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Plasma phthalate levels in children with speech delay

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ABSTRACT

Speech delay is a common developmental concern. Environmental pollutants like phthalates, recognized as endocrine disruptors, may be a risk factor. We aimed to investigate the relationship between phthalates and speech delay. The study comprised 50 children with isolated speech delay and 40 healthy children of similar ages. Children were assessed for speech delay risk factors and phthalate exposure sources. High-pressure liquid chromatography examined plasma di-(2-ethylhexyl) phthalate (DEHP), mono-(2-ethylhexyl) phthalate (MEHP) and dibutyl phthalate (DBP) levels. DEHP, MEHP, and DBP levels varied between study and control groups: 0.377 (0.003–1.224 μg/ml), 0.212 (0.007–1.112 μg/ml) ($p = 0.033$), 0.523 (0.031–2.477 μg/ml), 0.152 (0.239–2.129 μg/ml) ($p < 0.001$), and 0.395 (0.062–1.996 μg/ml) and 0.270 (0.006–0.528) ($p = 0.004$). Multiple linear regression was used to adjust phthalate levels and speech delay risk factors. DEHP levels were did not differ significantly between the groups ($p = 0.233$), whereas MEHP and DBP levels were considerably higher in the study group ($p < 0.001$). The statistically significant rise in plasma phthalate levels in children with speech delay implies phthalate exposure may be a risk factor, but further epidemiological research is needed.

1. Introduction

Speech delay, one of the most prevalent developmental disorders, is defined as a child's inability to attain the level of language development they should have reached by a certain age and falling behind their peers [1]. Delay in language acquisition without any identified cause is observed at a rate ranging from 2.3 % to 19 % in pre-school children between the ages of 2 and 5 [2]. Challenges encountered in language development lead to difficulties in attention, learning, and long-term memory [3]. Children with a speech delay are more likely to experience psychological and behavioral adjustment issues in preschool and later on [4,5].

Research indicates that neurodevelopmental disorders, including speech delay, arise from the interplay of hereditary and environmental risk factors [6,7]. Multiple risk factors, including maternal and pregnancy-related problems in the prenatal period, prematurity [8], hypoxic birth [9], hearing loss [10], male sex, family history and environmental factors such as low socioeconomic status, caregiver education level and lack of stimuli [11,12], have been linked to speech delay. Another risk factor for speech delay may be prenatal or postnatal exposure to environmental pollutants by the mother or child. Exposure to environmental pollutant chemicals may have a detrimental effect on neurological development [6,13], hence impacting language skills. Among those, phthalates attract significant attention due to their ubiquitous usage [14].

Phthalates are semi-volatile synthetic chemicals that are used as plasticizers, color and odor fixatives. Numerous products used in daily life, including medical devices, food packaging, cosmetics/personal care products, medical materials, and toys, may contain certain amounts of different phthalate derivatives [15,16]. Since phthalates are not covalently bound to the plastic matrix, they can readily leach out and contaminate the environment. Moreover, humans are exposed to these chemicals by different routes (oral, dermal, inhalation, and intravenous) [17,18]. In a number of investigations, phthalates were detected in

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different body fluids such as blood, urine, saliva, and breast milk [19,20]. Phthalates were additionally identified in amniotic fluid. This indicates that phthalates can cross the placental barrier and that humans are exposed to these plasticizers even during fetal development [21].

Phthalates, such as di-(2-ethylhexyl) phthalate (DEHP) and dibutyl phthalate (DBP), are the most commonly employed derivatives. These chemicals may affect many systems, particularly the reproductive system and hormones. They are suggested to have anti-androgenic properties [22,23]. Due to their effects on androgens, studies on male and female rats have shown that phthalates may interfere testosterone-dependent brain development during prenatal period [24]. Numerous studies assess the adverse consequences of phthalate exposure throughout important developmental stages on neurodevelopment in infancy and childhood, indicating reduced cognitive and psychomotor development scores [25–27]. The effects of phthalate metabolites on language development are understudied, with published studies yielding contradictory and vague conclusions [3,28,29].

