

Distribution of Persistent, Lipid-Soluble Chemicals in Breast and Abdominal Adipose Tissues: Lessons Learned from a Breast Cancer Study

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Abstract

Objective: We sought to determine differences between concentrations of persistent, lipid-soluble chemical contaminants in breast and abdominal adipose tissues and to explore whether concentrations measured in one tissue could predict concentrations in the other tissue. **Methods:** We analyzed surgical specimens and measured concentrations of prevalent dioxins, furans, polychlorinated biphenyls, organochlorine pesticides, and brominated diphenyl ethers to determine their partitioning between breast and abdominal adipose tissues of 21 women. The women constituted a subgroup, undergoing mastectomies with simultaneous breast reconstruction, of a case-control study evaluating links between breast cancer and body burdens of organohalogen contaminants. **Results:** For every contaminant, differences between concentrations in breast and ab-

dominal adipose tissues did not exceed the analytical error. Results indicated that, with some notable exceptions, measurements in breast and abdominal adipose tissues were correlated and that concentrations of target chemicals in one tissue could be derived from measurements in the other tissue. **Conclusions:** This information will allow comparison of results from body burden studies that used different tissues. It may also facilitate future breast cancer studies by allowing selection of controls among patients undergoing surgical procedures other than breast surgery, minimizing concerns about overmatching. We also observed large differences in the lipid content of surgical specimens. These differences underscore the need for lipid adjustment of concentrations to avoid misclassification. (Cancer Epidemiol Biomarkers Prev 2004;13(3):416–424)

Introduction

Persistent organic chemicals such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDDs/PCDFs or dioxins) are ubiquitous in the environment. Dioxins continue to be inadvertently formed during chemical and thermal processes (1). Chlorinated pesticides and PCBs have been used extensively throughout the world and, long after their use was discontinued, their residues persist in the food chain and in human tissues (2–4). Polybrominated diphenyl ethers (PBDEs), recently introduced and used as flame retardants, show increasing trends in biota,

including humans (5–8). Common features of all these chemical classes are their persistence in the environment, their solubility into fatty tissues (lipophilicity), their resistance to metabolism, and their bioconcentration potential through the food web. Measurements of body burdens of chemical contaminants (or their metabolites) are good indicators of exposures and strengthen our ability to examine associations between exposures and health outcomes.

Many of these contaminants are considered toxic and have been associated with adverse health effects. Several studies on the possible role of estrogenic or antiestrogenic chemicals in the development of breast cancer have assessed exposures to certain organochlorine compounds through the analysis of biological specimens. In each study, researchers selected a particular type of specimen such as adipose (9–22), serum (23–35), or plasma (36–39) that best fit their objectives within constraints of availability and convenience. As these studies resulted in conflicting conclusions, however, it is compelling to explore the possible role of certain laboratory approaches in the apparent discrepancies. In addition to the selected analytical methodologies that may affect precision and accuracy in the measurement of target analytes, auxiliary measurements of lipids and use of such measurements to adjust chemical concentrations may introduce bias in the results.

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Blood is much easier to collect than adipose; serum and plasma specimens have been used to assess exposures to lipophilic chemicals in many breast cancer studies (23–39). However, while measurements in adipose tissues reflect steady-state concentrations of lipophilic chemicals, measurements in whole blood, serum, and plasma may fluctuate with surges in blood lipids and, as such, may be biased. The main contributor to this bias is the transport mechanism and partitioning of each chemical in the various blood components according to its relative solubility. For example, <18% of dichlorodiphenyldichloroethylene (DDE) or dichlorodiphenyltrichloroethane (DDT), but over 40% of dieldrin, are distributed in the erythrocytes reflecting differences in cell membrane permeability (40). Similarly, most PCDD/PCDF congeners are found in the lipoprotein fraction of blood. The more highly chlorinated congeners (penta- through octa-substituted), however, do not appear to partition between the lipoprotein and the protein fractions according to the lipid content of these fractions (41). Instead, the percentage associated with the protein fraction increases with the degree of chlorination, possibly suggesting preferential binding (41). The adipose/serum partition coefficient is the ratio of the concentration of a chemical in adipose and in serum at equilibrium and determines the degree at which a chemical accumulates in fatty tissues. Because of interindividual and even intraindividual differences in absorption, distribution, metabolism, and elimination of contaminants, empirically derived adipose/serum partition coefficients have wide ranges (42). This makes direct comparisons of contaminant concentrations in adipose and blood samples problematic, even when all concentrations are adjusted for lipids. Common blood lipid determinations are either gravimetric (where lipids are chemically separated from blood and weighed) or enzymatic (where measurements of cholesterol, triglycerides, and, often, phospholipids are used to estimate total lipids in blood; 43, 44). Several studies have examined correlations between concentrations of organochlorine chemicals in adipose and in serum, plasma, or whole blood, with little consensus (42, 45–48). Besides physiological mechanisms, analytical error often adds to the discrepancy between measurements in adipose and in blood. This is particularly true when concentrations in small volumes of blood approach the limit of detection (47). Less variation may be expected in the distributions of lipophilic chemicals among various types of adipose tissues, but there are extremely few data in the literature. Current knowledge is hampered by the limited number of patients with measurements in more than a single tissue and by low body burdens that stretch analytical methodologies (45, 47) and increase analytical error.

