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Phthalate Exposure among Roma Population in Slovakia

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***Abstract*—**Phthalates are ubiquitous environmental pollutants well known because of their endocrine disrupting activity in human organism. The aim of our study was, by biological monitoring, investigate exposure to phthalates of Roma ethnicity group i.e. children and adults from 5 families (n=29, average age 11.8 ± 7.6 years) living in western Slovakia. Additionally, we analysed some associations between anthropometric measures, questionnaire data i.e. socio-economic status, eating and drinking habits, practise of personal care products and household conditions in comparison with concentrations of phthalate metabolites. We used for analysis of urine samples high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS) to determine concentrations of phthalate metabolites monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), mono-iso-butyl phthalate (MiBP), mono(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP), mono(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP) and mono(2-etylhexyl) phthalate (MEHP). Our results indicate that ethnicity, lower socioeconomic status and different housing conditions in Roma population can affect urinary concentration of phthalate metabolites.

***Keywords*—**Biomonitoring, ethnicity, human exposure, phthalate metabolites.

I. Introduction

SINCE first application phthalates became one of the ubiquitous environmental pollutants of a modern world [1]. These alkyl or alkyl aryl esters of 1,2-benzene dicarboxylic acid [2], [3] are used in an industrial production of plastics mostly as plasticizers and solvents [4]. Phthalates are not bonded covalently with molecules of polymers, so that they can be released into the environment [5] during their production, usage and removing [6]. Phthalates can be found in food and drinking water [7], personal care products (cosmetics, perfumes), food packaging, children toys, flexible PVC, building materials, paints and varnishes [8]. They can enter human body due to ingestion, inhalation or dermal absorption [3], [9], [5]. Special type is exposure in utero, when a fetus is exposed through maternal organism [10]. Phthalates do not bioaccumulate in tissues, but they are metabolized and excreted by urine and faeces [11]. Their presence in organism can cause various health disorders, especially in hormonal regulation. Phthalates have endocrine disrupting activity and potential impact on the reproductive and nervous system [12].

The alkyl chains length gives rise to different molecular weights and functionality between the different types of phthalates [5]. Low molecular weight (LMW) phthalates have fewer than eight carbon atoms and high molecular weight (HMW) phthalates have eight or more carbon atoms in an alkyl chain [11]. The simple monoesters are the major urinary metabolites of LMW phthalates, e.g. diethyl phthalate (DEP) or di-n-butyl phthalate (DnBP). HMW phthalates, e.g. di-(2-etylhexyl) phthalate (DEHP) and di-n-octyl phthalate (DnOP), are metabolized to their corresponding monoesters and could be further metabolized to several oxidative metabolites. Before excretion monoesters and oxidative metabolites may be glucuronidate [13].

Studies evaluating data from extensive human biomonitoring [14]-[17] consider ethnical origin of probands, living in one geographical area, as an important factor in metabolism of phthalates and work with their final concentrations of phthalate metabolites separately. In an analysis of National Health and Examination Survey (NHANES) 2001-2008 (USA) was found variation across the three major ethnic groups. Levels of phthalate metabolites in urine samples of probands self-reported as black were highest for monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), mono-iso-butyl phthalate (MiBP), mono-benzyl phthalate (MBzP) and ΣDEHP and for whites was highest level of mono-(3-carboxypropyl) phthalate (MCPP). Mexican-Americans probands had higher urinary concentrations of MnBP and MiBP than whites [17].

It is the reason why we have decided to realize biomonitoring of phthalates with probands of Roma ethnicity in Slovakia. In our study we measured the urinary concentrations of 6 phthalate metabolites in 29 probands of Roma ethnicity from 5 families considering race/ethnicity as important factor in a phthalate metabolism. Additionally, we analysed some associations between anthropometric measures, questionnaire data i.e. socio-economic status, eating and drinking habits, practise of personal care products and household conditions in comparison with concentrations of phthalate metabolites. Till nowadays, to the best of our knowledge, the study which explore an association between minority or ethnic group with specific habits and genetic history (Roma ethnicity) and exposition of phthalates was not done yet.

