
# BIOMONITORING OF URINARY PHTHALATE BIOMARKERS IN LACTATING WOMEN

**Pablo Dualde [A B C], Nuria Leon [B], Olga Pardo [A,B], Máximo Vento [D], Agustín Pastor [C], Vicent Yusà [A, B, C*]**

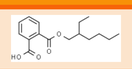
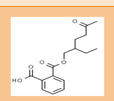
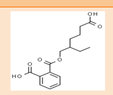
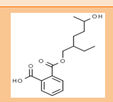
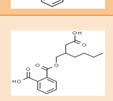
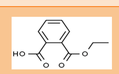
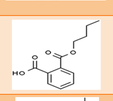
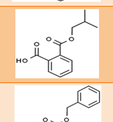
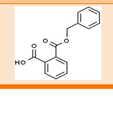
[A] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, 21, Avenida Catalunya, 46020, Valencia, Spain
[B] Public Health Laboratory of Valencia, 21, Avenida Catalunya, 46020 Valencia, Spain
[C] Analytical Chemistry Department, University of Valencia, Edifici Jeroni Muñoz, Dr. Moliner 50, 46100 Burjassot, Spain
[D] Neonatal Division at the University and Polytechnic Hospital La Fe, Avenida Fernando Abril Martorell, 106, 46026 Valencia, Spain

## Indroduction

Phthalates are high volume produced chemicals used as plasticisers in a wide range of applications [1]. Phthalates are frequently released into the environment by leaching and migration from the products to the air, food, water and dust. Therefore, general population is continuously exposed to phthalates through ingestion, inhalation or dermal absorption [2]. There is a high concern about exposure to phthalates since they are suspected endocrine disruptors for humans [3]. After entering in the human body, phthalates undergo metabolism to monoesters. A high percentage of the absorbed dose is excreted in urine during the first 24h as free or conjugated metabolites [1,4]. The purpose of the present study was to assess the urinary levels of phthalate metabolites in lactating women.

## Methodology

### A. Metabolites studied

| Phthalate (Acronym) | Metabolite (Acronym) | Structure |
|---|---|---|
| Di-2-ethylhexyl Phthalate (DEHP) | Mono-2-ethylhexyl phthalate (MEHP) |  |
| | Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) |  |
| | Mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP) |  |
| | Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) |  |
| | Mono[2-(carboxymethyl)hexyl] phthalate (2cx-MMHP) |  |
| Diethyl Phthalate (DEP) | Mono-ethyl phthalate (MEP) |  |
| Di-n-butyl phthalate (DBP) | Mono-n-butyl phthalate (MnBP) |  |
| Di-isobutyl phthalate (DiBP) | Mono-isobutyl phthalate (MiBP) |  |
| Benzylbutyl Phthalate (BzBP) | Mono-benzyl phthalate (MBzP) |  |

### B. Study design

- Lactating women (n=104)
- Urine samples
- Collected between 2 and 8 weeks after birth.
- All the participants signed an informed consent.

### C. Chemical analysis

**Sample treatment:**
- Enzimatic deconjugation
- Dilute & Shoot

**HPLC-MS/MS (QqQ) conditions**:
- Luna Omega (1.6 µm) Polar C18 (50 x 2.1mm) Phenomenex
- Flow rate:400 $\mu L \cdot min^{-1}$.
- Mobile phase A: HAc 0.1% in water .
- Mobile phase B MeOH:ACN (90:10) 0.1% HAc.
- Ionization mode: ESI neg.