In a related report, we presented the first systematically collected data on persistent halogenated contaminants from California (49). These chemicals were measured in breast adipose tissues of women participating in a breast cancer case-control study centered in the San Francisco Bay Area.⁵ In this article, we examine the distributions of persistent chemical contaminants in breast and abdominal adipose tissues in a subset of

these women who underwent mastectomies with concurrent breast reconstruction. The current analysis is designed to provide insight into a serious methodological restriction involving the selection of appropriate controls in epidemiologic studies. Adipose is the tissue of choice for assessing steady-state body burdens of lipophilic contaminants, but invasive techniques are required for sample collection. Use of breast adipose samples to assess body burdens in breast cancer studies limits the eligible pool of controls to subjects who were prompted to undergo a biopsy following some abnormal finding in their breasts and whose biopsies were benign. The selection of these women for use as controls may be problematic in that benign breast conditions may actually be a risk factor for breast cancer. Conversely, selecting control subjects among women undergoing plastic surgery for breast implants or breast reduction may introduce bias in terms of age, socioeconomic status, etc. Use of abdominal adipose would help avoid the concern that use of benign breast biopsy controls may result in overmatching. In addition, if concentrations are equivalent, use of more easily accessible abdominal adipose tissue samples will allow future studies to draw from a wider selection of subjects. Furthermore, it would be possible to compare results across studies that had used different adipose tissues. Unfortunately, aside from case reports on few individuals (50–53), no systematic effort has been reported on distributions of persistent organic chemicals in various types of adipose tissues. There is clearly a great need for a systematic study on this topic. We examined concentrations of major OCPs, PCBs, PCDDs/PCDFs, and PBDEs in breast and abdominal adipose tissues in an effort to address the question of contaminant distributions in different adipose stores. In addition, we report the great variation in lipid content of surgical specimens we observed.

Materials and Methods

Study Population. For our full study,⁶ we recruited women undergoing breast surgery for suspected breast cancer at Stanford University Hospital (Stanford, CA) and Kaiser Permanente Hospital (Oakland, CA). All patients signed informed consent and medical release forms allowing us to access medical records, including pathology reports and associated diagnostic data. Our use of human subjects in this study was reviewed by the California Health and Human Services Agency, Committee for the Protection of Human Subjects and was found to be in compliance with their ethical standards as well as with the U.S. Code of Federal Regulations, Title 45, Part 46, Protection of Human Subjects. We also obtained equivalent approvals from each participating hospital's institutional review board. We administered each subject an epidemiologic interview composed of a dietary questionnaire and a questionnaire on medical and reproductive history, family history, environmental exposures, health habits, and demographic characteristics. Surgeons collected small amounts (<1 g) of breast adipose tissue during surgery. To study the distribution

⁵Reynolds et al., unpublished observations.

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of chemicals in breast and abdominal adipose tissues, we focused on a subset of women ($n = 21$) undergoing mastectomies with simultaneous breast reconstruction. A transverse rectus abdominis myocutaneous (TRAM) flap uses abdominal skin, fat, and muscle to recreate the breast that is removed. Small amounts (1–2 g) of abdominal adipose were collected during the TRAM surgery in addition to the corresponding breast adipose tissue. To study the variability in lipid content of surgical specimens, we used the entire population of the full study ($N = 161$).⁷

Histopathology. The Department of Pathology at Stanford University and the Kaiser Permanente Hospital evaluated histologic sections of all breast lesions and coded diagnoses as invasive malignant disease, noninvasive malignant disease, ductal carcinoma *in situ* (DCIS), or benign histologic changes. For the purpose of this study, all 21 women undergoing the TRAM procedure were pooled irrespective of disease status (malignant, benign, or DCIS).

Sample Analysis. Samples were stored at -20°C until analysis. Samples were thawed, weighed, mixed with Na_2SO_4 , homogenized with dichloromethane/hexane (1:1, v/v), and fortified with ^{13}C -labeled internal standards [all 17 2,3,7,8-substituted PCDDs/PCDFs; PCBs 77, 126, 169, 28, 52, 47, 101, 105, 118, 153, 180, 194, and 209; hexachlorobenzene (HCB); β -hexachlorocyclohexane (β -HCH); DDE; DDT; dieldrin; mirex; and 3,3',4,4'-tetrabrominated diphenyl ether]. One-tenth of the extract was analyzed for OCPs, PCBs, and PBDEs and nine-tenths was analyzed for PCDDs/PCDFs and coplanar PCBs. Lipid content was determined in an aliquot of the extract by evaporating the solvent and weighing the remaining solids to constant weight (gravimetric determination). The extracted samples were serially processed through glass columns containing Na_2SO_4 and AX21 carbon. The first fraction off the carbon column was further cleaned up by gel permeation chromatography (Fluid Management Systems, Waltham, MA) and Florisil chromatography; recovery standards were added and the final extract was concentrated to 10 μl for PCB, OCP, and PBDE analysis (7). PCDDs/PCDFs and coplanar PCBs were eluted from the carbon column with toluene and the eluate was cleaned up through alumina and acid silica columns; recovery standards were added and the extract was concentrated to 10 μl .

PCDDs/PCDFs and PCBs were analyzed by high-resolution gas chromatography coupled with high-resolution mass spectrometry (Finnigan MAT90; Finnigan Corp., San Jose, CA) with a DB5ms (Agilent Technologies, Wilmington, DE) column (60 m, 0.25 mm ID, 0.25 μm film thickness). Perfluorokerosene was used to establish the lock masses and selected ions characteristic of the analytes were monitored. OCPs and PBDEs were analyzed by low-resolution mass spectrometry in negative chemical ionization mode (Finnigan 4510; Finnigan) with a DB5ms (Agilent Technologies) column (60 m, 0.25 mm ID, 0.25 μm film thickness). Methane was used as the reagent gas; the ion source pressure was 0.6 Torr and the

ion source temperature was 100°C . The electron energy was typically 70 eV and the electron current was kept at 0.3 mA.

In addition to the individual 2,3,7,8-substituted PCDD/PCDF congeners, their International Toxic Equivalents (I-TEQ; the sum of 17 toxic congeners weighed by individual toxicity factors) were calculated using the I-TEQ convention (54). Toxicity factors decrease with increasing number of chlorines; therefore, tetra- and penta-substituted congeners drive the I-TEQ. The sum of the five prevalent PBDE congeners (total PBDE) is often used as a summary index of PBDE body burden.

