Biomonitoring of di-(2-ethylhexyl) phthalate (DEHP) exposure in human

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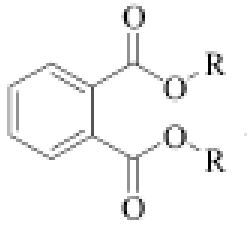
HUMAN BIOMONITORING

- ✓ In industrialized societies, humans are exposed to a wide spectrum of man-made chemicals
- ✓ Human biomonitoring includes monitoring of chemicals, their metabolites or specific reaction products in *blood, urine, faeces, hair, saliva, breast milk* or *human adipose tissue* => assessing environmental exposure (diseases and disorders of bodily functions)

PHTHALATES



- ✓ Alkyl diesters of phthalic acid
- ✓ Application

  Polymeric => plasticizers (DEHP, DiNP)

  Non-polymeric => fixatives, detergents, lubricating oils, and solvents
- ✓ Not bounded on polymeric chains => easily released (direct release, migration, evaporation, leaching, abrasion)

PHTHALATES


children toys
medical devices
foodstuff
CONSUMER
automotive products
cosmetics
building, household

HEALTH OUTCOMES

Reproductive system

- Developmental anomalies (cryptorchydism, premature thelarche, decreased AGD...) *(Chou et al., 2009; Swan et al. ,2005)*
- Endocrine disruptors (antiandrogenic effect, semen quality...) *(Duty et al. 2003 a, b; Hauser et al., 2007)*

Respiratory system

- Rhinitis, wheezing, higher risk of asthma, obstructive disorders of airways *(Hoppin et al., 2004)*

Thyroid

- Altered thyroid hormone males levels (decreased T3,T4) *(Mekker et al., 2007*

Metabolic

- Increased waist circumference *(Stahlhnut et al.,2007)*

Behaviour

- Children's after prenatal exposure (ADHD, sex dependent behaviour ♂) *(Engel et al.,2010)*

Di-(2-ethylhexyl) phthalate DEHP

- ✓ most substantial long chain phthalate in the environment (1-4 t per year)
- ✓ the use -clothing, toys, food containers, medical devices, building, household, automotive products
- ✓ human exposure

