Use of stored serum in the study of time trends and geographical differences in exposure of pregnant women to phthalates

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EDMaRC

Endocrine Disruption of Male Reproduction and Child Health

Key findings

- Serum phthalate metabolites may be used to assess prenatal exposure to some, but not all, phthalates if interpreted with caution.
- Phthalate exposure varies between countries and over time. European regulations appear to have resulted in decreasing exposure from the early 1990s to the 2010s in Denmark.

Background

Prenatal exposure to phthalates may have **adverse health effects** later in life. In the EU, the first temporary **restrictions on phthalates** were enacted in December 1999. These involved DEHP, BBzP, DnBP as well as the use of DiNP, DiDP, DNOP specifically in toys and child care articles.

Historical birth cohorts with adult offspring often only have maternal serum samples to asses prenatal exposure. There are **two main challenges** with measurements in serum compared to urine:

- Lower phthalate metabolite levels due to fast metabolism *in vivo*
- Risk of post-collection contamination

We wished to explore:

A. Whether prenatal maternal serum samples can be used to assess phthalate exposure.
B. Differences in phthalate exposure across three birth cohorts from different geographical regions and times.

Methods

Population: Pregnant women in three birth cohorts

The Raine cohort	**Cop1**	**Cop2**
1989-91	1997-2001	2012-14
n = 986, GA = 18/34	*n* = 235, GA = 23	*n* = 103, GA = 18

Outcomes: **32 phthalate metabolites** from 15 phthalate diesters measured by isotope-diluted liquid chromatography-tandem mass spectrometry (LC-MS/MS) in

- **Serum samples** from Raine and Cop1
- **Urine and serum samples** from Cop2

Statistics:

- Correlations were tested by Spearman's test
- Differences between the cohorts were tested by Mann-Whitney U test

Results & Discussion

A

Samples are at risk of being **contaminated** with phthalate diesters from the environment during sampling, handling and storage. Unlike urine, **serum contains esterases** that can catalyze the conversion of diesters to monoesters. Oxidation of diesters can only occur *in vivo*.

If samples have been contaminated, levels of primary diesters will generally be high and metabolite levels of primary and secondary metabolites will not correlate.

In our three cohorts, we found:

- Generally large inter-individual variation in serum phthalate levels.
- Significant correlations between many primary and secondary metabolites in serum (r = 0.153-0.710, $P < 0.01$).

Further, we found significant correlations between some metabolite levels in serum and urine samples (Table 1).

Overall, these findings support that serum levels of some phthalate metabolites originate from true phthalate exposure.

Table 1 | Correlations between selected serum and urine phthalate metabolite levels (ng/mL)

		Urine levels						
		MEP	MEHP	MECPP	MCMHP	MCPP	MiNP	MCiOP
Serum levels	MEP	0.580***	0.080	0.031	0.032	0.039	-0.041	-0.034
	MEHP	.221*	0.151	.218*	0.160	0.034	-0.001	0.064
	MECPP	.231*	.343***	.360***	.359**	.208*	0.108	0.185
	MCMHP	0.118	0.159	0.173	.268*	0.108	-0.060	0.089
	MCPP	.231*	.217*	0.181	0.167	.302**	.246*	0.114
	MiNP	0.028	0.032	0.096	0.142	-0.008	0.010	0.106
	MCiOP	0.060	0.083	0.089	.222*	.230*	.298**	.380***

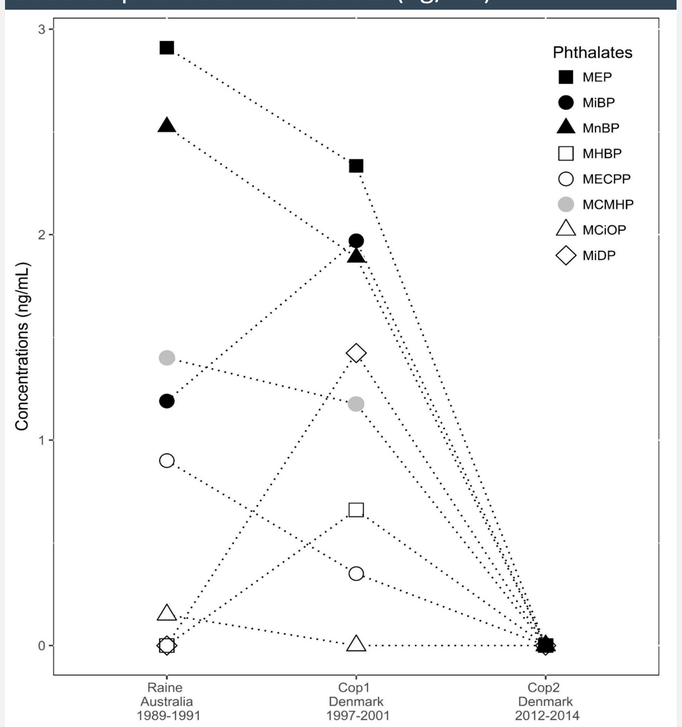
*Significant correlations: *P < 0.05, **P < 0.01, ***P < 0.001.*

B

Generally, serum phthalate metabolite concentrations and detection rates were higher in the Australian cohort compared to the two Danish cohorts. Levels in the older Danish cohort were overall higher than in the recent one which is congruent with regulations on phthalate-use in the EU since 1999.

- In Cop2, all phthalates were significantly lower than in the Raine cohort ($P < 0.05$) and, apart from MCiOP, also than in Cop1 ($P < 0.001$).
- In the Raine cohort, levels of MECPP, MCPP and MCiOP ($P < 0.001$) as well as MCMHP ($P < 0.01$) were significantly higher than in Cop1.
- Levels of MiBP, MiDP and MHBP were significantly higher ($P < 0.001$) in Cop1 than in the Raine cohort.

Figure 1 | Median maternal serum concentrations of selected phthalate metabolites (ng/mL)



Conclusion

- Historical biobank serum samples may be used for assessment of exposure to some, but not all, phthalates if interpreted with caution. Our data support that especially primary metabolites should be cautiously interpreted.
- Geographical regions seem to differ in phthalate exposure patterns, and European regulations appear to have resulted in a decreasing exposure from the early 1990s to the 2010s in Denmark. National/local production and consumer patterns also seem to affect exposure.

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Fetal, neonatal endocrinology and metabolism (to include hypoglycaemia)

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