworth L, Titus-Ernstoff L, *et al.* (1995) Organochlorine compounds and estrogen-related cancers in women. *Cancer Causes Control* **6**: 551–566.
28. Calle EE, Frumkin H, Henley SJ, Savitz DA, Thun MJ (2002) Organochlorines and breast cancer risk. *CA Cancer J Clin* **52**: 301–309.
29. Ahlborg UG, Lipworth L, Titus-Ernstoff L, *et al.* (1995) Organochlorine compounds in relation to breast cancer, endometrial cancer, and endometriosis: an assessment of the biological and epidemiological evidence. *Crit Rev Toxicol* **25**: 463–531.
30. International Agency for Research on Cancer. IARC. (1997) *Polychlorinated Dibenzo-para-dioxins and Polychlorinated Dibenzofurans. IARC Monograph* 69th edn. Lyon, France: World Health Organization.
31. Birnbaum LS, Fenton SE (2003) Cancer and developmental exposure to endocrine disruptors. *Environ Health Perspect* **111**: 389–394.
32. Krishnan V, Safe S (1993) Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs) as antiestrogens in MCF-7 human breast cancer cells: quantitative structure–activity relationships. *Toxicol Appl Pharmacol* **120**: 55–61.
33. Safe S, Astroff B, Harris M, *et al.* (1991) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds as antioestrogens: characterization and mechanism of action. *Pharmacol Toxicol* **69**: 400–409.
34. White TE, Gasiewicz TA (1993) The human estrogen receptor structural gene contains a DNA sequence that binds activated mouse and human Ah receptors: a possible mechanism of estrogen receptor regulation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Biochem Biophys Res Commun* **193**: 956–962.
35. Zacharewski T, Harris M, Safe S (1991) Evidence for the mechanism of action of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated decrease of nuclear estrogen receptor levels in wild-type and mutant mouse Hepa 1c1c7 cells. *Biochem Pharmacol* **41**: 1931–1939.
36. Manz A, Berger J, Dwyer JH, Flesch-Janys D, Nagel S, Waltsgott H (1991) Cancer mortality among workers in chemical plant contaminated with dioxin. *Lancet* **338**: 959–964.
37. Kogevinas M, Saracci R, Winkelmann R, *et al.* (1993) Cancer incidence and mortality in women occupationally exposed to chlorophenoxy herbicides, chlorophenols, and dioxins. *Cancer Causes Control* **4**: 547–553.
38. Kogevinas M, Becher H, Benn T, *et al.* (1997) Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study. *Am J Epidemiol* **145**: 1061–1075.
39. Lynge E (1985) A follow-up study of cancer incidence among workers in manufacture of phenoxy herbicides in Denmark. *Br J Cancer* **52**: 259–270.
40. Flesch-Janys D, Berger J, Gurn P, *et al.* (1995) Exposure to polychlorinated dioxins and furans (PCDD/F) and mortality in a cohort of workers from a herbicide-producing plant in Hamburg, Federal Republic of Germany. *Am J Epidemiol* **142**: 1165–1175.
41. Warner M, Eskenazi B, Mocarelli P, *et al.* (2002) Serum dioxin concentrations and breast cancer risk in the Seveso Women's Health Study. *Environ Health Perspect* **110**: 625–628.
42. Bertazzi A, Pesatori AC, Consonni D, Tironi A, Landi MT, Zocchetti C (1993) Cancer incidence in a population accidentally exposed to 2,3,7,8-tetrachlorodibenzo-para-dioxin. *Epidemiology* **4**: 398–406.
43. Bertazzi PA, Bernucci I, Brambilla G, Consonni D, Pesatori AC (1998) The Seveso studies on early and long-term effects of dioxin exposure: a review. *Environ Health Perspect* **106**(Suppl 2): 625–633.
44. Bertazzi PA, Consonni D, Bachetti S, *et al.* (2001) Health effects of dioxin exposure: a 20-year mortality study. *Am J Epidemiol* **153**: 1031–1044.
45. Revich B, Aksel E, Ushakova T, *et al.* (2001) Dioxin exposure and public health in Chapaevsk, Russia. *Chemosphere* **43**: 951–966.
46. Petreas MX, She J, Winkler J, *et al.* (2000) Organochlorine body burden in California populations. *Organohalogen Compounds* **48**: 17–21.

47. Petreas M, Smith D, Hurley S, Jeffrey SS, Gilliss D, Reynolds P (2004) Distribution of persistent, lipid-soluble chemicals in breast and abdominal adipose tissues: lessons learned from a breast cancer study. *Cancer Epidemiol Biomarkers Prev* **13**: 416–424.
48. Breslow NE, Day NE. (1980) *Statistical Methods in Cancer Research. Vol. I, The Analysis of Case-Control Studies*. Lyon, France: International Agency for Research on Cancer.