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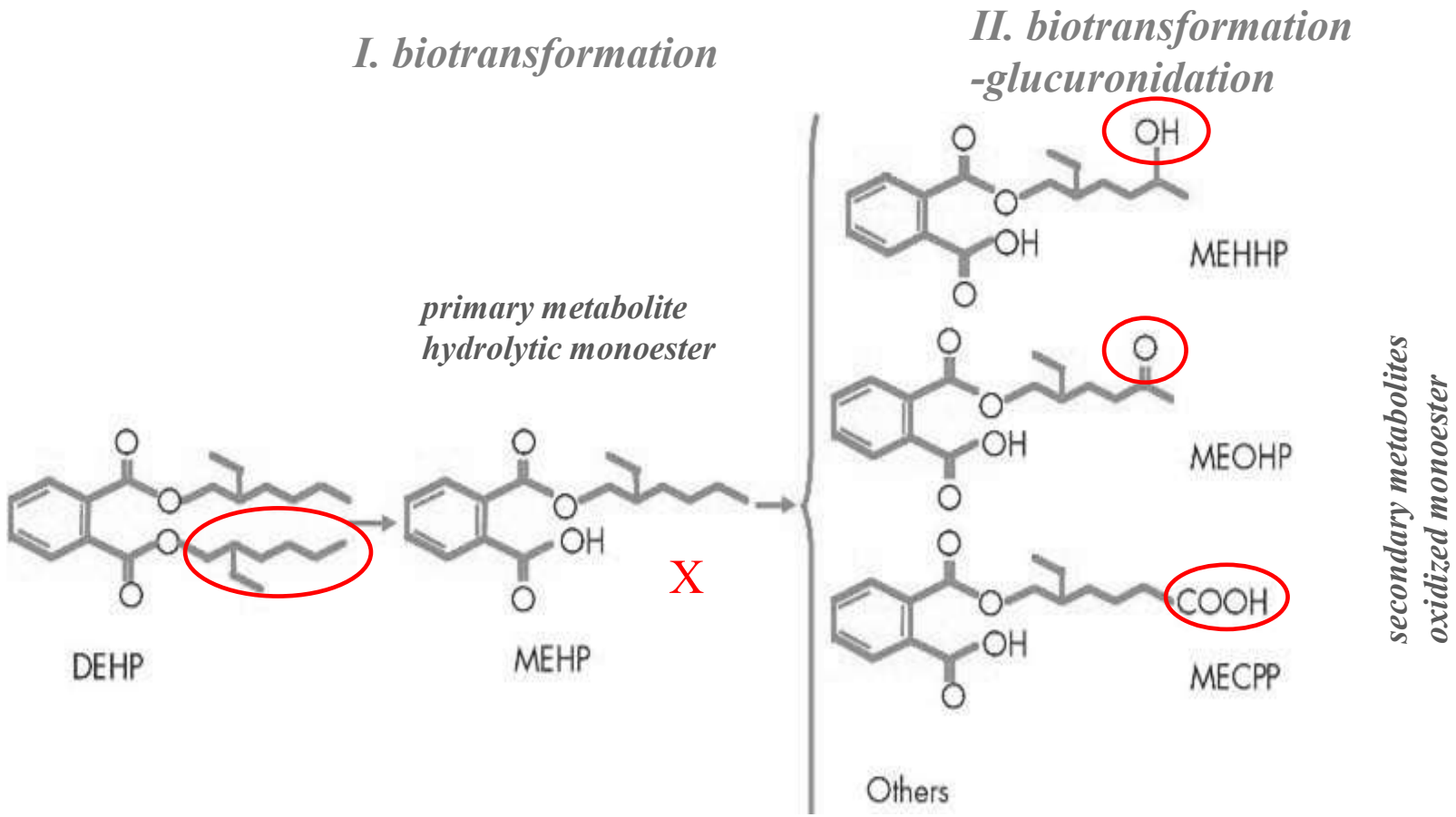
HUMAN EXPOSURE DEHP

Di-(2-ethylhexyl) phthalate DEHP

- ✓ most substantial long chain phthalate in the environment (1-4 t per year)
- ✓ the use -clothing, toys, food containers, medical devices, building, household, automotive products
- ✓ major source of exposure foodstuff
- ✓ metabolic pathway

METABOLIC PATHWAY DEHP



Primary and secondary phthalate metabolites used as reference standards in biological monitoring studies to quantify external exposure to the respective parent phthalates

(Hauser and Calafat , 2005;)

DEHP- HEALTH EFFECT

- ✓ reproductive and developmental toxicant in animals
  - hypospadias, cryptorchidism
  - decreased AGD, mating, pregnancy, fertility...

- ✓ human endocrine disruptor
  - reduction in sperm motility and chromatin damage, testosterone levels, AGD
  - disruption of foetal germ cell and Leydig cell development
  - potential to alter androgen-responsive brain development

Kavlock et al., 2002; Akingbemi et al., 2004; Borch et al., 2005; Foster, 2006; Hauser et al. 2000; Lambrot et al. 2009; Desdoits-Lethimonier et al., 2012; Swan et al., 2005; Marsee et al., 2006; Mendiola et al., 2012)

HUMAN EXPOSURE DEHP- TDI

We converted those excretion values of the phthalate metabolites to total daily intake (TDI) values for the parent phthalate applying the equation according to Koch et al. (2003).