Due to the paucity of studies examining the effects of phthalate exposure on language development, the present study sought to investigate the association between phthalate levels and speech delay in children aged 2–6 years and to shed light on further research in terms of causality.

2. Materials and methods

2.1. Study participants

This is a case-control investigation. In September of 2019, the Hacettepe University Ethical Review Board for Non-Interventional Clinical Research granted approval (approval number: GO 19/748).

The study subjects were chosen among children who applied to Hacettepe University İhsan Doğramacı Children's Hospital:

1. Study group (n = 50): Children aged 24–72 months admitted to the Developmental Pediatrics Outpatient Clinic with isolated speech delay comprised the study group.
2. Control group (n = 40): Age-matched healthy children between 24 and 72 months admitted with acute complaints to the General Pediatrics Outpatient Clinic were recruited as the control group.

Patients who had been previously diagnosed with a neurodevelopmental, genetic, or metabolic disorder or living in a stimuli poor environment related to speech delay were excluded from the study.

During the evaluation of the patient, the family was informed and written consent was obtained. A questionnaire was administered to evaluate the possible routes of phthalate exposure, demographic information, and risk factors for speech delay. This questionnaire primarily investigated whether or not mothers were exposed to phthalate-derivative-containing products during pregnancy and the postpartum period, as well as whether or not children's dietary patterns and environment could cause significant phthalate exposure.

2.2. Data collection

The data was collected from October 2019 to February 2020. Demographic data [child's age, gender, birth order, mother's and father's age and education level, family's socioeconomic status, and place of residence (urban/rural)] and breastfeeding status and duration were obtained from the patient's file. The missing information was added to the form by interviewing the family. The Hollingshead-Redlich Scale [30] was used to ascertain the socioeconomic status of the individuals.

The Ages and Stages Questionnaire (ASQ) was used to evaluate the language and other developmental domains, including gross motor, fine motor, problem-solving, and personal-social development, of children in the study and control groups. ASQ is a widely used screening instrument in large-scale screening programs and research, and it can be completed by parents or other caregivers on their own or with the assistance of a trained professional [31]. We utilized the Ages and Stages Questionnaire (ASQ-TR) in its Turkish translation. The ASQ-TR has a sensitivity of 0.94 and a specificity of 0.85 [32]. According to the ASQ scoring system, children who scored below the threshold in the language domain but were within the normal range in all other domains were classified as having an isolated speech delay and were followed. The control group consisted of children who scored within the normal range on all developmental domain evaluations.

On the same day that the ASQ was administered, blood samples were collected from the children.

2.3. Laboratory analyses of serum phthalates

2.3.1. Chemicals and reagents

All chemicals, including DEHP, DBP, acetonitrile, n-hexane, and tetrahydrofuran, were purchased from Sigma-Aldrich (Mannheim, Germany). MEHP was from Cambridge Isotope Laboratories (Tewksbury, MA).

2.3.2. Deplasticization of the glassware

All glassware was washed and kept in 10 % nitric acid for 24 h. The glassware was then cleansed four times and cleaned for two hours with n-hexane:tetrahydrofuran (50:50). They were dried at 37°C. To prevent plastic contamination, high-pressure liquid chromatography (HPLC) vials were retained at 400°C for four hours. Aluminum foil was used to prevent all glass materials' coverings from coming into contact with plastic.

2.3.3. Biological samples

5 ml of children's venous blood was collected into heparinized tubes utilizing the drip technique with a sterile needle tip without a plastic structure at the rear end. The samples were centrifuged for 10 min at 3500 rpm. Plasma samples were stored at −80 °C until analysis.

2.3.4. Extraction of DBP, DEHP, and MEHP from plasma

The Paris et al. [33] method with certain modifications [34] was employed to conduct an examination of plasma levels of DEHP, DBP, and MEHP. Briefly, after spiking plasma (200 µL) with phthalates (1 ppm in the last volume), sodium hydroxide (1 N, 400 µL), phosphoric acid (50 %, 100 µL) and acetonitrile (800 µL) were added and mixed. The mixture was vortexed and centrifuged. The supernatant (600 µL) was transferred to another tube and evaporated under a nitrogen stream until dry. Residues were kept at −20°C.

