Phthalate and DINCH metabolites in HBM4EU urine samples from teenagers and young adults in Czechia

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Introduction

- Phthalates - ubiquitous, high production volume industrial compounds, used as plasticizers and solvents.
- Low molecular weight (LMW) phthalates (shorter chains) used as solvents in personal care products (PCPs), children's toys, food packaging materials.
- High molecular weight (HMW) phthalates mainly used as plasticizers in polyvinylchloride (PVC).
- Phthalates are not bonded to products → can migrate into environment, enter human bodies or other organisms.
- Phthalates are considered endocrine disruptors → some phthalates even legislatively restricted, therefore alternatives are needed – e.g. DINCH, used to substitute HMW phthalates (DEHP and DiNP mainly).
- Human exposure to phthalates is assessed through urinary metabolites [1].
- Nearly all the European population is highly exposed to phthalates [2], [3].
- Phthalate and DINCH metabolites were assessed in two Czech cohorts (Teenagers cohort - TAC and Young adult cohort - YAC), under the framework of the HBM4EU project.

Methods

- Participants from the South Moravian region, Czechia completed questionnaires and supplied urine samples
- TAC = 300 urine samples, children (11 to 17 years old)
- YAC = 300 urine samples, young adults (18 to 37 years old)
- Urine samples analysed for both creatinine (CR and specific gravity (SG), SG correction was used for data analysis.
- Samples thawed, homogenized, 500 µL pipetted into 96-well plate, 500 µL of mixture with β-glucuronidase and isotopically labelled standards in acetate buffer added.
- Samples incubated (120 min, 55 °C), 50 µL of 1% acetic acid added.
- Samples precleaned using 96-well plate SPE (Oasis HLB; 3 mL, 60 mg), concentrated, redissolved in 500 µL of 50% methanol.
- HPLC conditions – Agilent 1290 Infinity II HPLC system, Acquity UPLC BEH C18 (100 x 2,1 mm; 1,7 µm), mobile phase A: 0,1mM ammonium fluoride in Milli-Q water, mobile phase B: 0,1mM ammonium fluoride in methanol.
- MS detection on Agilent QQQ6495, electrospray ionisation, negative mode.
- Isotopic dilution method used for quantification.
- YAC data divided into 2 seasons – season 1 (S1) from October to April, season 2 (S2) from May to September.
- TAC collected from October to December, not seasonally divided.
- We evaluated relationship between phthalate metabolites and determinants of exposure, including on seasonality, gender, time spent indoors, home renovations/redecorations in last two years, and PCP usage.

Results and discussion

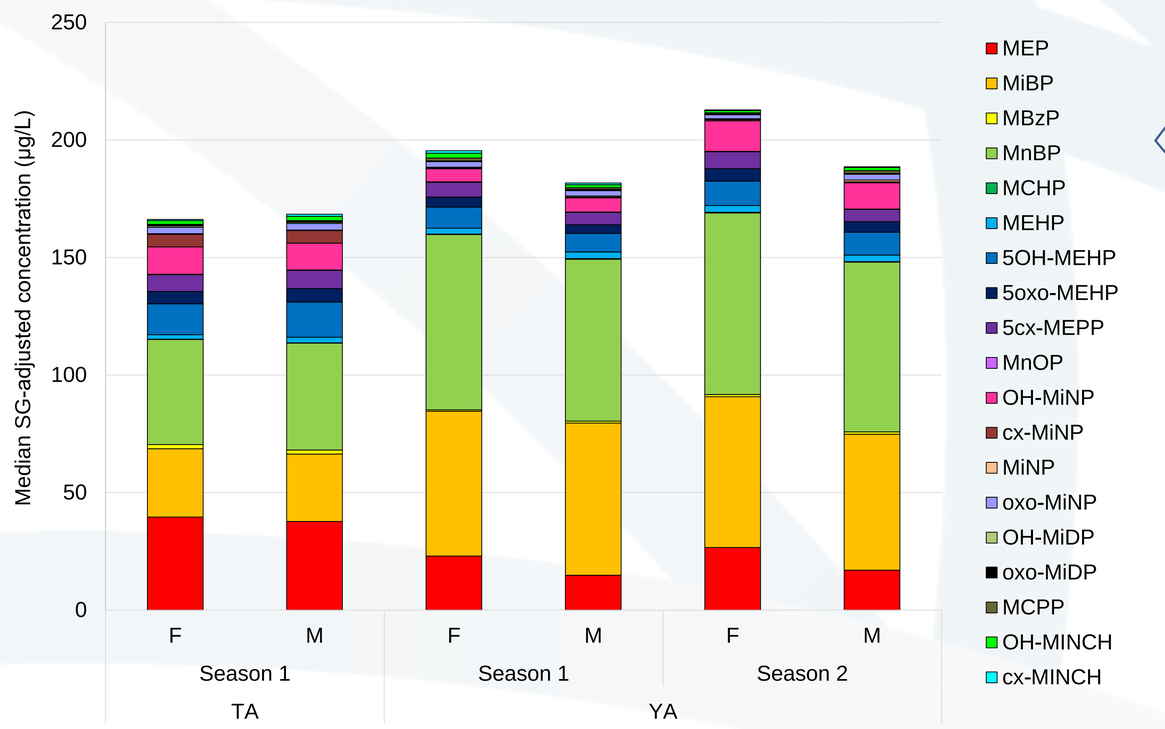


Figure 1 Median concentration of phthalate metabolites in both cohorts

Seasonality differences:

- Phthalates in outdoor air generally higher in warmer months (higher primary and secondary emissions → higher environmental concentrations → greater human exposure → higher urinary concentration).
- 5OH-MEHP, OH-MiNP and cx-MiNP – significantly higher concentrations in urine in S2.
- Only cx-MINCH significantly higher in S1 – potential difference between phthalates and their replacements.

Cohort differences:

- MiBP and MnBP higher in YAC
- MEP, MBzP and secondary DEHP metabolites (5OH-MEHP, 5oxo-MEHP, 5cx-MEPP) higher in TAC.

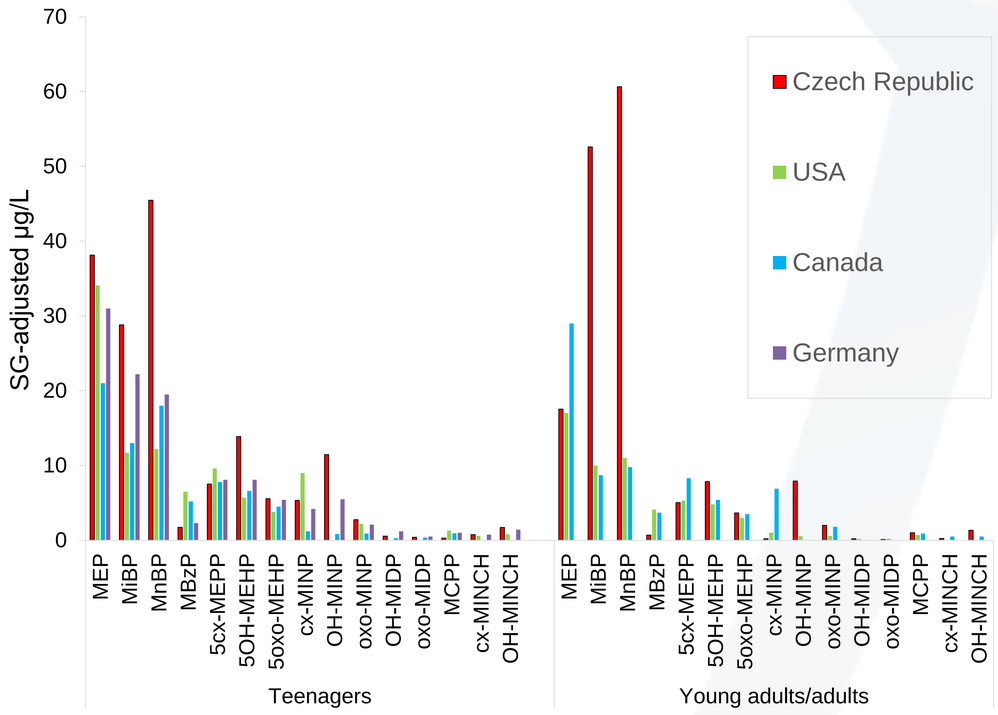
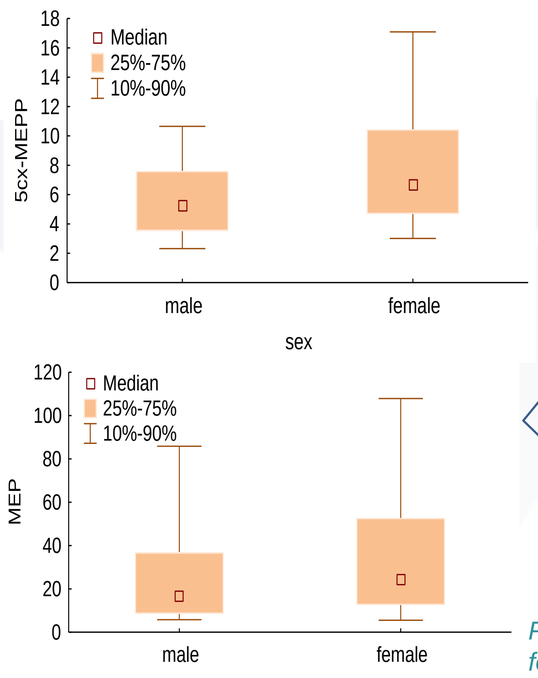


Figure 2 Comparison of urinary concentrations of phthalate metabolites in TAC and YAC from Czechia, Germany, Canada and USA

Comparison with other studies:

- MiBP and MnBP 5-6 times higher in Czechia (data from Europe in general higher than in North America).
- MBzP 5-6 times lower in Czechia.
- Differences attributed to combined effects of lifestyle, dietary exposure or chemical use patterns.



