# Levels of PCDD/PCDFs, PCBs and OC Pesticides in Breast Adipose of Women Enrolled in a California Breast Cancer Study

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## INTRODUCTION

The debate on whether certain PCBs and organochlorine pesticides (OCPs) contribute to breast cancer continues as a number of studies produce conflicting results (1-9). As more evidence becomes available on the estrogenic and antiestrogenic activity of certain PCB and PCDD/PCDF congeners, reliable analytical methods are necessary to conduct epidemiologic studies on the possible links of these chemicals to breast cancer. A challenge to analysts involved in such studies is the limited amount of material available for analysis. In this paper, an isotope dilution procedure is described for the analysis of PCDD/PCDF, PCBs and OCPs in 1 g of human breast adipose tissue.

## EXPERIMENTAL METHODS

Study population. Women undergoing breast surgery (biopsy, lumpectomy, or mastectomy) at Stanford University, California, were recruited in the study. Small amounts of breast adipose tissue were collected during surgery and women were interviewed regarding risk factors. The total study population consists of 50 cases and 50 controls and the study is expected to be completed by the time of this presentation.

Sample preparation. Samples were stored at -20 °C until analysis. Samples were thawed, weighed, mixed with $Na_2SO_4$ and homogenized with 1:1 dichloromethane:hexane. Following centrifugation, 1/10 of the volume was analysed for OCPs and PCBs and the remaining 9/10 analysed for PCDD/PCDFs and coplanar PCBs.

OCP and PCB analysis. Samples were processed through an automated GPC column (FMS) linked to a Florisil column. The extract was concentrated and recovery standards

were added. The same extract was injected into a HRGC/HRMS (EI) to measure PCBs and into a HRGC/LRMS (NCI) to measure OCPs.(10)

PCDD/PCDF and coplanar PCB analysis. Samples were serially processed through columns containing $Na_2SO_4$ and AX21 Carbon. PCDD/PCDFs and coplanar PCBs were eluted from the carbon column with toluene in the reverse direction and the eluant cleaned up with Alumina; recovery standards were added and the sample concentrated to 10 μL.

Lipid content. Lipid content was determined gravimetrically in an aliquot of the extract.

Internal Standards. Labelled internal standards were used for PCDD/PCDFs (all seventeen 2,3,7,8-substituted congeners), for PCBs (#77, 126, 169, 28, 52, 47, 101, 105, 118, 153, 180, 194, 209) and for OCPs (HCB, β-HCH, DDT, DDE, Mirex, Dieldrin).

HRGC/HRMS for PCDD/PCDF and PCBs PCDD/PCDFs and PCBs were analyzed by HRGC/HRMS (Finnigan MAT 90) with a 60m, 0.25 mm ID, 0.25 μm film thickness, DB-5ms column. The samples were introduced through a SPI injector. The temperature was held initially at 220 °C for 2 minutes, increased to 260 °C at 5 °C/min, and then to 300 °C at 1 °C/min. PFK was used for the lock masses and the MS was operated in an EI mode with multiple ion monitoring.

NCI-HRGC/LRMS for OCPs OCPs were analyzed by HRGC/LRMS in NCI mode (Finnigan 4500) with a 60m, 0.25 mm ID, 0.25 μm film thickness, DB-5ms column. The samples were introduced through a splitless injector and methane was the reagent gas. The ion source pressure was held at 0.6 Torr and ion source temperature was 100 °C. The electron energy was typically 70eV and the electron current was kept at 0.3 mA. A quantitative standard mixture of $^{13}C$ and $^{12}C$ OCPs at concentrations of 125-400 pg/μL was injected daily to evaluate instrument performance and obtain response factors.

QA/QC. Standard QA/QC procedures were followed (Method blanks, recovery standards, duplicate analysis). In addition SRM 1945 (PCBs and OCPs in whale blubber) was used to assess performance (10).

## RESULTS and DISCUSSION

A wide range (3-94%, with a mean of 66%) of lipid content was observed in the specimens received for analysis, making it imperative that results be expressed on a lipid basis to avoid misclassifications. Body burdens of major organochlorine analytes appeared overall similar, with a few exceptions, to data reported in other studies (4, 11,12). Preliminary data from 62 breast adipose samples are shown on Table 1 for the most prevalent PCDD/PCDFs, PCBs and OCPs. The most prevalent OCPs in this set of US women were p,p'-DDE, trans-nonachlor, oxychlordane, p,p'-DDT, HCB, β-HCH and dieldrin (10). Of the 84 PCB congeners measured, the 20 shown on Table 1 comprised over 75% of the Total PCBs. The most prevalent PCBs

were 153/132, 180, 74, 138, 182/187, 170, comprising over 50% of the Total PCBs measured. The four PCDD/PCDF congeners shown were measurable above detection in all subjects, while the remaining congeners were not systematically found. To adjust for missing values (below the DL), an adjusted TEQ (Adj-TEQ) based on only those four congeners is given in addition to the I-TEQ which uses half the DL for non-detects.

