Relative Abundance of Organochlorine Pesticides and Polychlorinated Biphenyls in Adipose Tissue and Serum of Women in Long Island, New York[1]

Steven D. Stellman,[2] Mirjana V. Djordjevic, Joshua E. Muscat, Lin Gong, David Bernstein, Marc L. Citron, Agnès White, Margaret Kemeny, Erna Busch, and Anne N. Nafziger

Divisions of Epidemiology [S. D. S., J. E. M., D. B.] and Cancer Etiology and Prevention [M. V. D., L. G.], American Health Foundation, New York, New York 10017; ProHEALTH Care Associates, LLP, Lake Success, New York 11042 [M. L. C.]; Long Island Jewish Medical Center, New Hyde Park, New York 11042 [A. W.]; North Shore University Hospital, Manhasset, New York 11030 [M. K., E. B.]; and Bassett Healthcare, Cooperstown, New York 13326 [A. N. N.]

Abstract

Some organochlorine pesticides (OCPs) and PCBs are under investigation as possible risk factors for breast cancer because of their estrogenic properties and widespread presence in the environment. It is important to know whether adipose tissue used by some investigators and serum assays used by others can provide comparable information on body burden. Concentrations of seven OCPs or their breakdown products as well as 14 PCB congeners were measured in the adipose tissue and serum of 293 women enrolled as controls in a case-control study of environmental factors for breast cancer in Long Island, New York, a high-risk region. Adipose OCP/PCB levels were measured using a supercritical fluid extraction method developed by the authors. 1,1-Dichloro-2,2-di(4-chlorophenyl)ethylene (p,p′-DDE) was detected in all adipose and serum samples; two chlordane derivatives, β-hexachlorocyclohexane (a lindane isomer) and hexachlorobenzene, were detected in at least 92% of adipose samples. The di-ortho hexachlorinated PCB congeners 2,4,5,2′,4′,5′-hexachlorobiphenyl and 2,3,4,2′,4′,5′-hexachlorobiphenyl were detected in all adipose and over 98% of serum samples. 1,1-Dichloro-2,2-di(4-chlorophenyl)ethylene comprised 77% of total pesticide residues in adipose and 71% in serum. 2,4,5,2′,4′,5′-Hexachlorobiphenyl comprised 24% of adipose and 21% of serum PCBs. The relative concentration patterns of the 14 PCB congeners were similar to those reported in other human studies and were also typical of patterns reported in environmental samples from various biota, including mammals and birds, but differed substantially from patterns reported in occupationally exposed workers. All adipose-serum correlations for pesticides and most PCBs were statistically significant. Either serum or adipose OCP/PCB levels of a variety of environmental organochlorine compounds may serve as useful biomarkers of body burden.

Introduction

Measurable levels of OCPs[3] and PCBs have been reported in human and animal tissue for several decades (1). Two broad groups of OCCs have been particularly targeted because some members of these groups exhibit estrogenic properties in specialized assays (2).

The first group consists of OCPs and their derivatives. These include β-HCH (Fig. 1), which is an isomer of lindane, a widely used environmental pest control product and scabicide; the fungicide HCB; breakdown products of the termiticide chlordane, *i.e.*, *trans*-nonachlor and oxychlordane; as well as p,p′-DDT and p,p′-DDE, which is the derivative of p,p′-DDT most commonly found in the environment and in humans.

The second class of organochlorinated compounds is PCBs. These are industrial mixtures that were widely used as insulators in electrical equipment, as hydraulic fluids, and in many other applications. Although their domestic manufacture has ceased, millions of pounds still remain in actively used equipment. Millions of additional pounds have been released into the environment, often through careless disposal. Fig. 1 shows the generic structure of PCBs, as well as one common PCB congener, BZ 153 [as given in the IUPAC system delineated by Ballschmiter and Zell (3)].

Within the past few years, at least 11 epidemiological studies have assessed possible associations of the body burden of these compounds with risk of breast cancer in women. Five studies have used adipose tissue (4–8), five have relied on levels measured in blood serum or plasma (9–13), and one study has used both sources (14). Significant associations between p,p′-DDE and/or PCB levels and breast cancer risk have been reported from studies that used media of either type (4, 7, 9, 13, 14), and lack of association with both adipose and blood measurements has also been reported by various authors (5, 8, 11, 12). The study by Krieger *et al.* (10) showed no overall

Received 11/5/97; revised 3/3/98; accepted 3/13/98.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by National Cancer Institute Grants CA-63021 and CA-72562.
[2] To whom requests for reprints should be addressed, at American Health Foundation, 320 East 43rd Street, New York, NY 10017. Phone: (212) 551-2521; Fax: (212) 687-2339; E-mail: stellman@compuserve.com.

[3] The abbreviations used are: OCP, organochlorine pesticide; OCC, organochlorine compound; PCB, polychlorinated biphenyl; β-HCH, β-hexachlorocyclohexane; HCB, hexachlorobenzene; p,p′-DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; p,p′-DDE, 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene; o,p′-DDD, 1,1-dichloro-2-(*ortho*-chlorophenyl)-2-(*para*-chlorophenyl)ethane; BZ, Ballschmiter and Zell; BZ 153, 2,4,5,2′,4′,5′-hexachlorobiphenyl; BZ 138, 2,3,4,2′,4′,5′-hexachlorobiphenyl; SFE, supercritical fluid extraction; GC-ECD, gas chromatography-electron capture detection.

