Biomonitoring of Mono(2-Ethylhexyl) Phthalate and Mono-N-Butyl Phthalate in Roma Children in Slovakia

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Abstract

Background: Di(2-ethylhexyl) phthalate and di-n-butyl phthalate (DnBP) presents two of the most commonly used phthalate plasticizers with a wide use mainly in production of PVC products, food packaging, medical devices, cosmetics and personal care products. Objectives: The aim of study was to assess exposure to phthalates based on the determination of urinary concentrations of mono(2-ethylhexyl) phthalate (MEHP)and monobutyl phthalate (MnBP) as primary metabolites of DEHP and DnBP in Roma children (n= 26; boys=12; girls= 14; average age 9.08 ± 3.57) in condition of Slovak republic. Methods: The urinary concentrations of MEHP and MnBP were determined by high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS). Results: The distribution of the MEHP levels ranged from 4.11 to 177.32 ng.ml-1 and from 56,41 to 628,52 ng.ml-1 in MnBP. The median value reached 14.53 ng.ml-1 in MEHP and 117.06 ng.ml-1 in MnBP. Statistically significant difference between the MEHP excretion and the age of a child (p≤0.0079) within boys' group was found. In addition, in the case of MnBP any statistically significant dependence of the MnBP concentration in the urine on the age of individuals (p≤0.7) was observed. Conclusions: Our results suggest that sociodemographic and habitual behavior and lifestyle could affect concentration of phthalate metabolite in specific type of cohort (Roma population).

Keywords: Biomonitoring. Exposure. MEHP. MnBP. DEHP. DBP. Roma children.

1. Introduction

Phthalates are a kind of synthetic chemicals widely used in the chemical industry. Because of their chemical properties they are used to enhance the flexibility of plastic polymers (e.g. PVC). The more phthalates are added into the material, the greater the flexibility is (Stanley et al. 2003). Since 1930 they have been used for various purposes: they have become an inseparable part of personal care products (perfumes, body lotions, and cosmetics), paints, industrial plastics

and of some medical devices and pharmaceuticals (Latini, 2005). Nowadays they can be found mainly in floor coverings, clothing, detergents, glues, technical lubricants, toys and cosmetics (nail polishes, nail polish removers, aftershaves, hair sprays and the like) (ATSDR, 1995, 2001). They are also used in the production of many other consumer products (for instance, building products, furniture, dentures, modelling clays, lubricants, insecticides and others) (Schettler, 2005). Because they are not tightly bound to polymers via covalent bond, and their molecules are loosely arranged among polymer chains, phthalates are able to migrate (this quality is achieved via their lipophilic character) and next migrate into the air inside buildings, atmosphere, food and other products, environments and materials and thus exposed man (Schettler, 2005; Pilka, Kolena, Petrovičová, 2012;). Although phthalates and their metabolites, unlike stable, persistent organic pollutants as for example organochlorine pesticides (DDT), do not accumulate in the environment, and they are present in living organisms only for a short time, there still presents a constant threat of exposure.

Phthalates are synthetised as esters of 1,2-benzenedicarboxylic acid (phthalic acid) and are appreciated especially for their ability to promote the stability of plastics (Genuis et al. 2012). Physical properties and distribution of phthalate esters depend on the length and branching of the dialkyl or alkyl-aryl side chains (ester alcohols) (Latini, 2005). Phthalates are classified as endocrine disruptors defined as exogenous agents influencing production, release, transport, metabolism, structure, influence and elimination of hormones responsible for the stability of homeostasis in body, reproduction, development and behaviour. This definition concerns rather the heterogeneous groups of molecules – from naturally occurring substances (phytoestrogens) to biochemically generated components, such as plastics, pesticides, industrial solvents, pharmaceutical agents (diethylsilbestrol), and heavy metals (Genuis et al., 2012). There are three ways of exposure to phthalates: inhalation (inspiration – phthalates present in air and in dust particulates – PVC floor coverings, cleaning and polishing products), ingestion (consumption – food and beverages), and dermal contact (absorption via skin – cosmetics, intravenous injection – medical material) (Latini, 2005). Due to *in utero* exposure abnormalities in the development of a male reproductive system can occur (phthalate syndrome). These abnormalities include reduced anogenital distance (AGD), hypospadias, cryptorchidism, malformation of epididymis or of vas deferens, as well as reduced level of the fetal testosterone (Meeker et al., 2009; Koch et al. 2011). According to Pan et al. (2006) exposure to a high dose of DEHP and DnBP in adult males disrupts the biosynthesis of testosterone in testes and also influences the normal regulation of feedback on the hypotalamic-pituitary-testicular axis. Other effects on the human health are the connection to an occurrence of the disease *diabetes mellitus*, influence on the synthesis of thyroid hormones, alteration of adipocyte differentiation (and thus possible impact on the occurrence of obesity), and possible relation to the occurrence of respiratory diseases (Hatch et al., 2008; Boas et al., 2010; Bertelsen et al., 2013).

