Screening of pollutants in human matrices – urine (and some more)

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Strategies

- Knowledge about use and emissions from products
- Literature studies and the “apple strategy”
- Targeted high-through put methods with detection of many compounds-”exponomics”
- Use of biobanks
- Untargeted methods- “metabolomics” and “adductomics”

High trough-put methods

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LC/LC/MS/MS

LC/MS/MS

- Possibility to analyse many compounds in one run
- E.g. proteomics in nasal lavage
- Analysis of 708 peptides from 244 different proteins in one injection

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Pesticide biomarkers

- Solid phase extraction in 96-well plates – LC-MS/MS
  - 2,4-D 2,4-dichlorophenoxy acetic acid
  - MCPA 4-chlor-2-metylphenoxy acetic acid
  - HMCPA metabolite of MCPA
  - 2,4,5-T 2,4,5-trichlorophenoxy acetic acid
  - 3-PBA 10 of 18 pyrethroids
  - 4-F-3-PBA Cyflutrin
  - DCCA Permethrin, cypermethrin, deltametrin, cyfluthrin
  - 2,4,6-T Procloraz
  - TCP Chlorpyrifos
  - OH-T Thiabendazole
  - CCC Chlormequat
  - MQ Mepiquat

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Pesticide biomarkers

- Direct injection of hydrolysed urine in 96-well plates – LC/LC-MS/MS
  - ETU metabolite to ethylenbis-dithiocarbamates (mancozeb)

- Dervivatization prior to LC-MS/MS
  - 3,4-DCA metabolite to anilides, diuron, propanil
  - 3,5-DCA metabolite to dicarboximider, vinclozolin, iprodione, procymidon

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Percentage of pesticides above the LOD in different groups

Biomarkers	General population 2004-05 N=100	General population 2005-07 N=116	Vegetarianes 2005-06 N=40	Imigrants 2006-09 N=50
	%	%	%	%
MCPA	6	17	10	19
HMCPA	17	12	5	17
2,4,5-T	1	>LOD	>LOD	2
2,4,6-T	ND	ND	ND	68
2,4-D	94	62	90	81
3,4-DCA	27	34	22	54
3,5-DCA	99	91	95	98
3-PBA	19	1	25	62
ETU	98	96	87	96
TCP	ND	ND	ND	74
CCC	100	ND	ND	ND
MQ	100	ND	ND	ND

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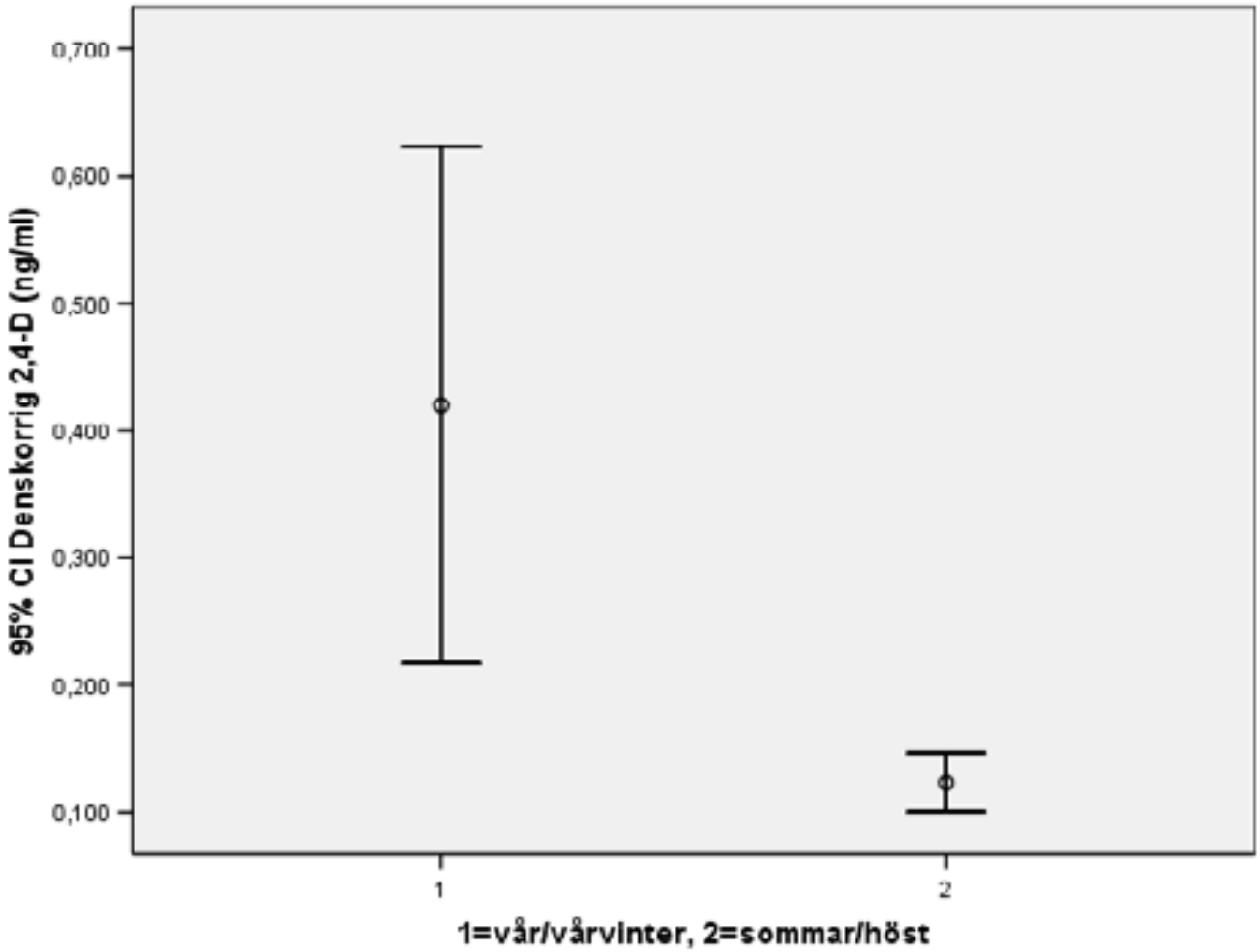
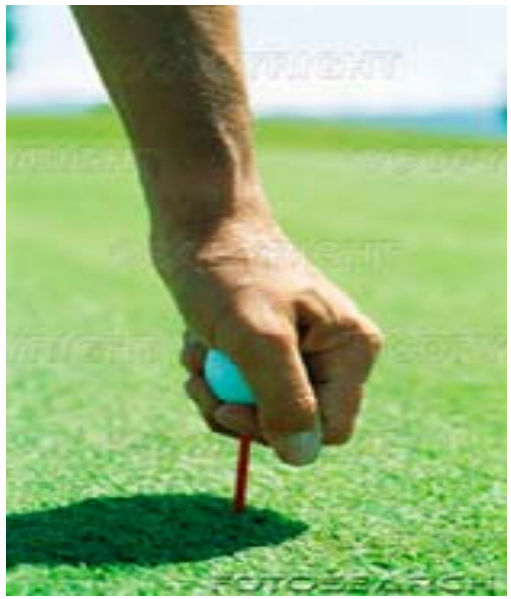
2,4-D during spring and summer/autum