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II. Methods

A. Study Population

The cohort consisted of Roma ethnicity probands from Slovakia (n= 29) i.e. 5 families mothers and children; all of them live in village Nemčiňany, Zlaté Moravce district (Slovak Republic). Participation was voluntary and there was a possibility to withdraw participation at any time during the study. All adult participants gave written informed consent, the legal representatives of all children until 15 years gave written informed consent prior to the study, to provide samples of urine, complete questionnaires and allow the researchers to take measurements and also to process their personal records and data. The anthropometric data was collected using standard anthropological methods; body height (by A 319 TRYSTOM, Ltd., Pasteurova 15, 772 00 Olomouc Czech Republic), body-mass index (BMI) was estimated and classified by WHO (1995) and by CDC growth charts (2000). Body weight was estimated by The Omron BF510 (Kyoto, Japan).

B. Phthalate Analysis

We collected spot urine samples (2x2ml) from all probands. Samples were stored in a transport box at 2-6°C, and then banked in the laboratory at -73°C until analysis. We measured urinary concentrations of mono ethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), mono-iso-butyl phthalate (MiBP), mono(2-etylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP) and mono(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP). We used high-performance liquid chromatography (HPLC) and tandem mass spectrometry (MS/MS) (Infinity 1260 and 6410 triplequad, Agilent) using method reported by [18], modification of [19].

We purchased analytical standards from Cambridge isotope laboratories (MA, USA). Briefly, 1ml of urine was thawed, buffered with ammonium acetate, spiked with isotope labeled phthalate standards, β-glucuronidase enzyme (Roche, Germany) and incubated (37°C). After deconjugation were samples diluted with phosphate buffer (NaH_2PO_4 in H_3PO_4) and loaded on SPE cartridges (ABS Elut Nexus, Agilent). Cartridges were conditioned with acetonitrile followed by phosphate buffer before extraction. To remove hydrophilic compound, SPE cartridges were flushed by formic acid and HPLC grade water. Elution of analytes was performed by acetonitrile and ethylacetate. Eluate was dried by nitrogen gas and reconstituted with 200µl of H_2O. For HPLC, an Agilent Infinity 1260 liquid chromatography equipped with ZORBAX Eclipse phenyl-hexyl column was used. Separation was done using nonlinear gradient program. Agilent 6410 triplequad with electro-spray ionisation was used for mass specific detection of phthalate metabolites. Instrumental settings were as follows: spray ion voltage (-3,800V), nitrogen nebulizer gas pressure (8psi), and nitrogen curtain gas pressure (7psi), capillary temperature (430°C), and collision gas (nitrogen) pressure (1.5mTor). Precursor and product ions, collision energies, retention times, and limits of detection (LOD) are showed in Table I.

C. Quality Control

Samples were analysed in batches of ten unknown samples one reagent blank and one quality control (QC) sample. Exceeding of LOD levels in reagent blank or deviation from 99th confidence interval in QC resulted into rejection of the batch and repeated analysis.

D. Statistics

To describe the urinary phthalate metabolites levels in the study population, means with SD, medians and the 5th to 95th percentiles of concentrations were computed for each metabolite. The non-parametric Mann-Whitney U (Wilcoxon rank-sum) test was used for all comparisons and a difference was considered to be significant when p value was ≤ 0.05. We used statistics program Statistica 8.0 (Stat Soft. Inc.).

TABLE I
PHTHALATE MONOESTERS: CHROMATOGRAPHIC AND MASS SPECTROMETRIC PARAMETERS

Compound name	Precursor ion	Product ion	F (V)	CE (V)	RT	LOD
MEP	193	77.1	60	15	6.7	4.41
MEP- labeled	197.1	79	60	15	6.7	
MiBP	221.1	76.9	90	10	9.4	
MnBP	221.1	76.9	90	10	9.8	1.01
MnBP- labeled	225.1	78.8	90	10	9.8	
MEHP	277.1	133.9	90	14	13.5	0.81
MEHP- labeled	281.1	137.1	90	14	13.5	
5OH-MEHP	291	121.2	90	10	9.3	0.54
5OH-MEHP labeled	294.9	124	90	10	9.3	
5oxo-MEHP	293.1	121.2	95	12	9.7	0.64
5oxo-MEHP labeled	297	124.1	95	12	9.7	