2.3.5. Chromatographic analysis

Residues were dissolved in 60 % ACN (300 µl). Standards (0.2, 0.5, 1, 2 and 5 ppm for DEHP; 0.2, 0.5, 1, 2 and 5 ppm for DBP; 0.2, 0.5, 1, 2.5 and 5 ppm for MEHP) and samples (100 µl) were injected into HPLC (Agilent 1100 series, Santa Clara, CA). HPLC columns were Spherisorb C18 ODS2 column (25 cm×5 µm x4.6 mm i.d.) (Waters, Milford, MA), and ODS C18 precolumn (4 cm) (Waters, Mil- ford, MA). Mobile phase was 0.1 % H_3PO_4 and ACN [pH 3.0, 80:20 (v/v)]. Flow rate was 1 ml/min. Retention times for DBP, DEHP, and MEHP were 4.1 min, 32.5 min and 4.5 min, respectively. Due to close retention times of DBP and MEHP, their analyses were performed separately.

Plasma concentrations of DBP, DEHP, and MEHP were calculated from standards and peak areas were used for quantification. Limit of detections (LODs) were 0.38 µg/ml for DBP, 0.09 µg/ml for DEHP, and 1.4 µg/ml for MEHP. Limit of quantifications (LOQs) were 1.15 µg/ml for DBP, 0.27 µg/ml for DEHP, and 4.26 µg/ml for MEHP.

Recovery studies were performed on blank samples of plasma spiked with levels of 9.1 µg/ml of DBP, 9.8 µg/ml of DEHP, and 10.1 µg/ml of MEHP. Within-day precisions were DBP 0.71 ± 0.40 % CV, DEHP 3.09 ± 1.29 % CV, and 3.27 ± 1.05 % CV for MEHP. Between- run precisions were 1.06 ± 0.56 % CV for DBP, 9.21 ± 1.19 CV for DEHP and

7.92 ± 2.11 % CV for MEHP.

2.4. Statistical analysis

IBM SPSS 21.0 (Chicago, IL) was utilized for the analyses. The assumption of normality was made using the Kolmogorov-Smirnov test and histogram. Data that complies to a normal distribution was represented by its mean and standard deviation. Data that deviated from a normal distribution were represented using the median, minimum, and maximum values. To compare groups, the significance test of the difference between two means or the Mann-Whitney *U* test was employed, according to the normality assumption. Categorical variables were compared using the chi-square test. Multiple regression analysis was used to elucidate the overall variance in the dependent variable while taking into account the varying independent variables across groups. The square root and logarithmic transformations were applied to achieve normality for regression modeling, as determined by visual inspection of histograms and the Kolmogorov-Smirnov test. Statistical significance was determined by considering p values less than 0.05.

3. Results

Sociodemographic characteristics and risk factors for speech delay in both groups are detailed in Tables 1 and 2. The number of boys ($p = 0.036$) and history of speech delay ($p < 0.001$) was higher in the study group. The father's education level ($p = 0.049$) and smoking during pregnancy ($p = 0.008$) was higher in the control group. Regarding other characteristics and factors, there was no statistically significant difference between the two groups.

The median values of detectable DEHP, MEHP and DBP levels were significantly higher in the study group compared to the control group ($p = 0.033$, $p < 0.001$ and $p = 0.004$, respectively) (Table 3). There was no difference in phthalate levels between boys and girls in either the study or control groups, based on comparisons between groups or within groups.