### D. Risk assessment

$$\text{Hazard Quotient (HQ)} = \frac{\text{P95}}{\text{Biomonitoring equivalent (BE)}}$$

- DEHP was related as the sum of 5 metabolites (MEHP, MEHHP, MEOHP, MECPP and 2cx-MMHP)
- DnBP was calculated as MnBP levels
- DEP was calculated as MEP levels
- BzBP was calculated as MBzP levels

## Results

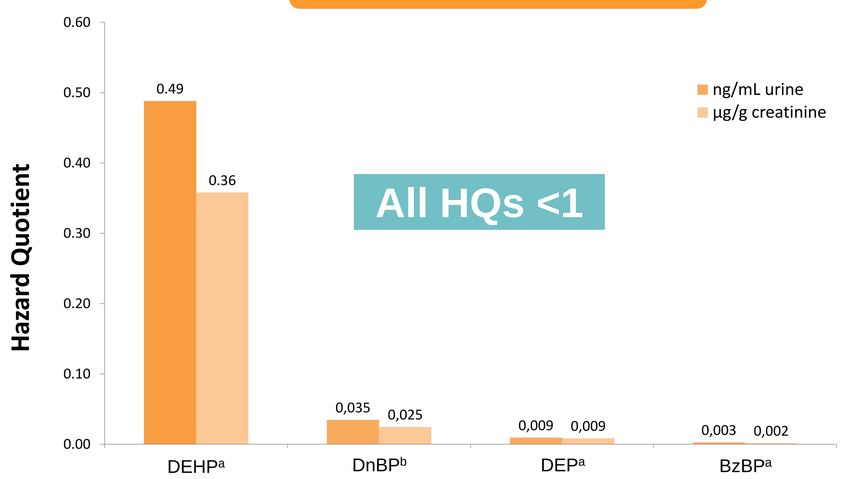
### A. Phthalate metabolite levels in urine

| Biomarkers | LoQ (ng/mL) | DF(%) | Arithmetic mean (ng/mL) | Geometric mean (ng/mL) | P50 (ng/mL) | P95 (ng/mL) |
|---|---|---|---|---|---|---|
| **MEHP** | 2 | 91.3 | 6.54 | 3.13 | 3.08 | 23.34 |
| **MEOHP** | 0.5 | 99 | 13.03 | 6.61 | 6.13 | 36.53 |
| **MECPP** | 1 | 100 | 30.73 | 15.52 | 14.40 | 93.82 |
| **MEHHP** | 2 | 96.2 | 17.00 | 8.86 | 8.50 | 42.96 |
| **2cx-MMHP** | 2 | 80.8 | 8.56 | 3.82 | 3.39 | 17.83 |
| **MEP** | 2 | 100 | 68.08 | 34.90 | 32.60 | 170.06 |
| **MnBP** | 0.5 | 99 | 17.47 | 11.34 | 12.21 | 48.66 |
| **MiBP** | 2 | 99 | 21.28 | 13.78 | 12.49 | 80.64 |
| **MBzP** | 1 | 85.6 | 3.20 | 2.19 | 2.20 | 10.25 |

Limit of quantification (LoQ); Detection frequency (DF); Arithmetic mean (AM); Geometric mean (GM); 50th percentile (P50); 95th percentile level (P95)

Analytes with detection frequencies <5%: Mono-isononyl phthalate, mono-cyclohexyl phthalate, mono-(3-carboxypropyl) phthalate, mono-n-octyl phthalate, and mono-methyl phthalate.

### B. Risk assessment




[a] BE derived from US EPA RfD; [b] BE derived from Health Canada TDI

## Conclusions

- ✓ MEP was the metabolite which presented the highest levels in urine.
- ✓ All HQ were lower than 1, therefore, the studied population exposure to phthalates is below the safety levels established in the literature.

## References

[1] Frederiksen et al., 2007. Metabolism of phthalates in humans. Mol. Nutr. Food Res. 51, 899 – 911.
[2] Weiss et al. 2018. Daily intake of phthalates, MEHP, and DINCH by ingestion and inhalation. Chemosphere 208, 40-49.
[3] Heudorf et al. 2007. Phthalates: Toxicology and exposure. Int. J. Hyg. Environ. Health 210, 623–634.
[4] Wittassek et al. 2011. Assessing exposure to phthalates – The human biomonitoring approach. Mol. Nutr. Food Res. 55, 7–31.