F-fragmentor, CE- collision energy, RT-retention time (min.), LOD- limit of detection ($\mu g.L^{-1}$)

III. Results

The cohort consisted of 29 Roma ethnicity participants, 11 males and 18 females in average age 11.8 ± 7.6 years. The baseline characteristics of study group are shown in Table III. Concentrations of detected phthalate metabolites above the limit of detection (LOD) were found in 100% of samples for MEP, MnBP, MiBP, 5OH-MEHP and 5oxo-MEHP and in 89.66% of samples for MEHP. Urinary concentrations of phthalate monoesters characterized by means and standard deviations (SDs), minimum, the 5th, 50th (medians), 95th percentiles and maximum are shown in Table II. From the three DEHP metabolites, 5OH-MEHP had the highest level-ratio (mean: 58%), followed by 5oxo-MEHP (36%) and MEHP (6%). The mean excretion-ratios of MEHP to 5OH-MEHP and MEHP to 5oxo-MEHP were 1:9.7 and 1:5.9.

We divided our cohort into two groups, children in age 3-15 years (n=23; average age 8.48 ± 2.97) and adults (n=6; average age 24.50 ± 6.35). In both groups phthalate metabolite concentrations were widely variable with large differences between mean and median concentrations. Medians in a group of children were consistently higher for each phthalate metabolite except MEP, for which the median for adults group was higher (22.32 $\mu g.L^{-1}$) in comparison with group of children (16.04 $\mu g.L^{-1}$) (Fig. 1).

TABLE II
URINARY CONCENTRATIONS OF PHTHALATES METABOLITES (μg.L⁻¹) IN STUDY GROUP

	Mean	SD	Min	Percentiles			Max
				5th	50th (median)	95th	
MEP	32.17	39.87	<LOD	<LOD	16.26	113.33	187.15
MnBP	164.40	117.11	42.43	44.99	111.71	402.03	469.36
MiBP	54.09	88.42	2.34	2.73	36.19	155.78	469.53
5OH-MEHP	61.76	78.24	10.33	12.76	26.87	244.88	330.96
5oxo-MEHP	37.62	50.59	5.07	5.18	14.95	165.90	200.45
MEHP	7.13	7.49	<LOD	<LOD	4.02	18.59	34.58
ΣDBP	214.49	169.11	48.58	62.86	151.54	533.4	799.86
ΣDEHP	99.38	128.71	15.40	18.9	40.63	410.78	531.42

ΣDBP= MnBP + MiBP; ΣDEHP= MEHP+ 5OH-MEHP+ 5oxo-MEHP;
% > LOD: MnBP, MiBP, 5OH-MEHP and 5oxo-MEHP 100%, MEP 89.66%, MEHP 79.93%

The comparison (Mann-Whitney U test) between groups of children and adults for the urinary metabolite levels did not show significant differences for MEHP, MnBP, MiBP and MEP while on the border of the statistical significance were urinary concentrations of secondary DEHP metabolites 5oxo-MEHP (p=0.059) and 5OH-MEHP (p=0.084).

After summarizing DEHP metabolites, we did not find any statistic significant difference between groups. When we divided our cohort into 5 groups based on household where probands live, we did not find any statistically significant difference between these 5 families. To further examine gender differences, any statistically significance was found.

In questionnaire were participants asked for information about their consumer practises and usage of plastic materials (Table III). There was difference only in a frequency of consumption of foodstuff in a plastic packaging, storing food in plastic containers and type of used perfumed cosmetics. Surprisingly, we did not find any statistical significance between levels of phthalates and consumer practises of probands.