Multiple linear regression analysis for risk factors including gender, smoking during pregnancy, father's education level, and family history of speech delay revealed no significant difference in DEHP levels between the study and control groups ($p = 0.233$). In contrast, MEHP and DBP levels in the study group were significantly higher ($p < 0.001$) (Table 4). Since the distribution of phthalate concentrations was highly skewed and did not conform to normality assumptions based on histogram inspection and Kolmogorov-Smirnov tests, data transformations were applied prior to regression analyses. Specifically, square root transformation was used for DEHP and DBP, and logarithmic transformation was applied to MEHP levels. These transformations aimed to stabilize variance, reduce skewness, and improve the interpretability and robustness of the multivariable linear regression models.

Tables 5 and 6 present the results of questionnaires used to evaluate potential phthalate exposure routes for children and their mothers in the study and control groups. According to potential phthalate exposure routes of mothers and children, DEHP, DBP, and MEHP levels of children in the study group were evaluated. We observed that plasma DEHP levels were higher in those whose mothers used hair dyes (0.574 ± 0.305 μg/ml) and conditioners (0.486 [0.211 – 1.224] μg/ml) at any time and those who used perfume/deodorant (0.535± 0.340 μg/ml) during pregnancy compared to those who did not (0.313 ± 0.173 μg/ml, 0.312 [0.003 – 1.096] μg/ml, and 0.364 ± 0.188 μg/ml, respectively) ($p = 0.001$, $p = 0.041$, and $p = 0.048$, respectively). No statistically significant difference was observed for any of the exposure routes with regards to DBP and MEHP.

Upon comparing the DEHP and DBP levels of children in the control group, it was observed that there was no significant statistical difference between the groups based on the exposure of their mothers to phthalates, either during pregnancy or at any other time. Those who stored food in plastic containers had higher plasma DBP concentrations (0.284 [0.076–0.468] g/ml, $p = 0.049$) than those who did not (0.062 [0.006–0.528] g/ml, $p = 0.049$). While plasma MEHP levels of children in the control group were higher than those whose mothers used fabric softeners at any time (0.313 [0.063–2.129] g/ml) and during pregnancy (0.267 [0063–2.129] g/ml) compared to those who did not (0.137 [0.024–0.348] g/ml and 0.124 [0.024–0.348] g/ml, respectively), plasma MEHP levels of children ($p = 0.010$, respectively $p = 0.011$). There was no difference in MEHP results regarding childhood phthalate exposure routes.

Table 1
Sociodemographic Characteristics of the Study and Control Groups.

Characteristics	Study Group (n = 50)	Control Group (n = 40)	p
Gender, n (%)	12 (24.0)	18 (45.0)	**0036**
Female	38 (76.0)	22 (55.0)	
Male			
Age (month)**	39 [24–70]	43.5 [24–72]	0.199
Mother's age (year)*	33.6 ± 5.28	34.73 ±5.39	0.322
Father's age (year)*	36.86 ± 4.93	37.85 ± 6.25	0.403
BMI Z scores, n (%)			
< −2 SD	3 (6)	1 (2.5)	0.16
≥ −2 SD- < −1 SD	6 (12)	4 (10)	
≥ −1 SD- < 1 SD	27 (54)	31 (77.5)	
≥ 1 SD- 2 SD	12 (24)	4 (10)	
≥ 2 SD	2 (4)	0 (0.0)	
Mother's educational level, n (%)			
8 years	10 (20.0)	10 (25.0)	0.724
9–12 years	16 (32.0)	10 (25.0)	
> 12 years	24 (48.0)	20 (50.0)	
Father's educational level, n (%)			**0.049**
8 years[a]	8 (16.0)	1 (2.5)	
9–12 years	17 (34.0)	12 (30.0)	
> 12 years	25 (50.0)	27 (67.5)	
Total breastfeeding duration (month), n (%)			
< 6 months	8 (16.0)	9 (22.5)	0.434
≥ 6 months	42 (84.0)	31 (77.5)	
Birth order, n (%)			
1	18 (36.0)	20 (50.0)	0.522
2	21 (42.0)	13 (32.5)	
3	8 (16.0)	4 (10.0)	
4 +	3 (6.0)	3 (7.5)	
Place of residence, n (%)			
Urban	45 (90.0)	40 (100.0)	0.063
Suburban	5 (10.0)	0 (0.0)	
Monthly income (x10^3 Turkish Lira)**	5 [2–18]	6 [1–16]	0.139
Social-economic level, n (%)			0.162
Higher education, profession or higher administrative positions	25 (50.0)	24 (60.0)	
Smaller business owners, government officials or skilled laborers, high school graduates	17 (34.0)	13 (32.5)	
Semi-skilled laborers; educational level below high school	4 (8.0)	3 (7.5)	
Semi-skilled laborers; primary school graduates or not educated	4 (8.0)	0 (0.0)	

