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Changes in Maternal Serum Chlorinated Pesticide Concentrations across Critical Windows of Human Reproduction and Development

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Abstract

Investigators often employ a single cross-sectional measure of *in utero* exposure when evaluating associations between organochlorine pesticides/ metabolites (OCs) and adverse reproductive outcomes. Few data are available on the stability of exposures to OCs over critical windows of human reproduction and development inclusive of the periconception window. Our objective was to measure changes in OC concentrations prior to conception and throughout pregnancy or after 12 unsuccessful months attempting pregnancy. Seventy-nine women planning pregnancy were prospectively enrolled and followed for up to 12 menstrual cycles. Blood specimens were obtained for toxicologic analysis of seven OCs from participating women at baseline (preconception, n=79), at the first prenatal visit following a positive pregnancy test leading to a live birth (n=54) or after pregnancy loss (n=10), at approximately 6 weeks post-partum (n=53), and after 12 unsuccessful cycles (n=9). Overall and daily rate of change in OCs concentration (ng/g serum) were estimated adjusting for serum lipids and baseline concentration. Significant ($P < 0.05$) decreases in the overall and daily rate of change in OCs concentrations (ng/mL serum) were observed from baseline to pregnancy for HCB (−0.032, −0.001, respectively) and trans-nonachlor (−0.050, −0.002, respectively) while oxychlordan demonstrated an increase during this critical window (0.029, 0.001, respectively). Significant decreases in aldrin (−0.002, -1.47×10^{-4} , respectively), HCB (−0.069, −0.003, respectively), and trans-nonachlor (−0.045, −0.002 respectively), and an overall increase for oxychlordan (0.015) were seen for women with pregnancy losses. Significant decreases also were observed among infertile women for aldrin (−0.003, -3.52×10^{-6} , respectively), DDE (−0.210, -4.29×10^{-4} , respectively), and HCB (−0.096, -2.03×10^{-4} , respectively), along with an increase for trans-nonachlor (0.034, 7.59×10^{-5} , respectively). These data, though limited by sample size and the possibility of laboratory measurement error, suggest that OC concentrations may change over critical windows. This underscores the importance of timing biospecimen collection to critical windows for development in the assessment of reproductive and/or developmental effects.

Keywords

critical windows; organochlorine pesticides; persistent organic pollutants; pregnancy; pregnancy loss

Introduction

Organochlorine pesticides persist in the environment and accumulate in lipids, facilitating their bio-magnification in aquatic food chains, and leading to human exposure (Humphrey 1987; National Research Council.1999). This broad chemical family includes, but is not limited to aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene (HCB), lindane, and mirex, as well as their contaminant isomers or metabolic byproducts such as β -hexacyclochlorohexane (β -HCH, a contaminant of lindane), dichlorodiphenyldichloroethylene (DDE; i.e., metabolite of DDT), oxychlordane (i.e., metabolite of chlordane), and trans-nonachlor (i.e., metabolite of chlordane) (Casarett et al. 1996). Despite banned or restricted use of these compounds, exposure appears ubiquitous in North America, likely a consequence of prior widespread use, environmental persistence, and consequent contamination of the food supply (Gunderson 1995).

The human embryo and fetus comprise potentially vulnerable subpopulations for developmental toxicants, in part, given the highly interrelated and timed sequence underlying successful human development as reflected in critical windows (Hruska et al. 2000). Maternal exposure as measured by serum or plasma concentrations of organochlorine compounds has been associated with adverse reproductive and developmental outcomes as recently summarized (Buck Louis et al. 2006; Toft et al. 2004).

Dietary organochlorine pesticide exposure also has been associated with adverse human reproductive outcomes, though with equivocal results (Hruska et al. 2000), possibly reflecting differences in cross-sectional exposure assessment and its timing, involving women with either clinically recognized pregnancies or deliveries (Axmon et al. 2004; Maervoet et al. 2007). This exposure assessment strategy is likely motivated by the reported long half-lives and environmental persistence of these compounds (Agency for Toxic Substances and Disease Registry 1994; 1995; 2002a–c; 2005), though recent findings suggest serum concentrations of a closely related group of persistent organochlorine pollutants, polychlorinated biphenyls (PCBs), actually decline across sensitive windows of human reproduction (Bloom et al. 2007).

An immediate question is whether the decline in concentrations is restricted to any one class of persistent compounds or inclusive of others such as organochlorine pesticides and their metabolites. Data addressing this question in the literature are sparse. While fairly recent publications have reported stable maternal organochlorine/metabolite blood concentrations across gestation (Jarrell et al. 2005; Longnecker et al. 1999), early evidence suggests otherwise (Curley and Kimbrough 1969; Roncevic et al. 1987). To address this critical data gap, we assessed concentrations of serum organochlorine pesticides/metabolites across critical windows of human reproduction and development commencing with preconception quantification.