Playing golf [medians (ng/ml)]

	Yes	No	P
2,4-D	0,28	0,12	0,01
3,5-DKA	2,9	1,1	0,05



Biomarkers of other environmental contaminants

- Direct injection of glucoronidas treated urine in 96-well plates – LC/MS/MS
  - Phthalates
    - monoethyl phthalate (MEP)
    - mono-n-butyl phthalate (MBP)
    - monobenzyl phthalate (MBzP)
    - mono-(2-ethylhexyl) phthalate (MEHP)
    - mono-(2-ethyl-5-hydroxylhexyl) phthalate (5-OH-MEHP)
    - mono-(2-ethyl-5-oxohexyl) phthalate (5-oxo-MEHP)
    - mono-(2-ethyl-5-carboxypentyl) (5-cx-MEPP)
    - mono-(4-methyl-7-hydroxyloctyl)phthalate (7-OH-MMeOP)
    - mono-(4-methyl-7-oxooctyl) phthalate (7-oxo-MMeOP)
    - mono-(4-methyl-7-carboxyheptyl)phthalate (7-cx-MMeHP)
  - Alkyl phenols
    - bisphenol A
    - nonylphenol
  - Cotinine

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Environmental contaminants in young Scanian men

Year	2000	2004	2006	2010	2013
Number	274 serum and urine	200 serum and urine	200 serum	314 serum and urine	200 serum and urine (or more?) Men and women
Compounds	PCB CB-153 p,p´-DDE Phthalates	PCB CB-153 p,p´-DDE Phthalates	PCB CB-153 p,p´-DDE PBDE, HBCDD	PCB CB-153 p,p´-DDE PBDE, HBCDD Phthalates Bisphenol A Nonylphenol PFCs	Phthalates Bisphenol A Nonylphenol PFCs Much more?
More	Determinants Sperm quality Biobank	Determinants Biobank	Determinants Biobank	Determinants Sperm quality Biobank	Determinants Biobank Sperm quality?