Gender differences:

- MEP higher in females in cold season (higher usage of PCPs)
- 5oxo-MEHP, 5cx-MEPP and cx-MiNP higher in females in warm season (longer time spent indoorsthan males, more house-cleaning, but also higher phthalate concentrations in outdoor air in warm season in general)
- OH-MINCH higher in females in cold season.

Figure 4 Gender differences in YAC for MEP and 5cx-MEPP

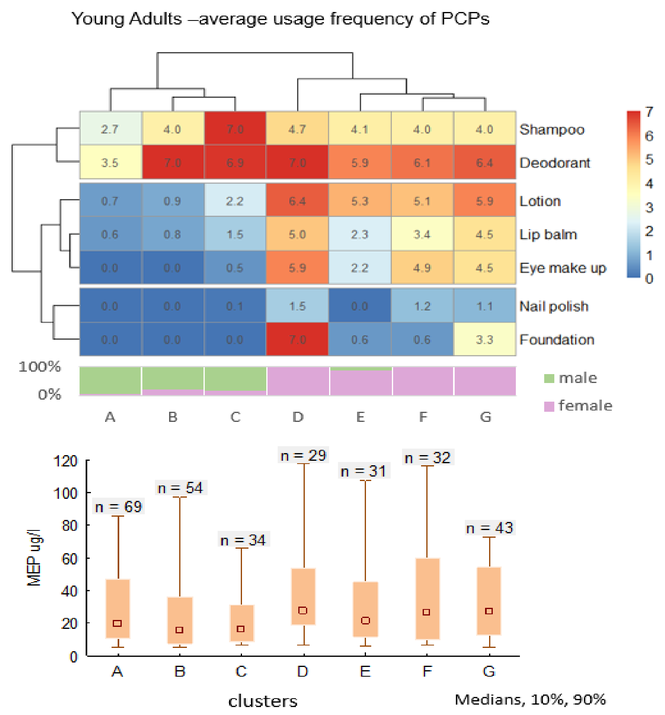


Figure 3 Cluster analysis - average frequency of PCP usage in YAC

Cluster analysis of YAC:

- Less PCPs are used mainly by males (usually only shampoo and deodorant) – left side of figure 3.
- More PCPs in higher frequency used mainly in females – right side of the figure 3.
- MEP increases with the amount and frequency of PCP usage, therefore it is higher in females.

Conclusions

We found seasonal differences between phthalate exposure in cold and warm months (5OH-MEHP, OH-MiNP and cx-MiNP higher in warm season; cx-MINCH higher in cold season), differences between both cohorts (MiBP and MnBP higher in YAC, MEP, MBzP and secondary DEHP metabolites higher in TAC) and gender differences (MEP, 5oxo-MEHP, 5cx-MEPP, cx-MiNP and OH-MINCH higher in females). There is a large variability in phthalate metabolites in human urine, due to different lifestyle, dietary composition, time spent indoors, cleaning frequency and many other factors. Czech levels of phthalate metabolites are generally similar to those measured in Germany, Canada and USA, except for MnBP and MiBP, which are higher in Czechia, and MBzP, which is lower in Czechia.

Sources

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Abbreviations

Dimethyl phthalate - DMP, monomethyl phthalate – MMP, diethyl phthalate - DEP, monoethyl phthalate – MEP, diisobutyl phthalate – DiBP, monoisobutyl phthalate – MiBP, butyl benzyl phthalate – BBzP, monobenzyl phthalate – MBzP, di-*n*-butyl phthalate – DnBP, mono-*n*-butyl phthalate – MnBP, dicyclohexyl phthalate – DCHP, monocyclohexyl phthalate – MCHP, di(2-ethylhexyl) phthalate – DEHP, mono(2-ethylhexyl) phthalate – MEHP, mono(2-ethyl-5-hydroxy-hexyl) phthalate - 5OH-MEHP, mono(2-ethyl-5-oxo-hexyl) phthalate - 5oxo-MEHP, mono(2-ethyl-5-carboxy-pentyl) phthalate - 5cx-MEPP, di-*n*-octyl phthalate – DnOP, mono-*n*-octyl phthalate – MnOP, diisononyl phthalate – DiNP, monoisononyl phthalate - MiNP, 7-hydroxy-(monomethyl-octyl) phthalate - OH-MiNP, 7-oxo-(monomethyl-octyl) phthalate - oxo-MiNP, 7-carboxy-(monomethyl-heptyl) phthalate - cx-MiNP, diisodecyl phthalate – DiDP, bis(2-propylheptyl) phthalate – DPHP, 6-hydroxy-(monopropyl-heptyl) phthalate - OH-MiDP, 6-oxo-(monopropylheptyl) phthalate - oxo-MiDP, mono-3-carboxypropyl phthalate – MCPP; 1,2-cyclohexanedicarboxylic acid diisononyl ester- DINCH, cyclohexane-1,2-dicarboxylic acid-mono(hydroxyl-isononyl) ester - OH-MINCH, cyclohexane-1,2-dicarboxylic acid-mono(carboxy-isooctyl) ester - cx-MINCH.

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