Materials and Methods

Study sample

Ninety-nine women were recruited from a larger cohort of anglers, whose fish consumption exposure had been characterized in 1991–1992, on the basis of reporting that they had not completed childbearing (Vena et al. 1996). Women were recruited approximately five years

later for the expressed purpose of assessing serum PCBs and organochlorine pesticides/metabolites concentrations in relation to sensitive reproductive (i.e., time to pregnancy) and developmental (i.e., pregnancy loss, birth size) outcomes. Upon enrollment in the prospective pregnancy cohort, women completed a baseline interview and were instructed in the completion of daily diaries for the capture of menstruation, sexual intercourse, home pregnancy test results, and time-varying covariates believed relevant for reproduction (i.e., cigarette smoking, alcohol and caffeine consumption, and multivitamin use). Women were instructed to test for pregnancy using the Clearblue Easy® home pregnancy kit on the day of expected menses and one week later. Blood specimens, collected from 79 women who completed the study, were timed to reflect exposure at sensitive windows as described in Figure 1: upon entering the cohort, prior to attempting to conceive (baseline) (n=73; 92% of the cohort); following a positive pregnancy test resulting in an early pregnancy loss (EPL) (n=10; 100% of early losses); following a positive pregnancy test resulting in a clinical pregnancy loss after entry into prenatal care (CPL) (n=3; 75% of clinical losses); following a positive pregnancy test resulting in a live birth (prenatal) (n=54; 100% of live births); and approximately 4–6 weeks following delivery (postnatal) (n=53; 98% of live births). An additional blood specimen was obtained from 9 (81% of infertile women) women who did not become pregnant despite 12 months attempting pregnancy with at least one act of intercourse during each estimated fertile window (infertile). Given that we did not have a biomarker of ovulation, the fertile window was estimated to comprise eight days (i.e., five days before ovulation, the day of and two days following) using the Ogino-Knaus method (Knaus 1929; Ogino 1930). The study protocol complied with the U.S. regulations on the protection of human subjects; all study participants gave written informed consent before participation in any aspect of the study.

Toxicologic measurement

Sera specimens were transported within 36 hours of collection to the Toxicology Research Center at the University at Buffalo (Buffalo, NY, USA) for the analysis of seven organochlorine pesticides/metabolites, and 76 single or co-eluting PCB congeners, using gas chromatography with electron capture detection (GC-ECD) as previously described (Bloom et al. 2007; Greizerstein et al. 1997). In brief, 4 mL specimens were run in batches of 14 including ten participant serum samples and four quality control (QC) samples (i.e., one reagent control, one serum control, a control with fifteen calibration standards at known values, and one duplicate participant sample). Individual compound concentrations were calculated from standard curves for the 15 calibration standards and the remaining compounds were calculated from response factors generated in the laboratory. Limits of detection (LOD) were determined as three standard deviations of 6–10 reagent blanks included in sample batches analyzed by time of specimen collection. Sample blank concentrations were removed from laboratory observed values which were subsequently corrected for recovery using surrogate standards added and equilibrated prior to sample extraction (Greizerstein et al. 1997). Values below the limits of detection following removal of the sample blank were replaced by zero during the generation of the data file.

Total serum lipids (TL) were quantified using enzymatic methods as the function of total cholesterol (TC) and triglycerides (TG) expressed in mg/dl as $TL = 2.27 TC + TG + 0.623$ (Bernert et al. 2007; Phillips et al. 1989). For analysis purposes, pesticides/metabolites concentrations are reported as ng/g wet weight with serum lipids entered as a covariate in the analytic model. This approach has been empirically demonstrated to minimize bias that may arise from misspecification of models that automatically incorporate lipid standardization of wet weight organochlorine concentrations (Schisterman et al. 2005).

Statistical analysis

Distributions for pesticides/metabolites and covariates were skewed even after Box-Cox transformations and were zero inflated due to the limit of detection; thus, no further transformations were undertaken. Bivariate analyses were performed to explore serum concentrations (ng/g serum) and lipids (mg/dl) across three critical windows dependent upon pregnancy outcome: baseline, prenatal or early loss, and infertile or postnatal. For example, the baseline concentration was compared with the prenatal or early pregnancy loss concentration and later with the postnatal or the infertility. There were 48 paired observations for the baseline-prenatal window, 10 for the baseline-EPL, 9 for the baseline-infertility, and 47 for the prenatal-postnatal. Concentrations were assessed in relation to relevant reproductive covariates: body mass index (kilograms/height in meters²), gravidity (number of pregnancies), parity (number of births), and lifetime duration of lactation in months (Glynn et al. 2003; Kostyniak et al. 1999). As only four women had clinical pregnancy losses, no further analyses were conducted in this subgroup. All analyses were carried out using SAS v. 9.1 (SAS Institute, Inc. Cary, NC USA).

