**In utero exposure to organochlorine pesticides and early menarche in the Avon Longitudinal Study of Parents and Children**

**Supplemental analyses result tables**

**Graph of Lipid adjusted detection limit for all organochlorine pesticides Vs. Gestational serum sample weight**

Study participants with a lipid-adjusted limit of detection (LOD) ≥13ng/g lipid were removed from the analyses to avoid possible bias as a result of small serum samples with high LODs. We selected this censoring level by reviewing the chart of the lipid-adjusted LOD vs available serum amount for the measurement (below). The LOD has been defined as the detectable amount of target analyte divided with the sample size. Hence, for low volume samples an increase in the LOD will result. To prevent inclusion of high LOD values for samples with a low sample amount we decided to censor any data having a LOD higher than 13ng/g lipid. This censoring minimizes detection bias of higher concentrations for low serum volume samples.

*Figure 1 Lipid adjusted detection limit for all organochlorine pesticides (ng/g) Vs. Sample weight (g)*

**Analysis using whole-weight concentrations**

Below, we present the median, and 25th and 75th percentiles for the maternal gestational serum concentrations of organochlorine pesticides using whole weight concentrations, and the association between these concentrations and early menarche.

*Gestational serum concentrations (whole weight pg/g serum) by case/control status and overall*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Whole weight (pg/g serum) |  |  |  |
|  | Total | Cases | Controls |  |
| Analyte | **N (%> LOD**a**)** | **Median (IQR)** | **N (%> LOD**a**)** | **Median (IQR)** | **N (%> LOD**a**)** | **Median (IQR)** | **p-valueb** |
| HCB | 372 (100) | 294.2 (220.5–366.8) | 184 (100) | 295.7 (215.7–366.8) | 188 (100) | 291.0 (220.9–365.5) | 0.95 |
| β - HCH | 374 (98.1) | 275.1 (201.6–373.1) | 186 (98.4) | 273.5 (194.5–368.1) | 188 (97.9) | 277.3 (210.4–373.4)  | 0.30 |
| p,p’- DDE | 428 (99.8) | 1885.1 (1148.6–2797.1) | 210 (99.5) | 1798.4 (1085.8–2889.5) | 218 (100) | 1956.5(1205.1–2688.6) | 0.53 |
| p,p’- DDT | 342 (92.5) | 67.5 (48.8–94.5) | 174 (92.3) | 63.7 (47.6–97.0) | 168 (92.7) | 68.5 (50.3–93.1) | 0.77 |

a Each biological sample had an individual limit of detection based on the sample’s weight

b Comparison between cases and controls using Wilcoxon Rank Sum Test

*Association between organochlorine gestational serum concentrations (logged organochlorine concentration pg/g serum) and early menarche*

|  |  |  |  |
| --- | --- | --- | --- |
| Organochlorine | Unadjusted OR (95% CI) a, e | Adjusted OR (95% CI) a, e | Adjusted p value  |
| HCB | 0.93 (0.59–1.46) | 0.88 (0.54–1.44) b | 0.61 |
| β-HCH | 0.87 (0.60–1.28) | 0.81 (0.56–1.18) b | 0.28 |
| p,p’ - DDE | 0.88 (0.67–1.15) | 0.89(0.68 –1.17) c | 0.42 |
| p,p’ - DDT | 0.98 (0.68–1.42) | 0.93 (0.62–1.38) d | 0.70 |

a Odds of early menarche for a unit increase of logged organochlorine pesticide concentration

b Adjusted for mother’s age at menarche and total lipids

c Adjusted for total lipids

d Adjusted for mother’s prenatal BMI and total lipids

e Number of cases, number of controls used in both unadjusted and adjusted models: HCB (184, 188); β-HCH (186, 188); PP’-DDE (210, 218); PP’-DDT (183, 179)

**Additional analyses using generalized additive models to detect departures from linearity**

We also explored the association between timing of menarche and organochlorine pesticides using only the set of daughters in the control group. This group consists of a random sample of eligible daughters and it is representative of the range of ages when menarche is most commonly attained. Results from linear regression models were consistent with those found in the case-control analysis in that we did not find evidence of an association between age of menarche onset and exposure to organochlorine pesticides. We also used generalized additive models to detect whether associations between exposure to organochlorine pesticides and age at menarche in the control group exhibited departures from linearity.1 Overall, the results of this analysis are suggestive of an earlier age of menarche attainment in those with higher exposure to organochlorine pesticides compared to the reference. However, the confidence intervals are wide and include the null, and in addition, there is no evidence of an exposure-outcome effect. These results suggest the presence of the subtle effects among subjects who are at the center of the age at menarche distribution, however, this evidence should be interpreted with caution and further replication in other large independent cohorts is important.