Sources for phthalate exposure

>90% softeners of plastics

Medical applications

Cars

Buildings

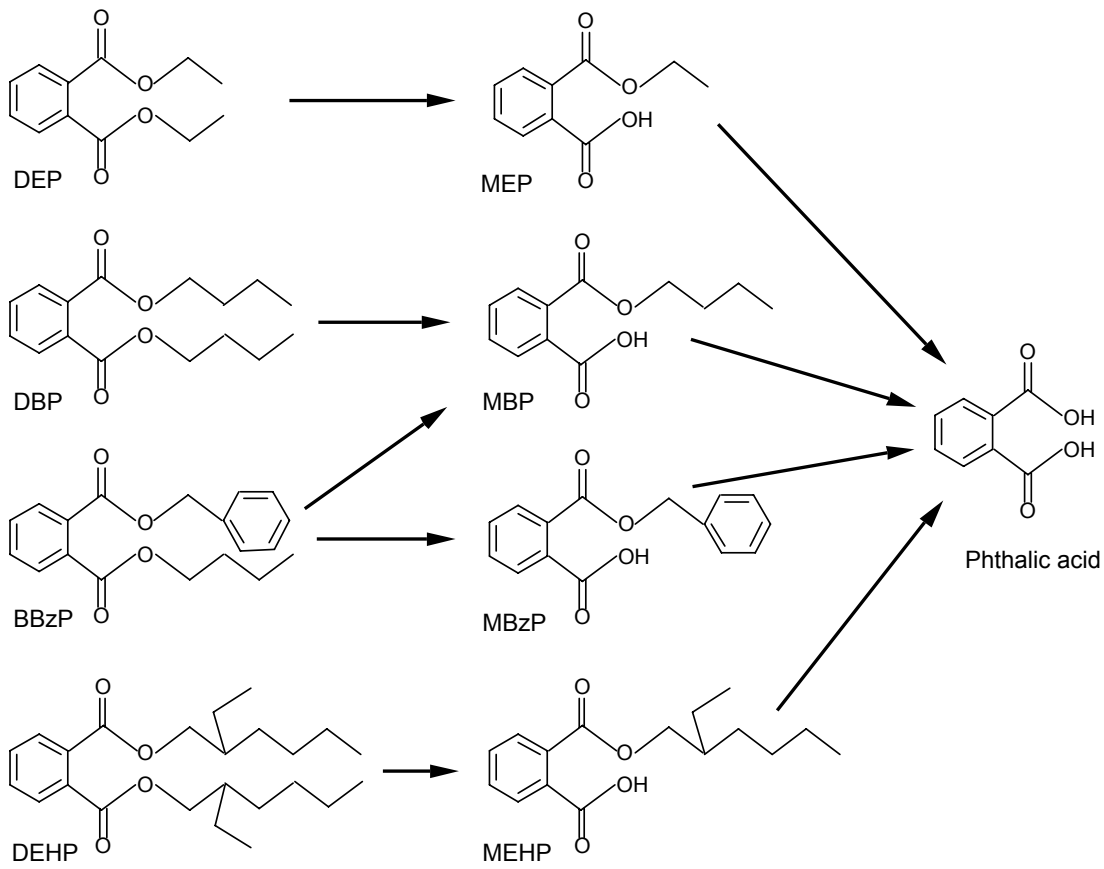
Floors

Cables

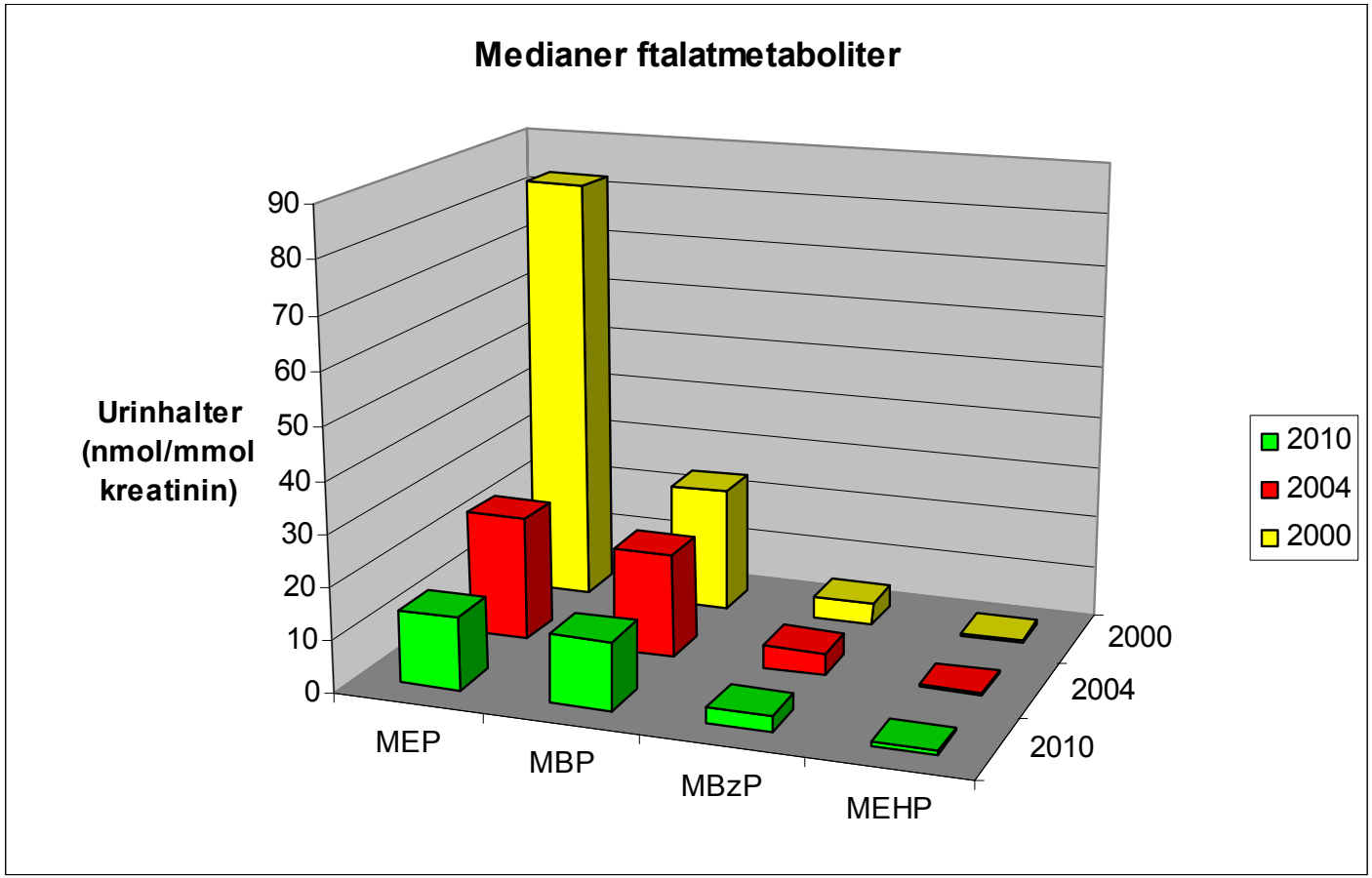
Sources of phthalate exposures