The overall and daily rate of change in serum pesticides/metabolites concentrations and total serum lipids were evaluated for paired observations by critical window. Overall change was estimated by the difference in concentrations between the measure at the second critical window and the first according to Equation 1 where Y_{ijk} represents measures for the i^{th} {1, ..., n } participant; for the j^{th} pesticide/metabolite (where 1= aldrin, 2= β -HCH, 3= DDE, 4= HCB, 5= mirex, 6= oxychlorthane, 7= trans-nonachlor, and 8= total serum lipids); demonstrating the k^{th} {1, 2, 3, 4} reproductive outcome (where 1= prenatal, 2= EPL, 3= infertility, and 4= postnatal; and Y'_{ijk} represents the baseline measurement for $k= 1, 2$, or 3, and the prenatal measurement where $k= 4$).

$$D_{ijk} = Y_{ijk} - Y'_{ijk} \quad \text{Equation [1]}$$

The daily rate of change was estimated as the difference in concentrations between the two critical windows divided by the number of days between specimen collections, given that women had varying time intervals for becoming or remaining pregnant. According to Equation 2 where t represents the duration in days, for the i^{th} {1, ..., n } participant, between the reported positive hCG pregnancy test date and the outcome specimen sample date, where $k=1, 2, 3$, and the date of the prenatal and postnatal specimens where $k=4$.

$$R_{ijk} = D_{ijk} / t_i \quad \text{Equation [2]}$$

Mean overall and daily rate of change in pesticides/metabolites concentrations by critical window was estimated using multiple linear regressions adjusting for baseline pesticides/metabolites concentration and the overall/daily rate of change in serum lipids, each centered by its mean. Centering covariates facilitated the interpretation of the model intercepts as the mean adjusted change over time (Kleinbaum et al. 1998). Adjusting for baseline pesticides/metabolites concentrations was necessary, since overall and daily changes varied in relation to baseline values (Figure 1). Due to the limited sample size, additional covariates were not considered in models, but were explored using non-parametric bi-variate correlations. Serum lipids could not be quantified for 10 women (8 live births, 1 pregnancy loss, and 1 infertile), given insufficient remaining blood and necessitating the use of an EM multiple imputation algorithm (Horton and Kleinman 2007).

For each regression model, residuals were examined employing Q-Q plots and comparison of predicted values with those generated employing the LOESS non-parametric regression

procedure, for evaluating the normality assumption for the distribution of model residuals. Statistical significance was declared as $p < 0.05$ for two-tailed tests and crude differences in overall change and daily rate of change in pesticides/metabolites concentration were evaluated using the Wilcoxon signed rank test. Non-parametric tests were favored, since changes in pesticides/metabolites concentrations deviated substantially from normality even with Box-Cox transformation.

Results

No significant differences were demonstrated for median baseline pesticides/metabolites concentrations or other study covariates by critical window (Table 1). The median interval between the collection of the two serum specimens varied by critical window: 151 days (interquartile range (IQR)=180.5) between baseline and prenatal specimens among women giving birth, and then 240 days (IQR = 21) between prenatal and postnatal specimens among those women; 63 days (IQR= 4) between baseline and early pregnancy loss specimens; 79 days (IQR=76) between baseline and clinical pregnancy loss specimens; and 446 days (IQR=127) between baseline and infertility specimens.

The vast majority of values for DDE (89–100%), mirex (89–100%), oxychlordane (100%), and HCB (89–100%) were measured above the LODs regardless of critical window (Table 2); considerable variation for aldrin (30–89%), β -HCH (0–43%), and trans-nonachlor (60–100%) was observed. The majority (57–100%) of β -HCH values, including all early loss and infertile specimens, were measured below the LOD, precluding assessment of changes in these critical windows for this compound. Baseline concentrations across pesticides/metabolites concentrations ($n=67$) were positively correlated at low to moderate strength (i.e. $r=0.17$ – 0.45 ; $P < 0.05$ to $P < 0.10$). However, a single inverse correlation was measured between concentrations of the dieldrin metabolite aldrin and the chlordane metabolite trans-nonachlor ($r_{sp} = -0.25$, $P = 0.038$).

