# EFFECTS OF ORGANOHALOGENS AND METALS ON THE MENSTRUAL CYCLE OF THE CREE OF JAMES BAY

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## Introduction

Measurable serum quantities of organohalogens (OHs), such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and pesticides are an inevitable consequence of modern life. However, these ubiquitous contaminants appear to be associated with disease. More specifically, the pesticide dichloro-diphenyl-trichloroethane (DDT) and its residue dichlorodiphenyldichloroethylene (DDE) have been linked to sterility, spontaneous abortion, stillbirth, and birth defects[1]. Exposure to DDT and PCBs is associated with decreased menstrual cycle length[2,3], luteal phase length[3] and increased rate of abnormalities in menstrual flow[4]. Higher levels of DDT are associated with lower levels of urinary estrone conjugates ($E_1C$) and pregnanediol 3-glucuronide (Pd3G), metabolites of estradiol and progesterone[5].

Organohalogens are not the only contaminants associated with compromised reproductive health. Increased blood levels of lead have been associated with higher follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels[6], irregular menstruation, and infertility[7].

The Cree peoples living on the western shore of James Bay have previously been shown to have higher blood levels of OHs and lead than inhabitants of lower latitudes[8]. The purpose of the present study was to examine whether women who experience more menstrual cycle dysfunction have different levels of OHs and metals in their blood compared to women who do not experience dysfunction.

## Materials and Methods

*Subjects.* Thirty-four women, ages 18-41 ($\bar{x}$ = 31.02, SD=6.61) from the First Nations Cree population participated in the study between July 2000 and October 2004. Participants had not breast fed or used an intrauterine device/oral contraceptives within the previous 3 months or used injectable contraceptives within the previous 12 months. Participants also had not been pregnant for at least 6 months prior to enrollment, and were free of chronic diseases, endocrine disorders or reproductive diseases. Women with menstrual irregularities were not excluded.

*Hormone and Menstrual Cycle Analyses.* Daily first-morning urine samples were collected using kits consisting of urine specimen vials, urine collection cups, and a storage box. Specimens were stored in the participants' home freezers, then in a central -20C freezer until transferred to the laboratory and stored at -80C. While collecting urine samples, participants were asked to keep a daily diary with information about their urine collections, menstrual bleeding and symptoms, illness, medications, physical exertion, and intakes of caffeine, alcohol, and tobacco.

LH and FSH were assayed using non-competitive time-resolved immunofluorometric assays[9] while the urinary metabolites, estrone 3-glucuronide ($E_1$3G) and Pd3G, were assayed using competitive time-resolved fluoroimmunoassays[10]. Creatinine levels were measured using a Vitros 250 Chemistry System (Ortho-Clinical Diagnostics)[11] to normalize hormonal levels for variability in urine production.

*Contaminant Analyses.* Blood samples were obtained from participants for plasma measurements of exposure. OHs were measured by GC-MS[8] and include: aldrin, alpha-chlordane, cis-nonachlor, trans-nonachlor, gamma-chlordane, hexachlorobenzene, β-hexachlorohexane, mirex, oxychlordane, PBDE congeners 47, 99, 100, and 153, PCB congeners 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 163, 170, 180, 183, and 187, p,p'-DDE, and p,p'- DDT. Aroclor 1260 was imputed from PCB congener abundance. Metals were measured by ICP-MS[12] and include: antimony, beryllium, bismuth, cadmium, cobalt, copper, lead, lithium, molybdenum, nickel, selenium, silver, tellurium, thallium, tin, arsenic, mercury, uranium, and zinc. OH values were corrected for lipid content, which was calculated by enzymatic analysis.

*Group Classification.* Graphs and cycle phase lengths were generated from the hormone levels and days of bleeding of each woman. Day of the LH surge onset and day of luteal transition defined the last day of the follicular phase[13]. Women were classified as having: No Dysfunction, Hypermenorrhea (>7 days bleeding, including 1-2 days of no bleeding if bracketed by bleeding day), Short Follicular Phase (<12 days), or Other Dysfunctions. Other Dysfunctions include long follicular phase (>23 days), long luteal phase (>16 days), short luteal phase (<10 days), long menstrual cycle (>35 days), short menstrual cycle (<25 days), and premature menstrual bleeding (Pd3G value on day of menses onset is >50% peak).

*Statistical Analyses.* In both the metals and OHs datasets, individual variates were often highly correlated. To reduce the large number of variables under investigation, principal components analysis (PCA) was employed for OHs and metal datasets, separately. PC scores for participants were only calculated using OHs and metals detected in greater than 70% of samples; values below the levels of detection were imputed as one-half the detection limit. The log(x+1) concentration of all contaminants measured was then used in separate PCAs of the correlation matrices of the metals and the OH variables to obtain two sets of PC scores for all participants. Using these PC scores, t-tests (*a priori* user-defined contrasts for either equal or unequal variances, as required) were performed to compare a control group (No Dysfunction) with each of the Hypermenorrhea, Short Follicular Phase, or Other Dysfunctions groups. We also compared the Short Follicular Phase group with the Other Dysfunctions group using the same method, but the primary focus was to compare the levels of contaminants between the No Dysfunction group and the three types of dysfunction.