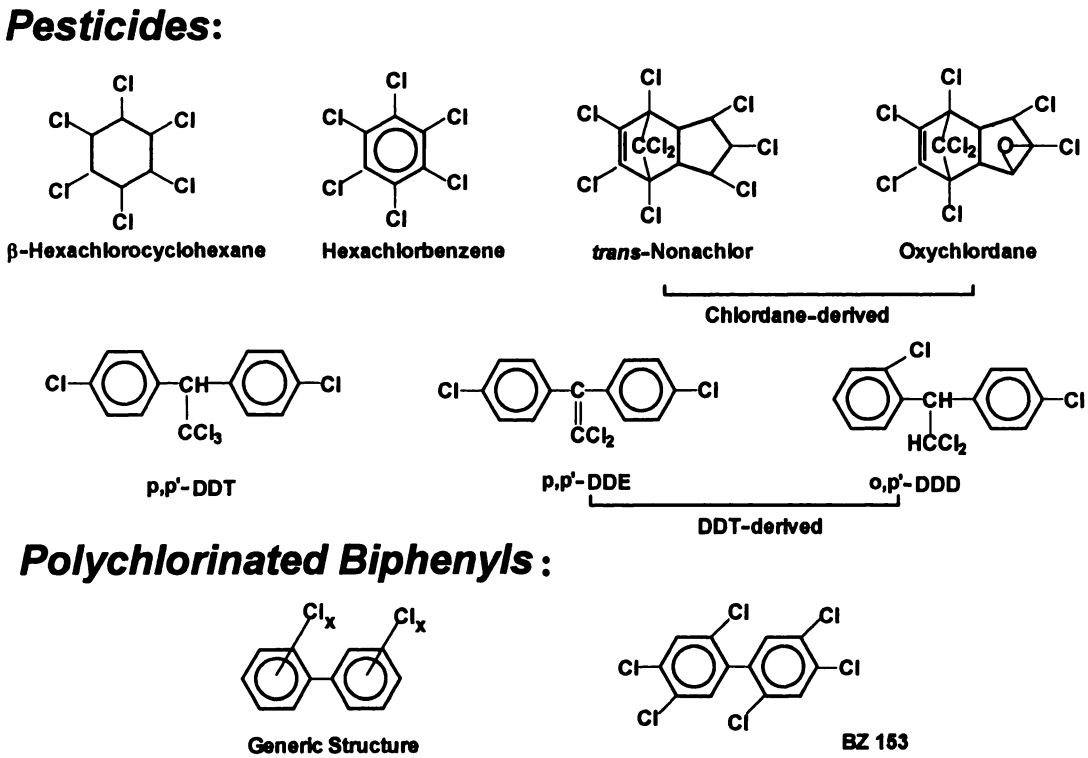

Fig. 1. Chemical structures of OCPs and PCBs.

elevation in risk, but Savitz (15) has raised the possibility that a significant association might exist within some subgroups. In one report, breast cancer risk was unrelated to adipose levels of p,p'-DDE or other pesticides but increased in a dose-related fashion with adipose levels of β-HCH (6).

A number of studies now in progress rely exclusively on serum, whereas others use adipose tissue. To compare results of the published studies with each other and with the results of other studies in progress, it is important to form a judgment as to whether serum and adipose tissue provide comparable information about body burden when used as long-term biomarkers of pesticide exposure. In this report, we used both serum and adipose tissue gathered from 293 women without breast cancer to ascertain the relative abundance of organochlorinated compounds in the two media; that is, we determined which were present in the highest concentrations, which were next highest, and so forth. We also investigated in the same population the correlations between adipose and serum concentrations of OCPs and PCBs. These comparisons provide a basis for judging the suitability of organochlorine levels measured in the two different media to represent body burden.

Materials and Methods

We have carried out a hospital-based, case-control study of breast cancer as part of the National Cancer Institute-sponsored Long Island Breast Cancer Study Project (16). The study used the patient populations of the two largest hospitals on Long Island: Long Island Jewish Medical Center, New Hyde Park, NY, and North Shore University Hospital, Manhasset, NY. Between October 1994 and October 1996, a total of 1033 female patients were interviewed in person by trained interviewers in the presurgical testing areas of the hospitals, under a protocol that was approved by the Institutional Review Boards of the hospitals and of the American Health Foundation. A serum sample was obtained from each patient during her scheduled presurgical testing appointment. Adipose tissue was collected in the operating room. Both serum and adipose tissue were immediately frozen after collection and kept in a hospital freezer at −20°C until ready for shipment on dry ice to the American Health Foundation laboratory for analysis. All laboratory personnel were blind to the identities of cases and controls.

Laboratory Methods. The levels of OCP/PCB in adipose tissue were determined by a SFE method developed expressly for this study and described by Djordjevic *et al.* (17). The assay consists of extraction of tissue by supercritical CO_2, followed by extraction with CO_2 modified with 5% dichloromethane, and simultaneous *in situ* removal of the bulk of fat on a partially deactivated neutral alumina sorbent. This is followed by additional cleanup of SFE extracts by adsorption column chromatography to remove the remaining traces of fat and analysis by GC-ECD.

Serum levels of OCP/PCB were determined using a modification of the method of Wolff *et al.* (18). A 1–2-ml serum aliquot, to which 50 μl of a 25-ppb γ-chlordane solution was added as internal control, was treated with 1.5 ml methanol and extracted three times with a 2.5-ml mixture of diethylether: hexane (1:1). The combined extract is reduced to 0.5 ml under nitrogen, chromatographed on the alumina column, and analyzed by GC-ECD.

Study Participants. A total of 362 women with pathologically confirmed breast cancer were enrolled, of whom 54 (15%) had carcinoma *in situ*. Two groups of controls were also enrolled: 470 women with benign breast disease and an additional 201 women who underwent surgery for conditions unrelated to the breast, such as hernia, cholecystectomy, and hip replacement.

Table 1 Selected characteristics of control patients

	n	(%)
Age		
<50	157	53.6
50–59	60	20.5
60–69	45	15.4
70–80	31	10.6
Total	293	100.0
Mean (yr)	50.0	
SD	13.1	
Menopausal status		
Premenopausal	136	46.4
Perimenopausal	26	8.9
Postmenopausal (natural)	88	30.0
Surgical menopause	43	14.6
Total	293	99.9
County of residence		
Nassau (Long Island)	132	45.1
Suffolk (Long Island)	24	8.2
Queens (New York City)	127	43.3
Other	10	3.4
Total	293	100.0
Education		
Through high school	95	32.4
Any college, college graduate	127	43.3
Postgraduate	71	24.2
Total	293	99.9
Mean (yr)	14.7	
SD	2.9	
Race		
White	264	90.1
Black	28	9.6
Asian	1	0.3
Total	293	100.0
Religion		
Protestant	27	9.2
Catholic	151	51.5
Jewish	101	34.5
Other, none, refused	14	4.8
Total	293	100.0
Diagnosis		
Benign breast disease	212	72.4
Surgical control	81	27.6
Total	293	100.0

Final designation of study participants as cases or benign breast disease controls was based upon pathological diagnosis as determined by post-discharge chart review and consultation with the surgeon and/or pathologist.