Non-parametric correlations for pesticides/metabolites, and total serum lipids, across critical windows are presented in Table 3. No significant correlations were measured between concentrations for aldrin or β -HCH regardless of critical window. The chlorinated insecticide metabolite DDE demonstrated strong positive correlations between measures, regardless of critical window (baseline-prenatal $r=0.85$, $P < 0.0000$, $n=48$; baseline-early loss $r=0.92$, $P=0.000$, $n=10$; baseline-infertile $r=0.80$, $P=0.010$, $n=9$; and prenatal-postnatal $r=0.76$, $P < 0.0000$, $n=47$), as did the cyclodiene pesticide mirex (baseline-prenatal $r=0.78$, $P < 0.0001$; baseline-early loss $r=0.92$, $P=0.000$; baseline-infertile $r_{sp}=0.70$, $P=0.035$; and prenatal-postnatal $r_{sp}=0.40$, $P=0.005$). For HCB, only concentrations for baseline and prenatal measures demonstrated a significant correlation ($r=0.29$, $P=0.043$). Oxychlordane (baseline-infertile $r=0.87$, $P=0.002$ and prenatal-postnatal $r=0.52$, $P=0.000$) and trans-nonachlor (baseline-infertile $r=0.79$, $P=0.012$) also each demonstrated significant correlations only for specific critical windows. For each pesticide/metabolite, correlations across critical windows were statistically similar except those for oxychlordane between baseline-infertility, and each baseline-prenatal ($P=0.005$) and baseline-early loss ($P=0.010$). Serum total lipids demonstrated moderate to strong positive correlations across critical windows however statistical significance was shown for only the baseline-prenatal measures ($r=0.48$, $P=0.002$) and the prenatal-postnatal measures ($r=0.58$, $P < 0.0001$). Following division of pesticides/metabolites by serum total lipids point estimates varied somewhat from those generated using serum wet weight values. However no substantial differences were noted between correlation coefficients expressed on a wet weight basis and those expressed on a lipid weight basis (data not shown).

Median values were considered for the overall (i.e., ng/g serum) and daily (i.e., ng/g serum per day) rates of change in pesticides/metabolites across critical windows (data not shown).

Between baseline and prenatal specimens (n=48), statistically significant decreases were demonstrated for the overall and daily rate of change in HCB (−0.035 and −0.001, respectively) and trans-nonachlor (−0.050 and −0.002, respectively). Conversely, increases were measured for oxychlordane (0.032 and 0.001, respectively). Between baseline and early loss specimens (n=10), significant decreases were observed for the overall and daily rate of change in HCB (−0.061 and −0.003, respectively) and for the overall change in trans-nonachlor (−0.054). No significant crude changes were observed for DDE or mirex among women who conceived, regardless of outcome. Significant overall and daily rates of change were demonstrated for DDE (−0.069 and -8.00×10^{-5} , respectively), HCB (−0.088 and −0.000, respectively), and for trans-nonachlor (0.045 and 1.90×10^{-4} , respectively) between baseline and infertility specimens (n=9). Excluding β-HCH, all measured pesticides/metabolites demonstrated significant overall and daily rates of change between prenatal and postnatal concentrations (n=47). These latter changes reflected decreases (aldrin, DDE, HCB, and oxychlordane) and increases (mirex and trans-nonachlor) between the prenatal and postnatal measurement. Expressed as mg/dL, total serum lipids showed median overall and daily rates of decrease from baseline to prenatal for women whose pregnancies ended in a live birth (−15.965 and −0.627, respectively) and for women experiencing early losses (−1.571 and −0.030, respectively), but an increase for infertile women (18.969 and 0.033, respectively). None of these results achieved significance (data not shown). However, statistically significant increases ($P < 0.0001$) were demonstrated for the overall and daily rates of change in total serum lipids between the prenatal and postnatal specimens (169.016 and 0.705, respectively).

Multiple linear regression models, describing the adjusted mean overall and daily rate of change for pesticides/metabolites by critical window are presented in Table 4. Means were adjusted for the overall or daily rate of change in serum total lipids and baseline pesticides/metabolites concentrations. Women who conceived during the study, those with live births (n=48) and those with early pregnancy losses (n=10), demonstrated significant reductions in the overall and daily rate of change in serum HCB (−0.032 and −0.001; −0.069 and −0.003, respectively), and trans-nonachlor (−0.050 and −0.002; −0.045 and −0.002, respectively) concentrations similar to the aforementioned unadjusted median values. Statistically significant increases in oxychlordane (0.029 and 0.001, respectively; 0.015 for overall change only) were observed, though somewhat more moderate than those observed in the aforementioned unadjusted medians analysis.

In contrast to women giving birth, women experiencing early pregnancy losses were observed to have significant adjusted changes in both the overall and daily rates of change in sera concentrations of aldrin (−0.002 and -1.47×10^{-4} , respectively) and mirex (0.006 and 3.32×10^{-4} , respectively). These pesticides/metabolites did not demonstrate significant changes in the unadjusted medians analysis. Changes in HCB (−0.069 and −0.003, respectively) as well as trans-nonachlor (−0.045 and −0.002, respectively) were significant with the former slightly greater and the latter slightly smaller than those demonstrated during the unadjusted medians analysis.