Abbreviations: SD, standard deviation
* Mean ± SD
** median [min – max]
[a] Difference in education level due to group with 8 years or less education.

4. Discussion

In studies on speech delay, male gender [35,36], education level of parents [11,36] and family history of speech delay [11] are some of the well-known risk factors. In our study, the males were diagnosed with isolated speech delay three times more. Moreover, the family history of speech delay was higher and the father's education level was lower in the study group compared to the control group.

In numerous studies, it is reported that exposure to smoking in

Table 2
Comparison of Risk Factors for Speech Delay in the Study and Control Groups.

	Study Group n (%)	Control Group n (%)	p
Prenatal period			
Smoking	5 (10.0)	13 (32.5)	**0.008**
Alcohol consumption	0 (0.0)	0 (0.0)	NA
Infection	10 (20.0)	8 (20.0)	1.000
Hypothyroidism	6 (12.0)	9 (22.5)	0.184
Radiation	2 (4.0)	0 (0.0)	0.501
Natal period			
Delivery type			
NSVD	24 (48.0)	16 (40.0)	0.448
C-section	26 (52.0)	24 (60.0)	
Birth weight (kg)*	3.33 ± 0.49	3.25 ± 0.43	0.429
Birth week*	39.12 ± 1.45	38.57 ± 1.17	0.058
Hypoxia history	3 (6.0)	2 (5.0)	1.000
Congenital anomaly	0 (0.0)	1 (2.5)	0.444
Postnatal period			
Disease/Disability	6 (12.0)	5 (12.5)	1.000
Hearing	0 (0.0)	1 (20.0)	
Visual	4 (66.7)	2 (40.0)	
Other	2 (33.3)	2 (40.0)	
Screen exposure			
0–1 h	8 (16.0)	11 (27.5)	0.322
1–2 h	21 (42.0)	12 (30.0)	
> 2 h	21 (42.0)	17 (42.5)	
Speech delay in the family	33 (66.0)	7 (17.5)	**< 0.001**

Abbreviations: NSVD, Normal spontaneous vaginal delivery; C-section, Cesarean section
* Mean ± SD.

Table 3
Plasma di-(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP) and mono-(2-ethylhexyl) phthalate (MEHP) levels of the children in the study and control groups.

Phthalate	Study Group	Control Group	P
DEHP (µg/ml)*	0.377 [0.003 – 1.224] (n = 47)	0.212 [0.007 – 1.112] (n = 35)	**0.03**
MEHP (µg/ml)*	0.523 [0.031 – 2.477] (n = 47)	0.152 [0.239 – 2.129] (n = 33)	**< 0.001**
DBP (µg/ml)*	0.395 [0.062 – 1.996] (n = 42)	0.270 [0.006 – 0.528] (n = 30)	**0.004**

Abbreviations: DEHP: di-(2-ethylhexyl) phthalate, DBP: dibutyl phthalate, MEHP: mono-(2-ethylhexyl) phthalate
* Median [min – max]

prenatal and postnatal periods have adverse effects on children's neurological development process and thus on language development [37–39]. Despite no differences between the two groups regarding mother smoking during the postpartum period and smoking at home, unexpectedly, smoking rate during pregnancy was reported to be higher in the control group. This outcome may be due to selection bias in self-reporting and because families of the case group may have refrained from disclosing smoking behavior at critical periods, such as pregnancy, due to the associated risks of smoking. Regarding smoking, which is known to have numerous obvious unfavorable health effects [37,40], this difference in the speech delay during the prenatal period should be reexamined with prospective and larger sample studies.