Quality Assurance. Samples were analyzed in batches of six, and both abdominal and breast adipose samples from a TRAM study patient were included in the same batch. The identity and disease classification of the samples were masked. In addition to the six samples, each batch contained a reagent blank. Duplicate analyses were performed and standard reference materials (SRM 1945, whale blubber; National Institute of Standards and Technology, Gaithersburg, MD) were incorporated in the analysis.

Data Tracking. All completed questionnaires, medical records, and pathology reports were kept in a secure filing cabinet. Questionnaire information was extracted, coded, and entered in a computerized database (55) with the patient's medical record number as the sole identifier. Chemical analysis results were compiled in spreadsheets. Questionnaire data and chemical analysis data were merged and subjected to statistical analysis using SAS (56).

Statistical Methods. Duplicate analysis is a basic procedure to assess the precision of a chemical method and relative percentage differences (RPDs) are routinely used to assess agreement between duplicate chemical analyses. The RPD is defined as the ratio of the absolute value of the difference between two measurements over the average of the two measurements. The Mann-Whitney-Wilcoxon test was used to compare RPDs for a given chemical from the TRAM pairs to RPDs from duplicate analyses of breast adipose tissues to assess whether differences between tissues exceeded differences within the same tissue (analytical error).

The first question we wanted to address with these data was: Are concentrations in abdominal and breast adipose tissues the same? This question is addressed quantitatively with the intraclass correlation, a measure of concordance corrected for chance agreement (57). In a plot of the pairs of points, with the breast tissue value on one axis and the abdominal tissue values on the other, the intraclass correlation measures the scatter of the data around the 45° line of perfect agreement; intraclass correlation = 1 indicates perfect agreement. Agreement may also be tested with the Wilcoxon matched-pairs test, a nonparametric equivalent of the matched-pairs t test (57). In this case, the null hypothesis is that the (lipid-adjusted) concentration of an analyte in breast adipose is equal to its (lipid-adjusted) concentration in abdominal adipose.

If concentrations are not equal, a second question may be addressed: Even if the data are not interchangeable, can they still be used to derive one from the other? This is assessed by regression analysis and by examining the

⁷Reynolds et al., unpublished observations.

resulting R^2 statistic, representing the amount of variance explained in one measure by accounting for the other. If the agreement between the two values is good, the R^2 will be close to the square of the intraclass correlation coefficient. However, even in situations of poor absolute agreement, the R^2 may be high if the data are well correlated. In that case, breast adipose concentrations could be derived from abdominal adipose concentrations using the regression equation.

Results

Table 1 shows the demographic characteristics of the 21 women who had the TRAM procedure. Most of the TRAM patients were non-Hispanic White (14 of 21, 66.7%) and between 41 and 59 years old (19 of 21, 90.7%) with an average age of 48.5 years. Most were born in the United States (66.7%), were married or living as married (90.5%), and were of high socioeconomic status. Most (17 of 21, 80.9%) were cases, although we also included one with benign disease and three with DCIS.

Table 2 shows concentration means and ranges of major contaminants measured in the TRAM specimens. These concentrations were similar to the overall concentrations measured in the entire study population.⁸ Whereas valid data for at least some analytes were contributed by all 21 women, not all 21 contributed complete sets of results. Analytical results were not included in this evaluation if either the breast or the abdominal measurement from a participant was compromised. Reasons for exclusion included insufficient sample for analysis or measurements below the limit of detection. Only analytes consistently measured above the detection limit were included in the comparison. To account for analytical error (*i.e.*, the uncertainty of the analytical procedure), the results from the TRAM analyses were compared with results from the analysis of duplicate samples. Whereas, for many analytes, mean RPDs for TRAM pairs appear greater than the RPDs for duplicate analyses, none of these differences were statistically significant (all Mann-Whitney-Wilcoxon tests had $p > 0.05$).

Table 3 summarizes the answers to our two study questions. On whether the data are interchangeable, the intraclass correlation coefficients and the outcome of the hypothesis testing (H_0 : abdominal concentration = breast concentration) are listed for each of the major analytes. Intraclass correlation coefficients were, in general, >0.8 with a few exceptions. The lowest intraclass correlation coefficients were estimated for the two most toxic PCDD/PCDF congeners, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (12378-PeCDD; 0.471 and 0.426, respectively). With the exception of 12378-PeCDD and the summary PCDD/PCDF measure (I-TEQ), the Wilcoxon signed rank test could not reject the hypothesis that concentrations in the two tissues were equal at the 5% confidence level. Similarly, results from the regression analysis address the question of whether breast adipose concentrations can be derived from abdominal tissue

Table 1. Demographic characteristics of the 21 women who underwent the TRAM procedure

Characteristic	No.	%
Age (yr)		
<40	1	4.8
41–50	12	57.4
51–59	7	33.3
≥60	1	4.8
Race/ethnicity		
Non-Hispanic White	14	66.7
Hispanic	3	14.3
Black	0	0.0
Asian/Pacific Islander	3	14.3
Other	1	4.8
Birthplace		
California	8	38.1
Other U.S. states	6	28.6
Foreign born	7	33.3
Marital status		
Married/lives as married	19	90.5
Never married	2	9.5
Family income (\$)		
<50,000	3	15.0
50,000–99,999	8	40.0
≥100,000	9	45.0
Education		
High school graduate or lower	5	23.8
College graduate	9	42.9
Master's degree	6	28.6
Doctoral degree	1	4.8

measurements. With the exception of 2378-TCDD, all intercepts were not significantly different from zero at the 5% confidence level. In addition, R^2 statistics varied across analytes ranging from 0.238 to 0.997. The lowest R^2 values were calculated for 2378-TCDD and 12378-PeCDD (0.238 and 0.275, respectively).

