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# Toddler's behavior and its impacts on exposure to polybrominated diphenyl ethers

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# Abstract

Children have higher polybrominated diphenyl ether (PBDE) body burdens than adults, which may be related to hand-to-mouth behavior. We investigate associations between children's behavior, including hand-to-mouth contacts, and markers of PBDE exposure. In addition, we investigate associations between characteristics of the home environment and exposure. Eightythree children aged 12-36 months were recruited from North Carolina (2009-2010). Children provided blood and handwipes samples, which were analyzed for PBDEs. Parents completed questionnaires, providing demographic, behavioral, and environmental data. More active children had higher levels of PBDEs on their hands and in their bodies. For example, children who spent more time sleeping had lower exposures to PBDEs; each additional hour of sleep resulted in a 30% decrease in handwipe BDE-99 levels (P < 0.001) and a 15% decrease in serum (P = 0.03). After accounting for handwipe PBDE levels, children who licked their fingers while eating had higher serum PBDEs. Other behaviors were not consistently associated with serum levels. Playing with plastic toys was associated with higher handwipe levels of PBDEs, while frequent vacuuming decreased handwipe PBDE levels. Characteristics of the home environment generally were not associated with serum PBDEs. Our results suggest that certain aspects of children's behavior and their environment impact exposure to PBDEs.

# Keywords

behavior; children; exposure; flame retardants; handwipes; house dust; polybrominated diphenyl ethers (PBDEs)

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# INTRODUCTION

At one time, polybrominated diphenyl ethers (PBDEs) accounted for a large proportion of flame retardants used in household products including polyurethane foam and electronics; however, regulatory action and concern over the persistence, bioaccumulation, and toxicity of the Penta and Octa commercial formulations led to a halt in production in the mid-2000s.<sup>1–3</sup> Despite this change, PBDEs continue to be ubiquitously detected in both the indoor and outdoor environments and exposures to the general population are widespread. Children have higher levels of PBDEs in their bodies than adults, with the highest body burdens being reported for children of approximately 3 years of age.<sup>4,5</sup>

Children's exposures to environmental chemicals are thought to be different, and in many cases, higher than adults.<sup>6</sup> Higher exposures may occur because children drink more water, breathe more air, eat more food per unit body weight, and have a higher surface area to volume ratio than adults.<sup>7</sup> Children's behavior and activity patterns are also much different than adults and may have an important role in environmental contaminant exposures.<sup>7,8</sup> For example, children often put their hands or other objects in their mouths and eat with their fingers, both of which been shown to increase exposure to lead and pesticides.<sup>9–15</sup> The types of microenvironments in which children spend their time may also contribute to environmental contaminant exposures, and are likely to change with age. For instance, very young infants often spend a large proportion of their time stationary (for example, in a crib or held by their parents), while toddlers have greater ability to interact with their environment and move from one microenvironment to another.<sup>6,8</sup>

Here, we investigate associations between children's behavior and activity patterns and exposure to PBDEs. In our previous research, we measured paired samples of house dust, handwipes, and serum collected from toddlers in North Carolina and found that PBDEs measured on handwipes were one of the strongest predictors of serum PBDE levels.<sup>16</sup> Although associations varied by congener, age, socioeconomic status, and breastfeeding were also significant predictors of exposure.<sup>16</sup> Here we build on this previous study and investigate potential pathways of hand-to-mouth exposure, investigating the associations between children's behavior and the levels of PBDEs on their hands and in serum samples. We also investigate characteristics of children's home environment (for example, the type of flooring and cleaning practices in the home) and their impact on children's PBDE levels. In addition, as we hypothesize that the relationships between hand-to-mouth behaviors and serum levels may be altered by the levels of PBDEs on children's hands or in their home, we explore interactions between children's behavior and their environment.

# METHODS

## Study Population

Families with children between 12 and 36 months of age (n = 83) were recruited from pediatric clinics in Durham, North Carolina from May 2009 to November 2010.<sup>16</sup> Upon enrollment, a study team visited each family's home (between 0900 and 1700 h) to collect environmental and biological samples (described below) and to administer a short questionnaire, which included information on demographics, children's behaviors and

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development, and the home environment. All aspects of this study were authorized by the Duke University Institutional Review Board before initiation, and all parents/guardians gave informed consent before sample collection. Study activities were approved by the Institutional Review Board at the Centers for Disease Control and Prevention (CDC) before any serum specimens were transferred to the Division of Laboratory Sciences for chemical measurements.