2. Material and Methods

The biomonitoring of the primary phthalate metabolites in urine (MEHP and MnBP) was realised in the Roma population on November 2012. Cohort consisted of 30 participants at the age 3 to 32 years (12 males and 18 females), Roma children and adolescents (n = 26) and their mother (n = 4) from segregated part of Nemcinany, district Zlate Moravce (Slovakia). The collection of urine samples were carried out in five Roma families differing from each other from the viewpoint of their sociodemographic situation and factors of life-style (financial and habitual behaviour and hygien). Participants were also asked to fill in a questionnaire related to personal and medical records (diagnosed diseases, current health status, smoking) but also monitored the frequency of use of plastics and plastic products, cosmetics and personal care products. The questionnaire also contained an informed consent. The participation in the research was voluntary - the participants could end their participation anytime during the research without stating the reason. The anthropometric data (height, weight, BMI) were obtained via standard anthropological methods. The urine samples were stored in a deep freezer at temperature -73 ºC until analyse. Concentration of MEHP and MnBP was determined by high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS) using the methodology of Pilka et al. (2003). Because of a small number of participants we used nonparametric Mann-Whitney U (Wilcoxon rank-sum) test by programme Statistica 8.0 (Stat Soft. Inc.).

3. Results and Discussion

We evaluated the levels of MEHP and MnBP in the urine of children at the age 3 to 15 years (n=24). The median concentrations of the MnBP was 117.06 ng.ml^{-1} and for MEHP 14.53 ng.ml^{-1}. When gender was taken into account we observed higher concentrations of MnBP in boys (194.47 ±199.87 ng.ml^{-1}), than in girls (158.08 ±106.54 ng.ml^{-1}). In the case of median this difference was not that significant and the median in girls (122.12 ng.ml^{-1}) was higher than in boys (99.42 ng.ml^{-1}). For MEHP the average concentration and the standard deviation in boys (36.44 ± 30.28 ng.ml^{-1}) was also

higher than in girls (31.88 ± 46.69 ng.ml^{-1}). These differences were, however, subtle when compared with the MnBP distribution. On the contrary, notable differences were observed within the median where the values in boys (25.98 ng.ml^{-1}) substantially exceeded the values of median in girls (13.94 ng.ml^{-1}).

We found statistically significant difference between the MEHP excretion and the age of the participant in boys ($p \le 0.0079$). On the other hand, any statistically significant dependence of MnBP concentration in urine on the age of the participants was observed ($p \le 0.7$).

Our research observed people from five different families because to estimated exposure by different habitual behavior and life-style factors.

The first monitored family consisted of five children (2 boys, 3 girls) and their mother. The median of the MEHP concentrations in urine was 91.03 ng.ml^{-1}, and 99.42 ng.ml^{-1} in MnBP. The research participants stated in the questionnaire that they occasionally consume food packed in plastic containers, and their mother also uses decorative cosmetics and make up. In this family, waste by incineration, including products containing PVC was recorded directly in their indoor environment. One boy from this family had the level of MnBP in urine lower than the limit of detection. The level of MEHP of a 10-year-old girl was higher within the whole monitored group (177.32 ng.ml^{-1}) and an 11-year-old girl had as the only one from the observed group BMI value characteristics for malnutrition (first interval of percentiles (0-5)).

The second family provided four urine samples – from 1 boy and 3 girls. The 7-year-old boy had the MEHP and MnBP level lower than LOD. This boy, as well as a girl whose MnBP metabolite level was higher than 300 ng.ml^{-1}, belong to the interval of overweight (the fourth interval of BMI percentile). In the case of MEHP the levels of this metabolite in urine ranged since 14.53 ng.ml^{-1} to 33.15 ng.ml^{-1}. This family stated they eat food packed in plastic containers at least once in a week and also that they use cosmetic products (eau de perfume). The median of MnBP concentrations in girls from this family was 306.58 ng.ml^{-1} and of MEHP 18.79 ng.ml^{-1}.

The third family provided 6 samples – 3 females and 3 males. In this family consume food packed in plastic containers at least once in a week; all of them use toilet water and burn plastic waste. The median of the whole group was for MnBP 89.02 ng.ml^{-1}, which is the lowest median out of the five observed families. In the case of MEHP the median reached the level 23.14 ng.ml^{-1}, which was the second highest median out of all five families.

The fourth family consisted of 4 males and 5 female members. In samples from two men and one woman showed to have the urine concentration of MnBP lower than LOD. The median of the measured MnBP concentrations was 178.53 ng.ml^{-1}; the median of MEHP was 11.91 ng.ml^{-1}. A relatively high concentration of MEHP metabolite was detected in an 8-year-old boy (41.26 ng.ml^{-1}). In this family, they eat food packed in plastic containers only occasionally and that except for shampoo they usually use soap.

Within the fifth family samples from 4 girls and one boy were analysed. Two sisters aged 15 used toilet water. Highest concentration of MnBP from the whole observed group was detected in the 7-year-old boy.