Many epigenetic factors may play a role in the etiology of speech delay [41]. Among these factors genetics, lifestyle, and environmental pollutants are suggested to be the main factors [42]. The development of the central nervous system in children is sensitive to the environment during the intrauterine period and first years of life [43]. Considering that language development is one of the primary markers of the neurodevelopmental process, studies investigating the role of phthalates, one of the environmental pollutants, in the etiology of speech delay attract more attention day by day. There are studies indicating that prenatal exposure to phthalates may be linked to cognitive impairments in infants and children [44,45]. As part of cognitive development, prenatal exposure to these chemicals may have substantial impact on speech [46].

In the current work, median plasma levels of DEHP, its metabolite MEHP and DBP were significantly higher in the study group compared to control group. After correcting for speech delay risk factors, which differed between the two groups, there was no significant difference between the two groups in terms of DEHP levels, while MEHP and DBP levels were found to be significantly higher in the study group. In a prospective study conducted in Denmark, the researchers examined the relationship between prenatal phthalate exposure and language development of children aged 20–36 months. In the study, high urinary diethyl phthalate (DEP), butyl benzyl phthalate (BBP) and DEHP metabolite levels in the third trimester of mothers were associated with low language scores (word count and complex language use) in boys. However, this relationship was not observed in girls [28]. A two-centered cohort study (Sweden and the United States) examined the relationship between language development and urinary phthalate metabolites. In the Swedish subjects, doubling of the prenatal DBP and BBP metabolite levels significantly increased the estimated relative risk (odds ratio, OD) for language delay by approximately 25–40 % after corrected analyses. However, no association with any DEHP metabolite was observed in either of the cohorts with language delay [29]. Conversely, an inverse relationship between MEHP and speech delay was observed in the present study most probably due to the difference in the biological samples. In a study published in Singapore, a large number of metabolites of different environmental chemicals were measured by gas chromatography-mass spectrometry (GC-MS) in hair samples taken from mothers at 26–28 weeks of gestation and their relationship with the developmental areas of 24-month-old children were examined. The researchers found that high phthalic acid levels were associated with low expressive language scores (univariate p value= 0.022) [47].

In our study, in which possible sources of exposure from the intrauterine period to early childhood were questioned, the plasma DEHP levels were found to be higher in the study group whose mothers reported using hair dyes and conditioners at any time and those who used deodorant during pregnancy. Plasma MEHP levels of children in the

Table 4a
Multiple linear regression analysis of DEHP levels in the study and control groups.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95,0 % Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	.985	.227		4.338	.000	.533	1.438
	Group	−.081	.067	−.156	−1.203	**.233**	−.215	.053
	Gender	.026	.061	.047	.421	.675	−.096	.148
	Father's education	−.036	.043	−.095	−.836	.406	−.121	.049
	Smoking during pregnancy	−.063	.076	−.096	−.824	.412	−.214	.089
	Speech delay history in family	−.074	.067	−.143	−1.101	.274	−.208	.060

[a]Dependent Variable: square root_ di-(2-ethylhexyl) phthalate (DEHP)

Table4b
Multiple linear regression analysis of MEHP levels in the study and control groups.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0 % Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	−.470	.822		−.572	.569	−2.108	1.168
	Group	−1.150	.243	−.573	−4.731	**.000**	−1.635	−.666
	Gender	−.045	.220	−.021	−.204	.839	−.482	.393
	Father's education	.162	.156	.110	1.043	.300	−.148	.473
	Smoking during pregnancy	.024	.275	.009	.087	.931	−.525	.573
	Speech delay history in family	.419	.245	.211	1.707	.092	−.070	.907

[a]Dependent Variable: logarithm_ mono-(2-ethylhexyl) phthalate (MEHP)

Table 4c
Multiple linear regression analysis of DBP levels in the study and control groups.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0 % Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	.763	.246		3.095	.003	.271	1.255
	Group	−.291	.078	−.525	−3.712	**.000**	−.447	−.134
	Gender	−.017	.068	−.029	−.248	.805	−.153	.119
	Father's education	.010	.046	.025	.220	.827	−.082	.102
	Smoking during pregnancy	.025	.078	.038	.322	.748	−.131	.181
	Speech delay history in family	.125	.075	.227	1.673	.099	−.024	.275

[a]Dependent Variable: square root_ dibutyl phthalate (DBP)

Table 5
Possible exposure routes of the mothers to phthalates in the study and control groups.