- Solvents in fragrances and hair care products

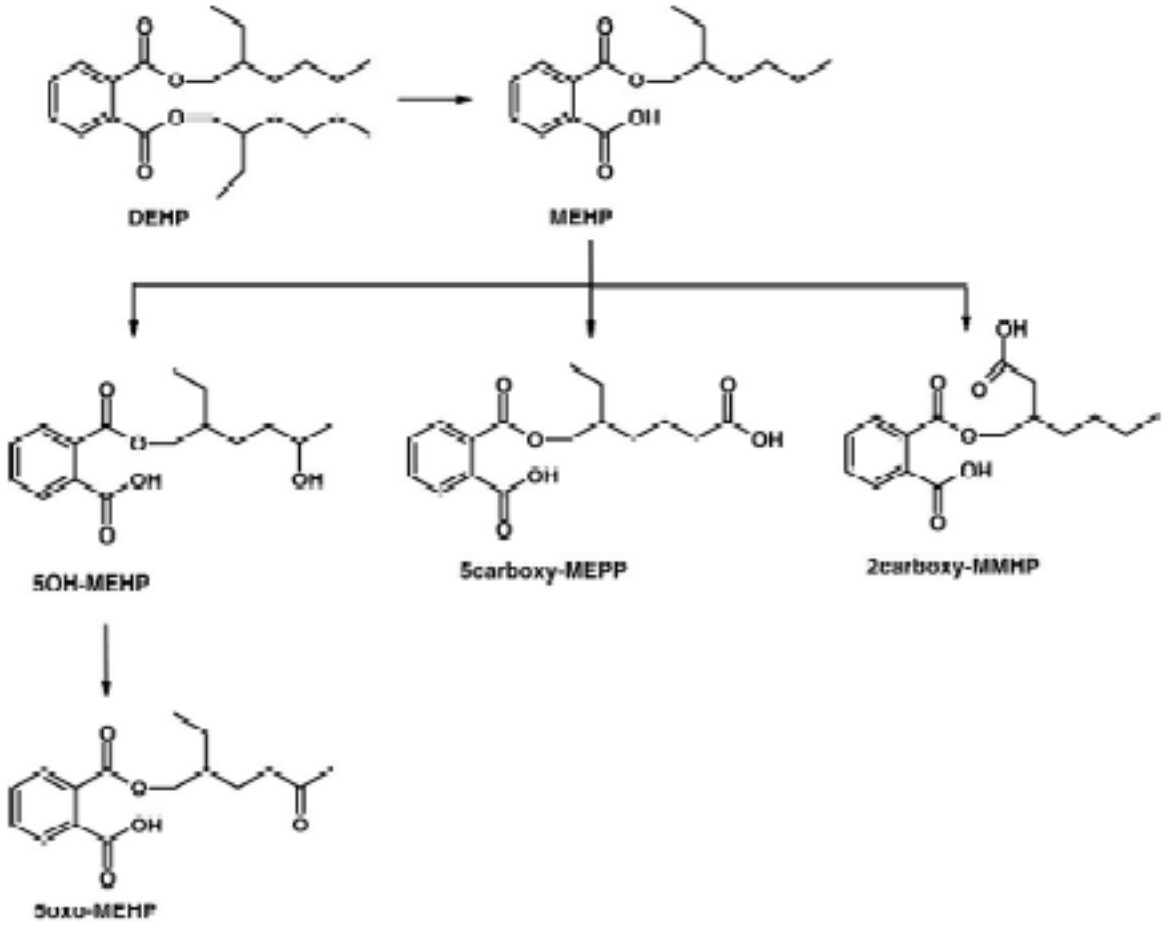
Metabolism phthalates



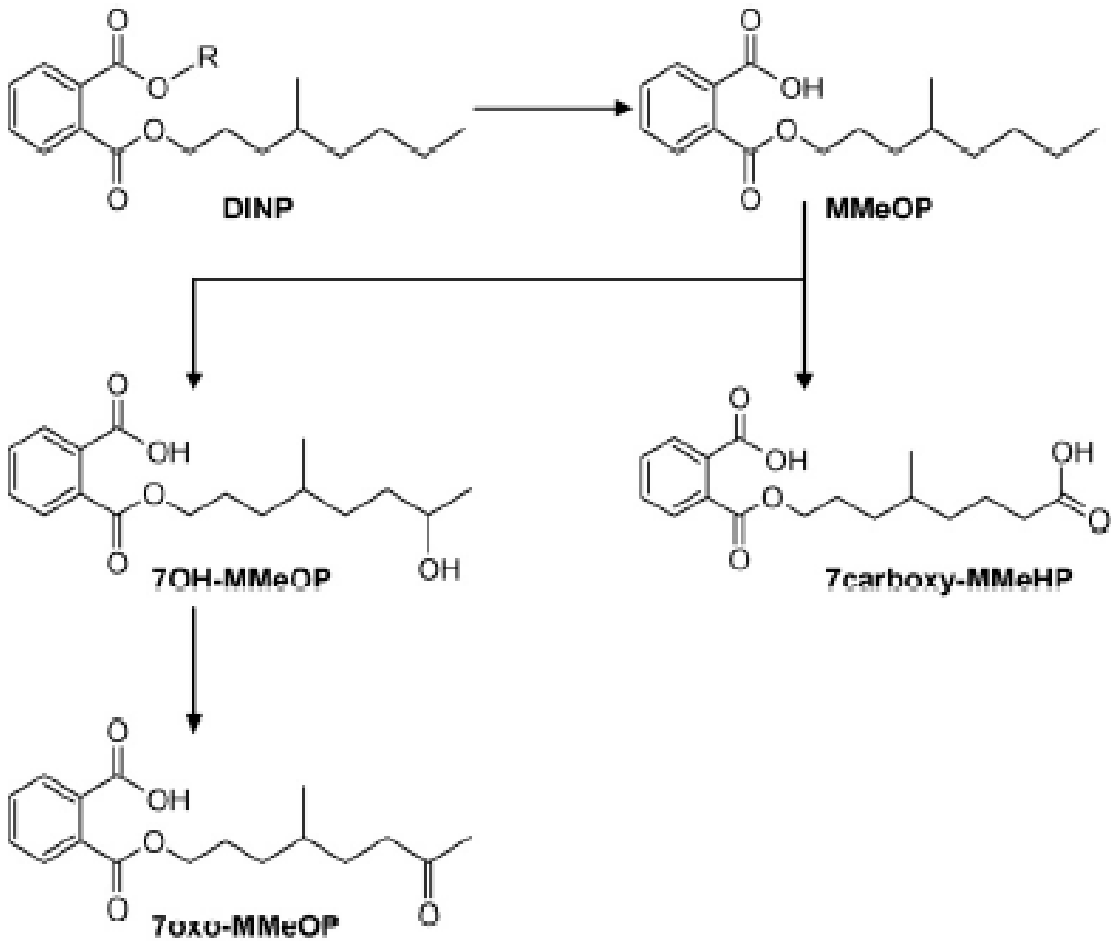
Phthalate metabolites 2000, 2004 and 2010