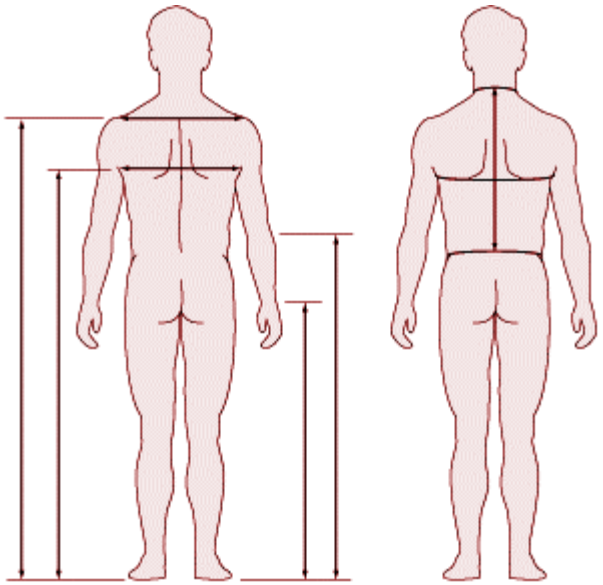
$$\text{Total daily intake } (\mu g/kg/day) = \frac{ME(\mu g/g) \text{ x } CE \text{ } (mg/kg/day)}{Fue \text{ x } 1{,}000 \text{ } (mg/g)} \text{ x } \frac{MWd}{MWm}$$

ME- urinary concentration of monoester per gram creatinine
CE- creatinine excretion rate normalized by body weight
Fue- molar fraction of the urinary excreted monoester related to parent diesters
MWd- molecular weight of phthalate diesters
MWm- molecular weight of phthalate monesters

MATERIAL AND METHODS

Anthropometry

- ✓ Body-mass index BMI
- ✓ Waist-to-height ratio WHTR
- ✓ Waist to hip ratio WHR
- ✓ Fat mass index FMI



Sampling

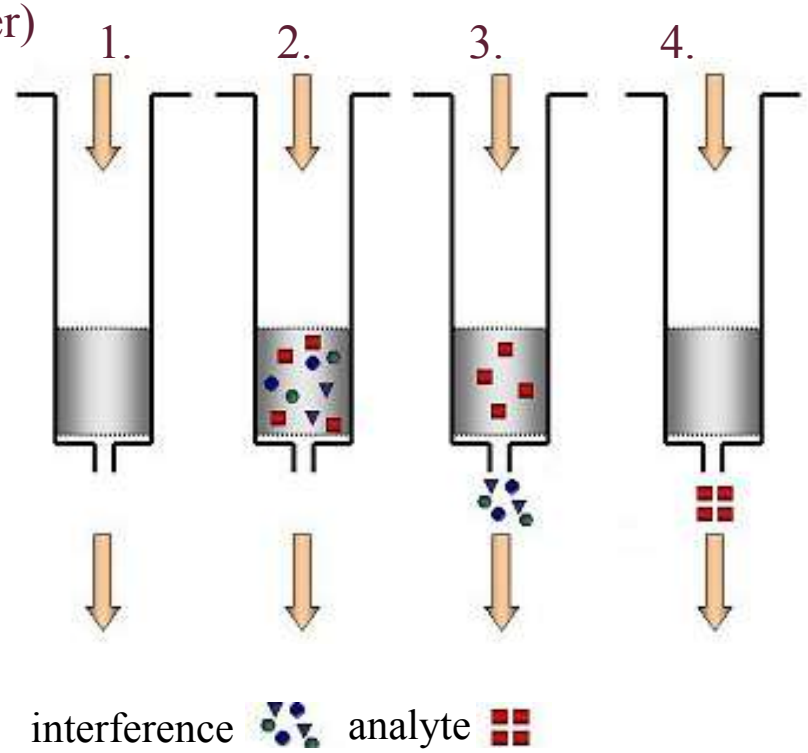
- ✓ Urine collection- 4 ml per proband
- ✓ Storage -73°C

Extensive questionnaire

MATERIAL AND METHODS

Urine analysis- sample preparation

- ✓ Deglucuronidation (β-glucuronidase; E.Coli, K12)
- ✓ Solid phase extraction (ABS Elut Nexus, Agilent)
  1. Conditioning (ACN/phosphate buffer)
  2. Sample adition
  3. Rinsing (formic acid, water)
  4. Elution (ACN/EtoAc)
- ✓ Evaporation and reconstitution