TABLE III
BASELINE CHARACTERISTIC OF COHORT AND CONSUMER PRACTISES OF PROBANDS (COUNT/%)

Male	11 (38)			
Female	18 (62)			
Age	3-15	17 <		
	23 (79.3)	6 (20.7)		
Mean (SD)	8.48 (±2.97)	24.50 (±6.35)		
BMI	underweight	normal weight	overweight	
	1 (3.5)	25 (86.2)	3 (10.3)	
Consumer practise	**Yes**	**No**		
Smoking	3 (10.3)	26 (89.7)		
Drinking drink from plastic bottle during last 24 hours	29 (100)	-		
PVC flooring at home	29 (100)	-		
Burning of plastic waste for heating	29 (100)	-		
Applying of perfumed cosmetics:	29 (100)	-		
- soap, shampoo	29 (100)	-		
- fragrances	18 (62)	11 (38)		
- makeup	1 (3.5)	28 (96.6)		
Consumer practise	**Daily**	**1-2x a week**	**Occasionally**	**Never**
Consumption of foodstuff in plastic packaging	-	14(48.3)	15 (51.7)	-
Heating food in plastic container	-	-	-	29 (100)
Drinking drinks from plastic bottle	29 (100)	-	-	-
Food stored in plastic container	-	-	6 (20.7)	23 (79.3)

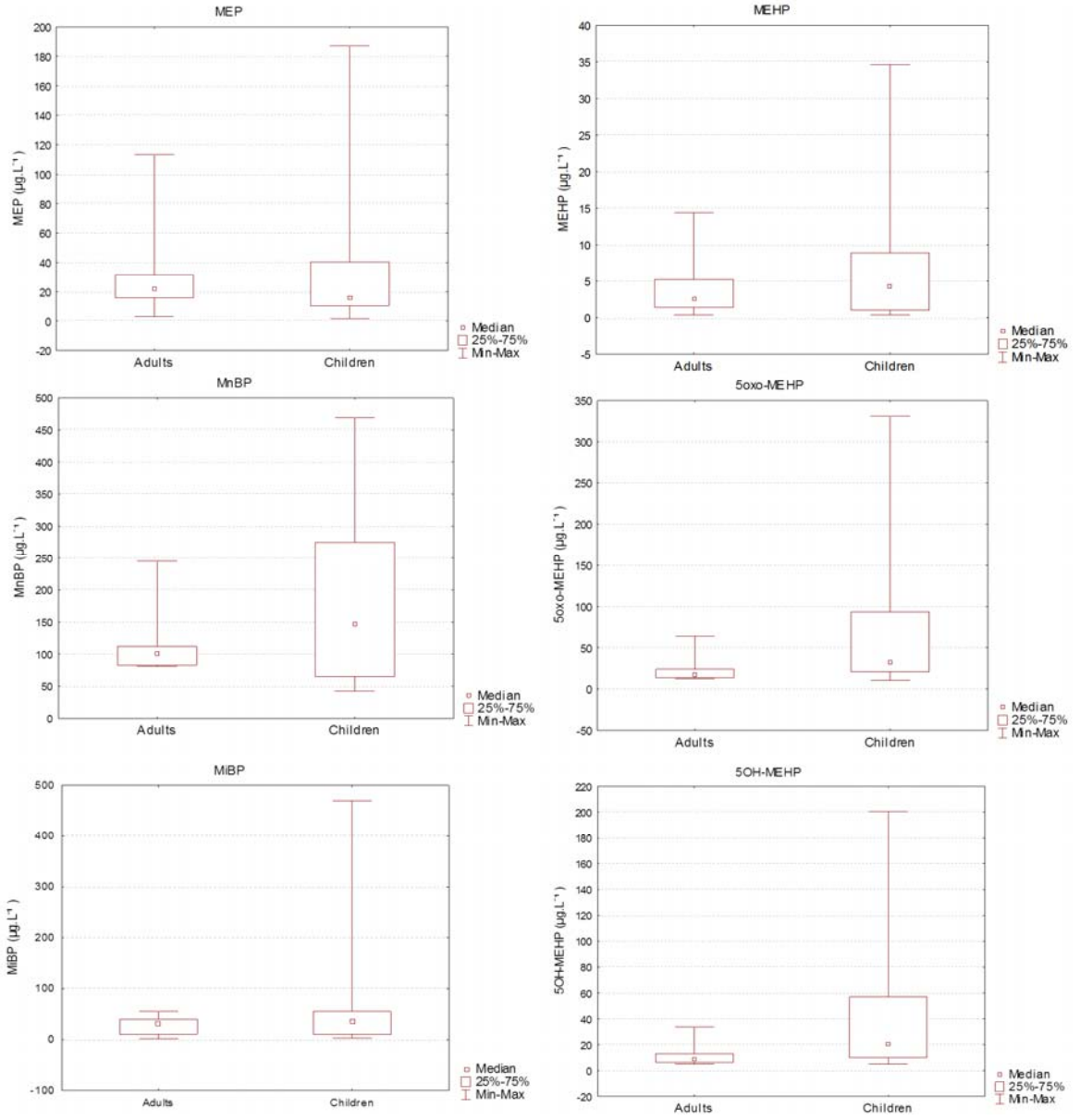

Fig. 1 Box-plots of urinary concentrations of phthalate metabolites in group of children and adults