DEHP metabolism

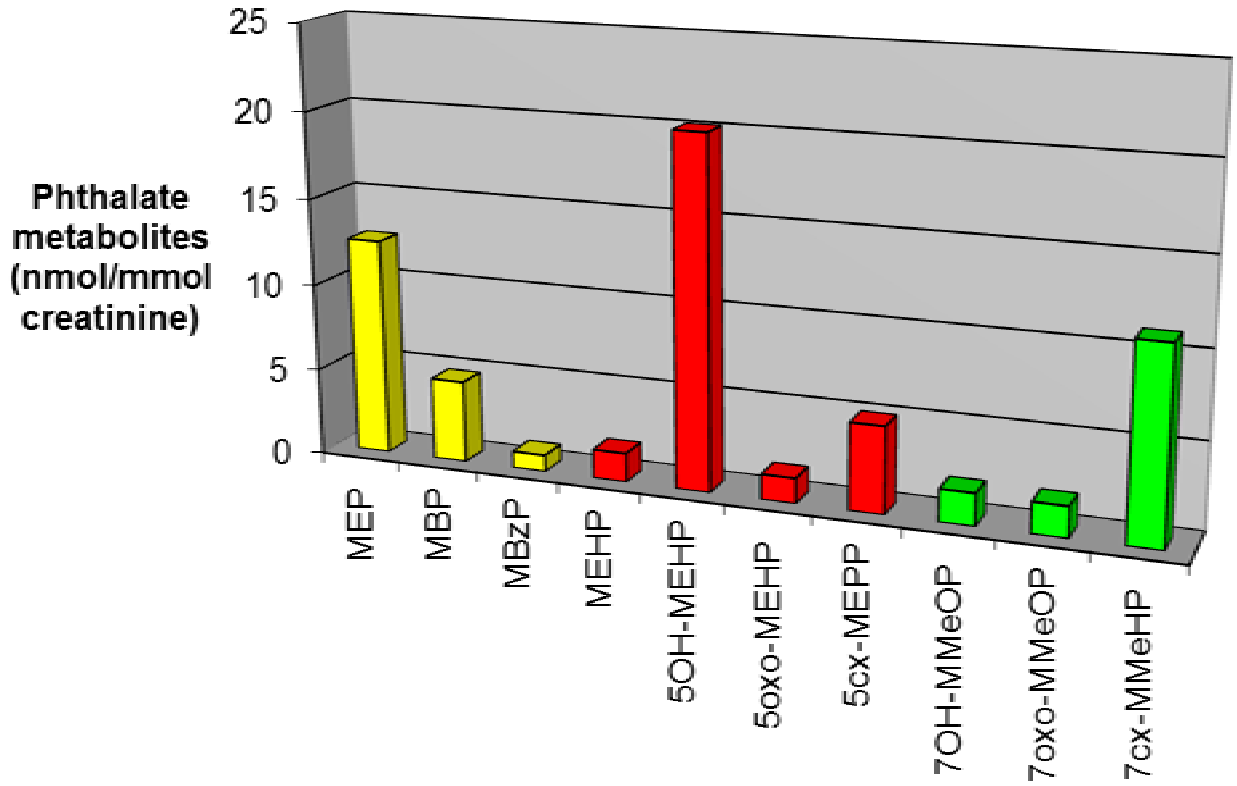


DiNP metabolism



R= iso-nonyl alkyl chain

More phthalate metabolites



Biobanks

- Possibility to screen for time-trends
- Possibility to study new contaminants in the past
- Possibility to perform prospective studies with samples from the past
- Seldom urine samples

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Pregnancy loss and phthalate exposure

- Urine from 128 women on day 10 after the first day of the last menstrual period before conception
- Phthalate metabolites for DEP, DBP, DBzP and DEHP analysed