MATERIAL AND METHODS

HPLC-MS/MS

- ✓ High performance liquid chromatography (HPLC)
  - ZORBAX Eclipse plus phenyl-hexyl column
- ✓ Tandem mass spectrometry (MS/MS Agilent 6410 triplequad)

Table 1. Phthalate monoesters: chromatographic and mass spectrometric parameters

Compound Name	Precursor Ion	Product Ion	Fragmentor (V)	Collision Energy (V)	RT (min)	LOD, $ng.ml^{-1}$
MEHP-C4	281.1	137.1	90	14	14.7	
MEHP	277.1	133.9	90	14	14.7	0.81

RESULTS

- ✓Cohort consist 108 adults - age range 19-69 years
- ✓general population from Nitra

Table 2. Baseline anthropometric characteristic of study subjects by gender

	Mean ± SD		-95%		+95%	
	Female (n=66)	Male (n=42)	Female (n=66)	Male (n=42)	Female (n=66)	Male (n=42)
BMI	24.5 ± 5.05	26.26 ± 3.36	23.26	25.21	25.74	27.30
FMI	8.65 ± 3.74	5.99 ± 2.21	7.73	5.30	9.57	6.68
WHTR	0.5 ± 0.09	0.52 ± 0.06	0.48	0.50	0.53	0.54
WHR	0.83 ± 0.09	0.92 ± 0.08	0.80	0.90	0.85	0.95