As presented in Table 4, significant decreases in the adjusted overall and daily rate of change between baseline and infertility measures (n=9), were demonstrated for aldrin (−0.003 and -3.52×10^{-6} , respectively), DDE (−0.210 and -4.29×10^{-4} respectively), and HCB (−0.096 and -2.03×10^{-4} , respectively). In contrast, significant increases were demonstrated for the overall and daily rate of change in trans-nonachlor (0.034 and 7.59×10^{-5} , respectively). Baseline pesticides/metabolites concentrations were generally strong and significant inverse predictors of the overall and daily rates of change in pesticides/metabolites concentrations among infertile women (data not shown). In general, as the baseline concentration for pesticides/metabolites increased, the rate of decrease in concentration between time points increased, or conversely, the rate of increase between time points decreased. Total serum lipids

were a significant predictor for only the overall and daily rate of change in oxychlordane (7.02×10^{-5} , 7.67×10^{-5} , respectively).

Baseline pesticides/metabolites concentrations were frequently significant and strong inverse predictors for both overall and daily rates of change (data not shown). However, the *change* in total serum lipids concentration was infrequent and generally weakly positive (data not shown). Serum lipids had little importance for pesticides/metabolites concentrations between the baseline and prenatal measures among the 48 women who went on to a live delivery; significant lipids coefficients were observed for only the overall and daily rate of change in DDE (0.001 and 0.002, respectively) and (daily) trans-nonachlor (1.11×10^{-4}). Among women with early losses significant lipids coefficients were observed for both the overall and daily rate of change in aldrin (4.68×10^{-5} , 7.59×10^{-5} , respectively) as well as (daily) trans-nonachlor (2.60×10^{-4}).

Table 4 also presents the overall and daily rates of change in pesticides/metabolites concentrations between the pre- and postnatal measures (n=47) adjusted for the change in serum total lipids, prenatal pesticides/metabolites concentrations, and the reported duration of breastfeeding between the delivery and the postnatal serum specimen. Significant decreases were demonstrated for the overall and daily rates of change in aldrin (-0.004 and -1.14×10^{-5} , respectively), β -HCH (-0.004 and -1.46×10^{-5} , respectively), DDE (-0.141 and -0.001 , respectively), HCB (-0.027 and -1.16×10^{-4} , respectively), and oxychlordane (-0.032 and -1.38×10^{-4} , respectively). In contrast, significant increases were demonstrated for the overall and daily rate of change in trans-nonachlor (0.110 and 4.64×10^{-4} , respectively) concentrations. Total serum lipids were generally weak positive predictors of change except for the daily rate of change in aldrin for which the lipids coefficient was negative (data not shown). Baseline pesticides/metabolites concentrations were significant and strong inverse predictors for all measures (data not shown).

Figure 1 illustrates the overall (Figure 1a) and daily rate of change (Figure 1b) in DDE by critical window, as a function of women's baseline concentrations. These figures illustrate the dependence of daily rate of change on baseline concentration using DDE as an example, especially for women becoming pregnant or experiencing early losses. A general pattern of a greater decrease in concentrations from baseline to the next critical window is observed for an increasing baseline concentration, supporting adjustment for baseline concentration when estimating rates of change.

Neither age nor BMI at baseline were significantly associated with any of the measured changes in pesticides/metabolites (data not shown) regardless of critical window nor was self reported duration of breastfeeding among women with a live birth. However, among women with a prior pregnancy (n=38), the overall ($r=0.38$, $P=0.020$) and daily ($r=0.38$, $P=0.017$) rates of change for mirex concentrations from baseline to prenatal collection were significantly associated with parity as was the daily rate of change in oxychlordane ($r=-0.45$, $P=0.005$).

Discussion

This prospective pregnancy study with preconception enrollment of women suggests that serum concentrations of organochlorine pesticides/metabolites are not stable over critical windows of human development including from conception. In the current study, decreases in serum concentrations of aldrin, HCB, and trans-nonachlor as well as increases for mirex and oxychlordane were observed over the peri-conception window in 58 women with conceptions during the study period. Among 47 women with live deliveries, decreases in aldrin, β -HCH, DDE, HCB, oxychlordane and increases in trans-nonachlor were observed between the prenatal

and postnatal time points. Decreases in aldrin, DDE, and HCB and increases for trans-nonachlor were observed for nine women who did not conceive during the study period.