**Results**

*PC Loadings.* Tables 1 and 2 show the PC loadings for the 12 OH variables and 17 metals, respectively. The axis loadings in PCA, combined with the raw concentration data for each participant, determine the PC axis scores—the new variable values—for each participant. The PC-1 axis for 12 OH variables is heavily loaded (greater than 0.8) in trans-nonachlor, oxychlordane, p'-DDE, PCB congeners 118, 138, 153, 163, 170, 180 and 187, and can be considered an overall measure of exposure to PCBs and pesticides, whereas PC-2 is a measure of exposure to PBDEs (PBDE congeners 47 and 153). For the metals, the PC-1 score was heavily determined by variation in the concentrations of thallium, antimony, cobalt and copper. Metals PC-2 score was a measure of the concentrations of lead, total arsenic, total mercury, nickel and zinc. Metals PC-3 score was determined mostly by total mercury, tin, selenium and cadmium concentrations. Finally, metals PC-4 score was a measure based upon molybdenum, bismuth and silver concentrations. Based on these loadings and the results of comparisons between dysfunction groups, we were able to see which contaminant groups may have a greater effect on observed variations in menstrual cycles.

*Contrast Tests.* Using t-tests at a significance value of $p = 0.05$, the PC scores described above were compared between the different dysfunction groups (No Dysfunction, Hypermenorrhea, Short Follicular Phase, Other Dysfunctions). Figure 1 shows the significant results. In tests of the PC axis scores of the 12 OH variables, the comparison for No Dysfunction vs. Other Dysfunctions ($t_{(30)} = 2.354$, $p=0.025$) and the comparison for the Short Follicular Phase group vs. Other Dysfunctions ($t_{(30)} = 2.167$, $p=0.038$) were significant (Figure 1A). The comparisons of the PC scores of metals produced significant results for the PC-1 score for the contrast of Short Follicular Phase vs. Other Dysfunctions ($t_{(28)} = -2.328$, $p=0.027$) (Figure 1B). There were also significant results for the PC-4 score between No Dysfunction and Short Follicular Phase ($t_{(28)} = 2.114$, $p=0.044$) and between Short Follicular Phase vs. Other Dysfunctions ($t_{(28)} = -2.299$, $p=0.029$) (Figure 1C).

**Discussion**

The results suggest that variation in exposure to environmental OHs and metals are associated with menstrual cycle dysfunction. More specifically, we found that women with Other Dysfunctions had significantly higher exposure to trans-nonachlor, oxychlordane, p'-DDE, PCB congeners 118, 138, 153, 163, 170, 180, and 187 collectively compared to women that had no menstrual cycle dysfunction. In addition, women with Short Follicular Phases had higher levels of molybdenum, bismuth and silver than the women with No Dysfunction.

This study was limited to a small population of First Nation Cree women. Owing to the small size of this group, the effects of the contaminants observed in this study may not be representative in all women or even all Cree women. It would be beneficial to conduct similar studies on a variety of populations to compare the results. All the women in

this study were exposed to environmental contaminants, including the control (No Dysfunction) group. For this reason, it would be helpful to conduct further studies with a control group that has been less exposed to these contaminants to obtain a better comparison of the actual effects. Because the Other Dysfunctions group encompasses numerous dysfunctions, it is hard to determine if any particular dysfunction is specifically associated with exposure to contaminants. However, our work suggests that future studies should look at the specific dysfunctions within this group. We found that the use of derived PC variables to represent variation in a suite of contaminants was a useful means for reducing the large number of variables for analysis. The PC analysis also served as a preliminary means for identifying contaminants that are more likely to have a significant effect on menstrual cycle function.

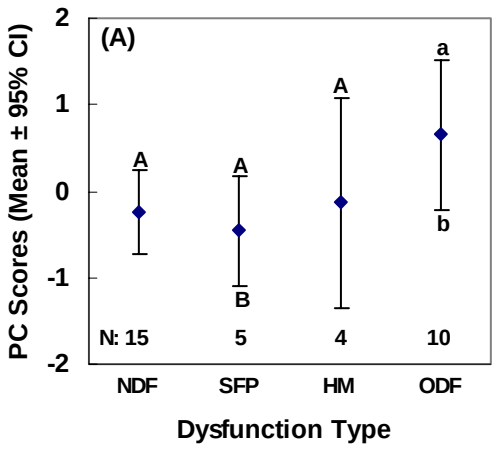
**Table 1: PC loadings for 12 OH variables**