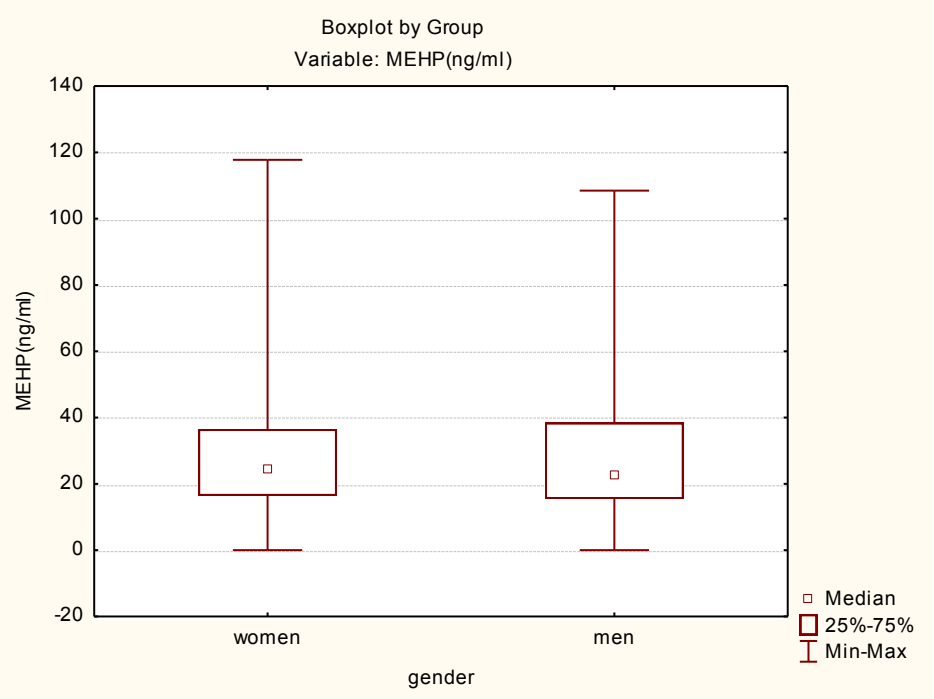
RESULTS

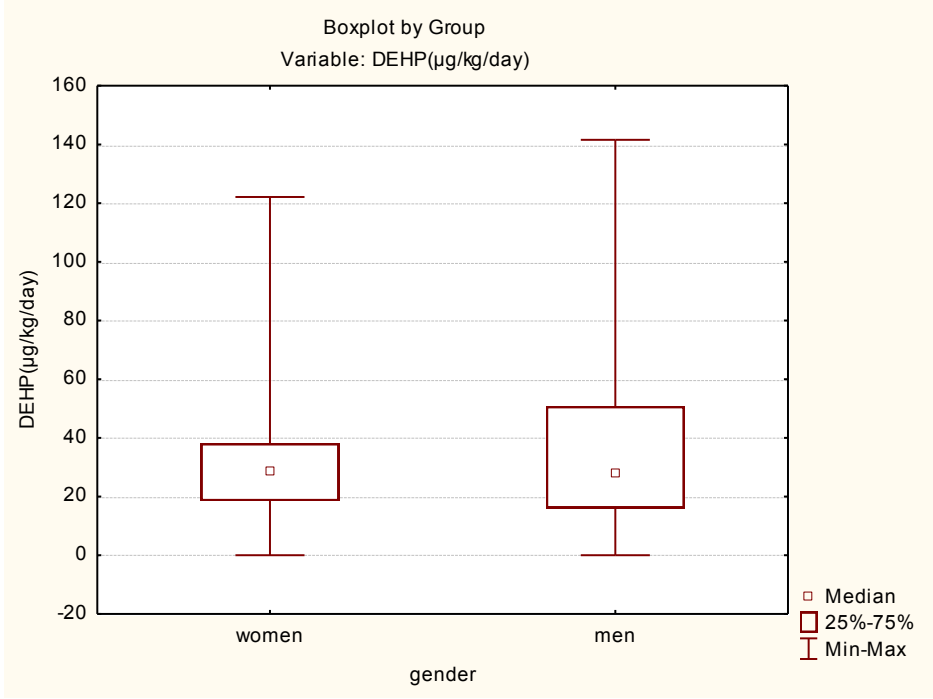
Table 4. Presence of urine metabolite in subjects and exceeded daily phthalate intake levels established by EFSA (2005) and US EPA

	Presence				
	MEHP	DEHP			
		TDI (EFSA)		RfD (US EPA)	
	n (%)	>50 µg/kg/day n (%)	Mean ± SD µg/kg/day	>20 µg/kg/day n (%)	Mean ± SD µg/kg/day
All n=108	100 (92.59)	18 (16.67)	75.55 ± 25.54	77 (71.30)	42.11 ± 23.43
Female n=66	60 (90.91)	7 (10.61)	77.4 ± 23.95	51 (85.00)	38.65 ± 19.52
Male n=42	40 (95.24)	11 (26.19)	74.38 ± 26.43	26 (61.90)	48.90 ± 28.43

RESULTS

Distribution of phthalate metabolite concentrations in subjects by gender (µg.L^{-1}) and estimated total daily intake TDI (µg/kg/day) based on (Koch et al. 2003)





RESULTS

Table 5. Comparison between excretion of the MEHP in the female (n=66) and male (n=42) set of our study

	Mean ± SD		Median		Min		Max		*P* value
	♀	♂	♀	♂	♀	♂	♀	♂	
MEHP ($\mu g.L^{-1}$)	28.51 ± 21.55	29.52 ± 20.74	25.82	21.73	4.54	7.4	117.77	108.46	0.8156

Mann-Whitney *U* tests (Wilcoxon rank-sum) confirmed that the two data sets were not significantly different ($P = 0.816$ for MEHP)

CONCLUSION

- ✓ general population from Nitra is exposed to DEHP to a higher extent in comparison with populations in other similar studies
- ✓ This is of great importance for public health since DEHP was not only the most important and ubiquitous phthalate in Europe over the last years, but also the phthalate with the greatest endocrine disrupting potency.

Limitations:

- single spot-urine measurements of MEHP that not reflect long-term exposure
- estimation of creatinine excretion by semiquantitative method that could affect the estimated values of TDI

Acknowledgments

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Thank you for your attention
Grazie per l'attenzione