#### **Behavioral and Environmental Assessment**

Parents were asked to complete a questionnaire to provide information on their child's behavior and habits. Questionnaires included information on children's hand-to-mouth behaviors (e.g., licking fingers while eating and thumb sucking), and pacifier use. Parents also provided information on the age at which their child began crawling and walking. This information, along with the child's age at the time of the home visit, was used to calculate the number of months that the child had been walking or crawling. In addition, parents reported the average amount of time their child spent in different microenvironments, including the average number of hours that the child spent sleeping, outside the home and in a car each week. Finally, parents provided information on the rooms in which children played the most (e.g., the type of flooring), the main type of toys that their child played with (plastic, metal, or wood), and home cleaning practices.

#### Handwipe Collection and Analysis

Handwipe samples were collected at home visits as described in Stapleton *et al.*<sup>16</sup> Briefly, parents were instructed not to wash their children's hands for at least 1 h before the home visit. When the study team arrived they wiped the entire surface of each child's hands (from fingers to the wrist) with sterile gauze wipes which had been soaked in exactly 3.0 ml isopropyl alcohol. Handwipes samples were extracted and analyzed for PBDEs using methods previously described in Stapleton *et al.*<sup>17</sup>

#### **Dust Collection and Analysis**

The study team collected a dust samples during the homes visit as described in Stapleton *et al.*<sup>16</sup> Briefly, parents reported which room in their homes the child spent most of their play time in and the equivalent of the entire floor-surface area of that room was vacuumed. Dust samples were analyzed for PBDEs as described in Allen *et al.*<sup>18</sup>

#### Serum Collection and PBDE Measurement

A small blood sample was collected from each child by venipuncture either in a pediatric clinic (n = 9) or by a trained phlebotomist at the home visit (n = 74) (as described in Stapleton *et al.*<sup>16</sup>). Blood samples were analyzed for 11 PBDE congeners (BDE-17, 28, 47, 66, 85/155, 99, 100, 153, 154, 183, and 209) and lipids at the CDC using established methods.<sup>19</sup>

#### Statistical Analyses

As reported previously,<sup>16</sup> with the exception of BDE-17, all congeners were detected in > 85% of dust samples. BDE-47, -99, -100, and -153, were also detected frequently in

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handwipe (> 82%) and serum samples (> 95%).<sup>16</sup> Because other congeners were detected less frequently in handwipes and serum,<sup>16</sup> we restricted analyses to BDE-47, -99, -100, and -153. Concentrations below the limit of detection (LOD) were substituted with the LOD/2.

As levels of PBDEs on handwipes and in serum were log-normally distributed we used log<sub>10</sub>-transformed values in analyses. We examined relationships between behaviors and environmental factors, and PBDE levels using linear regression models. To aid in the interpretation of results, we exponentiated  $\beta$ -coefficients (10<sup> $\beta$ </sup>), producing the multiplicative change in outcome. We considered confounding of behavior associations by several variables which our previous work indicated were associated with serum PBDE levels in our study cohort, including the child's sex and the father's educational attainment. In addition, as our previous work suggested that breastfeeding was related to BDE-153 levels in serum, models for BDE-153 were adjusted for the number of months that each child was breastfeed. Our previous work also indicated that age is related to PBDE levels in serum, suggesting it may confound associations between behavioral variables and serum PBDE levels. Where possible, we included age in adjusted models; however, for several behaviors which were strongly correlated with age, age was not included in models to avoid collinearity (e.g., time walking and time crawling which were strongly correlated with age;  $r_s > 0.90$ ). Finally, in models of serum PBDE levels, we considered effect modification by the levels of PBDEs in house dust and handwipes by adding an interaction term which consisted of a behavioral or environmental predictor and dichotomized dust or handwipe levels (above vs below the median). All statistical analyses were performed in SAS (version 9.2; SAS Institute Inc, Cary, NC, USA), with statistical significance defined as  $\alpha = 0.05$ .