IV. Discussion

According to LARES study of the WHO is considered that housing conditions are a strong determinant of population health which can negatively directly impact vulnerable and marginalized population groups, such as the Roma population [20]. Long-term bad economic situation, low educational level and incorrect lifestyle of the Romany minority is commonly considered as the main reason of a bad health status [21].

Biomarkers of phthalate exposure vary with socioeconomic factors [22], [23]. In a cohort of pregnant women characterized by low income and high social disadvantage, were found significantly higher urinary concentrations of DnBP and DiBP metabolites in comparison with other pregnant women in the USA [24]. Urinary concentrations of MnBP (median 147.64 µg.L^{-1}) for children in our study was three times higher than in study with Chinese children by medians 50.47 µg.L^{-1} [25], respectively 47.2 µg.L^{-1} [26] and Egyptian study median 47.5 µg.L^{-1} [27], likewise considerably higher than levels of each ethnic group in NHANES 2001-2008 medians 16.0-19.6 µg.L^{-1} [17]. Final concentrations of MnBP in our study were consistent with results of longitudinal cohort study of Danish (medians: males130 µg.L^{-1}, females 121 µg.L^{-1}) [28] and Norwegian children (GM 138.8 µg.L^{-1}) [5]. On the contrary in GerEs IV pilot biomonitoring, was median of MnBP higher than in Roma children (166 µg.L^{-1}) [31].

MiBP concentration for children in our study (median 31.69 µg.L^{-1}) was also much higher than in Egyptian children (17.6 µg.L^{-1}) [27], but lower than in Chinese (37.4 µg.L^{-1}) [26]. In NHANES 2001-2008 were medians of MiBP (3.5-6.4 µg.L^{-1}) [17] lower than in our adults (36.91µg.L^{-1}).

When we compared results for group of Roma children with group of children from general population living in Žilina region, Slovakia (unpublished data), we found significantly higher urinary concentration of MnBP (p=0.0058) and ΣDBP metabolites (p=0.02) in Roma children.

According to our data we assume that there exists other source of exposure (paintings, abrasion of PVC flooring, inhalation solvent) or specific condition in household (burning of plastic waste for heating), which could affect higher DBP exposure in our cohort.

There is a study realized with probands from our geographical area, Slovakia (SK), Czech Republic (CZ) and Hungary (HU) [29]. Data were obtained through the projects COPHES and DEMOCOPHES from 2009-2012. Comparison of participants from general Slovak population with our data is shown in Table IV. There is a difference in urinary concentrations of MEP which was in our study considerably lower. Considerable distinction was found between adults, where the results of mothers from SK, CZ and HU were twofold higher than concentrations of Roma adults. More than twofold higher concentrations of MEP were found also in Egyptian [27] and Swedish study [30]. Interestingly in Norwegian children [5] was GM for MEP (60.9 μg.L⁻¹) threefold higher than in our study. Authors suggest higher MEP concentrations in children of mothers with lower education which is in contrast with our suggestions.

In Chinese study urinary MEP median concentration was in accordance with our data [26].

Černá et al. [29] mentioned significant association between the frequency of using personal care products and higher concentrations of MEP, more strongly in adults. Based on the questionnaire and the low socioeconomic status of Roma population, lower urinary concentrations of MEP in adults were not surprising, because MEP is commonly used in cosmetics, personal care and sun screen products.