	Mother's exposure at any time			**Mother's exposure during pregnancy**		
	Study (n = 50.%)	**Control (n = 40.%)**	**p**	**Study (n = 50. %)**	**Control (n = 40.%)**	**p**
Shampoo	49 (98.0)	40 (100.0)	1.000	50 (100.0)	40 (100.0)	NA
Hair conditioner	17 (34.0)	24 (60.0)	**0.014**	19 (38.0)	24 (60.0)	**0.038**
Hair spray	4 (8.0)	7 (17.5)	0.206	3 (6.0)	4 (10.0)	0.695
Hair dye	24 (48.0)	25 (62.5)	0.170	4 (8.0)	4 (10.0)	1.000
Make up	26 (52.0)	27 (67.5)	0.138	21 (42.0)	23 (57.5)	0.144
Nail polish	7 (14.0)	9 (22.5)	0.295	2 (4.0)	5 (12.5)	0.235
Shower gel	19 (38)	19 (47.5)	0.365	13 (26.0)	18 (45.0)	0.059
Perfume/ deodorant	38 (76.0)	36 (90.0)	0.084	23 (46.0)	30 (75.0)	**0.005**
Detergents	50 (100.0)	40 (100.0)	NA	50 (100.0)	40 (100.0)	NA
Fabric softeners	37 (74.0)	24 (60.0)	0.158	37 (74.0)	26 (65.0)	0.355
Use of dishwashing gloves	6 (12.0)	5 (12.5)	1.000	6 (12.0)	6 (15.0)	0.677
Use of medicine	11 (22.0)	11 (27.5)	0.546	10 (20.0)	13 (32.5)	0.177

control group whose mothers used softeners (at any time and during pregnancy) were higher vs. control. Although it is stated that the prominent routes for exposure to phthalates are oral and inhalation [19], these results also show the importance of dermal exposure. In the control group, plasma DBP levels were higher in those whose mothers used plastic storage containers for food. DBP is a phthalate predominantly found in personal care and cosmetic products, and enteric-coated drug tablets [19], and no difference was found in both the study and control groups in the inquiries made in this regard. Therefore, more research should be done with DBP-related exposure sources and their possible undesired outcomes.

Our study has some limitations and strengths. First, although the adequate number of participants in the study and control groups was determined using the G-power 3.0.10 program with 0.80 effect size, 80 % power, and 5 % margin of error based on previous studies, repeating a similar study with larger groups will enhance the strength of the study. Although the original study was designed as a 1:1 matched case-control study, we encountered difficulty enrolling control children during the study period. We believe that, among other factors, this difficulty can largely be attributed to the reluctance of parents to have their children participate in a blood draw that would not directly benefit their child. However, this mismatch was adjusted when evaluating the results, along with other confounding risk factors that differed between the two groups. In our study, a single blood sample was taken and a cross-sectional evaluation was made. However, although the phthalate metabolite results of the children have not been compared with the blood samples of the mothers, it should be kept in mind that the exposure routes during pregnancy and afterward mostly continue in the same environment and by similar routes. However, prospective follow-up studies should be conducted in terms of long-term effects of endocrine disruptors. Although questioning about possible prenatal and childhood exposure routes in our study is one of the advantages of this study, it should be considered that the results may be affected by the possibility of incomplete or incorrect recall in the retrospective responses of the questionnaire studies. One of the strengths of this study is that it was designed as a case-control study, which enhances the practical applicability of the clinical assessment and enables a concurrent and comparative examination of various potential risk factors, including environmental chemicals, within a manageable sample size and limited time frame. Furthermore, the age group included the preschool age, the period when speech disorders are most frequently encountered and require early intervention. In our study, in order to prevent possible contamination during the collection of blood samples and experimental procedures, the use of plastic materials was avoided, and other materials used were pre-treated as described in the method section. In addition, although the early development inventory we used in our study was a

Table 6
Possible exposure routes to phthalates in the newborn and early childhood periods of children in the study and control groups.