# RESULTS

Parents frequently reported that children licked their fingers after eating (55.4% occasionally or frequently licking fingers while eating) and also reported that more than half the children put their hands in their mouths (55.4% puts hands in mouth at least two times per hour). Thumb sucking and current pacifier use were less common (12.5% and 26.5%, respectively); however, pacifier use in the past was reported by 47.7% of parents. On average children spent 12.2 ( $\pm$ 1.6) hours sleeping and 0.9 ( $\pm$ 0.8) hours in a car seat per day. Nearly all children were walking at the time of survey (n = 79; average age for starting to walk 11.5  $\pm$  2.2 months). Children's toys were primarily made of plastic (79.5%) as opposed to wood, metal or a combination of materials (20.5%). Carpet was the dominant flooring material in children's bedrooms (71.1%), playrooms (79.5%), and the home as a whole (50.6%). Parents reported frequent vacuuming, with 73.5% reporting vacuuming > 4 times per month.

#### Behavior, Activity, and Handwipes

Children's age was positively associated with PBDE levels on handwipes, although associations were small, imprecisely estimated and not statistically significant (Table 1). Although all effect estimates were all positive (indicating higher levels of PBDEs on handwipes with increased activity), associations between the number of months that a child had been walking or crawling and the levels of PBDEs on their hands at the time of the study visit were not statistically significant. Children spending more time sleeping per day

had significantly lower levels of BDE-47, -99, -100, and -153 on their hands. For instance, after adjusting for age, each additional hour of sleep resulted in a 30% decrease in the levels of BDE-47 on handwipes ( $10^{\beta} = 0.70$ ; 95% confidence interval (CI): 0.59, 0.83; Table 1). Similarly, the hours spent in a car each day was also inversely associated with the levels of PBDEs on children's hands, although associations did not reach statistical significance.

#### Behavior, Activity, and Serum

As we reported previously in this cohort,<sup>16</sup> age was positively associated with the levels of PBDEs in serum (Table 2). Associations for the number of months walking or crawling and serum PBDE levels were similar to those for age, suggesting that increased activity and mobility may lead to a higher body burden. Similarly, time spent sleeping, a measure of inactivity, was inversely associated with the levels of PBDEs in serum. For each addition hour of sleep, for example, children's serum levels of BDE-99 decreased by 15% ( $10^{\beta}$  = 0.85; 95% CI: 0.74, 0.98; Table 2). Associations for other congeners were generally less precise but also suggested inverse associations between time spent sleeping and serum PBDEs. Using a pacifier currently or in the past was associated with slightly lower serum BDE-47, -99, and -100 levels, although associations were imprecisely estimated. Licking fingers while eating was not significantly associated with higher levels of serum in adjusted models (age, sex, and father's occupation). However, as we hypothesize that the relationships between hand-to-mouth behaviors and serum levels may be altered by the levels of PBDEs on children's hands or in dust, we conducted analyses with investigating the interaction between PBDEs and hand-to-mouth behaviors. After accounting for the levels of PBDEs on children's hands, children that licked their fingers while eating had higher levels of PBDEs (BDE-47, P = 0.07; BDE-99, P = 0.04; BDE-100, P = 0.08; and BDE-153, P = 0.34; effect estimates not shown). Geometric mean BDE-99 levels in serum by handwipe and finger lick frequency are displayed in Figure 1. Patterns were similar, although less precisely estimated, when considering effect modification by house dust PBDE levels (results not shown).

#### **Environmental Characteristics and Children's Exposure**

Children that played with toys primarily made of plastic had higher levels of PBDEs on their hands; for example, children that played with primarily plastic toys had BDE-100 levels 1.98 times those of children that played with wood, metal, or multiple material toys (95% CI: 0.92, 4.26). Similarly, children with carpet in their homes tended to have higher levels of measured congeners; however, results did not reach statistical significance. Our results were also suggestive of an association between frequent vacuuming and/or sweeping in the home and decreased levels of PBDEs on children's hands (Table 3). Children living in homes that were vacuumed or swept frequently, for example, had handwipe BDE-99 levels 0.50 times those of children living in homes vacuumed less often (95% CI: 0.24, 1.04). In general, we did not observe associations between housing characteristics and serum PBDE levels in toddlers. One possible exception is BDE-153, which was lower in serum samples from children that played with mostly plastic toys.