The present analysis is based upon the first 293 control women for whom either adipose or serum analyses have been completed. There were 212 women with benign breast disease ("benign controls") and 81 surgical controls. There were 101 women among this group for whom both adipose and serum assays were available, making it possible to study serum-adipose correlations as well as partition coefficients. Samples were chosen for analysis in the order received by the laboratory and analyzed without regard to or knowledge of case or control status.

Demographic characteristics of the 293 control women are presented in Table 1. The mean age at diagnosis was 50 years. The group was highly educated, with 43% college graduates and an additional one-fourth having some education beyond college. The predominant religion was Catholic (52%), and the second most frequent was Jewish (35%); 90% were white.

Long Island has been a focal point for several studies because of its elevated breast cancer rates (19), and the hospitals were selected to ensure a large representation from this area. About one-half of the women were residents of the two New York State counties that make up Long Island, and most of the remainder were from adjacent Queens County in New York City.

Results

Adipose and Serum Concentrations of OCCs. Adipose levels of pesticides and PCBs were measured in 221 control women, and serum levels were measured in 173. Fig. 2 shows, side by side, the similarity of distributions of the concentrations of selected analytes in adipose and in serum. Table 2 displays adipose concentrations of all 21 target analytes (seven pesticides or their breakdown products and 14 PCB congeners) in relation to limits of detection. Analytes were considered observed if their concentrations exceeded limits of detection. Table 2 also displays median and quartiles for observed concentrations. p,p′-DDE was detected in all 221 adipose samples. All other pesticides were detected in at least 92% of samples. The two PCB congeners, *i.e.*, BZ 153 and BZ 138, were also detected in all samples.

Table 3 shows the corresponding concentration distributions in serum. p,p′-DDE was detected in all serum samples analyzed. HCB, oxychlordane, and *trans*-nonachlor were present in over 97% of serum samples, whereas p,p′-DDT was above detection limits in only 38.7% of samples. BZ 153 was measurable in all but two serum samples.

Relative Abundance. The relative contribution of each of the seven pesticide residues to total pesticides in both adipose tissue and serum are shown in Table 4. p,p′-DDE was the most abundant, comprising 77% of total pesticide residues in adipose and 71% of total serum pesticides. The next most common pesticides in adipose tissue were the chlordane derivatives *trans*-nonachlor and oxychlordane, whereas in serum the most abundant after p,p′-DDE was β-HCH. The remaining pesticide residues were present in approximately equal concentrations.

Table 5 shows the relative abundance of the 14 PCB congeners in our study participants and as reported by several other authors. The most common congener was the symmetrical, di-ortho hexachlorinated congener BZ 153. It was present in nearly identical proportions in adipose and serum, comprising 24.3 and 20.9%, respectively. The next most abundant congeners were BZ 74, BZ 118, BZ 138, and BZ 180, each of which comprised at least 10% of total PCBs measured.

For comparison purposes, Table 5 also shows the relative abundance of the same set of congeners reported in several other reported human studies, as well as reports from environmental samples collected for a variety of biota. A recent report on adipose tissue by Hardell shows the relative contribution of BZ 153 about the same as ours but also weighted somewhat toward higher molecular weight congeners (20).

The two highly dissimilar columns of Table 5 (columns 4 and 5) show the congener distribution in fat and serum reported by Wolff *et al.* (21). Those data were based upon a cohort of electrical equipment workers who were occupationally exposed to PCBs for extended periods of time, including the week that samples were obtained. In those workers, the lower molecular weight congener BZ 74 predominated. The clearance rate for BZ 74 is much more rapid than for the higher molecular weight compounds like BZ 153 (22). Columns 6 and 7 show data for human adipose tissue and breast milk reported by McFarland and Clarke