1. Hastie T, Tibshirani R. Generalized additive models: some applications. Journal of the American Statistical Association. 1987; 82 (398).

*Predicted means and regression Coefficients and 95% CI from linear regression models including only controls*

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| --- |
| **Predicted means, regression coefficients and 95% confidence intervals (CI) from linear regression models conducted only with the set of daughters in the control group** |
| **Organochlorine** | **Mean age at menarche unadjusted** | **Mean age at menarche (adjusted)** |
|  |  |  |  |  |
|  | *Mean* | *Regression Coefficient and 95% CI* | *Mean* | *Regression Coefficient and 95% CI* |
|  |  |  |  |  |
| **HCBa, e** | 13.30 | -0.12 (-0.48,0.25) | 11.25 | 0.05 (-0.29,0.40) |
| **β-HCHb, e** | 12.76 | 0.02 (-0.22,0.26) | 11.05 | 0.10 (-0.13,0.33) |
| **p,p’ – DDEc, f** | 13.35 | -0.08 (-0.29, 0.12) |  |  |
| **p,p’ – DDTd, g** | 13.45 | -0.23 (-0.54, 0.08) | 13.50 | -0.22 (-0.54,0.09) |
|  |  |  |  |  |
|  | *Predicted means*  | *Regression Coefficient and 95% CI* | *Predicted means*  | *Regression Coefficient and 95% CI* |
| **HCBa, e** |  |  |  |  |
| <25th  | 12.99 | Ref | 12.98 | Ref  |
| ≥25th – <50th  | 12.85 | -0.14 (-0.57, 0.28) | 12.75 | -0.23 (-0.63, 0.17) |
| <50th – ≥50th  | 12.73 | -0.27 (-0.67, 0.14) | 12.75 | -0.23 (-0.61, 0.16) |
| ≥75th  | 12.83 | -0.17 (-0.60, 0.28)  | 12.94 | -0.04 (-0.46, 0.38) |
|  |  |  |  |  |
| **β-HCHb, e** |  |  |  |  |
| <25th  | 12.93 | Ref | 12.88 | Ref  |
| ≥25th – <50th  | 12.73 | -0.19 (-0.62 ,0.23) | 12.70 | -0.18 (-0.58 ,0.22) |
| <50th – ≥50th  | 12.88 | -0.05 (-0.48 ,0.37) | 12.89 | 0.01 (-0.39 ,0.42) |
| ≥75th  | 12.83 | -0.09 (-0.53 ,0.33) | 12.90 | 0.02 (-0.39 ,0.43) |
|  |  |  |  |  |
| **p,p’ – DDEc, f** |  |  |  |  |
| <25th  | 12.91 | Ref  |  |  |
| ≥25th – <50th  | 12.87 | -0.04 (-0.41, 0.36) |  |  |
| <50th – ≥50th  | 12.95 | 0.04 (-0.37, 0.45) |  |  |
| ≥75th  | 12.80 | -0.12 (-0.52, 0.31) |  |  |
|  |  |  |  |  |
| **p,p’ – DDTd, g** |  |  |  |  |
| <25th  | 13.07 | Ref  | 13.07 | Ref  |
| ≥25th – <50th  | 12.74 | -0.33 (-0.77, 0.12) | 12.74 | -0.33 (-0.78, 0.12) |
| <50th – ≥50th  | 12.96 | -0.12 (-0.58, 0.36) | 12.97 | -0.10 (-0.57, 0.37) |
| ≥75th  | 12.76 | -0.31 (-0.76, 0.15) | 12.77 | -0.30 (-0.76, 0.16) |

aN= 173

bN= 173

cN= 200

dN= 164

eAdjusted for mother’s age at menarche

f Unadjusted p value. No potential confounders identified

gAdjusted for mother’s prenatal BMI

**Power analyses**

|  |  |  |
| --- | --- | --- |
| **Organochlorine** | **Sample size** | **Power** |
| **HCB** | 372 | 0.11 |
|  |  |  |
| **β-HCH** | 374 | 0.16 |
|  |  |  |
| **p,p’ – DDE** | 428 | 0.24 |
|  |  |  |
| **p,p’ – DDT** | 362 | 0.15 |