49. Hollander M, Wolfe DA (1973) *Nonparametric Statistical Methods*. New York: John Wiley & Sons, Inc.
50. North Atlantic Treaty Organization, Committee on Challenges of Modern Society (NATO/CCMS) (1988) International Toxicity Equivalent Factor (I-TEF) method of risk assessment for complex mixtures of dioxins and related compounds. Pilot Study on international information exchange on dioxins and related compounds. Brussels, Belgium: North Atlantic Treaty Organization, Committee on Challenges of Modern Society.
51. Moysich KB, Shields PG, Freudenheim JL, *et al*. (1999) Polychlorinated biphenyls, cytochrome P4501A1 polymorphism, and postmenopausal breast cancer risk. *Cancer Epidemiol Biomarkers Prev* **8**: 41–44.
52. SAS Institute, Inc. (2002) *SAS 8.2, 8.2* [computer program]. Cary, NC: SAS Institute Inc.
53. Hardell L, Lindstrom G, Liljegren G, Dahl P, Magnuson A (1996) Increased concentrations of octachlorodibenzo-*p*-dioxin in cases with breast cancer – results from a case-control study. *Eur J Cancer Prev* **5**: 351–357.
54. United States Environmental Protection Agency, National Center for Environmental Assessment (September 2000) *Draft Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* [web page]. Available from http://cfpub2.epa.gov/ncea/cfm/part1and2.cfm?ActType=default, (Accessed 23 November 2004).
55. National Center for Environmental Assessment, Exposure Assessment and Risk Characterization Group (2000) *Exposure to Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds*. Washington DC, USA: U.S. Environmental Protection Agency.
56. Petreas M, She J, McKinney M, *et al*. (2000) Dioxin Body Burdens in California Populations. In: Lipnick R, ed. *Persistent Bioaccumulative Toxic Chemicals*. Washington DC: American Chemical Society.
57. Saracci R, Kogevinas M, Bertazzi PA, *et al*. (1991) Cancer mortality in workers exposed to chlorophenoxy herbicides and chlorophenols. *Lancet* **338**: 1027–1032.
58. Bertazzi PA, Zocchetti C, Guercilena S, *et al*. (1997) Dioxin exposure and cancer risk: a 15-year mortality study after the ''Seveso accident''. *Epidemiology* **8**: 646–652.
59. Bertazzi PA, Zocchetti C, Pesatori AC, Guercilena S, Sanarico M, Radice L (1989) Ten-year mortality study of the population involved in the Seveso incident in 1976. *Am J Epidemiol* **129**: 1187–1200.
60. Mocarelli P, Patterson DJ, Marocchi A, Needham L (1990) Pilot study (Phase II) for determining polychlorinated dibenzo-p-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) levels in serum of Seveso, Italy residents collected at the time of exposure: future plans. *Chemosphere* **20**: 967–974.
61. Marshall LM, Hunter DJ, Connolly JL, *et al*. (1997) Risk of breast cancer associated with atypical hyperplasia of lobular and ductal types. *Cancer Epidemiol Biomarkers Prev* **6**: 297–301.
62. Vogel VG (2004) Atypia in the assessment of breast cancer risk: implications for management. *Diagn Cytopathol* **30**: 151–157.
63. Dupont WD, Page DL (1987) Breast cancer risk associated with proliferative disease, age at first birth, and a family history of breast cancer. *Am J Epidemiol* **125**: 769–779.
64. London SJ, Connolly JL, Schnitt SJ, Colditz GA (1992) A prospective study of benign breast disease and the risk of breast cancer. *JAMA* **267**: 941–944.
65. Bodian CA, Perzin KH, Lattes R, Hoffmann P, Abernathy TG (1993) Prognostic significance of benign proliferative breast disease. *Cancer* **71**: 3896–3907.
66. Wang J, Costantino JP, Tan-Chiu E, Wickerham DL, Paik S, Wolmark N (2004) Lower-category benign breast disease and the risk of invasive breast cancer. *J Natl Cancer Inst* **96**: 616–620.
67. Agency for Toxic Substances and Disease Registry (1998) *Toxicological Profile for Chlorinated Dibenzo-p-dioxins (CDDs)*. Atlanta, GA, USA: U.S. Department of Health and Human Services, Public Health Service.
68. Potischman N, Troisi R (1999) In-utero and early life exposures in relation to risk of breast cancer. *Cancer Causes Control* **10**: 561–573.
69. Okasha M, McCarron P, Gunnell D, Smith GD (2003) Exposures in childhood, adolescence and early adulthood and breast cancer risk: a systematic review of the literature. *Breast Cancer Res Treat* **78**: 223–276.