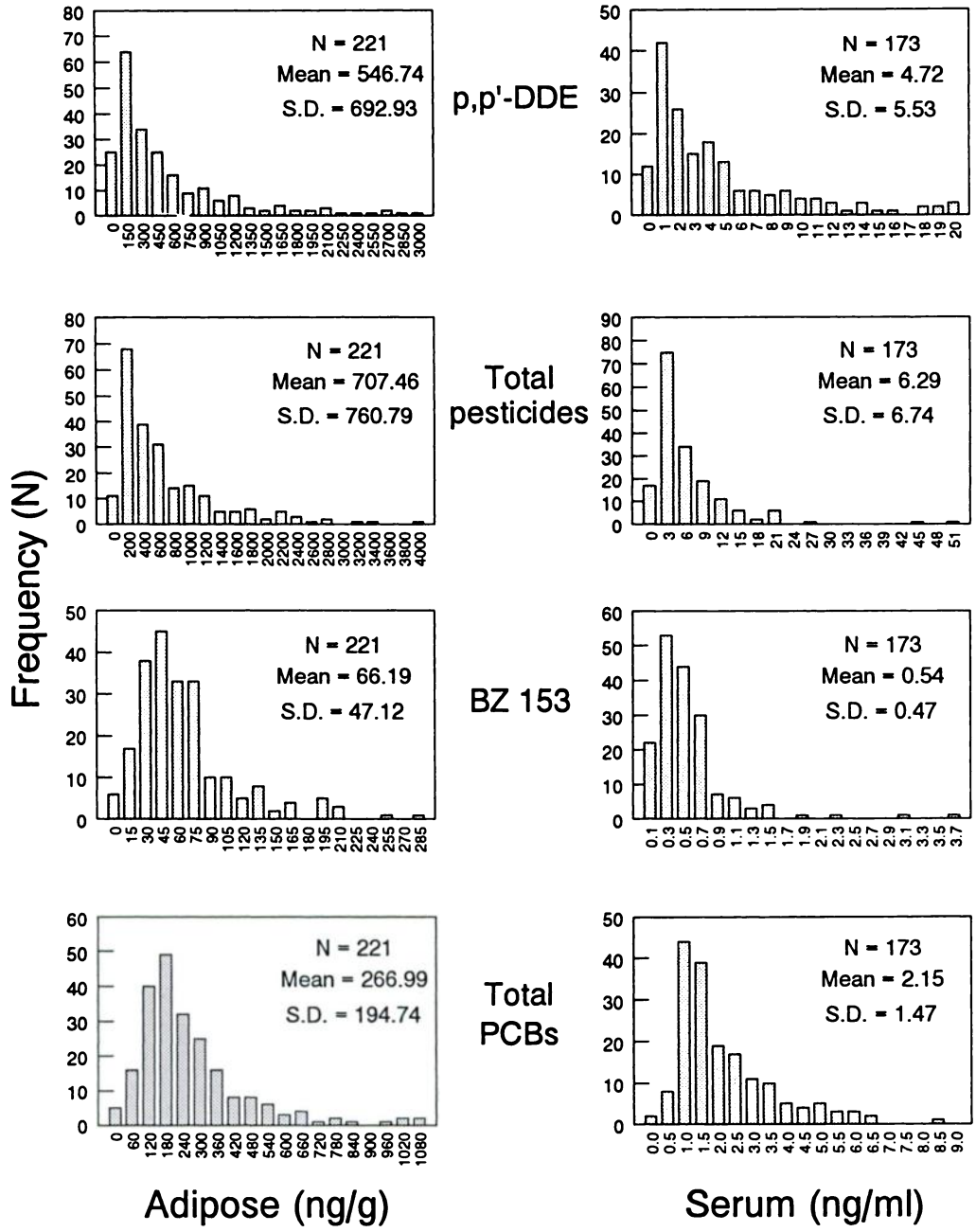

Fig. 2. Frequency distributions of concentrations of p,p'-DDE, total OCPs, total PCBs, and BZ-153 in adipose tissue and serum of Long Island women without breast cancer.

(23); these proportions are much closer to the present results.

As an additional comparison, we show the congener distributions for various species of fish, aquatic birds, aquatic mammals, oligochaetes (aquatic worms), and seston (aquatic fine particulate living matter; Table 5). The boldface entry is the congener with the highest concentration among the 14 assayed. BZ 153 was highest or nearly highest among all life forms except minnows.

Adipose-Serum Correlations. The correlation between adipose and serum concentrations is an important characteristic of lipophilic compounds such as the organochlorines, because it provides information about the steady-state partitioning of these compounds between the lipid and aqueous phases. If the concentrations in these two compartments are highly correlated, then measurements in either serum or adipose may be regarded as reliable indicators of body burden.

Fig. 3 shows scatterplots of serum *versus* adipose levels of four compounds or groups on a logarithmic scale: p,p'-DDE, total OCPs, BZ 153, and total PCBs. Each point on the graph represents serum and adipose levels of one woman. The slopes of the regression lines are sometimes called partition constants,

Table 2 Adipose tissue concentration distributions (ng/g) of 7 OCPs and 14 PCB congeners in 221 Long Island women without breast cancer

Concentration in adipose, ng/g	*n*	Detection limit	Observed[a]		Mean	SE	Percentile			Maximum
			n	%			25th	50th	75th	
p,p'-DDE	221	0.231	221	100.0	546.7	46.6	140.3	331.5	720.8	6326.3
o,p'-DDD	221	0.463	213	96.4	19.9	1.6	9.5	13.9	22.0	277.8
p,p'-DDT	221	0.602	209	94.6	17.0	1.4	6.6	10.7	19.6	171.9
β-HCH	221	0.231	203	91.9	22.2	1.6	8.8	15.2	27.1	150.5
HCB	221	0.116	216	97.7	19.7	1.8	10.7	15.9	21.3	340.4
Oxychlordane	221	0.069	217	98.2	40.9	1.8	23.2	34.2	52.8	156.6
trans-Nonachlor	221	0.069	220	99.5	45.9	2.4	23.6	35.8	53.5	211.7
Total pesticides	221				707.5	51.2	225.0	472.7	924.1	6670.0
BZ 74[b]	221	0.579	217	98.2	30.1	1.6	14.3	23.9	40.0	146.1
BZ 99	221	0.289	214	96.8	17.6	1.2	7.6	12.1	20.4	148.1
BZ 118	221	0.434	218	98.6	33.0	2.4	12.5	22.4	38.8	279.9
BZ 138	221	0.072	221	100.0	34.7	1.9	18.0	27.2	41.6	203.0
BZ 146	221	0.072	212	95.9	8.6	0.5	4.3	6.3	10.3	38.5
BZ 153	221	0.723	221	100.0	66.2	3.2	35.9	53.9	79.1	279.9
BZ 156	221	0.231	211	95.5	9.1	0.4	4.7	7.2	12.3	33.0
BZ 167	221	0.072	152	68.8	1.9	0.1	0.8	1.4	2.2	8.5
BZ 170	221	0.289	216	97.7	11.9	0.5	6.6	9.8	15.3	54.2
BZ 172	221	0.072	149	67.4	2.8	0.2	1.3	2.0	3.6	16.9
BZ 178	221	0.072	187	84.6	4.1	0.3	2.0	3.1	4.8	25.3
BZ 180	221	0.723	219	99.1	32.1	1.6	16.6	26.7	41.8	178.3
BZ 183	221	0.116	198	89.6	5.7	0.4	2.7	4.2	7.0	43.6
BZ 187	221	0.174	216	97.7	15.2	0.9	7.1	11.0	19.4	117.5
Total PCBs	221				267.0	13.1	141.8	213.9	329.0	1103.6

[a] Samples, the concentrations of which equal or exceed the limits of detection.
[b] See Refs. 3 or 23 for definitions of PCB congeners.