Lipid content in abdominal and breast adipose samples of the TRAM subset ($n = 21$) ranged from 58% to 96% with a mean of 82.7% (data not shown). Among participants in the full case-control study ($N = 161$), the lipid content in breast adipose was even more variable ranging from <10% to almost 100% with a mean of 76.4%. Figure 1 shows the lipid content of breast adipose specimens of all participants ($N = 161$) of the full study as a function of their age. Younger women appear to have a wider range of lipid content with many specimens below 40%. When all the women in the study were divided into two groups according to the median age, those women who were younger than 50 years had lower lipid content than those who were 50 years or older (Mann-Whitney-Wilcoxon test, $p < 0.05$).

Discussion

Very few data exist on the distribution of persistent organic chemicals in various types of adipose tissue. Analyses of specimens from two autopsies showed that DDE and selected PCB and PCDD/PCDF congeners were at similar levels in intra-abdominal and subcutaneous (*s.c.*) adipose tissue of two cadavers, while DDT and HCB appeared at higher concentrations in intra-abdominal adipose (51, 52). A recent study on autopsied females reported strong correlations among concentrations of DDE, DDT, HCB, and β -HCH in breast and

⁸Petreas et al., unpublished observations.

Table 2. Analyte concentrations and RPD in the analyses of major contaminants in TRAM (abdominal and breast pairs) and duplicate breast adipose samples

	TRAM analysis				Duplicate analysis			
	No. (pairs)	Concentration ^a		Variation		No. (pairs)	Variation	
		Mean	Range	RPD mean	RPD SD		RPD mean	RPD SD
PCDDs/PCDFs								
I-TEQ	19	20.5	8.8–52	11.9	12.1	3	7.9	6.6
2378-TCDD	19	3.9	0.3–9.5	47.5	40.7	3	21.9	8.8
12378-PeCDD	19	8.1	0.8–19	31.6	35.5	3	19.0	21.0
123678-HxCDD	18	46.0	17–160	10.5	9.7	3	5.0	3.8
1234678-HpCDD	19	68.7	2.6–290	9.0	6.5	3	6.1	4.9
12346789-OCDD	19	470	10–1410	16.1	23.3	3	7.1	5.7
23478-PeCDF	19	9.1	3.8–19	12.5	8.5	3	12.0	4.3
123478-HxCDF	19	4.8	1.8–13	13.7	12.3	3	5.3	1.9
123678-HxCDF	18	4.0	1.0–9.9	15.5	17.9	3	5.3	4.1
1234678-HpCDF	19	7.0	1.0–19	20.6	19.5	3	23.8	27.5
Average	–	–	–	19.8	19.2	–	11.4	8.9
OCPs								
DDE	13	1120	350–2700	17.1	13.1	4	15.9	10.0
<i>Trans</i> -nonachlor	12	100	50–190	21.7	18.4	3	24.2	21.6
Oxychlorodane	12	43	20–88	19.9	17.9	3	22.8	17.6
DDT	10	46	6–160	23.8	19.9	4	10.4	5.6
β -HCH	9	120	14–620	39.0	40.3	4	24.5	13.0
HCB	13	59	21–190	13.2	12.9	4	12.4	13.9
Dieldrin	13	24	17–46	6.7	5.4	4	19.7	14.1
Average	–	–	–	20.3	18.1	–	18.6	13.7
PBDEs								
PBDE-47	8	38	6–150	18.7	11.5	4	12.3	9.8
PBDE-99	7	8.8	2–31	30.1	23.1	4	28.3	29.2
PBDE-100	7	11	1–61	26.5	23.4	4	11.5	9.3
PBDE-153	8	24	2–190	30.6	28.5	4	11.1	10.4
PBDE-154	8	10	3–26	33.6	32.0	4	6.2	3.5
Sum of PBDEs	8	91	17–440	17.0	15.7	4	12.1	5.9
Average	–	–	–	26.1	22.4	–	13.6	11.4
PCBs								
PCB 153	12	160	51–350	19.2	23.0	3	15.4	13.4
PCB 138	12	100	33–230	24.4	30.0	3	22.7	18.9
PCB 180	12	110	52–240	21.8	22.2	3	20.3	20.5
PCB 118	12	34	9–81	17.7	20.3	3	5.2	3.8
Average	–	–	–	20.8	23.8	–	15.8	14.0

^aConcentrations of PCDDs/PCDFs are in pg/g lipid. Concentrations of PCBs, OCPs, and PBDEs are in ng/g lipid.

abdominal adipose tissues (50). Another study examining the distribution of PCDD/PCDF in liver (53), muscle, and fat components of a single bull calf reported quite similar concentrations in s.c., perirenal, and peritoneal adipose of the animal. Recent work on four bull calves fed PCDD/PCDF mixtures (58) showed equivalent concentrations in perirenal, s.c. (back fat), and rib-eye tissue for most congeners with the exception of 2378-TCDD, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (1234678-HpCDD), 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (12346789-OCDD) and 1,2,3,4,6,7,8,9-octachlorodibenzofuran (1,2,3,4,6,7,8,9-OCDF), where two to three times higher concentrations were measured in the rib-eye samples.

In our study, with some notable exceptions, we could not detect significant differences between concentrations in abdominal and breast adipose tissues, and high intraclass correlation coefficients indicated good agreement in the measurements. Exceptions were observed with certain dioxin congeners. Both 2378-TCDD and 12378-PeCDD had low intraclass correlation coefficients (0.471 and 0.426, respectively), poor R^2 (0.238 and 0.275, respectively), and slopes quite different from 1 (0.448 and 0.674, respectively), indicating preferential deposition of

these chemicals in abdominal adipose tissue. In addition, the intercept for 2378-TCDD was statistically different from zero.