# DISCUSSION

Cumulatively, our results suggest that certain aspects of children's behavior impact their levels of exposure to flame retardants. More active children, those who have been crawling or walking longer and slept less, had higher levels of PBDEs in their bodies. Although crawling and walking time were strongly associated with children's age, making it difficult to disentangle which factor (i.e., activity or some other age related behavior or characteristic) is driving associations, time spent sleeping was not strongly related to age in our study cohort. Adjusting analyses of sleeping time for age had little impact on associations with handwipe or serum PBDE levels, suggesting that more active children may have higher exposures to PBDEs, independent of their age. With respect to relationships between walking and crawling and measures of personal exposure, statistical associations were generally more precisely estimated for serum PBDEs than for handwipes. Increased day-to-day variation in the handwipe samples, relative to the serum samples, may have impacted the statistical stability of handwipe estimates.

After accounting for the levels of PBDEs on children's hands, we found significant associations between children's licking their fingers while eating and their serum PBDE levels, a finding which supports previous research indicating hand-to-mouth contact may be an important pathway of exposure.<sup>15,16,20</sup> In our study cohort, for example, we previously reported that children with the highest levels of PBDEs on their hands had serum PBDE levels 3.66 times those of children with the lowest levels on handwipes (reported results are for the sum of BDE-47, -99, and -100; 95% CI: 2.22, 6.05, comparing the third tertile to the first).<sup>16</sup> We did not find evidence that other hand-to-mouth contact frequency); however, our results were suggestive of an association between pacifier use and decreased serum BDE-47, -99, and -100 levels. It is possible that children that use pacifiers are less likely to put their hands or other objects in their mouths and, as a result, have reduced exposure to PBDEs via incidental ingestion. Frequency of hand washing may also modify the relationships between hand-to-mouth contact and serum PBDE levels, as has been reported in adults.<sup>20</sup>

In our cohort, children that played primarily with plastic toys had higher levels of PBDEs on their hands. Recent work suggests that plastic toys, which are commonly made from recycled materials, may contain PBDEs.<sup>21,22</sup> It is possible that PBDEs in children's toys are leaching out of these products and onto children's hands as they play. Alternatively, PBDEs present in indoor air/dust may adhere to plastics more readily than to other materials. However, playing with plastic toys was not associated with serum PBDE levels in our cohort. These results may reflect non-differential measurement error or a lag time between accumulation of PBDEs on children hands and in their serum. Similar patterns, demonstrating associations between environmental variables and handwipes but not serum, were also observed for flooring materials and cleaning frequency. For contaminants like PBDEs which have a long biological half-life, assessment behavior and the environment over time may be particularly important in understanding dominant pathways of exposure. Future assessment may be able to address this issue by conducting assessments of children's behavior and environment at multiple time points.

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Strengths of our study include a relatively large sample size for an exposure study of children and the use of several types of information: behavioral data, housing characteristics, house dust (an important source of exposure to PBDEs), a personal measure of exposure (handwipes), and a biomarker of exposure (serum). Weaknesses include a lack of information on specific dietary exposures (other than via breastfeeding) and sampling dust and handwipes at one point in time. Relying on parental reports of children's behaviors and activities is both a strength and a limitation of our research strategy. Information from parents is relatively easy to collect and may provide more information on a child's average behavior in different settings, but may also be biased by parents' perceptions of normal behavior. In our cohort, the reported frequency of children putting their hands in their mouths was relatively low (44.6% of parents reported that their children put their hands in their mouths two or less times per hour) compared with other previous assessments. For example. Tulve et al.<sup>23</sup> reported that children 10-60 months of age put their hands in their mouths an average of 16 times per hour. Although parents in our cohort may be under reporting hand-to-mouth contact, we do not anticipate that reporting errors are related to PBDE levels, particularly as reporting of hand-to-mouth contact did not differ by socioeconomic status (i.e., father's educational attainment). These data suggest that misclassification of hand-to-mouth behaviors may have biased results toward the null. More information is needed to determine how well parental reports capture average behavior patterns. In addition, using developmental milestones, such as walking and crawling, as indicators of children's interaction with the environment is problematic because there can be substantial variability between when a child first meets a milestone (e.g., takes a step) and when the preform the milestone on a regular basis (e.g., walks independently). Our analyses were also limited by our reliance on a few indicators of behavior. Other behaviors such as mouthing toys or other objects and hand washing are likely to influence exposure.