- Subclinical embryonal loss was identified by repeated measurement of urinary hCG
- OR for subclinical embryonal loss (n = 32) was 40.7 (95% CI: 4.5, 369.5) for MEHP

Toft et al. EHP, 2012;120:458-63

Phthalates and PFOS in human amniotic fluid

- Amniotic fluid from 300
- Danish pregnancy-screening biobank
- Covering 1980 to 1996
- Analyses of oxidized phthalate metabolites, PFOS and cotinine by LC/MS/MS

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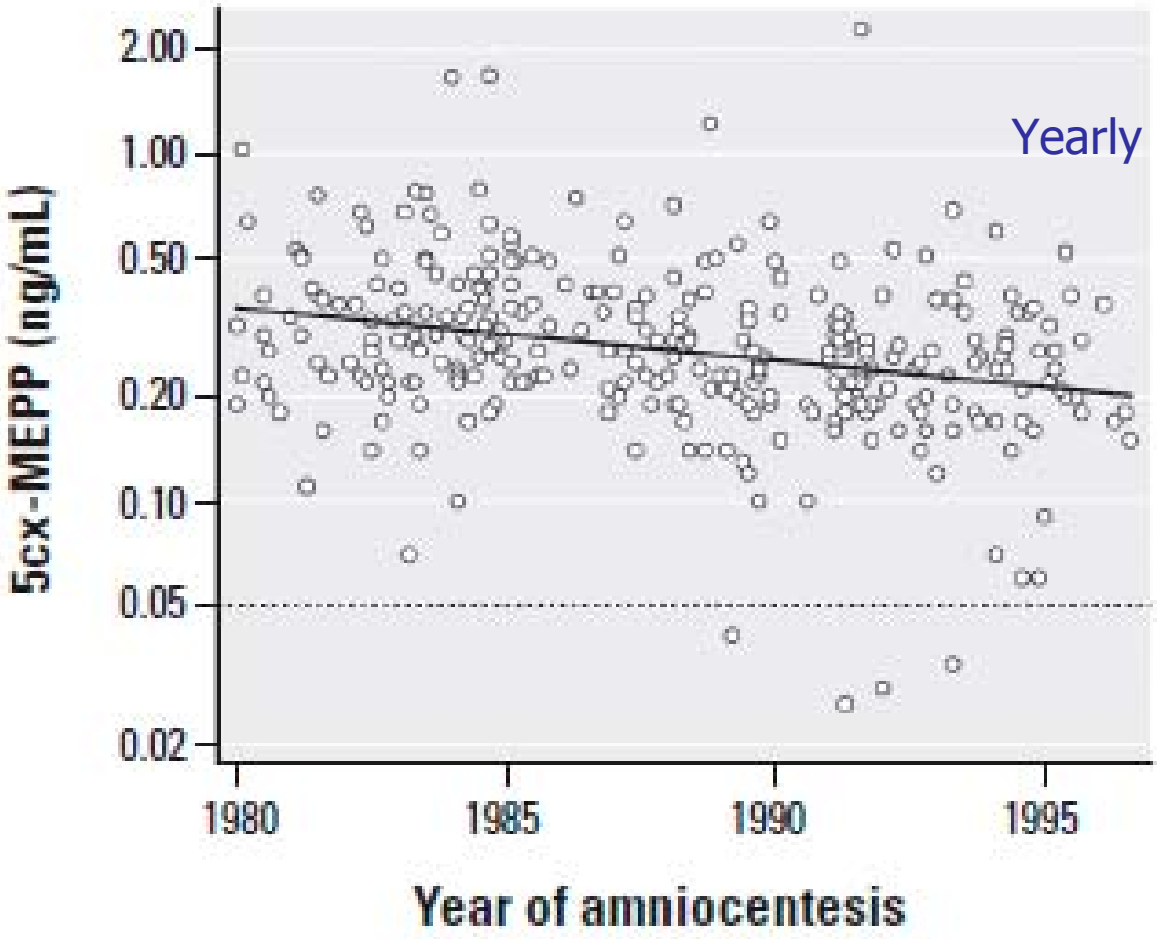
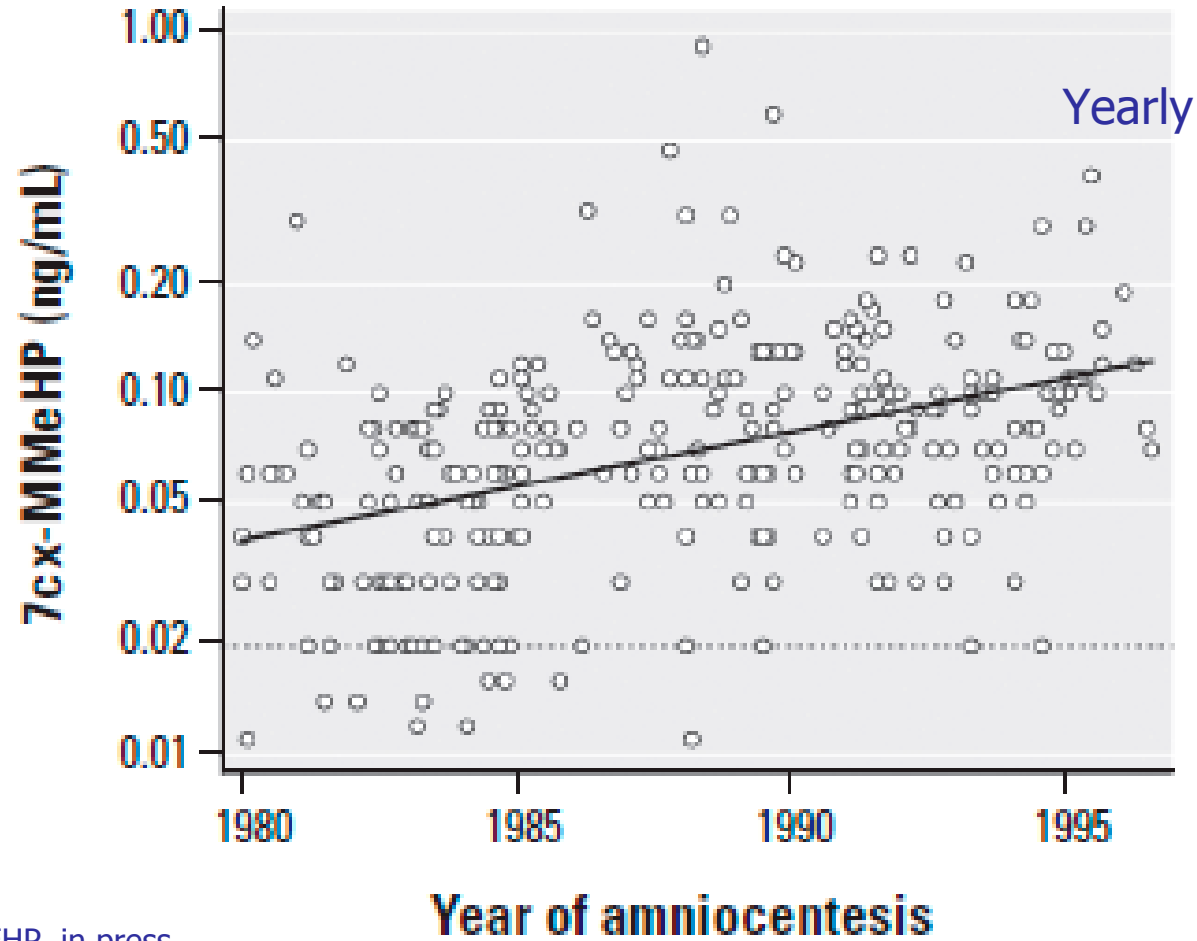
Time-trend of DEHP metabolite