The hypothesis that concentrations were equivalent in the two types of tissues was rejected for 12378-PeCDD and barely accepted for 2378-TCDD at the 5% significance level. The summary index of dioxin exposure (I-TEQ), a weighed sum of the 17 most toxic PCDD/PCDF congeners, which is driven greatly by these two dioxin members, was also affected. Although analytical error was high as the measurements were near the detection limit for these two analytes, we cannot discount the possibility that these two tetra- and pentachlorinated congeners (and possibly other similar molecules) partition differently than the more chlorinated and, therefore, bulkier congeners. The implication for breast cancer may be lower concentrations of 2378-TCDD and 12378-PeCDD in breast tissues and, possibly, less opportunity for toxicity. The hypothesis that concentrations are equivalent in the two types of tissues could not be rejected for any of the remaining target chemicals. In other words, given the uncertainty of the analytical method, the interindividual variability, the magnitude of the difference in concentrations in the two tissues, and

Table 3. Tests to assess agreement between abdominal and breast measurements of major analytes (intraclass correlation coefficient and Wilcoxon signed rank test) and linear regression to predict breast concentrations from abdominal concentrations

	Agreement between measurements		Correlation between measurements (breast = $a + b \times$ abdominal)		
	Intraclass correlation coefficient	Wilcoxon signed rank test (H_0 : abdominal = breast)	Intercept (a)	Slope (b)	R^2
PCDDs/PCDFs					
I-TEQ	0.843	Reject	2.053	0.844	0.731
2378-TCDD	0.471	Accept	1.913^a	0.448	0.238
12378-PeCDD	0.426	Reject	1.478	0.674	0.275
123678-HxCDD	0.930	Accept	3.614	0.921	0.867
1234678-HpCDD	0.976	Accept	0.584	1.049	0.957
12346789-OCDD	0.968	Accept	13.200	0.968	0.936
23478-PeCDF	0.942	Accept	0.372	0.926	0.894
123478-HxCDF	0.837	Accept	0.902	0.821	0.708
123678-HxCDF	0.824	Accept	0.801	0.764	0.685
1234678-HpCDF	0.763	Accept	0.527	0.968	0.614
OCPs					
DDE	0.934	Accept	167.000	0.927	0.885
<i>Trans</i> -nonachlor	0.671	Accept	49.260	0.485	0.538
Oxychlordane	0.804	Accept	18.500	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
PBDEs					
PBDE-47	0.987	Accept	-0.903	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
Sum of PBDEs	0.983	Accept	4.241	0.865	0.994
PCBs					
PCB 153	0.878	Accept	-0.263	0.920	0.790
PCB 138	0.884	Accept	11.110	0.896	0.781
PCB 180	0.800	Accept	32.810	0.649	0.697
PCB 118	0.906	Accept	4.685	0.820	0.834

^aAll intercepts are not significantly different from zero ($p > 0.05$), except for 2378-TCDD.

the number of paired samples examined, no statistically significant differences could be detected. However, there may be real differences that our study did not have the power to detect. We did observe low R^2 and deviations from a slope of 1 in the regression measurements for *trans*-nonachlor and oxychlordane. Whereas these two chemicals, both metabolites of chlordane, may indeed behave in a different way than other OCPs, the observed deviations are probably due to increased analytical error. This explanation is supported by the relatively high mean RPD for duplicate analysis for these two chemicals. Relatively poor fit ($R^2 = 0.41$) was also observed for 2,2',4,4',5,6'-hexabrominated diphenyl ether (PBDE-154), a minor PBDE constituent. Interestingly, PBDE-154 had the lowest RPD of all PBDEs in the duplicate analyses and the highest RPD in the TRAM analyses with an almost statistically significant difference in RPDs ($P = 0.06$). In addition, the regression slope for this congener (0.56) indicates that it appears to partition more in the abdominal than in the breast adipose. Both PBDE-154 and 2,2',4,4',5,5'-hexabrominated diphenyl ether (PBDE-153), which are hexabrominated congeners and, hence, bulkier than the tetra- and penta-PBDE congeners, have similar slopes, perhaps reflecting steric hindrance in transport through membranes. The other (tetra- and

pentabrominated) PBDE congeners and the total PBDEs (arithmetic sum of the five individual constituents), however, showed good fit and the latter was not affected by the erratic PBDE-154.

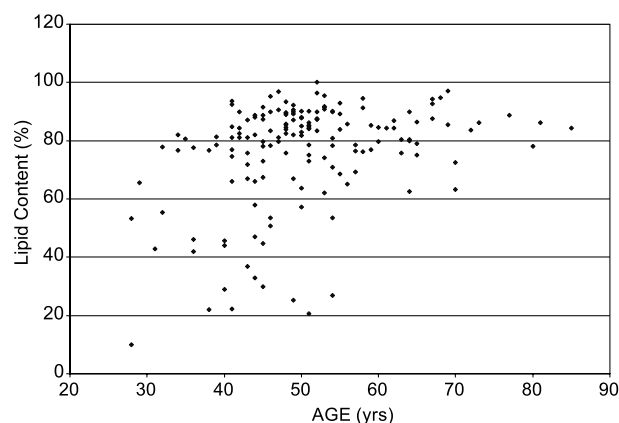


Fig. 1. Lipid content of breast adipose specimens as a function of age ($y = 46.6 + 0.59x$, $R^2 = 0.1159$, $N = 161$).