Among the few reports published to date concerning the toxicokinetics of organochlorine pesticides/metabolites across gestation, at least one study (Curley and Kimbrough 1969) reports statistically significant ($P < 0.05$) changes in concentrations over time. Decreases in plasma DDE and β -HCH concentrations were observed among five women providing specimens approximately 240 days (mean=8.4 and 4.5 parts per billion (ppb), respectively) prior to and approximately six days (mean=2.4 and 1.2 ppb, respectively) following delivery. In a study of 14 gravid women conducted in the former country of Yugoslavia (Roncovic et al. 1987), women providing blood specimens six and three months prior to and at, delivery demonstrated increases in geometric mean sera concentrations for β -HCH (1.28, 1.41, 1.45 $\mu\text{g/L}$ serum, respectively) and DDE (6.61, 6.14, and 7.57 $\mu\text{g/L}$ serum, respectively), though none achieved statistical significance. More recently, a Canadian study of 105 pregnant women with sera concentrations of DDE and HCB similar to those measured in the current study (Jarrell et al. 2005) reported no statistically significant difference in arithmetic/geometric mean concentrations measured during the 2nd trimester (i.e., 0.30/0.26 and 0.12/0.10 ng/g serum, respectively) and at delivery (i.e., 0.33/0.27 and 0.13/0.10 ng/g serum, respectively). The simple correlation among measures for DDE between the pre- and post-natal specimens in the current study is similar to that reported by the Canadian study ($r = 0.87$, $P = 0.000$) except we did not observe a significant correlation for HCB between measurements. An earlier American study of 67 women participating in the Collaborative Perinatal Project between 1959 and 1965 (Longnecker et al. 1999) reported strong correlations ($r \geq 0.77$) for serum DDE concentrations starting at approximately 12 weeks and continuing throughout gestation and approximately six weeks post-partum. Median 1st trimester DDE concentrations equal to 17 $\mu\text{g/L}$ serum in that study exceeded that equal to 1 ng/g serum ($n = 48$) in the current study, possibly a reflection of temporal trends.

Some significant differences were observed across critical windows for aldrin, DDE, HCB, and trans-nonachlor concentrations among the nine women who did not conceive during the study period. These changes are possibly indicative of laboratory error, such as drift between specimen batches analyzed by sampling time, changing exposure through diet (MacIntosh et al. 1996), or changes in body weight (Glynn et al. 2003; Wolff et al. 2007). The significant positive correlations measured among pesticides/metabolites concentrations at baseline are consistent with the mixed nature of background exposures to organochlorine compounds (Carpenter et al. 2002). The single negative correlation for aldrin and chlordane concentrations measured at baseline may reflect the inflation of the type-1 error rate stemming from numerous independent statistical tests. *A priori* we decided not to correct for multiple comparisons consistent with the exploratory nature of this work and our intent to try to globally assess patterns of change in organochlorine pesticides/metabolites by critical windows. Baseline concentrations impacted rates of change in a negative fashion consistent with previous findings (Ryan et al. 1993). While interesting, the observed patterns offer no clear direction for how best to model chemical mixtures when estimating effects on human reproduction and development. However, ignoring the varying directions of changes in individual pesticides may be problematic for interpreting the results for a single exposure study.

The reasons for observed changes between baseline and hCG detected pregnancy are unknown and somewhat puzzling given the alleged persistence of these chemicals in the body and absent earlier reports. We speculate that early homeostatic changes following human conception and early embryonic development may impact chemical mobilization from lipid reserves, or possibly as a result of the many physiologic changes accompanying pregnancy such changes in thyroid hormone bioavailability (Glinioer 1999), carbohydrate and protein metabolism (Baird 1986), hepatic triglyceride synthesis and clearance (Biezenski 1974), or expanded plasma

volume (Bernstein et al. 2001). Decreases may represent dilution (Glynn et al. 2007) concomitant with pregnancy associated weight gain (Wolff et al. 2007) or other ensuing metabolic changes that may impact the absorption, distribution, metabolism, and excretion of exogenous compounds (Casarett et al. 1996). Both increases and decreases may be reflective of individual exposures profiles such as dietary or occupational exposures. The extent to which pregnancy intention affects changes in behavior remains to be established.

Irrespective of the underlying mechanisms, the findings underscore the potential for variability arising from epidemiologic studies that rely on a single blood measurement of exposure for organochlorine pesticides/metabolites across the continuum of human reproduction, especially if timing of biospecimen collection is ignored. Our analytic approach may have introduced bias via collider stratification, given that we stratified on time dependent reproductive events (Hernan et al. 2004). This stratification may spuriously alter the distribution of exposures by outcome and, thereby, introduce selection bias stemming from unmeasured confounding. However, it is unlikely that this type of bias would explain the differences observed in our study. The extent to which this source of variability could affect the interpretation of study results requires further empirical evaluation, particularly prospective designs with longitudinal captures of biospecimens for toxicologic quantification. Our results discourage continued reliance on a single measure for organochlorine pesticide/metabolites concentrations for estimating exposure over the long term or across gestations. These results suggest that sampling efforts be timed to specific critical window for development, those most relevant for the specific reproductive outcome of interest. This will help to facilitate accurate exposure classification and reduce bias.

We are unaware of any prospective pregnancy studies with preconception enrollment that quantified serum pesticides/metabolites concentrations across critical windows. In light of the inherent limitations associated with this study (viz., use of GC-ECD for analysis and limited cohort size), we offer empirical data regarding median and mean changes in serum concentrations of pesticide/metabolites from preconception to the postnatal critical window of human development among women with background exposure to these compounds. Every attempt was made to capture all pregnancies to the extent possible and to retain women in the analysis who failed to achieve pregnancy. To address the varying times women required for becoming pregnant or for maintaining pregnancies until delivery, we estimated the interval between specimen collections and adjusted our analyses accordingly. By design, we were restricted to one blood collection during pregnancy and cannot assess changes during established pregnancies. In sum, our findings suggest a relatively dynamic nature of serum organochlorine pesticides/metabolites concentrations across critical windows and underscore the importance of collecting timed specimens for the assessment of sensitive reproductive and developmental toxicants.

