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## Exposure to PBB-153 and Digit Ratio

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### Abstract

The ratio between the second and fourth digits is a sexually dimorphic measure, established in utero and linked to prenatal sex steroid levels. An association was found between prenatal levels of Polybrominated Biphenyls, a synthetic chemical suspected to disrupt the endocrine system function, and the digit ratio in adult women.

### Keywords

Digit ratio; Endocrine disruptors; In-utero exposure; Polybrominated Biphenyl

## 1. Introduction

The ratio between the second and fourth digits, defined as the 2D:4D digit ratio, is a sexually dimorphic measure, established in utero and linked to prenatal sex steroid levels [1–3]. This ratio is stable throughout life from at least the age of two years [4]. The prenatal period is known to be critical for development and determination of future health. Digit ratio has been suggested as a marker of in-utero hormonal environment, and human and animal studies have found an association between the digit ratio and adverse health outcomes, including Autism [5], coronary heart disease [6] and breast cancer [7]. Synthetic environmental contaminants known as endocrine disrupting agents have been shown to interact with or mimic endogenous endocrine hormones, possibly affecting the digit ratio [8].

Following a tragic accident in the state of Michigan in the 1970s polybrominated biphenyl (PBB), a persistent brominated flame retardant chemical, was introduced into the food chain in MI, causing state-wide exposure. Animal studies suggest that PBB may interfere with endocrine function through blocking and/or mimicking natural hormones [9,10]. The Michigan Long-Term PBB Cohort, initially established by the Michigan Department of Public Health, now called the PBB Registry, was established to study possible health

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consequences of this exposure. PBB can transfer from mother to child in utero and through breast milk [11]. Since PBB is lipophilic, stored in adipose tissue and with a long half-life, children born years after the contamination episode are exposed. We have studied whether the digit ratio is associated with exposure to PBB in humans.

## 2. Methods

Beginning in the year 2012 attempts were made by the study team and by the Michigan Public Health Institute to contact all original PBB Registry members and their families, in order to engage them in research activities. Eligibility criteria for participation were: MI residents (current or past) who were potentially exposed to PBB directly (F0 generation) or in-utero (F1 generation). The estimated start date of the MI contamination is July 1st, 1973, therefore those born before this date were exposed directly, as children or adults and are referred to as (F0). Individuals potentially exposed during at least the first trimester of pregnancy were born after February 1974, and are referred to as (F1). Those born between July 1973 and February 1974 were exposed later in pregnancy and were excluded from the analyses.

Direct measurements of length of right hand digits 2 and 4 were performed twice by the same researcher, using a caliper (Swiss Precision Instruments, dial caliper, accuracy Range Per Revolution, Decimal Inch: 0.001), while participants were sitting and their hand placed palm up on a flat surface. The measurements were according to Manning et al. protocol [12]. Correlation between the repeated measurements was high ( $r$ , spearman = 0.89,  $p = 0.01$ ), and mean of the two measurements was calculated. Blood samples were collected and processed to isolate the blood serum, and the main PBB congener PBB-153 was measured at the Emory University Rollins School of Public Health laboratories. For F1 generation, who were exposed in-utero, the historic maternal enrollment PBB levels and an elimination model [13] were used to estimate PBB-153 maternal serum levels (i.e., their in-utero exposure level) during the first trimester.

### 2.1. Statistical Analysis

Linear regression models were used to study the association between PBB-153 exposure and 2D:4D digit ratio, separately for males and females. Participants age, height and weight were tested as potential confounders (i.e., if they were associated with both the digit ratio and the PBB-153 level) [14–16].

The distributions of PBB-153, both current and estimated in-utero levels, were highly skewed and were therefore log-transformed when examined as continuous variables, and were also dichotomized above and below the median PBB-153 level for males and females separately (cut points were: 0.812 parts per billion (ppb) and 0.554 ppb for F0 males and females, respectively; and median in-utero levels were 1.966 ppb and 1.852 ppb for males and females, respectively). Tertiles of in-utero levels were also examined, since endocrine disrupting chemicals are expected to manifest a non-monotonic association [17]. The cut points were: 1.5606 ppb and 3.8832 ppb for males, 1.0359 ppb and 2.8318 ppb for females. All analyses were performed using SPSS version 21.

### 3. Results

Measurements for right hand digits for ratio calculation was available for 258 participants, 207 were born prior to the exposure (F0 participants, 82 males, 125 females), and 51 individuals were born after February 1974 (F1 participants, 19 males, 32 females). Mean  $\pm$  SD ages of F0 participants were:  $49.83 \pm 4.43$  and  $49.87 \pm 4.49$  for males and females, and for F1 participants:  $30.45 \pm 7.6$  and  $33.38 \pm 6.83$  for males and females. Among both F0 and F1 groups the Rt. hand digit ratio was lower among males compared to females (Among F0:  $0.9821 \pm 0.038$  vs.  $0.9943 \pm 0.033$ ,  $p = 0.016$ ; and among F1:  $0.9697 \pm 0.0299$  vs.  $0.9938 \pm 0.043$ ,  $p = 0.039$ ).