Table 4. Summary of findings from studies on organochlorines and breast cancer

Tissue	Lipid adjustment	Analytes	Reference	
Adipose	Gravimetric	–	Unger et al. (9)	
	Gravimetric	β-HCH	Mussalo-Rauhama et al. (10)	
	Gravimetric	DDE, PCB	Falck et al. (11)	
	Gravimetric	12346789-OCDD	Hardell et al. (12)	
	Gravimetric	PCB77, HCB	Liljegren et al. (13)	
	Gravimetric	DDE, PCB	Guttes et al. (14)	
	Gravimetric	–	Zheng et al. (15–18)	
	Gravimetric	PCB	Aronson et al. (19)	
	None	DDE, PCB	Dewailly et al. (20)	
	None	–	van't Veer et al. (21)	
	None	–	Stellman et al. (22)	
	Serum	Cholesterol + triglycerides + phospholipids	–	Wolf et al. (23)
		Cholesterol + triglycerides	PCB	Moysich et al. (24)
		Cholesterol + triglycerides	Dieldrin	Hoyer et al. (25)
Cholesterol + triglycerides		–	Helzlsouer et al. (26)	
Cholesterol + triglycerides		–	Dorgan et al. (27)	
Cholesterol + triglycerides		DDE	Romieu et al. (28)	
Cholesterol + triglycerides		–	Ward et al. (29)	
Cholesterol + triglycerides ^a		Total DDT, HCB	Charlier et al. (30)	
Gravimetric		–	Lopez-Carrillo et al. (31)	
Gravimetric		–	Zheng et al. (18)	
None		DDE	Wolf et al. (33)	
None		–	Krieger et al. (34)	
None		–	Mendonca et al. (35)	
Plasma		None	HCB	Dewailly et al. (20)
	Cholesterol	–	Hunter et al. (37)	
	Cholesterol + triglycerides	–	Millikan et al. (38)	
	Cholesterol + triglycerides + phospholipids	–	Demers et al. (39)	

Note: Analytes found to have a significant association with the development of breast cancer are shown along with the tissue sampled and the type of lipid adjustment, if any.

^aFasting subjects, adjustment not used.

In summary, this analysis indicates that, with some interesting exceptions, measurements of many contaminants in abdominal and breast adipose tissues are highly correlated and that concentrations measured in one type of tissue could be used to estimate the concentration in the other tissue. The poor correlations we observed with the 2378-TCDD and 12378-PeCDD congeners, as well as with PBDE-154 and the two chlordane metabolites, may detract from the conclusion that concentrations in one type of tissue can predict concentrations in another type of tissue and, therefore, require further study. It is, however, reasonable to conclude that both abdominal and breast adipose tissues could be used to measure concentrations of many persistent lipophilic contaminants, greatly facilitating future epidemiologic studies. Additional work to validate these findings and, perhaps, predict partitioning based on structure-activity relationships of many lipophilic contaminants would guide the selection of appropriate biomonitoring plans in future studies.

These findings suggest that epidemiologic studies may be able to substitute measurements on one tissue for another, but we caution that the implications of such a decision be considered. Over half the compounds listed in Table 3 had intraclass correlations ≥ 0.9 , but any correlation < 1 will attenuate or reduce an observed relative risk estimate compared with a perfect measurement. For example, an intraclass correlation of 0.9 for a surrogate measurement can reduce a true odds ratio (OR) of 2.0 to an observed OR of 1.75–1.9 depending on how the OR is defined (quantiles, continuous measurement, etc.; 59). This will have the effect of reducing the study's statistical power. Conversely, to achieve the same power,

the study's sample size would have to be increased by 23% (59). In addition, we note that the specific correlation values we report are dependent on the ranges of the concentrations obtained, and their applicability to other studies will require similar ranges. The concentration ranges (Table 2), however, are representative of those expected in contemporary U.S. populations⁹ (8, 60).

An important finding of our study was the high variability in lipid content observed in the adipose specimens. This variability may be explained by breast tissue anatomy, where adipose is interspersed within nonfatty connective tissue. It may also reflect differential presence of blood or other nonlipid components in the sample submitted for analysis. Given the small size of these samples (often < 1 g), these nonlipid components may contribute to the weight of the specimen but not to the extractable lipids. If the actual lipid content of the specimen is not measured and contaminant concentrations are expressed on the basis of the total weight of the specimen, measurements in many patients will be underestimated. We should note that, given the minuscule size of the specimens and following common practice, we did not attempt to characterize the composition of lipids.

Figure 1 shows the percentage of lipid content of all the breast adipose specimens ($N = 161$) in our full study as a function of age. As shown in Fig. 1, younger women had a broader range of lipid content. Given that age is a known risk factor for breast cancer, use of non-lipid-adjusted

⁹Petreas et al., unpublished observations.

adipose tissue concentrations may lead to misclassifications and distort the ORs for disease. Table 4 summarizes the major studies examining organochlorine contaminants as risk factors for breast cancer, the type of sample used (adipose, serum, and plasma), the analytes, if any, which were associated with breast cancer, and whether lipid adjustment was performed. It should be noted that some of these studies (21, 22) did not use lipid-adjusted adipose measurements, which may partially explain the contradictory findings. We firmly believe that tissue concentrations must be adjusted for lipid content to make valid comparisons from subject to subject within a study or to compare different studies.