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Human Subjects Protection

The study protocol complied with the U.S. regulations on the protection of human subjects; all study participants gave written informed consent before participation in any aspect of the study.

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Figure 1.

Numbers of participants and blood specimen captured and analyzed for organochlorine pesticides/metabolites as part of the New York State Angler Cohort Prospective Pregnancy Study

CPL, clinical pregnancy loss; EPL, early pregnancy loss; hCG+, positive urine test for human chorionic gonadotropin on the day of expected menses.

Figure 2.

Average overall (a) and daily rate of change (b) in DDE concentration, as a function of baseline concentration, by critical windows. New York State Angler Cohort Prospective Pregnancy Study.

DDE, dichlorodiphenyltrichloroethane.

Outcomes:		Live birth (n=48)
	Early pregnancy loss (n=10)
	Infertility (n=9)

Table 1

Description of study cohort at baseline by critical window. New York State Angler Cohort Prospective Pregnancy Study.

Characteristic	Prenatal (n = 48) Median (range)	Pregnancy Loss (n = 10) Median (range)	Infertility (n = 9) Median (range)
Pesticides/metabolites (ng/g serum)			
Aldrin	0.00 (0.00, 0.02)	0.00 (0.00, 0.01)	0.00 (0.00, 0.02)
β-HCH	0.00 (0.00, 0.04)	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)
DDE	0.92 (0.39, 2.34)	1.06 (0.41, 1.58)	0.80 (0.47, 3.59)
Hexachlorobenzene	0.09 (0.02, 0.19)	0.12 (0.07, 0.14)	0.12 (0.06, 0.26)
Mirex	0.02 (0.00, 0.31)	0.02 (0.01, 0.09)	0.02 (0.00, 0.09)
Oxychlorodane	0.02 (0.00, 0.11)	0.01 (0.00, 0.11)	0.01 (0.00, 0.04)
Trans-nonachlor	0.05 (0.00, 0.15)	0.05 (0.03, 0.08)	0.06 (0.00, 0.10)
Total lipids (mg/dl serum)	530.17 (357.57, 915.38) ^a	529.05 (307.91, 829.94)	475.39 (414.45, 723.67) ^b
Age (in years)	30.5 (26, 34)	29.5 (26, 33)	30.0 (26, 32)
BMI (kg/m ²)	22.18 (17.7, 39.9)	22.39 (17.54, 34.56)	21.46 (17.28, 31.15)
Gravidity (# pregnancies)	1 (0, 5)	1 (0, 3)	0.5 (0, 2) ^c
Parity (# births)	1 (0, 3)	1 (0, 3)	0 (0, 2) ^d
Duration breastfeeding (months) ^e	6.0 (1, 28)	10.5 (2, 13)	13.0 (6, 20)

Note: No statistically significant differences for distributions across critical windows ($P > 0.10$). Zero values for pesticides/metabolites concentrations correspond to measures below the limits of detection.

^a n=6 missing serum lipids values

^b n=1 missing serum lipids value

^c n=1 missing value gravidity

^d n=2 missing parity values

^e Restricted to the 39 women reporting a history of breastfeeding at baseline.

β-HCH, beta-hexachlorocyclohexane; DDE, dichlorodiphenyltrichloroethane; HCB, hexachlorobenzene.

Mean limit of detection (LOD) values and percentage of concentrations above for organochlorine pesticides/metabolites (ng/g serum), by critical windows. New York State Angler Cohort Prospective Pregnancy Study.

Table 2

Pesticide/Metabolite	Baseline (n=73)		Prenatal (n=53)		Postnatal (n=52)		EPL (n=10)		Infertility (n=9)	
	Mean LOD	n (%)	Mean LOD	n (%)	Mean LOD	n (%)	Mean LOD	n (%)	Mean LOD	n (%)
Aldrin	0.016	37 (51)	0.010	33 (62)	0.008	33 (63)	0.010	3 (30)	0.008	8 (89)
β-HCH	0.008	31 (43)	0.014	18 (34)	0.013	14 (27)	0.014	0 (0)	0.013	0 (0)
DDE	0.022	73 (100)	0.067	53 (100)	0.037	52 (100)	0.068	10 (100)	0.037	8 (89)
Hexachlorobenzene	0.014	73 (100)	0.026	53 (100)	0.002	47 (90)	0.026	10 (100)	0.002	8 (89)
Mirex	0.007	72 (99)	0.015	53 (100)	0.034	52 (100)	0.016	10 (100)	0.034	8 (89)
Oxychlorane	0.013	67 (100) ^d	0.010	53 (100)	0.005	40 (100) ^b	0.010	9 (100) ^c	0.005	7 (100) ^d
Trans-nonachlor	0.005	70 (96)	0.004	38 (72)	0.008	52 (100)	0.004	6 (60)	0.007	8 (89)

NOTE: Characterization of measured pesticides/metabolites concentrations values in regard to the limits of detection encompasses all subjects with measured specimens including those for which paired specimens were not available for analysis. Boldface denotes pesticides/metabolites with ≥50% of measured values above the limit of detection.