Table 3 Serum concentration distributions (ng/ml) of 7 OCPs and 14 PCB congeners in 173 Long Island women without breast cancer[a]

Concentration in serum, ng/ml	*n*[a]	Detection limit	Observed[b]		Mean	SE	Percentile			Maximum
			n	%			25th	50th	75th	
p,p'-DDE	173	0.028	173	100.0	4.72	0.42	1.33	3.03	6.20	46.00
o,p'-DDD	137	0.056	121	88.3	0.22	0.03	0.10	0.16	0.23	3.12
p,p'-DDT	173	0.072	67	38.7	0.22	0.02	0.11	0.14	0.24	1.00
β-HCH	137	0.028	133	97.1	0.82	0.19	0.28	0.43	0.75	25.34
HCB	173	0.014	171	98.8	0.21	0.02	0.12	0.17	0.25	2.24
Oxychlordane	173	0.008	168	97.1	0.25	0.01	0.16	0.23	0.31	0.92
trans-Nonachlor	173	0.008	172	99.4	0.24	0.01	0.13	0.19	0.28	1.48
Total pesticides	173				6.29	0.51	2.31	4.03	8.21	51.20
BZ 74[c]	137	0.069	132	96.4	0.37	0.02	0.21	0.31	0.46	1.39
BZ 99	137	0.035	126	92.0	0.16	0.01	0.09	0.12	0.19	0.60
BZ 118	173	0.052	159	91.9	0.30	0.03	0.12	0.18	0.33	2.78
BZ 138	173	0.009	171	98.8	0.31	0.02	0.16	0.26	0.37	1.31
BZ 146	173	0.009	126	72.8	0.09	0.01	0.05	0.07	0.10	0.56
BZ 153	173	0.087	170	98.3	0.55	0.04	0.28	0.44	0.69	3.62
BZ 156	137	0.028	101	73.7	0.09	0.01	0.04	0.07	0.11	0.32
BZ 167	137	0.009	56	40.9	0.04	0.003	0.02	0.03	0.06	0.14
BZ 170	173	0.035	141	81.5	0.11	0.01	0.06	0.08	0.12	1.61
BZ 172	137	0.009	10	7.3	0.13	0.10	0.02	0.02	0.04	1.06
BZ 178	137	0.009	62	45.3	0.05	0.003	0.03	0.04	0.06	0.17
BZ 180	173	0.087	145	83.8	0.29	0.03	0.14	0.24	0.35	3.66
BZ 183	173	0.014	85	49.1	0.06	0.004	0.03	0.05	0.07	0.30
BZ 187	173	0.021	150	86.7	0.11	0.01	0.05	0.09	0.15	0.53
Total PCBs	173				2.15	0.13	1.12	1.68	2.88	8.61

[a] Concentrations of selected analytes were not available for 36 subjects due to use of 30-m column for initial samples.
[b] Samples, the concentrations of which equal or exceed the limits of detection.
[c] See Refs. 3 or 23 for definitions of PCB congeners.

in analogy with the concept in physical chemistry of equilibrium established between two phases (24). These slopes, shown as straight lines in Fig. 3, were 131 for p,p'-DDE and 110 for total pesticides. PCB partition constants were shallower than for pesticides; the partition slope for BZ 153 was 27 and for total PCBs was 72.

Table 6 presents the serum-adipose correlation coefficient (ρ) for each chemical species that we measured. Values of ρ for

Table 4 Mean concentration of individual pesticides in serum and adipose tissue and the percentage each makes of total concentration

Pesticide residue	Adipose[a]		Serum[b]	
	Mean concentration (ng/g)	% of all pesticides	Mean concentration (ng/ml)	% of all pesticides
HCB	19.74	2.8	0.214	3.2
β-HCH	22.21	3.1	0.824	12.3
Oxychlordane	40.89	5.7	0.249	3.7
trans-Nonachlor	45.90	6.4	0.236	3.5
p,p'-DDE	546.74	76.8	4.72	70.7
o,p'-DDD	19.85	2.8	0.218	3.3
p,p'-DDT	16.98	2.4	0.216	3.2
Total OCPs	712.30	100.0	6.677	100.0

[a] $n = 221$.
[b] $n = 173$.

most analytes were nearly identical whether or not samples that contained individual OCP/PCB below limits of detection were included. All OCP serum-adipose correlation coefficients were statistically significant ($P < 0.001$ except for oxychlordane $P < 0.01$). The highest correlation coefficient among the OCPs was observed for HCB ($\rho = 0.84$). For DDE (detected in 100% of samples) $\rho = 0.73$, for β-HCH (second most abundant pesticide in serum) $\rho = 0.58$, and for *trans*-nonachlor (second most abundant in adipose) $\rho = 0.66$.

Significant positive serum-adipose correlation coefficients ($P < 0.05$) were obtained for 10 of the 14 PCB congeners (counting detected levels only) or for 12 of 14 (counting all samples). Actual values of ρ tended to be lower for PCB congeners than for OCPs; the highest observed was for BZ 118 ($\rho = 0.69$). For BZ 153 (the most abundant congener), it was $\rho = 0.29$ ($P < 0.01$).

Discussion

The consistent and statistically significant correlations between serum and adipose levels of the large majority of OCP/PCBs suggest that either medium may be used with confidence as a biomarker of body burden of these organochlorinated compounds. The finding of detectable but low levels of p,p'-DDE and PCBs in all women studied is typical of human sampling studies but provides no clues to sources of exposure in individual study participants. The p,p'-DDE that we measured in our study subjects is more likely to have resulted from ingestion of p,p'-DDE from exogenous sources than as a metabolic end-product of past exposure to the parent compound, p,p'-DDT (25). The principal source of exposure to p,p'-DDE and other organochlorinated compounds for most Americans is through the food chain, notably meat, poultry, fish, and dairy products (1). p,p'-DDT has been banned from commercial usage in the United States since 1972, but its environmental persistence, notably as its derivative p,p'-DDE, has given it a ubiquitous presence in animals and humans more than two decades later.

Our ability to detect with confidence very small concentrations of OCP/PCBs, especially in serum, is attributable in part to exploitation of analytical methods based upon advanced chromatographic technology. For instance, the first 36 serum samples that were analyzed by GC-ECD on a 30-m fused silica capillary column did not allow for separation of β-HCH, o,p'-DDD, or 6 of the 14 PCB congeners. Upon switching to a 60-m column with a film thickness of 1 μm, we could detect even minute amounts of these residues; β-HCH now had a detection limit of 0.03 ng/ml and was observed in 97.1% of serum samples, whereas both BZ 74 and BZ 99 were detected in >90% of serum samples. By contrast, in a recent report by Archibeque-Engle *et al.* (26), only the most abundant pesticide residue (p,p'-DDE) and PCB congeners BZ 153 and BZ 118 were quantified in serum. Their choice of chromatographic columns (either packed column or capillary column with the lower resolution capacity), together with detection limits 1–2 orders of magnitude higher than we reported in Table 3, prevented the assessment of the whole spectrum of serum OCP/PCBs. Therefore, it was not surprising that Archibeque-Engle *et al.* (26) found poor or no correlation between the adipose and serum levels of OCP/PCBs. Improved GC resolution and overall method sensitivity for OCP/PCB assessment in both serum and