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References

- U.S. Environmental Protection Agency (U.S. EPA), National Center for Environmental Assessment. Dioxin and related compounds. Washington (DC): U.S. EPA; 2003 [cited 2003 Jun 17]. Available from <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm>.
- Kutz FW, Wood PH, Bottimore DP. Organochlorine pesticides and polychlorinated biphenyls in human adipose tissue. *Rev Environ Contam Toxicol*, 1991;120:1–82.
- Robertson L, Hansen L. PCBs. Recent advances in the environmental toxicology and health effects. Lexington (KY): University Press of Kentucky; 2001.
- Smith D. Worldwide trends in DDT levels in human breast milk. *Int J Epidemiol*, 1999;28:179–88.
- De Wit C. An overview of brominated flame retardants in the environment. *Chemosphere*, 2002;46:583–624.
- Noren K, Meironyte D. Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20–30 years. *Chemosphere*, 2000;40:1111–23.
- She J, Petreas M, Winkler J, Visita P, McKinney M, Kopec D. Polybrominated diphenyl esters (PBDEs) in the San Francisco Bay Area: measured in harbor seal blubber and human breast adipose tissue. *Chemosphere*, 2002;46:697–707.
- Petreas M, She J, Brown FR, et al. High body burdens of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in California women. *Environ Health Perspect*, 2003;111:1175–9.
- Unger M, Kiaer H, Blichert-Toft M, Olsen J, Clausen J. 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*, 1984;34:24–8.
- Mussalo-Rauhamaa H, Hasanen E, Pyysalo H, Antervo K, Kauppila R, Pantzar P. Occurrence of -hexachlorocyclohexane in breast cancer patients. *Cancer*, 1990;66:2124–8.
- Falck F Jr, Ricci A Jr, Wolff MS, Godbold J, Deckers P. Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. *Arch Environ Health*, 1992;47:143–6.
- Hardell L, Lindstrom G, Liljegren G, Dahl P, Magnuson A. Increased concentrations of octachlorodibenzo-*p*-dioxin in cases with breast cancer—results from a case-control study. *Eur J Cancer Prev*, 1996;5:351–7.
- Liljegren G, Hardell L, Lindstrom G, Dahl P, Magnuson A. Case-control study on breast cancer and adipose tissue concentrations of congener specific polychlorinated biphenyls, DDE and hexachlorobenzene. *Eur J Cancer Prev*, 1998;7:135–40.
- Guttes S, Failing K, Neumann K, Kleinstein J, Georgii S, Brunn H. Chlororganic pesticides and polychlorinated biphenyls in breast tissue of women with benign and malignant breast disease. *Arch Environ Contam Toxicol*, 1998;35:140–7.
- Zheng T, Holford TR, Mayne ST, et al. β -benzene hexachloride in breast adipose tissue and risk of breast carcinoma. *Cancer*, 1999; 85:2212–8.
- Zheng T, Holford TR, Mayne ST, et al. DDE and DDT in breast adipose tissue and risk of female breast cancer. *Am J Epidemiol*, 1999;150:453–8.
- Zheng T, Holford TR, Mayne ST, et al. Environmental exposure to hexachlorobenzene (HCB) and risk of female breast cancer in Connecticut. *Cancer Epidemiol Biomarkers & Prev*, 1999;8:407–11.
- Zheng T, Holford TR, Tessari J, et al. Breast cancer risk associated with congeners of polychlorinated biphenyls. *Am J Epidemiol*, 2000;152:50–8.
- Aronson KJ, Miller AB, Woolcott CG, et al. Breast adipose tissue concentrations of polychlorinated biphenyls and other organochlorines and breast cancer risk. *Cancer Epidemiol Biomarkers & Prev*, 2000;9:55–63.
- Dewailly E, Dodin S, Verreault R, et al. High organochlorine body burden in women with estrogen receptor-positive breast cancer. *J Natl Cancer Inst*, 1994;86:232–4.
- van't Veer P, Lobbezoo IE, Martin-Moreno JM, et al. DDT (dicophane) and postmenopausal breast cancer in Europe: case-control study. *BMJ*, 1997;315:81–5.
- Stellman SD, Djordjevic MV, Britton JA, et al. Breast cancer risk in relation to adipose concentrations of organochlorine pesticides and polychlorinated biphenyls in Long Island, New York. *Cancer Epidemiol Biomarkers & Prev*, 2000;9:1241–9.
- Wolff MS, Zeleniuch-Jacquotte A, Dubin N, Toniolo P. Risk of breast cancer and organochlorine exposure. *Cancer Epidemiol Biomarkers & Prev*, 2000;9:271–7.
- Moysich KB, Ambrosone CB, Vena JE, et al. Environmental organochlorine exposure and postmenopausal breast cancer risk. *Cancer Epidemiol Biomarkers & Prev*, 1998;7:181–8.
- Hoyer AP, Grandjean P, Jorgensen T, Brock JW, Hartvig HB. Organochlorine exposure and risk of breast cancer. *Lancet*, 1998;352:1816–20.
- Helzlsouer KJ, Alberg AJ, Huang HY, et al. Serum concentrations of organochlorine compounds and the subsequent development of breast cancer. *Cancer Epidemiol Biomarkers & Prev*, 1999;8:525–32.
- Dorgan JF, Brock JW, Rothman N, et al. Serum organochlorine pesticides and PCBs and breast cancer risk: results from a prospective analysis (USA). *Cancer Causes Control*, 1999;10:1–11.
- Romieu I, Hernandez-Avila M, Lazcano-Ponce E, Weber JP, Dewailly E. Breast cancer, lactation history, and serum organochlorines. *Am J Epidemiol*, 2000;152:363–70.
- Ward EM, Schulte P, Grajewski B, et al. Serum organochlorine levels and breast cancer: a nested case-control study of Norwegian women. *Cancer Epidemiol Biomarkers & Prev*, 2000;9:1357–67.
- Charlier C, Albert A, Herman P, et al. Breast cancer and serum organochlorine residues. *Occup Environ Med*, 2003;60:348–51.
- Lopez-Carrillo L, Blair A, Lopez-Cervantes M, et al. Dichlorodiphenyltrichloroethane serum levels and breast cancer risk: a case-control study from Mexico. *Cancer Res*, 1997;57:3728–32.
- Zheng T, Holford TR, Mayne ST, et al. Risk of female breast cancer associated with serum polychlorinated biphenyls and 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene. *Cancer Epidemiol Biomarkers & Prev*, 2000;9:167–74.
- Wolff MS, Toniolo PG, Lee EW, Rivera M, Dubin N. Blood levels of organochlorine residues and risk of breast cancer. *J Natl Cancer Inst*, 1993;85:648–52.
- Krieger N, Wolff MS, Hiatt RA, Rivera M, Vogelstein J, Orentreich N. Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. *J Natl Cancer Inst*, 1994;86: 589–99.
- Mendonca GA, Eluf-Neto J, Andrada-Serpa MJ, et al. Organochlorines and breast cancer: a case-control study in Brazil. *Int J Cancer*, 1999;83:596–600.
- Lagueux J, Pereg D, Ayotte P, Dewailly E, Poirier GG. Cytochrome P450 CYP1A1 enzyme activity and DNA adducts in placenta of women environmentally exposed to organochlorines. *Environ Res*, 1999;80:369–82.
- Hunter DJ, Hankinson SE, Laden F, et al. Plasma organochlorine levels and the risk of breast cancer. *N Engl J Med*, 1997;337:1253–8.
- Millikan R, DeVoto E, Duell EJ, et al. Dichlorodiphenyldichloroethane, polychlorinated biphenyls, and breast cancer among African-American and white women in North Carolina. *Cancer Epidemiol Biomarkers & Prev*, 2000;9:1233–40.
- Demers A, Ayotte P, Brisson J, Dodin S, Robert J, Dewailly E. Risk and aggressiveness of breast cancer in relation to plasma organochlorine concentrations. *Cancer Epidemiol Biomarkers & Prev*, 2000;9:161–6.
- Morgan DP, Roan CC, Paschal EH. Transport of DDT, DDE, and dieldrin in human blood. *Bull Environ Contam Toxicol*, 1972;8: 321–6.