Taken together, our results suggest that certain aspects of children's behavior and their environment impact exposure to PBDEs. Future studies should consider these pathways in assessing exposure to PBDEs and other environmental compounds.

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Figure 1.

Geometric mean serum BDE-99 levels by handwipe BDE-99 category (below *vs* above the median, 13.36 ng finger licking frequency while eating to children with lower BDE-99 levels and lower reported finger licking frequency.

Table 1

Characteristic	BDE-47	a	BDE-996	a	BDE-100	)a	BDE-1536	
	10 <sup>β</sup> (95% CI)	Ρ	10 <sup>β</sup> (95% CI)	Ρ	10 <sup>β</sup> (95% CI)	Ρ	10 <sup>β</sup> (95% CI)	Ρ
Demographics								
Age (months)	$1.04\ (0.98,\ 1.11)$	0.16	1.02 (0.96, 1.09)	0.45	1.02 (0.96, 1.08)	0.55	1.03 (0.97, 1.09)	0.31
Activity								
Walking (number of months walking)	1.02 (0.96, 1.08)	0.47	1.02 (0.96, 1.08)	09.0	1.00 (0.94, 1.06)	1.00	1.03 (0.97, 1.08)	0.34
Crawling (number of months crawling)	1.04 (0.99, 1.10)	0.12	1.03 (0.98, 1.09)	0.24	1.03 (0.97, 1.09)	0.37	1.03 (0.98, 1.09)	0.18
Microenvironments								
Time spent sleeping (h/day)	$0.70\ (0.59,\ 0.83)$	< 0.0001	$0.69\ (0.58,\ 0.82)$	< 0.0001	$0.70\ (0.59,\ 0.83)$	< 0.0001	0.77 (0.66, 0.91)	0.002
Time spent in car seat (h/day)	$0.78\ (0.54,1.13)$	0.19	0.82 (0.56, 1.20)	0.30	$0.79\ (0.54,1.15)$	0.21	$0.80\ (0.57,1.11)$	0.18

ve to the reference group for categorical variables, or the per unit change for continuous variables.

 $^{a}$ Unadjusted except for the time spent in different microenvironments which is adjusted for age.

Table 2

Regression analyses for behavioral predictors of serum PBDE levels.

Characteristic	BDE-47 <sup>a</sup>		BDE-99a		$BDE-100^{a}$		BDE-153 <sup>b</sup>	
	10 <sup>β</sup> (95% CI)	Р	10 <sup>β</sup> (95% CI)	Ρ	10 <sup>b</sup> (95% CI)	Ρ	10 <sup>β</sup> (95% CI)	Ρ
Demographics								
Age (months)	1.05 (1.00, 1.10)	0.06	$1.03\ (0.98,\ 1.08)$	0.25	1.06(1.01,1.11)	0.02	1.05 (1.00, 1.10)	0.04
Activity								
Walking (number of months walking)	1.04 (0.99, 1.08)	0.11	1.02 (0.97, 1.06)	0.41	1.05 (1.00, 1.09)	0.03	1.04 (0.99, 1.08)	0.09
Crawling (number of months crawling)	1.05 (1.00, 1.09)	0.05	$1.03\ (0.99,\ 1.08)$	0.18	$1.06\ (1.01,\ 1.10)$	0.02	$1.06\ (1.01,\ 1.10)$	0.01
Microenvironments								
Time spent sleeping (h/day)	0.88 (0.76, 1.02)	0.09	$0.85\ (0.74,\ 0.98)$	0.03	0.91 (0.78, 1.05)	0.19	0.90 (0.77, 1.04)	0.15
Time spent in car seat (h/day)	1.09 (0.82, 1.44)	0.54	$1.14\ (0.87,1.51)$	0.34	$1.10\ (0.83,\ 1.45)$	0.52	1.09 (0.82, 1.43)	0.55
Hand-to-mouth behavior								
Currently using a pacifier (yes vs no)	0.61 (0.35, 1.06)	0.08	$0.68\ (0.39,1.18)$	0.17	0.66 (0.38, 1.15)	0.14	0.93 (0.55, 1.58)	0.78
Used a pacifier in past (yes vs no)	0.76 (0.43, 1.33)	0.33	0.73 (0.42, 1.27)	0.26	0.61 (0.36, 1.05)	0.07	0.83 (0.48, 1.44)	0.51
Hand in mouth occurrences (2 time per hour vs none)	0.98 (0.61, 1.56)	0.93	0.96 (0.60, 1.54)	0.85	0.95 (0.59, 1.51)	0.82	0.87 (0.56, 1.36)	0.54
Lick fingers while eating (occasionally/ frequently vs seldom/never)	1.07 (0.67, 1.72)	0.78	1.07 (0.67, 1.71)	0.79	0.95 (0.59, 1.51)	0.81	0.96 (0.61, 1.50)	0.85
Abbreviations: CI. confidence interval: PBD]	E. polvbrominated c	liphenyl	ether. Exponentiate	d B-coe	fficients represent th	e multi	olicative change in s	erum rel