Table 5 Relative abundance of 14 PCB congeners in adipose tissue and serum of Long Island women without breast cancer in comparison with other environmentally and occupationally exposed populations and in comparison with relative abundance reported in aquatic mammals, birds, fish, and other biota

	Human (%)							Porpoise (%)	Carp (%)	Duck (%)	Fathead minnow (%)	Oligochaete (%)	Seston (%)	Shrimp (%)
	Fat	Serum	Fat	Fat	Serum	Fat	Milk							
Reference	This study		20	21	21	23	23	23	23	23	23	23	23	23
Congener[a,b]														
BZ 74	11.0	14.1	1.8	**47.2**	**51.1**	0.0	16.7	0.0	3.2	3.7	**55.2**	2.6	0.0	0.0
BZ 99	6.4	6.0	2.1	7.3	7.3	2.9	7.3	5.7	0.0	0.0	15.2	0.0	10.0	21.1
BZ 118	12.1	11.3	4.8	14.5	14.6	8.2	9.9	3.6	5.8	6.9	9.0	3.8	6.7	10.6
BZ 138	12.7	11.6	23.7	7.3	7.3	21.2	15.2	27.1	14.1	13.5	0.0	14.2	**23.3**	22.5
BZ 146	3.2	3.4	0.0	1.3	0.6	4.1	2.9	0.0	3.2	3.8	0.0	2.4	0.0	0.0
BZ 153	**24.3**	**20.9**	**26.6**	9.1	7.3	**32.6**	**18.2**	**36.5**	**27.0**	**26.7**	10.5	**23.8**	20.0	**22.5**
BZ 156	3.3	3.3	2.7	3.6	2.9	3.0	7.5	0.0	0.9	1.2	1.4	0.9	0.0	0.0
BZ 167	0.7	1.5	1.1	0.9	0.6	0.7	1.3	0.0	0.0	0.0	0.5	0.0	0.0	0.0
BZ 170	4.4	4.1	9.2	1.8	1.5	5.9	8.1	5.7	12.7	13.0	1.1	13.5	13.3	4.9
BZ 172	1.0	4.8	1.1	0.0	0.0	1.8	0.5	0.0	1.2	1.3	0.0	1.3	0.0	0.0
BZ 178	1.5	1.7	1.5	0.0	0.0	1.4	0.0	0.0	0.0	0.0	4.1	0.0	0.0	0.0
BZ 180	11.8	10.8	18.3	5.4	4.4	11.7	8.1	12.2	17.1	17.6	2.9	16.8	16.7	7.0
BZ 183	2.1	2.3	3.1	0.5	0.7	1.2	2.1	2.9	5.4	5.7	0.0	7.7	3.3	0.7
BZ 187	5.6	4.3	4.0	1.1	1.5	5.3	2.3	6.5	9.5	6.7	0.0	13.0	6.7	10.6
Total[c]	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Most abundant[d]	BZ 153	BZ 153	BZ 153	BZ 74	BZ 74	BZ 153	BZ 153	BZ 153	BZ 153	BZ 153	BZ 52	BZ 153	BZ 77	BZ 52

[a] Boldface shows the congener with the highest concentration among the 14 assayed.
[b] See Refs. 3 or 23 for definitions of PCB congeners.
[c] Data from McFarland and Clarke (23) normalized to 100% for 14 congeners shown.
[d] Includes congeners other than those shown above.

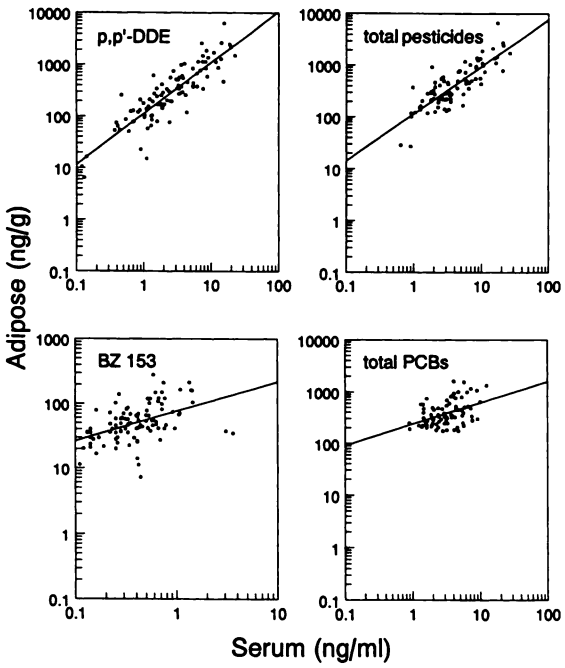

Fig. 3. Scatter plots (log-log scale) of concentrations of p,p'-DDE, total OCPs, total PCBs, and BZ-153 in adipose tissue *versus* serum of Long Island women without breast cancer.

adipose tissue may prove crucial in attempts to validate previous reports regarding the association of organochlorinated compounds with breast cancer, especially the role of β-HCH (6), pesticides other than p,p'-DDE, and perhaps specific PCB congeners.

The mean serum concentration of p,p'-DDE reported in various United States populations has clearly declined over time. The mean level of p,p'-DDE in 50 white women who served as controls in the Oakland cohort and whose blood was collected during 1964–1971 was 35.0 ppb (10). It was 11.0 ppb in 171 controls (80% of whom were white) in the study of women screened in New York City during 1985–1991 (9). The mean level in our own controls was 4.7 ppb, based on serum that we collected in 1994–1996. This temporal decline in human blood levels of p,p'-DDE since 1964 is almost certainly due to the cessation of active spraying of p,p'-DDT in the 1970s. Our study documents the persistence of OCCs in Long Island women, years after these compounds were banned from most commercial uses in the United States.