Digit ratio was not associated with height or weight, among both males and females, and among both F0 and F1. Digit ratio was negatively associated with age, only among F0 females (beta =  $-0.001$ ,  $p = 0.032$ ) and age was also associated with PBB-153 levels (above or below median PBB-153).

For both study groups and among either sex, there was no association between current PBB-153 levels and the digit ratio, either in the continuous format or as above/below the median PBB-153 level, including in the age-adjusted model (beta =  $0.002$ ,  $p = 0.73$  for above or below median PBB-153).

Estimated in-utero PBB-153 levels, which was available for 51 participants, were divided into tertiles of exposure. As presented in Fig. 1, among female participants only, exposure above the lowest tertile was associated with higher digit ratio, however this finding was with borderline significance: mean digit ratio  $\pm$  SD  $0.9702 \pm 0.046$ ;  $1.005 \pm 0.039$ ;  $1.004 \pm 0.041$  for lowest, median and highest tertiles, respectively (beta =  $0.393$ ,  $p = 0.062$  and beta =  $0.364$ ,  $p = 0.082$  for 2nd and 3rd tertiles, respectively, as compared to the lowest tertile,  $p$  for trend =  $0.11$ ). When comparing above or below median in-utero PBB-153 the results remained similar (beta =  $0.34$ ,  $p = 0.059$ , for above compared to below median level). Among males there was a non-statistically significant increase between first and second tertiles of PBB-153 levels (beta =  $0.024$ ,  $p = 0.16$ ) and to the third tertile beta =  $0.005$ ,  $p = 0.78$ ), with similar results when comparing above and below median in-utero PBB-153.

### 4. Discussion

The PBB Registry presents an opportunity to study the possible effect of in-utero exposure to endocrine disrupting chemicals. The direct measurements of the digits performed in this study is a strength, as indirect finger measurements (from scans, photocopies) may distort 2D:4D's [18]. Our findings suggest exposure to PBB-153 in-utero is associated with a higher, more feminized digit ratio. This main finding supports the possible estrogenic or anti-androgenic properties of PBB-153, as suggested in previous studies [7,14,15,19,20]. Previous studies based on the F1 daughters of the PBB Registry have found earlier pubic hair development and earlier menarche [21], and a dose-related increase in the risk of spontaneous abortion [22]. These studies support an alteration in reproductive hormone levels due to in utero exposure to PBB-153.

While an association between 2D:4D ratio and early life hormone levels has been observed in animals and humans [4,19], this is the first human study of 2D:4D ratio associated with in utero exposure to an environmental chemical capable of disrupting the endocrine system. Our findings regarding the timing of exposure (in-utero vs. current levels of PBB-153) supports the critical window of exposure in which development can be affected. Measuring prenatal hormones is a challenge and therefore measuring digit ratio has been suggested as a marker of exposure, and our findings provide support for the use of this measure.

Our small sample of F1 participants is a limitation, yielding lack of power to study the association between PBB-153 exposure and digit ration among sub groups in our study, specifically the F1 male participants. Future studies with a larger sample of participants with exposure measurement of an endocrine disrupting chemical in critical times of development are required, to clarify their effect on health and reproductive outcomes, and possible associations with digit ratio.

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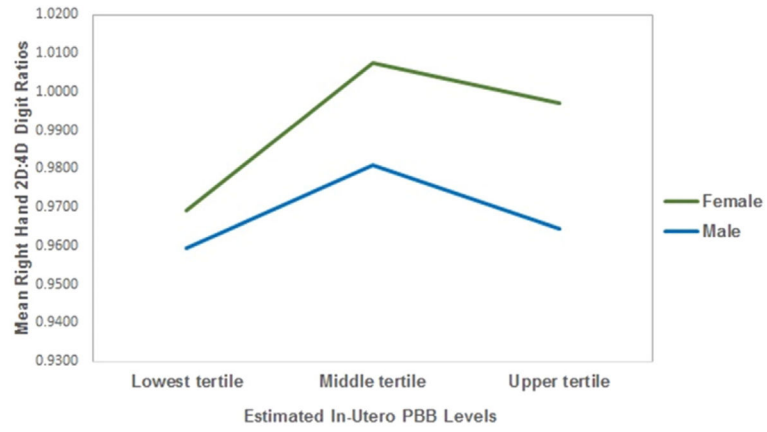
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**Fig. 1.** Mean Right Hand Digit Ratio by Tertiles of Estimated In-Utero PBB Exposure.