41. Patterson DG Jr, Fürst P, Henderson LO, et al. Partitioning of in vivo bound PCDDs/PCDFs among various compartments in whole blood. *Chemosphere*, 1989;19:135–42.
42. Mussalo-Rauhamaa H. Partitioning and levels of neutral organochlorine compounds in human serum, blood cells, and adipose and liver tissue. *Sci Total Environ*, 1991;103:159–75.
43. Akins JR, Waldrep K, Bernert JT Jr. The estimation of total serum lipids by a completely enzymatic “summation” method. *Clin Chim Acta*, 1989;184:219–26.
44. Phillips DL, Pirkle JL, Burse VW, Bernert JT Jr, Henderson LO, Needham LL. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Arch Environ Contam Toxicol*, 1989;18:495–500.
45. Needham LL, Burse VW, Head SL, et al. Adipose tissue/serum partitioning of chlorinated hydrocarbon pesticides in humans. *Chemosphere*, 1990;20:975–80.
46. Pauwels A, Covaci A, Weyler J, et al. Comparison of persistent organic pollutant residues in serum and adipose tissue in a female population in Belgium, 1996–1998. *Arch Environ Contam Toxicol*, 2000;39:265–70.
47. Archibeque-Engle SL, Tessari JD, Winn DT, Keefe TJ, Nett TM, Zheng T. Comparison of organochlorine pesticide and polychlorinated biphenyl residues in human breast adipose tissue and serum. *J Toxicol Environ Health*, 1997;52:285–93.
48. Lopez-Carrillo L, Torres-Sanchez L, Lopez-Cervantes M, Blair A, Cebrian ME, Uribe M. The adipose tissue to serum dichlorodiphenyldichloroethane (DDE) ratio: some methodological considerations. *Environ Res*, 1999;81:142–5.
49. Petreas MX, She J, Winkler J, et al. Organochlorine body burden in California populations. *Organohalogen Compounds*, 2000;48:17–21.
50. Waliszewski S, Gomez-Arroyo S, Infanzon R, Villalobos-Pietrini R, Hart M. Comparison of organochlorine pesticide levels between abdominal and breast adipose tissue. *Bull Environ Contam Toxicol*, 2003;71:156–62.
51. Ryan JJ, Schecter A, Lizotte R, Sun WF, Miller L. Tissue distribution of dioxins and furans in humans from the general population. *Chemosphere*, 1985;14:929–32.
52. Schecter A, Mes J, Davies D. Polychlorinated biphenyl (PCB), DDT, DDE and hexachlorobenzene (HCB) and PCDD/F isomer levels in various organs in autopsy tissue from North American patients. *Chemosphere*, 1989;18:811–8.
53. Startin JR, Wright C, Kelly M. Depletion of PCDDs in bull calf tissues. *Organohalogen Compounds*, 1994;21:33–7.
54. North Atlantic Treaty Organization, Committee on Challenges of Modern Society (NATO/CCMS). 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 (Report 176). Brussels (Belgium): NATO/CCMS; 1988.
55. Filemaker, Inc. FilemakerPro, ver. 6.0. Santa Clara (CA): Filemaker, Inc.; 2003.
56. SAS Institute, Inc. SAS, ver. 8.0e. Cary (NC): SAS Institute, Inc.; 2000.
57. Bland M. An introduction to medical statistics. 3rd ed. Oxford: Oxford University Press; 2000.
58. Feil VJ, Huwe JK, Zaylskie RG, et al. Chlorinated dibenzo-*p*-dioxin and dibenzofuran concentrations in beef animals from a feeding study. *J Agric Food Chem*, 2000;48:6163–73.
59. de Klerk NH, English DR, Armstrong BK. A review of the effects of random measurement error on relative risk estimates in epidemiological studies. *Int J Epidemiol*, 1989;18:705–12.
60. Petreas M, Rogers E, Zhao G, Windham G, Bhatia R, Charles M. Organohalogen body burdens in California women. *Organohalogen Compounds*, 2002;55:259–62.