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Table 1. Concentrations of OCPs, Dioxins and Furans, co-planal PCBs and non-coplanar PCBs among all study participants with complete data enrolled to date (n=62). Data expressed as ng/g fat (OCPs, non-coplanar PCBs) or pg/g fat (Dioxins and Furans, co-planal PCBs).

| Chemical | n | Mean | Std. Dev. | Median | Min. | Max. |
|---|---|---|---|---|---|---|
| OCPs (ng/g fat): | | | | | | |
| DDE | 60 | 745 | 364 | 682 | 120 | 2200 |
| trans-nonachlor | 60 | 136 | 148 | 87 | 20 | 690 |
| Oxychlordane | 59 | 72 | 57 | 56 | 17 | 340 |
| DDT | 59 | 50 | 43 | 40 | 8 | 260 |
| HCB | 60 | 46 | 28 | 35 | 14 | 170 |
| β-HCH | 61 | 41 | 38 | 32 | 1 | 210 |
| Dieldrin | 59 | 34 | 30 | 28 | 8 | 230 |
| DDE/DDT | 58 | 21 | 21 | 16 | 1.5 | 129 |
| Total OCPs | 58 | 1140 | 484 | 1090 | 253 | 2680 |
| PCBs (ng/g fat): | | | | | | |
| 153/132 | 61 | 159 | 97 | 131 | 44 | 559 |
| 180 | 61 | 139 | 78 | 123 | 55 | 497 |
| 74 | 61 | 86 | 107 | 56 | 12 | 790 |
| 138 | 61 | 98 | 68 | 80 | 16 | 402 |
| 182/187 | 61 | 47 | 31 | 40 | 15 | 212 |
| 170 | 61 | 60 | 34 | 50 | 21 | 165 |
| 196/203 | 61 | 36 | 20 | 33 | 12 | 132 |
| 194 | 61 | 42 | 23 | 36 | 16 | 117 |
| 199 | 61 | 29 | 17 | 27 | 9 | 103 |
| 156 | 61 | 34 | 28 | 26 | 4 | 160 |
| 118 | 61 | 27 | 16 | 24 | 6 | 86 |
| 206 | 61 | 22 | 19 | 15 | 6 | 117 |
| 183 | 61 | 18 | 13 | 15 | 6 | 79 |
| 99/113 | 61 | 18 | 13 | 13 | 5 | 89 |
| 177 | 61 | 18 | 14 | 14 | 3 | 85 |
| 28 | 61 | 10 | 7 | 8 | 2 | 47 |
| 105/127 | 61 | 6 | 4 | 5 | 1 | 18 |
| 128/162 | 61 | 6 | 4 | 5 | 0 | 21 |
| 157 | 61 | 5 | 5 | 4 | 0 | 25 |
| 101 | 61 | 4 | 4 | 3 | 1 | 28 |
| Total PCBs | 61 | 1120 | 585 | 983 | 451 | 3830 |
| Coplanar PCBs(pg/g fat) | | | | | | |
| PCB 77 | 46 | 37 | 73 | 23 | 4 | 501 |
| PCB 126 | 51 | 91 | 73 | 72 | 1 | 345 |
| PCB 169 | 52 | 62 | 40 | 55 | 2 | 204 |
| PCB_TEQ | 60 | 38 | 23 | 34 | 7 | 110 |
| Dioxins & Furans (pg/g fat): | | | | | | |
| OCDD | 62 | 573 | 568 | 404 | 136 | 3290 |
| HpCDD | 62 | 72.2 | 48 | 62 | 13 | 293 |
| 123678-HxCDD | 62 | 61 | 38 | 54 | 10 | 232 |
| 23478-PeCDF | 62 | 10.4 | 5.3 | 9.5 | 3 | 26 |
| I-TEQ | 62 | 17.8 | 13 | 14 | 6 | 78 |
| Adj-TEQ * | 62 | 12.6 | 6.9 | 10 | 5 | 42 |

* Adj-TEQ: Based only on the 4 congeners listed