TABLE IV
COMPARISON OF URINARY PHTHALATE METABOLITES OF ROMA CHILDREN AND ADULTS (OUR STUDY) AND GENERAL SLOVAK POPULATION [29]

	Romas (SK)		SK	
	children	adults	children	mothers
MEP	**18.17**	**20.83**	**39.64**	**54.81**
	1.5-34.8	-10.9-52.6	33.4-47.1	44.2-68.1
5OH-MEHP	**42.73**	**21.26**	**49.30**	**22.40**
	7.9-77.5	5.8-36.7	43.4-56.0	19.7-25.5
5oxo-MEHP	**23.9**	**9.93**	**33.26**	**14.21**
	1.5-46.3	1.3-18.6	29.2-37.9	12.5-17.2
MEHP	**3.37**	**2.49**	**3.95**	**3.96**
	0.1-6.6	-1.7-6.7	3.4-4.7	3.4-4.7
Σ DEHP	**66.86**	**31.25**	**82.75**	**36.72**
	9.7-124.03	7.18-55.3	77.8-94.0	32.3-41.8

Geometric mean (GM) and 95% confidence interval GM (CI GM) in μg.L⁻¹;
Σ of DEHP metabolites 5oxo-MEHP+5OH-MEHP

Concentrations of DEHP metabolites, 5OH-MEHP (GM 78.6 μg.L⁻¹), 5oxo-MEHP (49.8 μg.L⁻¹) and MEHP (7.8 μg.L⁻¹) [5] were higher in Norwegian than in our Roma children. We found similar results after comparison with GerEs IV pilot biomonitoring (Table V) [31]. Median of MEHP (4.33 μg.L⁻¹) was much lower in our children than in Chinese studies (24.72 [25] and 21.1 μg.L⁻¹ [26]) and in Swedish study (9 μg.L⁻¹) [30].

In study of Moreira et al. [32] was found increased migration of phthalates from plastic material during microwave operation, i.e. with prolonged time of usage plastic container and increasing heating time. We hypothesize lower intake of phthalates from food in plastic containers could be caused by absence of microwave heating in our cohort.

However, urinary concentration of 5oxo-MEHP in our study in group of children (median 21.06 μg.L⁻¹) was consistent with medians of children from Chinese studies (medians 20.56 μg.L⁻¹ [25] and 22.9 μg.L⁻¹ [26]) and also with NHANES 2003-2004 participants (Table V) [14]. Median of each one DEHP metabolite in a cohort of Egyptian research [27] and of MEHP in NHANES 2003-2004 [14] was lower than in our Roma participants what is in contrast with our suggestion.

We consider potential difference in environment and in sources of phthalate exposure in Egypt, USA and Slovakia. There could be also a partial factor of different ethnicity. The highest level-ratio (mean: 58%) from three DEHP metabolites was found in 5OH-MEHP, followed by 5oxo-MEHP (36%) and MEHP (6%). The mean excretion-ratios of MEHP to 5OH-MEHP and MEHP to 5oxo-MEHP were 1:9.7 and 1:5.9. These data are unequal with the excretion characteristic of primary and secondary DEHP metabolites in other studies [33]-[35] where the excretion ratio was for 5OH-MEHP threefold higher and for 5oxo-MEHP twofold higher. We speculate about ethnical specific metabolism of DEHP and ratios relation with this factor. This speculation has yet to be examined in larger samples.

TABLE V
COMPARISON OF OUR RESULTS WITH BIOMONITORING FROM GERMANY [31] AND USA [14] (μg.L⁻¹)

Study	Roma children		GerEs IV pilot		NHANES	
Year	2014		2001-2002		2003-2004	
Age	3-15 y		3-14 y		6-11 y	
	Median	P95	median	P95	Median	P95
MnBP	147.64	402.03	166	624	36.7	191
5OH-MEHP	33.67	244.88	52.1	188	36.5	318
5oxo-MEHP	21.06	165.90	41.4	139	25.8	197
MEHP	4.33	18.59	7.2	29.7	2.7	27.6

Our study has limitations. Single spot-urine measurement of phthalates does not necessarily reflect the whole exposure history of each participant. The small number of participants (n = 29) could distort results. Exposure sources may vary over time based on dietary intake, use of personal care products, and other factors.

V. CONCLUSION

We conclude that ethnicity, lower socioeconomic status and different housing conditions in Roma population can affect urinary concentrations of phthalate metabolites.

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