^a n=6 missing values
^b n=12 missing values
^c n=1 missing value
^d n=2 missing values.

β-HCH, beta-hexachlorocyclohexane; DDE, dichlorodiphenyltrichloroethane; HCB, hexachlorobenzene.

Table 3

Spearman correlation coefficients among paired sera specimens (ng/g serum) by organochlorine pesticides/metabolites and critical windows. New York State Angler Cohort Prospective Pregnancy Study.

Pesticides/metabolites	Baseline Measure, Prenatal (n=48)	Baseline Measure, Early Loss (n=10)	Baseline Measure, Infertility (n=9)	Prenatal Measure, Postnatal (n=47)
Aldrin	0.04	0.47	0.20	-0.22
β-HCH	0.09	-	-	-0.22
DDE	0.85 ^{***}	0.92 ^{**}	0.80 ^{**}	0.76 ^{***}
Hexachlorobenzene	0.29 [*]	0.09	0.20	-0.01
Mirex	0.78 ^{***}	0.92 ^{**}	0.70 [*]	0.40 ^{**}
Oxychlordane	0.21	0.04	0.87 ^{**}	0.52 ^{**}
Trans-nonachlor	0.13	-0.44	0.79 [*]	-0.07
Serum total lipids ^a	0.48 ^{b, **}	0.53 ^b	0.62 ^c	0.58 ^{d, ***}

NOTE: All β-HCH sera concentrations were zero for specimens sampled following an early pregnancy loss and after 12 at risk menstrual cycles with no conception.

^a Total lipids = (2.27 * total cholesterol) + triglycerides + 0.623

^b n=8 missing values

^c n=1 missing value

^d n=2 missing values

β-HCH, beta-hexachlorocyclohexane; DDE, dichlorodiphenyltrichloroethane; HCB, hexachlorobenzene

* P<0.05

** P<0.01

*** P<0.0001.

Table 4

Mean adjusted overall and daily rates of change in serum pesticides/metabolites concentrations by critical windows. New York State Angler Cohort Prospective Pregnancy Study.

Grouping/component	Critical Windows			
	Baseline-Prenatal (n=48) ^d	Baseline-Early Loss (n=10) ^d	Baseline-Infertility (n=9) ^d	Prenatal-Postnatal (n=47) ^b
Overall change (ng/g serum)				
Aldrin	-0.001	-0.002	-0.003	-0.004
β-HCH	0.002	-	-	-0.004
DDE	-0.039	-0.016	-0.210	-0.141
Hexachlorobenzene	-0.032	-0.069	-0.096	-0.027
Mirex	0.004	0.006	0.004	0.019
Oxychlorodane	0.029	0.015	-0.004	-0.032
Trans-nonachlor	-0.050	-0.045	0.034	0.110
Daily rate of change(ng/g serum)				
Aldrin	-5.50×10^{-5}	-1.47×10^{-4}	-3.52×10^{-6}	-1.14×10^{-5}
β-HCH	8.35×10^{-5}	-	-	-1.46×10^{-5}
DDE	-0.002	-0.002	-4.29×10^{-4}	-0.001
Hexachlorobenzene	-0.001	-0.003	-2.03×10^{-4}	-1.16×10^{-4}
Mirex	4.21×10^{-5}	3.32×10^{-4}	5.67×10^{-6}	6.93×10^{-5}
Oxychlorodane	0.001	4.60×10^{-4}	-7.89×10^{-6}	-1.38×10^{-4}
Trans-nonachlor	-0.002	-0.002	7.59×10^{-5}	4.64×10^{-4}

NOTE: All β-HCH sera concentrations were zero for specimens sampled following an early pregnancy loss and after 12 at risk menstrual cycles with no conception.

^a Adjusted for overall (mg/dl serum) or daily (mg/dl serum per day) rate of change in total serum lipids and prenatal pesticide/metabolites concentration (ng/g serum) at baseline

^b Adjusted for overall (mg/dl serum) or daily (mg/dl serum per day) rate of change in total serum lipids, prenatal pesticide/metabolites concentration (ng/g serum) at baseline, and duration of breastfeeding between delivery and the postnatal sample (days)

β-HCH, beta-hexachlorocyclohexane; DDE, dichlorodiphenyltrichloroethane; HCB, hexachlorobenzene

* P<0.05

** P<0.01

*** P<0.001.