PCBs have also been banned from domestic production since 1977 but are highly persistent in the environment even today. The major source of ambient exposure is environmental cycling of PCBs introduced previously into the environment. The general population may be exposed to PCBs by inhaling contaminated air and ingesting contaminated water and food; food consumption has accounted for the major body burden in the past (2, 27). Comparison of the distribution of congeners in the present study, which were weighted toward high molecular weight species with data reported for an industrial cohort by Wolff *et al.* (21), who observed more congeners of lower molecular weights, strongly supports the hypothesis that the exposures in the women in our case-control study were largely from environmental sources such as food and air rather than occupational sources.

Concern has been raised that serum levels of OCCs may be affected by hormonal or chemotherapeutic treatment for breast cancer, and that this may in turn bias comparisons with untreated controls. This concern, of course, does not apply to cohort or nested case-control studies, such as those of Krieger *et al.* (10), or Wolff *et al.* (9) in which serum was obtained

Table 6 Correlation coefficients of adipose *versus* serum concentrations of OCPs and selected PCB congeners

	Ratio of adipose to serum	Correlation coefficient: serum *vs.* adipose[a]	No. of observations[a]	P^a	Correlation coefficient: serum *vs.* adipose[b]	No. of observations[b]	P^b
Pesticides							
p,p'-DDE	115.8	0.728	101	<0.001	0.728	101	<0.001
o,p'-DDD	91.0	0.791	67	<0.001	0.758	85	<0.001
p,p'-DDT	78.6	0.700	31	<0.001	0.702	101	<0.001
β-HCH	27.0	0.584	78	<0.001	0.581	85	<0.001
HCB	92.2	0.841	99	<0.001	0.834	101	<0.001
Oxychlordane	164.2	0.332	97	<0.01	0.361	101	<0.001
trans-Nonachlor	194.5	0.657	99	<0.001	0.661	101	<0.001
PCBs[c]							
BZ 74	80.5	0.305	83	<0.01	0.326	85	<0.01
BZ 99	109.8	0.524	76	<0.001	0.522	85	<0.001
BZ 118	109.9	0.688	90	<0.001	0.689	101	<0.001
BZ 138	112.9	0.508	100	<0.001	0.515	101	<0.001
BZ 146	96.8	0.318	69	<0.01	0.198	101	<0.05
BZ 153	119.5	0.271	99	<0.01	0.289	101	<0.01
BZ 156	104.3	0.203	60	NS[d]	0.249	85	<0.01
BZ 167	45.2	0.052	30	NS	0.280	85	<0.01
BZ 170	109.2	0.234	77	<0.05	0.296	101	<0.01
BZ 172	22.2	−0.454	6	NS	−0.265	85	NS
BZ 178	90.8	−0.337	35	<0.05	0.048	85	NS
BZ 180	111.9	0.325	80	<0.01	0.354	101	<0.001
BZ 183	94.3	0.358	43	<0.05	0.343	101	<0.001
BZ 187	133.6	0.392	83	<0.001	0.386	101	<0.001

[a] Based only on samples the concentrations of which exceeded the limits of detection.
[b] Based on all samples.
[c] See Refs. 3 or 23 for definitions of PCB congeners.
[d] NS, not significant.

months or years before development of clinical disease requiring treatment. There is some evidence that this may not even be an important problem in case-control studies. In a pilot study, Gammon *et al.* (28) found similar levels of p,p′-DDE in breast cancer cases before and after chemotherapy. Virtually all of the samples in the present report were obtained from patients prior to any treatment, and many were obtained before a definitive breast cancer diagnosis was made.

Case-control comparisons and estimation of breast cancer risk in relation to OCC levels in both adipose and serum are presently under way in this population. Considerations of statistical power and relatively small differences in OCC levels between cases and controls underscore the need to analyze samples from large numbers of individuals. Both case-control and cohort studies in the past may have been limited by high costs associated with extraction and clean-up of samples. The adipose concentrations reported here were obtained by SFE, a technique that is more efficient than the traditional Soxhlet extraction method and that also has the advantage of consuming considerably smaller quantities of organic reagents. A similar method has been applied recently to analysis of PCBs by Hardell *et al.* (20).

To summarize, the relative abundance of the OCPs and PCBs was nearly identical in serum and adipose tissue, and the concentrations of most chemical species in adipose tissue were highly correlated with their concentrations in serum. Collection of serum in population studies is ordinarily considered less invasive than adipose tissue, although at least one group has shown that large-scale adipose studies are also feasible (8, 29). Our data thus support the assertion that either adipose or serum assays can be useful measures of human body burden of environmental organochlorinated compounds in epidemiological studies of breast cancer and increase confidence that data gathered in serum-based studies reflect body burden of OCCs in a quantitative manner suitable for use in assessment of cancer risk.

Acknowledgments

We are grateful to Dr. Vincent Vinciguerra of North Shore University Hospital for continuing encouragement and cooperation. We thank Dr. Dietrich Hoffmann for providing essential resources and guidance in developing analytic methods. We especially thank Belle Hecht and Elizabeth Sellman for outstanding efforts in identifying patients, encouraging their participation, conducting the interviews, and obtaining the adipose and serum specimens; Anna Mondora for organzing and supervising the field work and quality control; Pat Lamb for administrative support; and Vicki Liang and Preciosa Ong for data and computer support. We thank the many physicians and other professional staff of North Shore University Hospital and Long Island Jewish Medical Center for making their patients available for the study, and we thank the more than 1000 patients for their cooperation.

References

1. Lordo, R. A., Dinh, K. T., and Schwemberger, J. G. Semivolatile organic compounds in adipose tissue: estimated averages for the US population and selected subpopulations. Am. J. Public Health, *86:* 1253–1259, 1996.

2. Ahlborg, U. G., Lipworth, L., Titus-Ernstoff, L., Hsieh, C. C., Hanberg, A., Baron, J., Trichopoulos, D., and Adami, H. O. 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, 1995.

3. Ballschmiter, K., and Zell, M. Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography-composition of technical Aroclor- and Clophen-PCB mixtures. Fresenius Z. Anal. Chem., *302:* 20–31, 1980.

4. Wasserman, M., Nogueira, D. P., Tomatis, L., Mirra, A. P., Shibata, H., Arie, G., Cucos, S., and Wasserman, D. Organochlorine compounds in neoplastic and adjacent apparently normal breast tissue. Bull. Environ. Contam. Toxicol., *15:* 478–484, 1976.