Yearly decrease 4%

Jenssen et al. EHP, in press

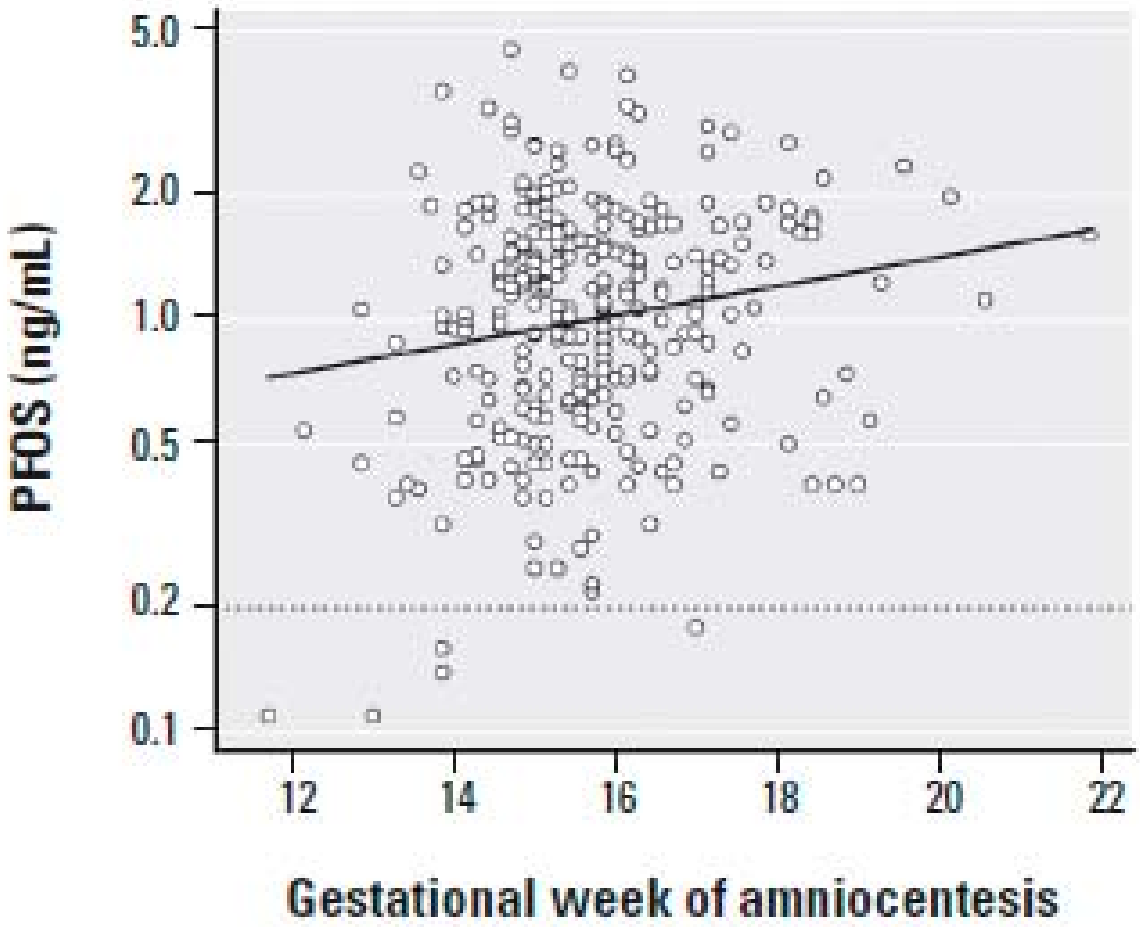
Time-trend of DiNP metabolite



Yearly increase 7%

Jenssen et al. EHP, in press

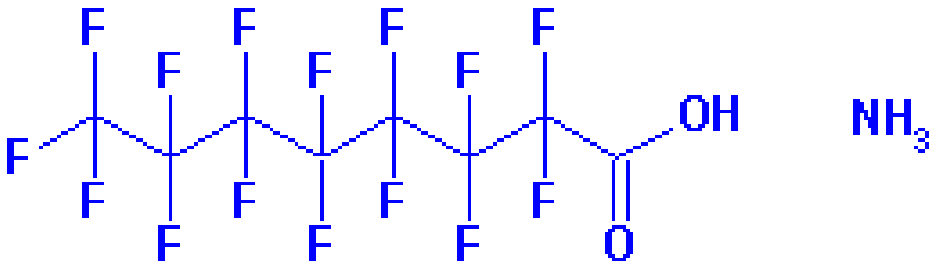
Relationship between gestational week and PFOS



Jensen et al. EHP, in press

Perfluorinated compounds (PFCs) in women between 1987 and 2007

- Biobanked serum samples from 80 women
- Sampled between 1987 and 2007
- Women born between 1934 and 1967
- Mean age 48 years (range 36-56)
- Analysis of 6 PFCs