ive to the reference group for categorical variables, or the per unit change for continuous variables.

<sup>a</sup>Adjusted for sex, father's education, and age (except for time walking and time crawling which were not adjusted for age).

<sup>b</sup>Adjusted for sex, father's education, breastfeeding duration, and age (except for time walking and time crawling which were not adjusted for age).

# Table 3

Regression analyses for environmental predictors of handwipe and serum PBDE levels.

Housing characteristic		Handv	vipes			Ser	m	
	BDE-47	BDE-99	BDE-100	BDE-153	BDE-47	BDE-99	BDE-100	BDE-153
	10 <sup>β</sup> (95% CI)	10 <sup>β</sup> (95% CI)	10 <sup>β</sup> (95% CI)	10 <sup>b</sup> (95% CI)				
Dominant toy material <sup>4</sup> (plastic vs wood/metal/mix)	2.06 (0.98, 4.32)	2.29 (1.07, 4.88)#	1.98 (0.92, 4.26)	1.57 (0.82, 3.01)	0.91 (0.50, 1.67)	1.01 (0.55, 1.86)	0.93 (0.51, 1.70)	0.60 (0.33, 1.08)
Home's flooring <sup>b</sup> (carpet vs wood/tile/ mixed)	1.23 (0.67, 2.26)	1.23 (0.67, 2.26)	1.09 (0.59, 2.00)	1.07 (0.63, 1.83)	0.92 (0.57, 1.48)	1.11 (0.70, 1.78)	1.00 (0.62, 1.63)	0.87 (0.55, 1.40)
Bedroom's flooring <sup>b</sup> (carpet vs wood/ tile)	1.11 (0.57, 2.15)	1.60 (0.83, 3.11)	1.44 (0.74, 2.8)	1.39 (0.78, 2.49)	0.64 (0.38, 1.07)	0.80 (0.48, 1.32)	0.66 (0.39, 1.11)	0.88 (0.53, 1.46)
Playroom's flooring $^{b}$ (carpet vs wood/ tile)	1.09 (0.59, 2.01)	1.47 (0.80, 2.72)	1.24 (0.67, 2.31)	1.15 (0.67, 1.97)	0.83 (0.51, 1.35)	0.93 (0.58, 1.50)	0.91 (0.56, 1.49)	0.84 (0.52, 1.35)
Vacuuming frequency $b(4 \text{ vs } >4 \text{ times})$ month)	0.59 (0.28, 1.23)	0.50 (0.24, 1.04)	0.57 (0.27, 1.21)	0.68 (0.35, 1.32)	0.77 (0.43, 1.38)	0.73 (0.41, 1.30)	0.69 (0.38, 1.24)	0.95 (0.53, 1.70)
Abbreviations: CI, confidence interval; PBD categorical variables, or the per unit change	E, polybrominated d for continuous varial	liphenyl ether. Expone oles.	sntiated β-coefficien	its represent the mul	tiplicative change in	handwipes or serum	relative to the refer	ence group for

 $^{\#}P < 0.05.$ 

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 $^{a}$ Adjusted for sex, father's education, and age.

 $b_{
m Adjusted}$  for father's education.