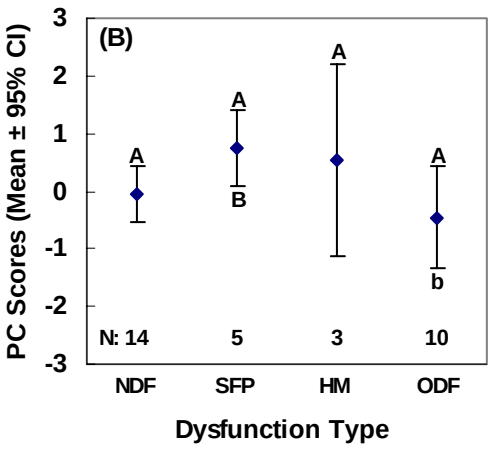
| Component Matrix (variance explained in parenthesis) | | |
|---|---|---|
| | **Component** | |
| | 1 (80.7%) | 2 (11.4%) |
| trans-Nonachlor | 0.932 | -0.146 |
| Oxychlordane | 0.892 | -0.145 |
| p'-DDE | 0.921 | -0.141 |
| PBDE 47 | 0.304 | 0.908 |
| PBDE 153 | 0.628 | 0.682 |
| PCB 118 | 0.951 | -0.0604 |
| PCB 138 | 0.985 | -0.00770 |
| PCB 153 | 0.982 | -0.0188 |
| PCB 163 | 0.984 | -0.0568 |
| PCB 170 | 0.982 | -0.0701 |
| PCB 180 | 0.981 | -0.0472 |
| PCB 187 | 0.977 | -0.0560 |

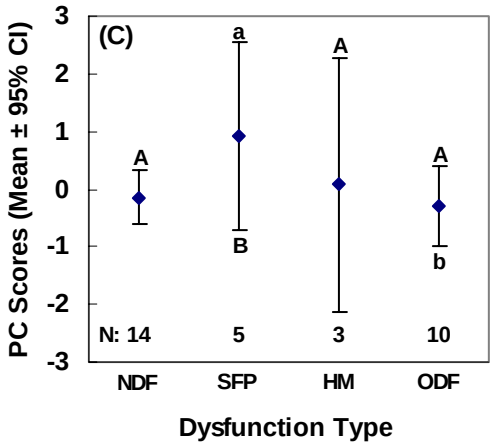
**Table 2: PC loadings for metals**

| Component Matrix (variance explained in parenthesis) | | | | |
|---|---|---|---|---|
| | **Component** | | | |
| | **1 (15.8%)** | **2 (15.5%)** | **3(12.4%)** | **4(11.3%)** |
| Thallium | -0.790 | 0.082 | -0.125 | -0.173 |
| Antimony | 0.594 | 0.196 | -0.157 | 0.340 |
| Cobalt | 0.523 | 0.226 | 0.162 | -0.482 |
| Lead | -0.365 | 0.718 | -0.065 | -0.271 |
| Total Arsenic | -0.329 | 0.694 | 0.345 | 0.291 |
| Nickel | 0.431 | 0.678 | 0.253 | 0.241 |
| Total Mercury | -0.369 | 0.619 | -0.520 | 0.050 |
| Zinc | -0.294 | -0.526 | 0.024 | -0.134 |
| Tin | -0.291 | 0.102 | -0.726 | 0.188 |
| Selenium | -0.098 | -0.031 | 0.656 | 0.087 |
| Molybdenum | 0.124 | -0.191 | -0.008 | 0.736 |
| Bismuth | -0.037 | 0.178 | 0.190 | 0.546 |
| Silver | 0.403 | 0.427 | -0.131 | -0.529 |
| Lithium | -0.137 | 0.388 | 0.332 | 0.103 |
| Uranium | 0.424 | -0.019 | -0.344 | 0.178 |
| Cadmium | -0.069 | -0.047 | 0.516 | -0.283 |
| Copper | 0.556 | 0.080 | -0.277 | -0.163 |

Figure 1. Comparison of the PC scores (means ± 95% CI) across menstrual cycle dysfunction types: No Dysfunction (NDF), Short Follicular Phase (SFP), Hypermenorrhea (HM), & Other Dysfunctions (ODF). (A) PC-1 scores for 12 OH variables. (B) PC-1 Scores for 17 metal variables. (C) PC-4 scores for 17 metal variables. The respective PCs account for 80.7% (A), 15.8% (B), and 11.3% (C) of the data variance. The notations "A" and "a" adjacent to the upper confidence limit designate results of *a priori* comparisons between NDF and each of the other groups; "B" and "b" designate the *a priori* comparison between SFP and ODF. Differences in the case of the letter indicate a significant difference ($p < 0.05$). N = number of observations.



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