	Study (n = 50. %)	Control (n = 40. %)	p
Baby bottle usage	34 (68.0)	29 (72.5)	0.643
Pacifier use	13 (26.0)	18 (45.0)	0.059
Plastic toy	47 (94.0)	40 (100.0)	0.251
Baby shampoo	50 (100.0)	40 (100.0)	NA
Hospitalization in intensive care	6 (12.0)	5 (12.5)	1.000
Mechanic ventilation	4 (8.0)	1 (2.5)	0.377
Dialysis by peritoneum or hemodialysis	1 (2.0)	0 (0.0)	1.000
Blood transfusion	2 (4.0)	0 (0.0)	0.501
Frequent infections/use of antibiotics (≥6/year)	3 (6.0)	7 (17.5)	0.102
Plastic plate/spoon	9 (18.0)	9 (22.5)	0.596
Plastic storage container	34 (68.0)	25 (62.5)	0.585
Plastic bottle/carboy	44 (88.0)	37 (92.5)	0.726
Frozen food consumption	6 (12.0)	10 (25.0)	0.109
Canned food consumption	3 (6.0)	5 (12.5)	0.458
Packaged food consumption	47 (94.0)	37 (92.5)	1.000
PVC products at home	40 (80.0)	29 (72.5)	0.403
PVC products in the nursery	25 (50.0)	20 (50.0)	NA
Smoking mother	11 (22.0)	16 (40.0)	0.064
Smoking at home	19 (38.0)	23 (57.5)	0.065

Abbreviation: PVC. Polyvinyl chloride; NA. Not applicable

parent-centered and easy-to-use screening questionnaire, each child in our study was evaluated by the same clinician, taking into account the parents' views. Consequently, the test findings were assessed with a higher objectivity.

5. Conclusion

Plasma DEHP, MEHP, and DBP levels were found to be significantly higher in children with isolated speech delay compared to healthy controls. After the adjusted analysis of the factors differing between the two groups in terms of delayed speech risk factors, there was no significant difference in terms of DEHP levels, while MEHP and DBP levels were markedly higher in the study group. The statistically significant higher phthalate levels in children with speech delay indicate that these subjects are more exposed to phthalates through different routes.

In conclusion, the precise understanding of how endocrine-disrupting chemicals impact to the development of speech delay is still incomplete. While our study did not aim to demonstrate a causal link, it does contribute to the scarce amount of research in this topic. Additional epidemiological and pathophysiology investigations with larger sample sizes are necessary to corroborate this association.

CRediT authorship contribution statement

Nihal Yaman Artunç: Conceptualized and designed the study and the data collection instruments, collected data, drafted the initial manuscript, and reviewed and revised the manuscript.

Pınar Erkekoğlu: Planned and supervised the plasma phthalate measurement experiments, and critically reviewed and revised the manuscript.

Gizem Yıldıztekin and Anıl Yirün: Performed the plasma phthalate measurement experiments, worked out the technical details and performed the numerical calculations for the suggested experiments and revised the manuscript.

Pınar Zengin Akkuş, Evin İlter Bahadur and Gökçenur Özdemir: Developmental assessment and data collection.

Elif N. Özmert: Conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed and revised the manuscript for important intellectual content.

Ethics approval

Ethical Review Board of Hacettepe University Non-Interventional Clinical Research in September 2019 (Approval number: GO 19/748).

Consent to participate

Approval is appropriate (include appropriate statements).

Consent for publication

Approval is appropriate (include appropriate statements).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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