Sources for exposure to PFC

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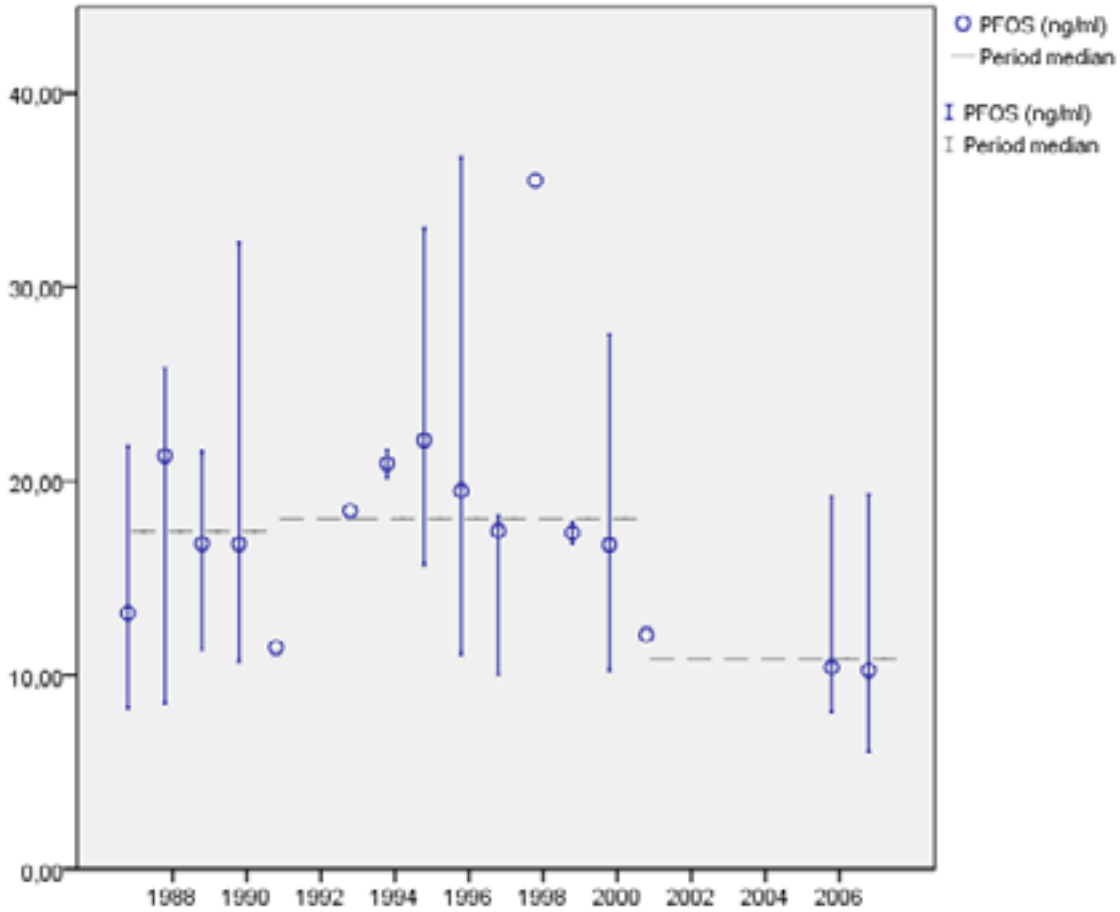
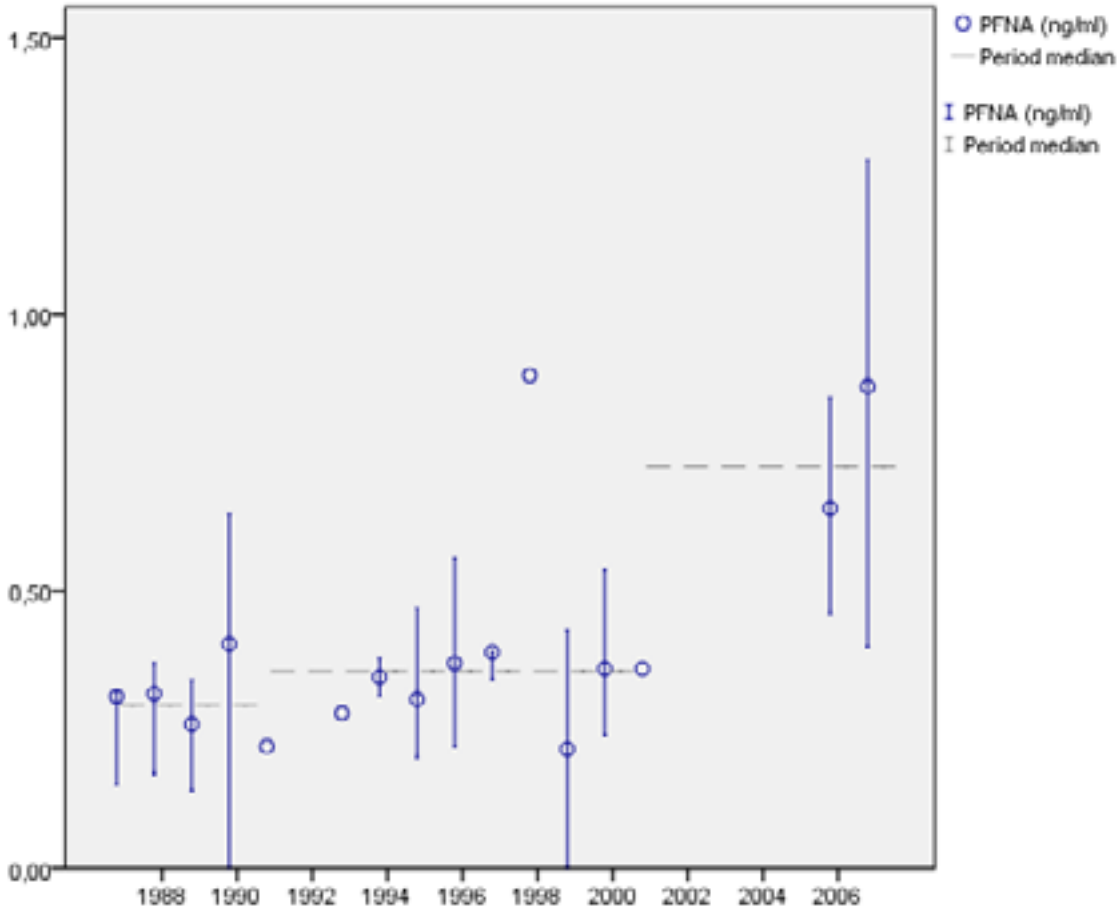
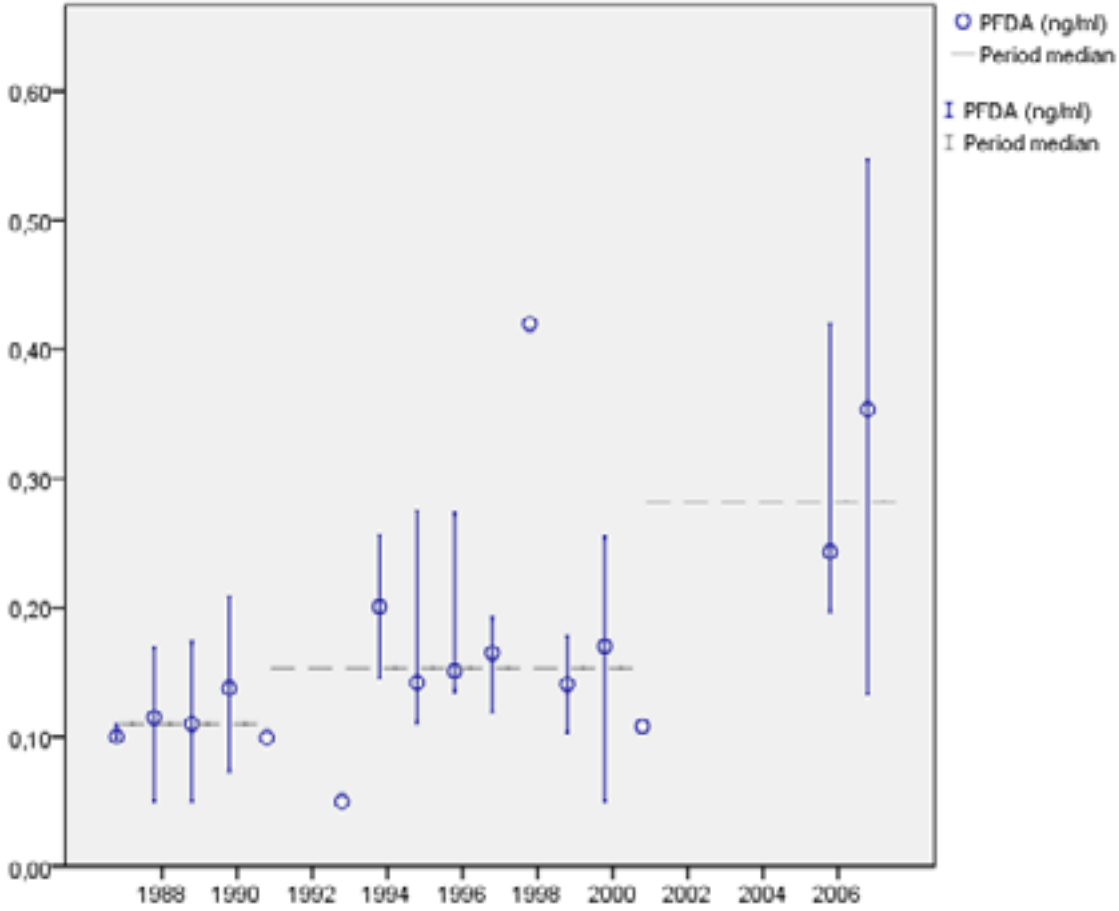
Time-trend for PFOS



Time-trend for PFNA



Time-trend for PFDA



Metals

- Possibility for untargeted screening of many different elements
- Targeted analysis of specific elements
- Time-trends from biobanks

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Metabolomics

- Untageted analysis using GC/MS or LC/MS
- Using the chromatogram as a fingerprint
- Analysis using e.g. principal component analysis (PCA) or related methods
- Mainly used for diseases but some data on time-trends
- Problem to identify substances using LC/MS since there are no good libraries

Adductomics

- Reactive compounds or metabolites
- Urine
  - E.g. mercapturic acids
- Hemoglobin
  - E.g. N-terminal valine
- Neutral loss LC/MS/MS
- IDA (information dependent acquisition)
- Still problem with libraries

Thank-you for listening