5. Unger, M., Kiaer, H., Blichert-Toft, M., Olsen, J., and 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., *34:* 24–28, 1984.

6. Mussalo-Rauhamaa, H., Hasanen, E., Pyysalo, H., Antervo, K., Kauppila, R., and Pantzar, P. Occurrence of β-hexachlorocyclohexane in breast cancer patients. Cancer (Phila.), *66:* 2124–2128, 1990.

7. Falck, J. F., Ricci, A., Jr., Wolff, M. S., Godbold, J., and Deckers, P. Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. Arch. Environ. Health, *47:* 143–146, 1992.

8. van't Veer, P., Lobbezoo, I. E., Martin-Moreno, J. M., Guallar, E., Gomez-Aracena, J., Kardinaal, A. F., Kohlmeier, L., Martin, B. C., Strain, J. J., Thamm, M., van Zoonen, P., Baumann, B. A., Huttunen, J. K., and Kok, F. J. DDT (dicophane) and postmenopausal breast cancer in Europe: case-control study. Br. Med. J., *315:* 81–85, 1997.

9. Wolff, M. S., Toniolo, P., Lee, E., Rivera, M., and Dubin, N. Blood levels of organochlorine residues and risk of breast cancer. J. Natl. Cancer Inst., *85:* 648–652, 1993.

10. Krieger, N., Wolff, M. S., Hiatt, R. A., Rivera, M., Vogelman, J., and Orentreich, N. Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. J. Natl. Cancer Inst., *86:* 589–599, 1994.

11. Lopez-Carrillo, L., Blair, A., Lopez-Cervantes, M., Cebrian, M., Rueda, C., Reyes, R., Mohar, A., and Bravo, J. Dichlorodiphenyltrichloroethane serum levels and breast cancer risk: a case-control study from Mexico. Cancer Res., *57:* 3728–3732, 1997.

12. Hunter, D. J., Hankinson, S. E., Laden, F., Colditz, G. A., Manson, J. E., Willett, W. C., Speizer, F. E., and Wolff, M. S. Plasma organochlorine levels and the risk of breast cancer. N. Engl. J. Med., *337:* 1253–1258, 1997.

13. Moysich, K. B., Ambrosone, C. B., Vena, J. E., Shields, P. G., Mendola, P., Kostyniak, P., Greizerstein, H., Graham, S., Marshall, J. R., Schisterman, E. F., and Freudenheim, J. L. Environmental organochlorine exposure and postmenopausal breast cancer risk. Cancer Epidemiol. Biomark. Prev., *7:* 181–188, 1998.

14. Dewailly, E., Dodin, S., Verreault, R., Ayotte, P., Sauve, L., Morin, L., and Brisson, J. High organochlorine body burden in women with estrogen receptor-positive breast cancer. J. Natl. Cancer Inst., *86:* 232–234, 1994.

15. Savitz, D. A. Re: breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. J. Natl. Cancer Inst., *86:* 1255, 1994.

16. Wittenberg, C. Long Island breast cancer studies move forward. J. Natl. Cancer Inst., *86:* 1501–1503, 1994.

17. Djordjevic, M. V., Hoffmann, D., Fan, J., Prokopczyk, B., Citron, M. L., and Stellman, S. D. Assessment of chlorinated pesticides and polychlorinated biphenyls in adipose breast tissue using a supercritical fluid extraction method. *15:* 2581–2585, 1994.

18. Wolff, M. S., Rivera, M., and Baker, D. B. Detection limits of organochlorine pesticides and related compounds in blood serum. Bull. Environ. Contam. Toxicol., *47:* 499–503, 1991.

19. Kulldorf, M., Feuer, E. J., Miller, B. A., and Freedman, L. S. Breast cancer clusters in the Northeast United States: a geographic analysis. Am. J. Epidemiol., *146:* 161–170, 1997.

20. Hardell, L., van Bavel, B., Lindstrom, G., Fredrikson, M., Hagberg, H., Liljegren, G., Nordstrom, M., and Johansson, B. Higher concentrations of specific polychlorinated biphenyl congeners in adipose tissue from non-Hodgkin's lymphoma patients compared with controls without a malignant disease. Int. J. Oncol., *9:* 603–608, 1996.

21. Wolff, M. S., Thornton, J., Fischbein, A., Lilis, R., and Selikoff, I. J. Disposition of polychlorinated biphenyl congeners in occupationally exposed persons. Toxicol. Appl. Pharmacol., *62:* 294–306, 1982.

22. Matthews, H. B., and Anderson, M. W. Effect of chlorination on the distribution and excretion of polychlorinated biphenyls. Drug Metabol. Dispos., *3:* 371–380, 1975.

23. McFarland, V. A., and Clarke, J. U. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. Environ. Health Persp., *81:* 225–239, 1989.

24. Menzer, R. E. Water and soil pollutants. *In:* M. O. Amdur, J. Doull, and C. D. Klaassen (eds.), Casarett and Doull's Toxicology, Ed. 4, pp. 872–902. New York: Pergamon, 1991.

25. Morgan, D. P., and Roan, C. C. Absorption, storage, and metabolic conversion of ingested DDT and DDT metabolites in man. Arch. Environ. Health, *22:* 301–308, 1971.

26. Archibeque-Engle, S. L., Tessari, J. D., Winn, D. T., Keefe, T. J., Nett, T. M., and Zheng, T. Comparison of organochlorinated pesticide and polychlorinated biphenyl residues in human breast adipose tissue and serum. J. Toxicol. Environ. Health, *52:* 285–293, 1997.

27. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Polychlorinated Biphenyls (Update). Atlanta, GA: United States Department of Health and Human Services, Public Health Service, 1996.

28. Gammon, M. D., Wolff, M. S., Neugut, A. I., Terry, M. B., Britton, J. A., Greenebaum, E., Hibshoosh, H., Levin, B., Wang, Q., and Santella, R. M. Treatment for breast cancer and blood levels of chlorinated hydrocarbons. Cancer Epidemiol. Biomark. Prev., *5:* 467–471, 1996.

29. Kohlmeier, L., and Kohlmeier, M. Adipose tissue as a medium for epidemiologic exposure assessment. Environ. Health Persp., *103* (Suppl. 3)*:* 99–106, 1995.