Moreover, this boy had the lowest MEHP concentration (4.11 ng.ml^{-1}) from all participants. The median of MnBP for this family, which stated that they consume food packed in plastic containers twice a week, was 90.22 ng.ml^{-1}. For MEHP the median of this family was the lowest from all five families (8.94 ng.ml^{-1}). Therefore, members of this family showed significantly lower levels of MEHP than other families.
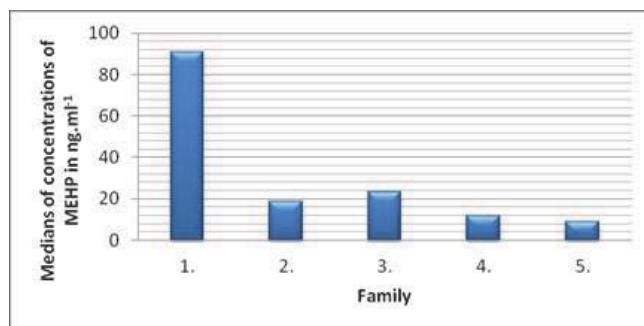
Graf 1: Medians of concentrations of MEHP in every single family
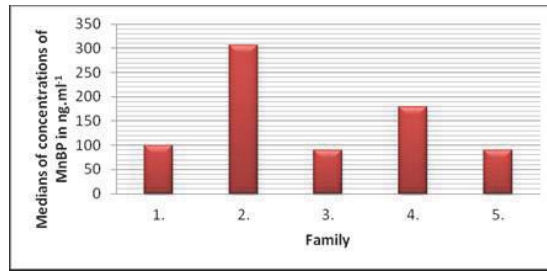
Graf2: Medians of concentrations of MnBP in each family

Based of results we can summarised, that in our research the highest MEHP metabolite levels (from 50.03 to 177.32 ng.ml^{-1}) were detected in the Roma family with children aged 6-11 years. These data are consistent with CDC (2003).

The CDC research brought a fusion of geometric means of demographic groups with regard to the ethnic origin, age and gender. Although a statistically significant dependence of participants' age on the metabolite concentrations was not determined, a statistical analysis of the MEHP metabolite levels points at a higher level of phthalate in urban populations, in groups with low income and in men. The above mentioned facts are thus in accordance with our research for our monitoring was realised in the ethnic group showing a chronically low financial income. Adibi et al. (2010) also notes situation, when women with lower income and lower social status were associated with significantly higher levels of phthalate metabolites (mainly of those with lower molecular weight). In our study we also assume on effect of socioeconomic status which could affected the MnBP exposure in Roma ethnicity.

Within our group one has to take into account the possible sources of exposure, as for instance a presence of floor coverings containing PVC in the participants' households, or plastic waste incineration provided in the questionnaire. These facts might have greatly affected the concentrations of phthalate metabolites in our group.

The medians concentrations of the MEHP and MnBP in the urine samples analysed in our study were compared to several studies realised in other countries. In general it can be observed that metabolite levels were higher in participants of our research than in participants from other studies, with certain exceptions (Tab.1). We presume that these differences might have been caused by the already mentioned sources of exposure, or by the lower social status of the families.

Study	Country	Age	n	MEHP in ng.ml⁻¹	MnBP in ng.ml⁻¹
Our research (2014)	Slovakia	3-15 y.	24	14.53	117.06
Becker et al., 2004	Germany	6-7 y.	50	6.91	X
Koch et al., 2011	Germany	5.6-6.7 y.	11	4.7	36.8
Kim et al., 2014	Korea	9-12 y.	39	13.2	X
Boas et al., 2010	Denmark	4-9 y.	342 ♀	3.6	121
			503 ♂	4.5	130
Wang et al., 2013	China	8-11 y.	144	X	40.7
Colacino et al., 2011	Egypt	10-13 y.	29 ♀	X	47.5
GerEs IV (pilot) (Becker et al., 2004)	Germany	3-14 y.	254	7.2	166
NHANES 1999-2000	USA	6-11 y.	328	4.9	41.4 (GM)
NHANES 2001-2002	USA	6-11 y.	393	4.4	31.1 (GM)

Tab. 1: Comparison of the MEHP and MnBP concentrations´ medians in the group monitored in our research with medians from other studies

4. Conclusion

This paper brings new informations about biological monitoring of mono(2-ethylhexyl) phthalate (MEHP) and of mono-*n*-butyl phthalate (MnBP) in Roma children, based on analysing their urine samples by using HPLC and MS/MS methods. It is pilot study where comparison concentrations of MEHP and MnBP in Roma children from the viewpoint of their age, gender and social environment were realised in condition of Slovak republic.

Our results indicate that sociodemographic status and also habitual behavior play important role for phthalate exposure in cohort of Roma children from Slovakia. In future higher incidence of some health outcomes resulting from phthalates exposure are possible in this population.

In the future, greater number of participants is necessary to verify our result.

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